Breeding biology of the alpine swift *Apus melba* in Sofia, Bulgaria
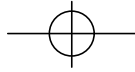
Anton Antonov and Dimitrinka Atanasova

Laying date, clutch-size and breeding success of alpine swifts *Apus melba* were compared over three seasons (1999–2001) in two colonies in the city of Sofia, Bulgaria. In order to study geographical variation, data were compared to the only long-term study of this species, carried out further north in Solothurn, Switzerland. The peak of laying occurred in the first half of May. Laying dates differed significantly between seasons. In 2000 swifts laid earlier than 1999 and 2001, with a more compressed laying period. The average clutch-size was 3.08 ± 0.55 eggs (n = 108) and it did not differ significantly among years. Breeding success was 1.85 ± 1.13 fledged young per pair and the main factor causing nestling mortality was falling outside the nesting cavities due to the limited space surrounding the nest. No relationship was found between clutch size and hatching rate or survival of the offspring. Compared to Solothurn, laying dates in Sofia were earlier and the laying period more protracted. Clutch-sizes were significantly higher than in Switzerland. Thus the alpine swift seems to follow the trend in swifts of laying larger clutches in the south of the range compared to the north. Despite earlier laying and larger clutch-size, breeding success in Sofia was lower than in Switzerland. This was attributed to higher nestling losses in Sofia due to the specific nest-situations.
Key words: alpine swift, *Apus melba*, clutch size, laying date, latitude.

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Aerial feeders and especially swifts depend on flying arthropods as a food source, whose abundance, spatial and temporal distribution is highly dependent on weather and ultimately on climate (Cucco & Malacarne 1996). As a result, the breeding of aerial insectivores is strongly influenced by climatic conditions and the laying dates, clutch-size and breeding success of swifts have been shown to follow annual variation of temperatures and rainfall (Lack & Arn 1947, Lack & Lack 1951, Cramp 1985, Cucco et al. 1992, Cucco & Malacarne 1996, Thomson et al. 1996). Cucco et al. (1992) suggested that for aerial feeders in the Mediterranean region these annual variations mostly affect laying dates while the clutch-size remains relatively stable, which probably results from the longer favourable period available for breeding. Further north in the temperate region, however, aerial feeders cannot post-

pone laying for long and annual weather variation affects mostly clutch-size rather than laying dates. Cucco et al. (1992) demonstrated that the clutch-size of pallid swifts *Apus pallidus* in northwestern Italy did not differ among years, while Lack & Lack (1951) showed that there was a marked annual variation of clutch-size in the common swift *Apus apus* at Oxford following the average daily maximum temperature in the second half of May. Furthermore, birds breeding at lower latitudes start nesting earlier, have longer breeding periods and are often multi-brooded compared with their conspecifics breeding in the north of the range. In most birds, clutch-size increases with increasing latitude (Lack 1947, Kulesza 1989). Given the strong dependency of aerial feeders on climate which in turn follows a latitudinal gradient, pronounced geographical trends in reproductive traits of swifts should also be expected.

Clutch-size in swifts, however, decreases with increasing latitude, which is a reversal of the general trend found in most bird species (Lack 1947, Cucco & Malacarne 1996). This trend is based largely on data on the common swift, which has an extensive range from Mediterranean to above the Arctic Circle (Cramp 1985) and for which several studies are available from different parts of the range. The peculiar trend of the swifts is thought to be the consequence of the more unpredictable aerial insect abundance further north due to sudden and prolonged cold spells (Lack & Lack 1951). Geographical trends in clutch-size and other reproductive features in the other two European species are not clear. Thus, the pallid swift, which is confined to southern Europe, tends to lay larger clutches near the northern boundary of its range than further south (Antonov & Atanasova 2001a). The alpine swift *Apus melba* is rather more widely distributed in southern Europe (Hagemeijer & Blair 1997) but extensive and long-term studies on its breeding biology only relate to some urban colonies in Switzerland (Lack & Arn 1947, Arn 1960). Very little information is available from other parts of the alpine swift's range to allow comparisons with Swiss data and with the other swift species whose breeding ecology has been studied more extensively (e.g. Boano & Cucco 1989, Cucco et al. 1992 for the pallid swift, Lack & Lack 1951, Carere & Alleva 1998 for the common swift).

In this paper we provide recent data on the breeding biology of the alpine swift in Sofia, Bulgaria. Laying dates, clutch-size and breeding success were recorded in two colonies with marked differences in numbers and nesting situation over three consecutive seasons. First, our aim was to explore the effects of season on the breeding parameters of alpine swifts and, second, to provide evidence for latitudinal effects on breeding parameters of this species in Europe by comparing data presented here with Solothurn, Switzerland (Lack & Arn 1947), which is further north in the species' range.

Methods

Study area and colonies

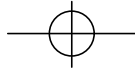The study was carried out from 1999 to 2001 in Sofia, Bulgaria (42°40' N, 23°20' E, altitude 580 m a.s.l.). Sofia was the first city in Bulgaria where the alpine swift formed urban colonies (Yankov 1983, Nankinov 1986). Since 1979, when it was first recorded, a rapid colonisation has been taking place linked both with establishment of new colonies and increasing density of existing ones (own unpubl. data). Observations were conducted at two colonies with substantial differences in numbers of breeding pairs and the situation of nests.

Most data were collected at colony A, the largest colony in the city and also the largest urban colony of alpine swifts in Bulgaria (own unpubl. data). During the study period it consisted of 75–80 pairs. It was situated on a 7-storey building covered with facing tiles. Swifts had their nests in the space between the external wall and the tiles but only 35 nests placed at ventilation apertures were visible from inside the building. Ventilation apertures were horizontal cylindrical holes in the top part of the wall (8 cm diameter and 16 cm length) beneath the tiles. A nest built there adjoined two vertical surfaces – the external wall beneath the tiles on the outside and a low wall (20–40 cm) from the inside loft. Additional data were collected at colony B in the eaves of a 5-storey building, a very different breeding situation where the nests were placed on a flat surface with plenty of space around. It held three breeding pairs in each year of the study.

Data collection and breeding parameters

Nests were visited daily or every other day during the laying period and also when estimated hatching and fledging dates approached. Colonies were visited twice a week when parents were feeding nestlings.

Mean air temperature and rainfall in May were obtained from the National Institute of Meteorology and Hydrology, Sofia, for the three years of the study in an attempt to explain seasonal variation in laying dates. Comparisons of laying dates and clutch-size among years and between colonies included only first clutches. The laying period was defined as the interval between the earliest and latest laying dates of first clutches at a colony or for a particular clutch-size in a season. Laying dates differed significantly among seasons (see Results), so in order to compare the laying dates of different clutch-sizes, they were standardised by subtracting the seasonal mean from each laying date. Replacement clutches were defined as those that followed the loss of the first clutches at the same nests.

Table 1. Laying dates of alpine swift *Apus melba* in Sofia, Bulgaria.

	Mean May temp.	May rainfall (mm)	Mean laying date ± s.d.	Earliest laying date	Latest laying date	Laying period (days)	n
1999	15.3	81	20 May ± 13.6	9 May	11 July	63	34
2000	16.4	12	9 May ± 4.6	2 May	25 May	23	38
2001	15.2	109	13 May ± 9.3	6 May	26 June	51	36
All years			14 May ± 11.4	2 May	11 July	70	108

We recorded hatching rate, survival of young in the nest, and overall breeding success. Hatching rate was the number of chicks hatched as a proportion of the number of eggs laid and survival of chicks in the nests was the number of chicks fledged as a proportion of the number of chicks hatched. Breeding success was recorded both as the mean number of fledglings per pair and per successful pair. Pairs that produced at least one 45-day old young were considered successful. We considered all chicks that disappeared before their 45th day as having died, since none of 48 young from the study colonies with exactly known age fledged before the age of 49 days (own unpubl. data).

Colony A suffered heavy predation in 2001 and most data on the breeding success of first clutches that year had to be omitted from the analysis. Only 8 nests that escaped predation by virtue of their inaccessibility were included in the calculation of total breeding performance. We did so because predation is an unusual cause of mortality in swifts, which normally choose nest-sites inaccessible to predators (O'Connor 1984). Hence,

comparisons of breeding success between seasons were performed only on 1999 and 2000 data.

The causes of breeding failure (excluding predation) and the stage at which losses occurred (eggs or chicks) were also noted. Nestling losses were categorised in the following way: (1) falling outside nesting cavities: the disappearance of chicks before their minimal accepted fledging age (45 days). They were not found inside the loft and no evidence of predation was recorded; some of these chicks were found dead near the building; (2) ungluing and collapsing of nests: this occurred at nests glued between the external wall and facing tiles without substantial support from below.

To investigate latitudinal variation in reproductive traits, data from the Sofia colonies were compared to the only European long-term study, which was carried out in Solothurn, Switzerland (Lack & Arn 1947). Both cities are situated at the foot of a mountain at a similar altitude (Sofia: 550 m a.s.l; Solothurn: 439 m a.s.l. but different latitudes (Sofia, 42°40' N; Solothurn 47°13' N).

Statistical procedures were performed using SPSS 10.0 software (SPSS/WIN Inc. 1999). None of the variables was normally distributed and could not be transformed to normal distribution, so non-parametric procedures were used to test between-year and between-colony differences. Means are reported with their standard deviations.
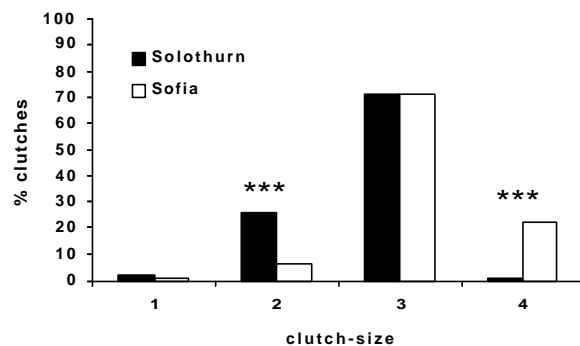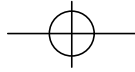

Figure 1. Clutch-sizes of alpine swifts in Sofia (n = 108) and Solothurn (n = 1070). Only first clutches are included and all the years are pooled. *** = P < 0.001, χ^2-tests.

Results

Laying date and clutch-size

The laying period of alpine swifts ranged from the beginning of May to the middle of August. The latest laying dates were found to be the result of genuine

Table 2. Laying dates of different clutch sizes by alpine swifts in Sofia, Bulgaria.

Clutch size	Mean laying date ± s.d.	Min.	Max.	range	n
2	+14.55 ± 16.26	−1	+52	54	9
3	−0.26 ± 8.15	−11	+44	56	77
4	−4.35 ± 3.41	−11	+1	13	20

Note: Data from all the three seasons were pooled. Each laying date is given as the signed difference from the respective mean laying date for the season in question. Laying dates differed significantly between clutch-classes (Kruskal-Wallis test $\chi^2 = 20.8$, df = 2, P < 0.0001).

second breeding attempts (Antonov & Atanasova 2001). The mean laying dates of first clutches varied significantly among the three years of study (Kruskal-Wallis test $\chi^2 = 37.130$, df = 2, P < 0.0001) and were earliest in 2000 (Table 1). Laying periods also differed among the seasons. In 2000 laying was not only the earliest but was also the most synchronised. This was the season with the highest mean air temperature and the lowest rainfall in May.

Clutches of alpine swifts in Sofia contained 1 to 4 eggs (Fig. 1). The mean size of first clutches was 3.08 ± 0.55 eggs (n = 108). It did not differ significantly between years (Kruskal-Wallis test $\chi^2 = 1.375$, df = 2, P = 0.503), nor did year have any effect on the frequencies of clutch-sizes ($\chi^2 = 2.08$, df = 2, P = 0.519).

First clutches decreased significantly in size during the course of the season ($r_s = -0.42$, n = 106, P < 0.001). Clutches of four eggs were initiated earliest with least

variation in their laying dates while clutches of two eggs were latest and the most variable in date (Table 2). Three-egg clutches were intermediate and on average were laid closest to the seasonal mean.

Most of the replacement clutches were recorded in 2001 and were induced by the heavy predation on colony A. The mean replacement clutch-size was 2.55 ± 0.51 eggs (n = 18). They contained either two eggs (n = 10, 55.6 %) or three eggs (n = 8, 44.4 %) and were significantly smaller than first clutches at the same nests (Wilcoxon Signed Rank test, T = 4.5, P = 0.013, n = 15). Of 16 replacements where the stage of original loss was known, the great majority were laid at nests that had lost chicks (13, 81.2 %); those following egg losses were much rarer (n = 3, 18.8 %).
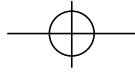
Breeding success

Over the period of study alpine swifts produced on average 1.85 ± 1.13 fledged young per pair overall and 2.31 ± 0.73 fledged young per successful pair (Table 3). The hatching rate was very similar between the 1999 and 2000 seasons (85.7 % v. 89.5 %, $\chi^2 = 0.09$, df = 1, n. s.), as was survival to fledging (72.2 % v. 60.6 %, $\chi^2 = 0.93$, df = 1, n. s.). No significant difference was found between seasons in the mean number of young per pair (Mann-Whitney test, U = 520, P = 0.257).

Hatching rate was highest in 3-egg clutches and lowest in 2-egg ones (Table 4) but it did not differ significantly among clutch sizes (Kruskal-Wallis test, $\chi^2 = 2.884$, df = 2, P = 0.236). Clutches of two, three and four eggs did not differ significantly in survival of young in the nest ($\chi^2 = 2.03$, df = 2, P = 0.36) or in the mean num-

Table 3. Breeding success of alpine swifts in Sofia, Bulgaria.

	No. of breeding pairs	No. of success ful pairs	Eggs laid	Eggs hatched	No. of young fledged	parameters				
						No. of unsuccessful pairs	% hatching	% fledging	Fledged/ pair	Fledged/ successful pair
1999	35	25	105	90	65	10	85.7	72.2	1.86	2.60
2000	35	29	105	94	57	6	89.5	60.6	1.63	1.96
total [a]	81	65	245	218	150	16	88.9	68.8	1.85	2.31

[a] Data on 11 nests from 2001 are also included (8 nests from colony A and 3 nests from colony B)

Table 4. Hatching rate and survival of nestling alpine swifts from different clutch sizes.

Clutch size	No. of eggs laid	No. of eggs hatched	No. of young fledged	Hatching rate (%)	Survival of chicks in the nest (%)	No. of fledglings per pair
2	16	12	9	75.0	75.0	1.12
3	168	153	112	91.1	73.2	2.00
4	60	53	29	88.1	54.7	1.93

ber of fledged young per pair (Kruskal-Wallis test, $\chi^2 = 4.83$, df = 2, P = 0.089).

Swifts in colony B produced more young per successful pair than those in colony A. (3.00 ± 0.53, n = 8 v. 2.20 ± 0.73, n = 61; Mann-Whitney U = 105.5, P = 0.005).

Mortality factors

Predation was observed only at colony A. In 2001 most nests (28, 77.8 %) suffered heavy predation, probably by black rats *Rattus rattus*. Mortality factors reported below do not include predation.

Of 59 dead chicks whose cause of mortality was known, 53 (89.8 %) fell outside the nesting cavities and six (10.2 %) died after ungluing and collapsing of two nests. There was no evidence of substantial chick starvation in the study colonies during the period of study. Distribution of chick mortality showed that most losses occurred at age 21–30 days but those happening at 11–20 days were also frequent (Fig. 2).

Comparison of breeding parameters with Swiss data

Laying dates in Sofia were earlier than those recorded in Solothurn. The earliest mean laying date recorded for 13 seasons in Switzerland was 21 May (laying period 11 May – 4 June), compared to 9 May in the present study. Swiss birds also had less variable and more synchronous laying periods. The mean duration of the laying period over five seasons in Solothurn was 24.5 ± 3.51 days (range 23–28) whilst in Sofia the laying periods in 1999 and 2001 were more than twice as long as in 2000 (Table 1) and also twice as long as the Solothurn mean.
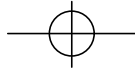
Clutch size was significantly larger in Sofia than in

Switzerland (3.08 ± 0.55, n = 108 v. 2.65 ± 0.54, n = 1070; z-test, z = 14.29, P < 0.001). This reflects the different proportions of clutch size classes in the two places ($\chi^2 = 158.25$, df = 2, P < 0.001). In Sofia 2-egg clutches were significantly rarer than in Solothurn ($\chi^2 = 17.35$, df = 2, P < 0.001) and 4-egg clutches were more abundant than expected ($\chi^2 = 140.69$, df = 1, P < 0.0001). Clutches of 3 eggs were the most common in both places and did not show a significant difference ($\chi^2 = 1.65$, df = 1, n. s.). In both Sofia and Solothurn clutch-size decreased during the course of the season.

The mean replacement clutch-size in Sofia was significantly larger than in Solothurn (2.55 ± 0.51, n = 18 v. 2.09 ± 0.52, n = 33, respectively; Mann-Whitney test, U = 174, P = 0.04).

Although alpine swifts in Sofia laid larger clutches, their breeding success (1.85 fledged young/pair) was lower compared to Solothurn (2.01 fledged young/pair). Hatching rate was slightly lower in Sofia (88.9 %) than Solothurn (94.4 %) but the difference was not significant ($\chi^2 = 0.71$, df = 1, P = 0.40). Thus lower

n = 64
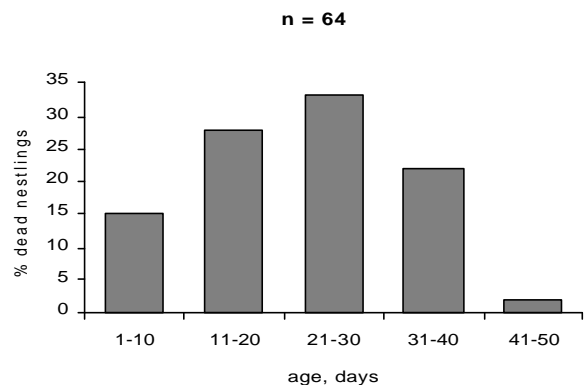
Figure 2. Nestling mortality of alpine swifts in relation to age (n = 64).

overall success in Sofia could be accounted for mostly by differences in nestling survival, which approached significance ($\chi^2 = 3.68$, df = 1, P = 0.055).

Discussion

Laying date and clutch-size

There were significant annual differences in the laying dates of alpine swifts in Sofia that were partly explained by the mean May temperature and amount of rainfall. With only a few years' data, however, the relationship could not be quantified. The long-term study on the same species in Solothurn also recorded significant annual differences in the onset of breeding which co-varied with the weather in May. Among-year differences are also known for the other European swift species (Lack & Lack 1951, Cucco et al. 1992) and other aerial feeders, e.g. house martins *Delichon urbica* (Bryant 1975) and barn swallows *Hirundo rustica* (Banbura & Zielinski 1998).

Although we have only three years' data, our results for the alpine swift seem generally to support Cucco et al.'s (1992) hypothesis that aerial feeders show little annual variation in clutch-size in southern Europe. The more southerly Bulgarian population had a very stable clutch-size over the study years, whereas Lack & Arn (1947) reported significant annual variation in clutch-size in Switzerland, though they did not test it statistically.

Clutch-size decreased significantly during the course of the breeding season. This phenomenon has been recorded in all the breeding studies on European swift species, on other aerial feeders and also on other temperate bird species, both passerine and non-passerine (Perrins & Moss 1975, O'Connor 1984). The seasonal decrease in clutch-size in swifts has commonly been attributed to young birds, which usually lay smaller clutches, breeding later in the season (Lack & Arn 1947) and also to the presumed deteriorating food supply as the season progresses (Lack & Lack 1951). No data were available on the ages of alpine swifts in Sofia but most clutches of 2 eggs were laid in newly built nests which were probably first clutches of first-time breeders (own unpubl. data). The decreasing food supply seems a less likely explanation since Cucco & Malacarne (1996) found that aerial arthropod abundance in

northwestern Italy was high and relatively stable from June through September, decreasing sharply only in November. Breeding later in the season, however, also imposes energy costs on parents due to the increased likelihood of overlap between reproduction and moult (Siikamäki et al. 1994).
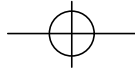
Breeding success and mortality factors

In the two years of available data, there were no obvious differences in breeding success between years, nor among broods of different sizes. Our data do not contradict the general pattern that breeding success of swifts in southern Europe is less variable than in northern Europe (Lack & Arn 1947, Lack & Lack 1951, Cucco et al. 1992, Thomson et al. 1996).

Nearly all chick losses resulted from chicks falling outside nesting cavities in colony A, suggesting that the nest situation affected the risk of falling. Each nest was surrounded by a very narrow level space adjoining two vertical surfaces (see Methods) that was not large enough to accommodate a brood of three grown chicks. Nests became progressively cramped as the young grew, so that some chicks were forced to stay outside the nest attached to the wall and thus more likely to fall. The observed peak in nestling mortality (21–30 days; Fig. 2) coincided with the period of increased nestling mobility, when the young were not yet fully proficient at crawling on vertical surfaces (own unpubl. data). In the pallid swift the peak of nestling mortality (40 % of losses) was at 1–10 days, lowering progressively with age (Malacarne & Cucco 1991). The same trend might well be expected in the alpine swift but the difference in age distribution of chick losses in the current study suggests that some extraneous factor was involved. Indeed, pairs breeding in colony B, nesting in the eaves with more extensive level surfaces available, produced significantly more fledged young.

Geographical trends in reproductive traits

Alpine swifts in Sofia laid earlier than their Swiss counterparts, as expected from the trend of earlier onset of breeding in the south of a species' range and later in the north. Further, the breeding season was longer in Sofia where, in addition, some pairs reared second broods (Antonov & Atanasova 2001b).

Clutch-size was significantly larger in Sofia compared to Solothurn, suggesting that clutch-size decreases with increasing latitude in this species. Such a reversal of the common trend of clutch-size observed in other birds is known for common swift and is attributed to its aerial feeding ecology (Lack & Lack 1951, Cucco & Malacarne 1996). More studies from other parts of the alpine swift's range are needed to substantiate this, however, because reproductive traits in swifts are known to be highly influenced at a small scale by local climatic conditions, which might obscure geographical trends (Cucco et al. 1992).

Alpine swifts in Sofia laid a significantly greater proportion of 4-egg and smaller proportion of 2-egg clutches than in Switzerland, whereas the frequency of 3-egg clutches did not differ. It is likely that the 3-egg clutch is the optimum clutch-size in the alpine swift since it was equally frequent at these two latitudes and, although not significantly so, was also the most productive in terms of fledged young per pair. Latitude and local conditions seem to influence the frequencies of more extreme clutch-sizes more strongly.
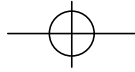
Given the earlier laying dates and larger clutch-size, a higher breeding success at Sofia colonies might have been expected but this was not the case. Hatching success was very similar between the two places but chicks had reduced survival in the nests in Sofia. We suggest this is probably a result of the specific situation of nests at our larger colony (see above), which increased the risk of chicks falling out of nest cavities. Nests in the Solothurn colonies seem to have been placed in safer situations as 80 % of them were built on flat surfaces in the eaves (Arn 1959), perhaps explaining the lower chick mortality there.

In conclusion, earlier laying dates, larger first clutches, a higher renesting potential and the occurrence of second clutches among alpine swifts in Sofia provide strong indirect evidence that feeding conditions for alpine swifts are more favourable there than in Solothurn. On the other hand, the breeding success of urban nesting alpine swifts may be locally limited by the choices birds make in positioning their nests.
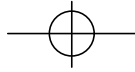
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Age-related changes in morphological characters in the pied flycatcher *Ficedula hypoleuca*

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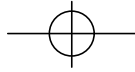
In birds, growth is usually assumed to have stopped when sexual maturity is reached, or even earlier when young birds become independent, after which few changes are expected. We tested whether male pied flycatchers *Ficedula hypoleuca*, in a Norwegian population studied over 15 years, showed age-related changes in four morphological characters: plumage colour, body mass, tarsus length, and wing length. Age-related changes were analysed for 321 males that were present in at least two consecutive years. Males became darker between their first and second year as adults (decrease of 1.1 Drost scores) and their wing length increased on average by 1.0 %, thus supporting findings of previous studies. Furthermore, body mass also increased (1.3 %), and, contrary to common belief, there was also a significant increase in tarsus length between the first and second year as adults (0.6 %). Changes occurring between the second and third years as adults were smaller, but at least wing length showed a significant increase of a further 0.3 %. Thus, pied flycatchers showed evidence of delayed growth in a number of morphological characters. On the other hand, there was no evidence of regression of morphological characters in old age. Males did not attain a duller plumage during their last years of life, nor did they decrease in body size or mass. Further, survival rate did not decrease significantly with age. This suggests that the physical condition of male pied flycatchers did not decline with old age. The results are discussed in relation to the short life span and hole-nesting habits of this species.
Key words: pied flycatcher, *Ficedula hypoleuca*, ageing, plumage colour, wing length, tarsus length.

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In a number of animal taxa, including birds, empirical evidence and theoretical models indicate that growth should occur until sexual maturity is reached, after which resources should be allocated to survival and reproduction (Kozlowski & Wiegert 1987, Roff 1992, Stearns 1992, Daan & Tinbergen 1997). In many species of birds, however, morphological changes also occur after sexual maturity has been reached. Delayed plumage maturation is well known, whereby the female-like plumage of young but sexually mature males may later develop into typical bright male coloration (Rohwer et al. 1980). Explanations of delayed plumage maturation focus on the benefits of female-like plumage in relation to predation and reduced social aggression for males that are at a temporary disadvantage in resource defence and mate attraction (Rohwer et al. 1980, Lyon & Montgomerie 1986, Rohwer & Butcher 1988, Slagsvold & Sætre 1991; but see Greene et al. 2000 for an example of high fitness of female-like males). Thus, delayed plumage maturation may represent an optimal trade-off between survival and reproduction.

Delayed growth in birds has been shown with respect to development of the flight feathers. Young, but sexually mature birds often have shorter wings than older birds (Alatalo et al. 1984 and references therein). This pattern has been explained by young birds being nutritionally stressed so that wing feathers cannot develop fully (Van Balen 1967), or that young birds have an optimal wing shape differing from older birds due to differences in flying experience (Alatalo et al. 1984). The size of secondary sexual characters may also increase up to several years after reproduction has started (e.g. Møller & de Lope 1999). On the other hand, the conventional view that body size in birds, especially when measured as skeletal size, does not change after fledging or independence, or at the latest once sexual maturity has been reached (Rising & Somers 1989, Alatalo et al. 1990, Daan & Tinbergen 1997, Gosler et al. 1998, Chinsamy & Elzanowski 2001), has been challenged in only a few studies (Smith et al. 1986). There is a possibility that the distinction between determinate and indeterminate growers (Daan & Tinbergen 1997) is not clear-cut, and that growth may occur during the reproductive phase of life even among birds, as is known among other vertebrate groups (see e.g. Ng et al. 1997, Wikelski & Thom 2000).

Individuals reaching old age may show signs of deterioration or regression of morphological characters, and such deterioration in physical condition is likely to be a manifestation of senescence. Senescence is an increase in mortality rates or a reduction in reproductive performance late in life (Rose 1991, Forslund & Pärt 1995, Holmes & Austad 1995, Martin 1995). A recent study of the barn swallow *Hirundo rustica* indicated that a number of measures reflecting individual performance (including morphological characters) deteriorated with age (Møller & de Lope 1999). In this species a secondary sexual character, wing length and migratory performance peaked at 2–4 years of age, and began to decline at 4–5 years of age. On the other hand, studies of other bird species have not found a decrease in old age in the size of morphological characters, especially wing length (Brown & Bhagabati 1998, Merom et al. 1999).
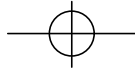
The likelihood of observing morphological changes in old age may be related to the life span of the species in question, provided that changes are linked to an ageing process. Lack (1954) suggested that mortality rates of many species of birds in the wild are so high that nearly all individuals die before the effects of ageing become apparent. This view has later been supported by others (e.g. Comfort 1979, Williams 1992). On the other hand, birds have a relatively long life span compared, for example, to mammals (except bats), and it has therefore been suggested that even relatively short-lived birds should show regular signs of ageing (Holmes & Austad 1995). The study made by Møller and de Lope (1999) concerned the barn swallow which has high mortality rates and, hence, a short life span.

Testing hypotheses dealing with long-term changes in traits is not straightforward. Simple comparisons of age groups cannot be used because of the possibility of trait-related differences in mortality and, hence, that the older age group may be a biased set of survivors. One therefore needs long-term studies of changes within individuals. We tested whether there were age-related changes in morphological characters in a population of pied flycatchers *Ficedula hypoleuca* that we have followed for 15 years. The pied flycatcher is a small passerine that winters in tropical Africa and migrates to Europe to breed. Males defend nest holes for breeding and usually return to the same area if they survive to the next year (Lundberg & Alatalo 1992). Yearly mortality rates are above 50 % and consequently few birds become more than five years old (Sternberg 1989, Lundberg & Alatalo 1992).

Some young pied flycatcher males have delayed plumage maturation (Lundberg & Alatalo 1992) which may be interpreted as a trade-off between mate attraction and survival (Sætre et al. 1994, Slagsvold et al. 1995). Further, young flycatchers have shorter wings than older ones (Alatalo et al. 1984). We tested whether these previously reported changes were apparent in our population, and we also tested whether tarsus length and body mass showed evidence for delayed growth.

In the pied flycatcher there is some evidence indicating that survival rates decrease with age (Sternberg 1989). In the closely related collared flycatcher *Ficedula albicollis* there is evidence for reduced reproductive performance of old females (Gustafsson & Pärt 1990), although such a pattern was not found in a study of pied flycatchers carried out by Sanz & Moreno (2000). There have been suggestions that old pied flycatcher males show signs of regression in plumage coloration (i.e. become duller; Winkel et al. 1970, Potti & Montalvo 1991). Thus, we also analysed changes of the four morphological characters (plumage colour, body

mass, tarsus length, and wing length) to test whether there was any evidence for a physical deterioration in old age.

Methods

Study area and material

The study was carried out during the breeding seasons of 1985–1999 in Sørkedalen near Oslo, south-eastern Norway. The study area consisted of three plots; Sinober, Tangen and Brenna (see Slagsvold et al. 1988 for a map). The Sinober plot consisted of mixed forest, Tangen was mostly coniferous and Brenna was deciduous forest. Males were captured and measured soon after arrival and were given a numbered metal ring and unique combinations of colour rings to permit identification of individuals in the field. Previously ringed males returning in later years were also captured with few exceptions. All unringed males appearing in the study areas were captured with only a few exceptions in the Brenna plot. A total of 1080 different males were captured and, of these, 338 were caught in at least two different years. However, due to a few cases of males not being recorded in one year (see below) or males having been present in one year without being captured, there were 321 individuals that had been caught in two or more consecutive years. Females were not included in the analyses due to high breeding dispersal in our population (low return rate; T. Slagsvold et al. unpublished data; see also Lundberg & Alatalo 1992).

Morphological measurements

The plumage colour of males was scored on a scale from black-and-white (score 1) to brownish and female-like (score 7; Drost 1936). Half-scores were used for intermediate types. Body mass was measured to the nearest 0.1 g with a Pesola spring balance and standardised to 0900 h (the regression line for body mass in relation to time of day increased with 0.043 g per hour). Tarsus length (with bent toes) was measured with calipers to the nearest 0.1 mm and wing length (flattened and straightened) to the nearest 0.5 mm. Mean values were used in cases of repeat measurements during the same year.

There were significant differences in mean measurements of some male characters between some of the 22 people that were involved in the field work during the 15-year study period. Since we have too few repeat measurements by two different persons of the same male in the same year to test rigorously for inter-personal differences in measurements, we relied on differences in mean values between different persons in the same year to correct values. Values were corrected for fieldworkers who had means significantly higher or lower than the overall mean and the mean of the person with the most measurements (TS, n = 573 measurements). Thus, tarsus length was corrected with 0.1–0.3 mm for two people (0.7 mm in the first year of field work for one of these who used a different measuring method in that year), wing length was corrected with 0.5–1.7 mm for 12 people, whereas measurements of plumage colour and body mass were not corrected due to small differences between people. Remaining differences in measurements between workers that were not corrected by the above method should not introduce systematic errors in the data set because, as far as we can judge, the identity of the person measuring each male was random with respect to male age. Thus, somebody with deviant measurements would have been just as likely to measure a specific bird before or after someone else, thereby removing any systematic effect on age-related changes. In addition, the consistency in measurement values of the same males in different years (see Fig. 3) when birds were often measured by different people, suggests that measurement errors were relatively small compared to between-male variation. Finally, the large sample sizes in many analyses will also reduce the problem that measurement errors can make it difficult to detect age-related changes of morphological characters.

Age determination

Male age was determined according to the method described by Karlsson et al. (1986) which is based on the colour and wear of the outermost greater wing coverts, the wear of wing and tail feathers and the colour of the inside of the upper mandible. Previously unringed males were thereby classified as first year (= second calendar year) or second year (= third calendar year), i.e. first and second year as adults. Of all unringed males, 52 % were classified as first year and 48 % as second
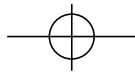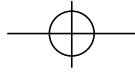
year (n = 988). We assumed that few males appeared in the study area for the first time as third year birds or older (see also Lundberg & Alatalo 1992). The return rate of nestlings ringed in our study area was too low to allow unbiased checking of the accuracy of age determination. However, among the 80 adult males that had been ringed as nestlings in the study area, 79 % returned in their first year, 16 % in their second year, and the remaining 5 % in their third to fifth year (some cases of late appearance in our study area may have been due to breeding dispersal of males that had been outside our study areas at younger ages). The proportion of males that were classified as second year was significantly larger for unringed males than for males ringed as nestlings ($\chi^2_1 = 19.92$, P < 0.001). This difference, however, need not indicate wrong age determination if locally recruited males tended to appear more often as first year birds than immigrants (cf. Pärt & Gustafsson 1989). We therefore tested the accuracy of age determination further within the sample of returning nestlings. This comparison gives an upper limit to accuracy since the field-

workers measuring the birds could have had some knowledge of which year specific ring numbers were used on nestlings. The age of 16 % of the males (13 of 80) was determined incorrectly. In particular, the ages of males that had not returned in their first year were classified incorrectly in 41 % of the cases, due to a strong tendency to classify birds with only a numbered metal ring as first year (80 % compared to 52 % for other birds, see above). In contrast, of the males that returned in their first year 90 % were classified correctly. Lundberg and Alatalo (1992) reported that they determined the age correctly for 90 % of the males when using the method of Karlsson et al. (1986). Thus, because male age may have been incorrectly determined for some birds, we checked overall patterns of age-related changes in morphology against the pattern shown by a restricted sample size consisting of males of known age (i.e. those that had been ringed as nestlings), of which 27 had been measured in two or more consecutive years.
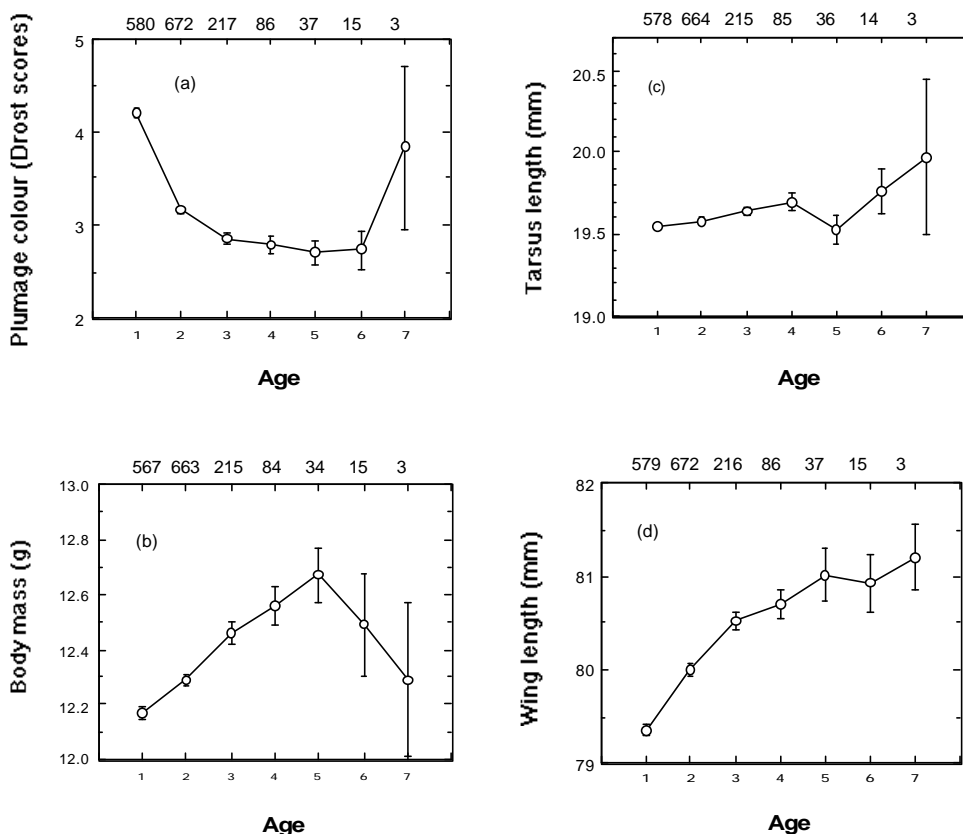
Figure 1. Mean values of morphological characters of male pied flycatchers in relation to age. (a) Plumage colour, (b) body mass, (c) tarsus length, and (d) wing length. Sample sizes are shown at the tops of the graphs. Vertical lines represent standard errors. Drost score 1 = black-and-white plumage colour (bright), score 7 = brown and female-like (dull).

Table 1. Correlations between plumage colour (higher values indicate decreasing plumage darkness) of male pied flycatchers and body mass, tarsus length and wing length.

Age class	Body mass		Tarsus length		Wing length	
	n	r	n	r	n	r
All ages	1584	−0.15***	1600	−0.04	1612	−0.19***
First year	567	−0.03	578	−0.09*	579	−0.04
Second year	663	−0.10*	664	0.03	672	−0.07
Third year	215	−0.07	215	−0.04	216	−0.05
Fourth year	84	−0.14	85	−0.12	86	−0.15
Fifth year	33	−0.21	36	−0.11	36	−0.25
Sixth year	15	−0.27	14	−0.28	15	0.00

* indicates $P < 0.05$ and *** indicates $P < 0.001$ without Bonferroni corrections; only the latter were significant with Bonferroni correction.

Survival rates

To assess changes in survival with age we analysed data on return rates. The use of return rate as a measure of survival is based on the assumption that males that did not return were dead. This assumption requires that all males in the study areas are discovered and that breeding dispersal is spatially restricted. These assumptions are nearly fulfilled in the Sinober and Tangen study plots where return rates of males were unusually high compared to other studies of pied flycatchers (Slagsvold & Lifjeld 1990). Males from the Brenna plot were excluded from analyses of survival, however, because a male not returning to this plot could not be assumed to be dead. This was because in some years (1) late-arriving males were excluded as all vacant nestboxes were closed before the end of migration, and (2) females were removed soon after settling, thus reducing the breeding success of males which may promote male dispersal (Greenwood & Harvey 1982, Slagsvold & Lifjeld 1990). In the present data set, 269 males in the Sinober and Tangen plots were recorded in at least two different years (mean 2.65 years), and in 259 of these cases the time series were continuous without 'holes' (e.g. not recorded in year y, but recorded in years $y - 1$ and $y + 1$). The very low frequency of discontinuous time series of males implies that estimates of survival based on the use of return rates will be almost identical to estimates based on capture-recapture models (Lebreton et al. 1992). Hence we used return rate as a measure of survival rate. Survival rate at age y was calculated as the number of males returning at age $y + 1$ divided by the number of males present at age y. The standard error of the survival estimate was calculated by assuming that survival rate was a variable with a binomial distribution (Sokal & Rohlf 1981).

Statistical analyses

Parametric tests were used throughout. Body mass, tarsus length and wing length were normally distributed, but plumage colour was log-transformed to obtain a normal distribution. All tests are two-tailed.

Results

Age-related changes: overall patterns

Analyses of overall changes in morphological characters indicated that plumage colour became darker ($r = -0.38$, $n = 1610$, $P < 0.001$) and that body mass, tarsus length and wing length increased with age ($r = 0.21$, $n = 1581$, $P < 0.001$; $r = 0.07$, $n = 1595$, $P = 0.007$; $r = 0.31$, $n = 1608$, $P < 0.001$, respectively; Fig. 1). Polynomial regressions were used to test for curvilinearity. This was apparent for plumage colour (first-degree term: $t = 15.33$, $P < 0.001$; second-degree term: $t = 10.91$, $P < 0.001$), body mass (first-degree term: $t = 4.63$, $P < 0.001$; second-degree term: $t = 2.28$, $P = 0.023$), and wing length (first-degree term: $t = 7.76$, $P < 0.001$; second-degree term: $t = 4.07$, $P < 0.001$), but
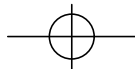
not for tarsus length (first-degree term: t = 1.04, P = 0.30; second-degree term: t = 0.26, P = 0.79). Analyses based on longitudinal data of individuals are used below to test whether the overall changes were due to selective mortality or changes within individuals.
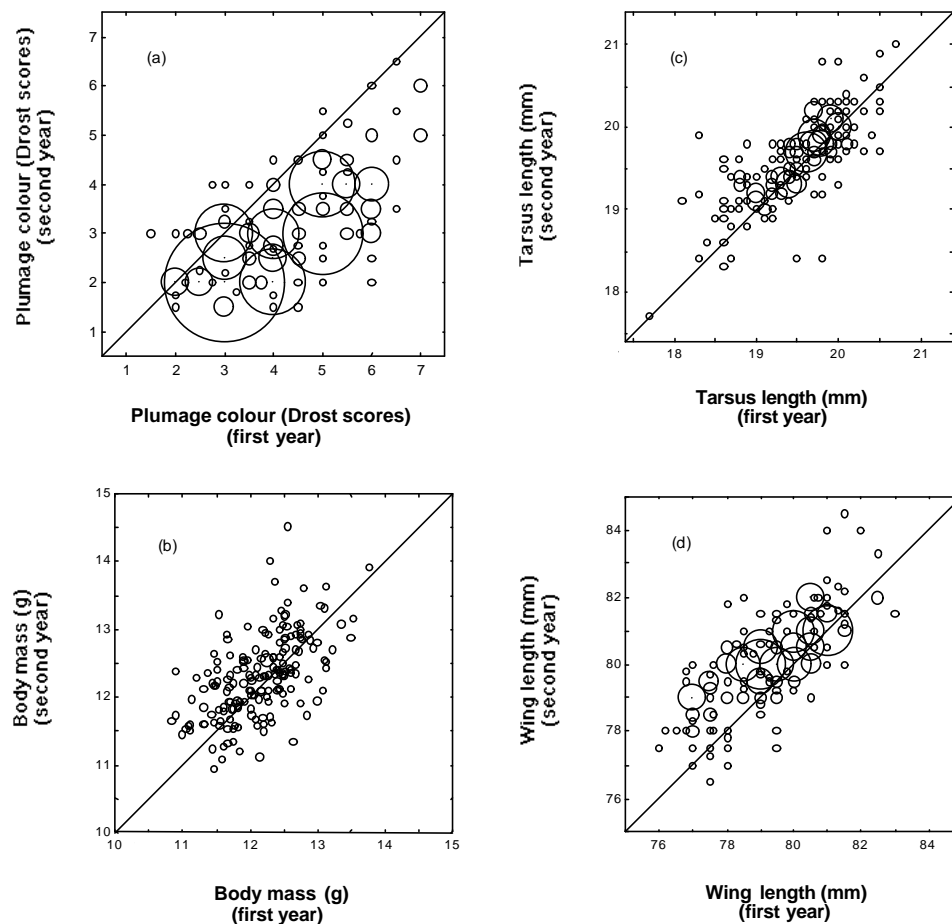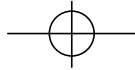
Correlations between characters

There were usually significant positive correlations between body mass, tarsus length and wing length both for all age classes combined and within age classes (P

< 0.05 in 14 of 21 tests). The mean correlation coefficient was 0.29 (range 0.13–0.51). Thus, birds with long tarsi were in general also heavy and had long wings. Correlations between plumage colour and body mass, tarsus length and wing length indicated that for all ages combined, dark males were heavier and had longer wings (Table 1). Within age classes there were generally negative correlations between plumage colour and body size characters (i.e. dark males were largest), but the correlations were mostly weak and non-significant (Table 1).
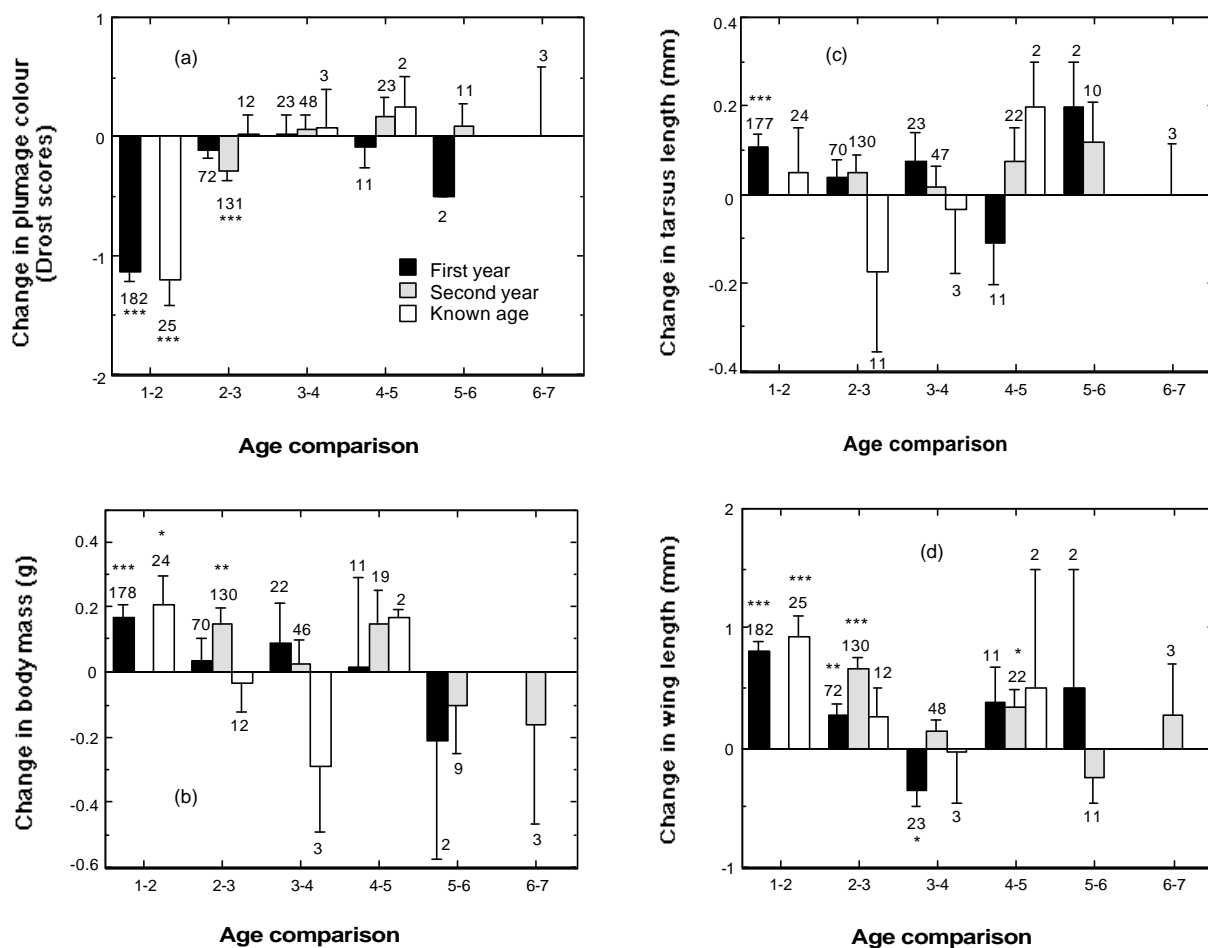
Figure 2. Mean changes in morphological characters between years for three groups of male pied flycatchers in relation to age: (a) plumage colour, (b) body mass, (c) tarsus length, and (d) wing length. The three groups of males are those that appeared in the study area in their first year as adults (= first year), those appearing in their second year (= second year), and those ringed as nestlings in the study area (= known age). Sample sizes are shown on top of the error bars. Vertical lines represent 1 s.d. Significance level of paired t-tests are shown as: * P < 0.05, ** P < 0.01, *** P < 0.001 (without Bonferroni corrections; only those with ** or *** were significant after Bonferroni corrections). Plumage colour is scored in such a way that negative changes indicate increased plumage darkness.

Figure 3. Correlations between measurements of morphological characters of male pied flycatchers in their first and second year as adults: (a) plumage colour, (b) body mass, (c) tarsus length, and (d) wing length. Larger symbols indicate overlapping data points.

Age-related changes: pairwise comparisons

Pairwise comparisons of measurements of the same individuals in subsequent years were used to control for possible biases in samples at different ages, e.g. due to differential mortality in relation to plumage colour and body size. The results showed that males became darker and larger between their first and second year (comparisons of birds appearing in the study area as one year old; Figs. 2 and 3). This group of males continued to become darker and larger when their second and third years were compared, but only the increase in wing length was significant (Fig. 2). At older ages there were only non-significant changes between years (Fig. 2).

Among males that appeared in the study area at two years old there were significant increases in plumage darkness, body mass and wing length from their second to third year as adults, but the increase in tarsus length

was not significant (Fig. 2). Further, the magnitude of these changes were larger than for those males appearing as one year old in the study area. Given the possibility that a proportion of these males were in reality only one year old when appearing in the study area (see Methods), the observed changes may have been partly due to changes occurring among this subset of males. Hence, significant changes between second and third year of life inferred from this comparison must be treated with caution. At higher ages there were only non-significant changes between years (Fig. 2).

The patterns reported above were compared to those for ringed nestlings, for which age was known with certainty. These analyses confirmed the changes occurring between the first and second year as adults (Fig. 2), except that the increase in tarsus length was not significant for this reduced sample size. The increase in tarsus length would have been significant at $\alpha = 0.05$ with a sample size of 296, i.e. less than our total sample size,
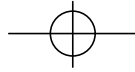
Table 2. Repeated-measures analyses of variance of morphological characters of male pied flycatchers in relation to age. Male group (appeared in the study area as one year old, appeared in the study area as two years old, or males of known age; see also Fig. 2) was included as a covariate. Tests of effects in the model are reported with F-values, degrees of freedom and corresponding P-values (upper part of table). Post-hoc t-tests of changes between different ages are reported with t-values and corresponding P-values (lower part of table).

Variance component	d.f.	Plumage colour		Body mass		Tarsus length		Wing length	
		F/t	P	F/t	P	F/t	P	F/t	P
Among individuals	335	4.47	<0.001	3.12	<0.001	6.11	<0.001	7.52	<0.001
Male group	2	0.48	0.62	0.88	0.41	0.92	0.40	1.00	0.37
Age	6	65.22	<0.001	9.30	<0.001	3.67	0.001	40.11	<0.001
1y–2y		−17.42	<0.001	3.80	<0.001	3.32	0.001	9.89	<0.001
2y–3y		−3.59	<0.001	3.31	0.001	1.23	0.22	6.94	<0.001
3y–4y		−0.40	0.69	1.43	0.15	0.55	0.58	0.19	0.85
4y–5y		0.64	0.52	0.91	0.36	−0.27	0.79	2.08	0.038
5y–6y		−0.14	0.89	−0.18	0.85	1.54	0.13	0.16	0.87
6y–7y		0.09	0.93	0.18	0.86	−0.22	0.83	0.51	0.61

given the observed magnitude of change. There were no significant changes between second and third year as adults although sample sizes were small (Fig. 2). The magnitude of the change in wing length was, however, similar to that of males assumed to be one year old when appearing in the study area. At higher ages sample sizes were too small to assess changes between years reliably (Fig. 2).

Repeated-measures ANOVA of all males present in two or more years with male category (i.e. appeared as first year, appeared as second year, and known age) as a covariate showed that there were significant changes both between one and two years of age and between two and three years of age for plumage colour, body mass and wing length (Table 2). The change in tarsus length between one and two years of age was also significant (Table 2). There is a possibility that the inclusion of a number of wrong age determinations might bias the changes between two and three years of age as noted above. However, there were no significant differences between the three groups of males in any of the analyses (Table 2), suggesting that this bias, if it is present, is small. Thus, based on all three groups of males, we conclude that males became darker and larger between their first and second year as adults, while there was a further increase in at least wing length, and possibly also plumage darkness and body mass, between their second and third year as adults.

Patterns of change through all ages for three groups of long-lived individuals were similar to those reported above (Fig. 4). Repeated-measures ANOVA with cohort group as a covariate showed that there were age-related changes for both plumage colour, body mass, and wing length (plumage colour: $F_{5,132} = 7.68$, $P < 0.001$; post-hoc t-tests indicated that the increase between ages 1 and 2 was significant at $P < 0.001$; body mass: $F_{5,126} = 3.59$, $P = 0.005$; post-hoc t-test of change between ages 1 and 2: $P = 0.044$; wing length $F_{5,131} = 12.34$, $P < 0.001$; post-hoc t-tests: change between ages 1 and 2: $P = 0.09$; change between ages 2 and 3: $P < 0.001$; change between ages 4 and 5: $P = 0.05$). For tarsus length, however, the effects in this small sample of males were not significant ($F_{5,130} = 1.54$, $P = 0.19$; post-hoc t-test of change between ages 1 and 2: $P = 0.15$).

Correlations between changes in characters

There was no evidence that changes in one character between years were correlated with changes in other characters (tests done for changes between first and second year (n = 177–182) and between second and third year (n = 197–202); $-0.11 < r < 0.14$, $P > 0.06$ in all of twelve correlation analyses).
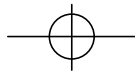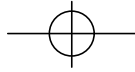
Changes in old age

In Figure 1 there seemed to be a decrease in plumage darkness and body mass at higher ages. However, changes between years at old age were not significant in pairwise comparisons (Fig. 2), suggesting that there was no regression in any of the characters we analysed. An alternative test is to compare measurements in the next-to-last year present in the study area with measurements in the last year present. In such comparisons we still did not find any significant decrease in plumage darkness or body size ($P > 0.08$ in all tests of males becoming at least 6 years old, n = 14). We also expanded this comparison to males present until at least 5 years old (n = 36) and to those present until at least 4 years old (n = 79), but no significant changes were found ($P > 0.10$ in all tests). Furthermore, we compared the changes occurring between the third year as adults (assuming that males were at a peak in performance at this middle age; cf. Møller & de Lope 1999) and the last year present in the study area. There was no evidence that any characters deteriorated (pairwise t-tests for males becoming at least four years old plus separate tests for those males becoming at least five years old or six years old). In fact, the only significant changes found were that wing length increased ($P < 0.015$ in each of three tests, although these tests were not significant after Bonferroni correction).
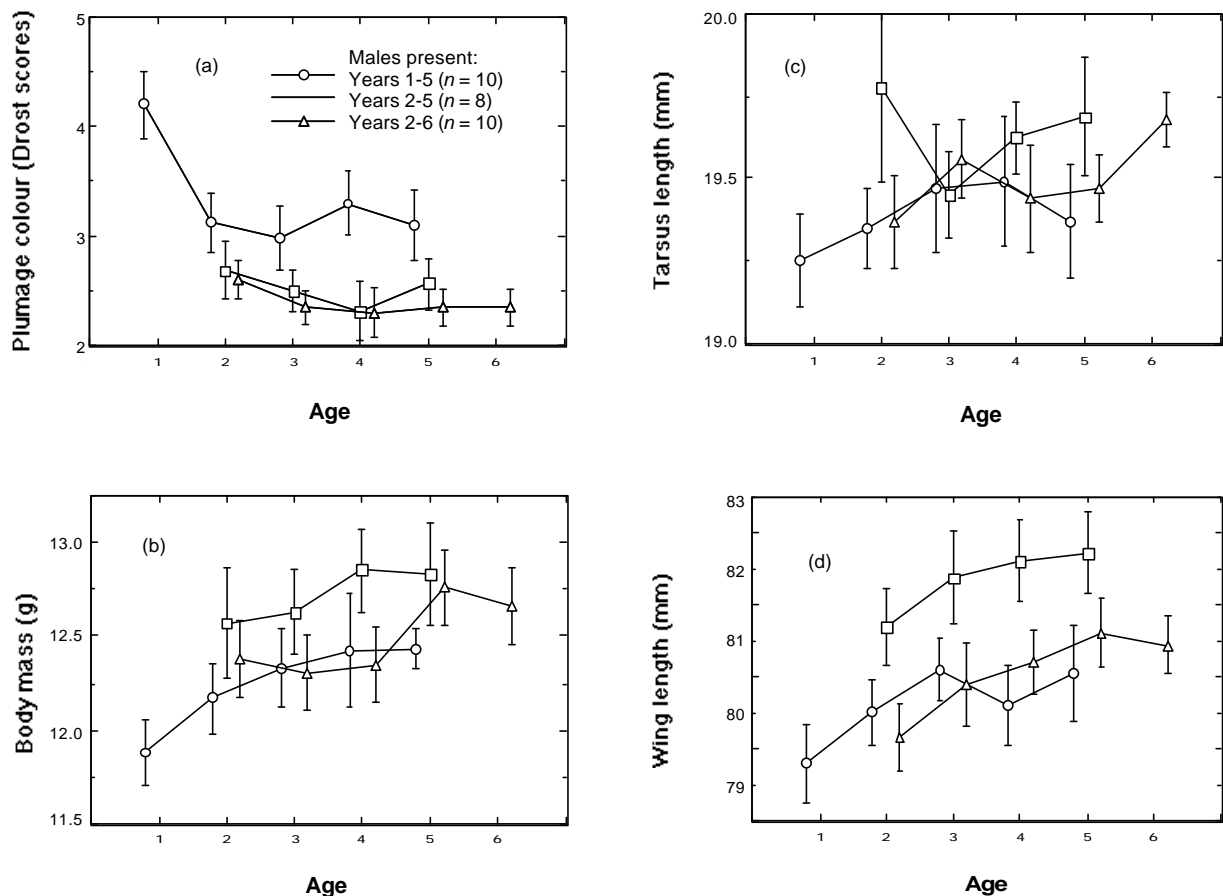
Figure 4. Mean values of morphological characters for three groups of male pied flycatchers in relation to age: (a) plumage colour, (b) body mass, (c) tarsus length, and (d) wing length. The three groups of males are those present at ages 1–5 (circles, n = 10), 2–5 (squares, n = 8), and 2–6 (triangles, n = 10). Vertical lines represent 1 s.e. Drost score 1 = black-and-white plumage colour (bright), score 7 = brown and female-like (dull).
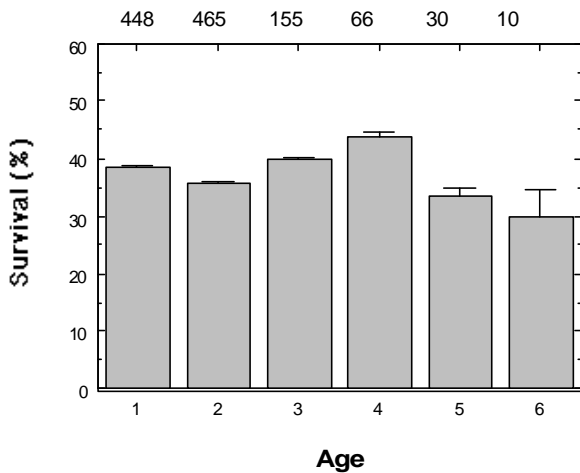
Figure 5. Survival rate (measured as return rate) of male pied flycatchers in relation to age. Sample sizes are shown at the top of the graph. Vertical lines represent 1 s.e.

Survival rates

There was no evidence that survival rate (measured as return rate) of males declined significantly with age (logistic regression with age as independent variable and return as dependent variable (n = 1174 observations): $\chi^2_5 = 2.71$, P = 0.74; Fig. 5).

There was no evidence for selective mortality in relation to morphological characters at any age (four separate logistic regressions with age and each of four morphological characters as independent variables, and return as dependent variable, including an interaction term between age and character (n = 1146–1172 observations): effect of age P > 0.46 in each analysis, effects of plumage colour, body mass, tarsus length and wing length: all P > 0.52, interaction term P > 0.38 in each analysis).
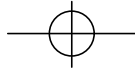
Discussion

In this study, we analysed age-related changes in morphological characters. For most birds, age was estimated since a low return rate of nestlings ringed in our study area meant that age was known with certainty for fewer than 8 % of males. Our analysis of age determination (see Methods) indicated that age may have been incorrectly classified for roughly 10 % of the males. One may ask whether such uncertainty in age would af-

fect our interpretations of the results. The most likely error is that some males recorded as appearing in our study area as two years old actually appeared when one year old. We believe that our conclusions are robust against some proportion of incorrect age determination for the following reasons. First, we analysed changes occurring at young age. Our findings that characters increased between first and second year as adults would not change even if we included many or all observations of changes occurring between the first and second year present in the study area for males assumed to have appeared in the study area as two years old. Plumage colour became darker and body size increased in paired comparisons of all individuals in their first and second year present (plumage colour, body mass and wing length: P < 0.001, tarsus length: P < 0.001, n = 307–313), and wing length increased between second and third year present (P = 0.002, plumage colour, body mass and tarsus length: P > 0.33, n = 116–120). Second, our analyses of changes at old age were done in several ways and with several different combinations of age classes. None of these analyses showed significant deterioration in any of the characters. Thus, we are confident that the patterns reported would not change even if we had known the exact age of every indivdual.

Changes at young age

We found evidence that full development of the four morphological characters (plumage colour, body mass, tarsus length and wing length) was delayed beyond the age of sexual maturity in male pied flycatchers. Our study confirmed previous findings that males are often duller and more female-like in their first year as adults (Lundberg & Alatalo 1992), and parallels changes in another plumage character, the white forehead patch (Dale et al. 1999). This delay in plumage maturation may be related to trade-offs between survival and reproduction (Slagsvold & Sætre 1991, Sætre et al. 1994, Slagsvold et al. 1995). Even though many first-year males, especially those that are dull-coloured, remain unmated under natural conditions they are clearly sexually mature as shown by experiments with releases of extra females, which led to nearly all males becoming mated and breeding (e.g. Dale & Slagsvold 1990, Sætre et al. 1995). Further, young and dull-coloured males also successfully fertilise eggs, as shown by DNA-fingerprinting (Lifjeld et al. 1997).

Our results also repeat the well-known pattern that young birds have shorter wings than older birds (Alatalo et al. 1984). However, we found that the increase in wing length continued even between the second and third years as adults, with tendencies for further increases even later in life. Thus, there is at least a two-year delay in the full growth of wing length. Such a pattern has rarely been documented in birds before (but see Brown & Bhagabati 1998, Merom et al. 1999, Møller & de Lope 1999). Our findings that even body mass and tarsus length increased after the first year as adults suggest an alternative to the explanation that shorter wings in young than old birds is due to differences in flying experience (Alatalo et al. 1984). The increase in wing length can be seen as a necessary consequence of the increase in body mass in order to avoid increasing wing loading. However, the two hypotheses are not mutually exclusive.
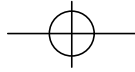
We found that tarsus length increased between the first and second years as adults. To our knowledge, this is the first study to show that tarsus length of a bird increases as late in life as after the age of sexual maturity. One previous study has reported increases in tarsus length (Smith et al. 1986), but these changes occurred before sexual maturity. The increase we documented was not very large (0.6 %), but statistically significant, and was not very different from the proportional increase in wing length (1.0 %). The increase in body mass may be directly related to the increase in tarsus length, and the finding that the proportional increase in body mass (1.3 %) was larger than that of tarsus length may be because tarsus length is a one-dimensional character whereas body mass may be viewed as a three-dimensional character. On the other hand, we expected that there would be positive correlations between changes in both tarsus length, body mass and wing length within individuals. We did not find evidence for this, but it is possible that measurement errors may have obscured significant positive correlations between such proportionally small changes in each character.

We suggest that at least part of the general increase in morphological characters after sexual maturity might be due to a primary increase in skeleton size (provided that tarsus length is proportional to skeleton size, see below) with secondary, allometric increases in body mass and wing length, although we cannot exclude the possibility of adaptive adjustments of the latter characters independently of tarsus length. Alternatively, selection might primarily work on body mass, in which case there may be secondary, allometric changes in tarsus and wing length. The proximate mechanisms for the increase in tarsus length may be both growth of cartilage and connective tissue (especially in joints which are included in our measure of tarsus length) and bone deposition. The relative contribution of these factors in the case of the pied flycatcher is not known. The ultimate question of why there is a delay in growth remains open to speculation. According to life-history theory, delayed growth may promote survival and this may represent an optimal strategy for young male pied flycatchers. Competition for nest sites in hole-nesting species is intense, and young birds may fail to find nest sites completely or only obtain inferior ones, so that attracting a female and breeding is uncertain (Lundberg & Alatalo 1992). In a German study, it was estimated that only 17 % of first-year males bred, compared to 64 % of older males (Sternberg 1989). Thus, young males may allocate more resources to survival at the expense of competitive ability through body size. The fitness consequences of different growth patterns is another question which would be the next issue to study. One could argue that the increases in wing length, tarsus length and body mass that we observed were small and might be trivial. However, even small changes in morphology may have significant costs and benefits and the differences in, for example, wing length between young and old birds have received wide attention from ecologists (see e.g. Alatalo et al. 1984), although they are of the same magnitude as the differences in tarsus length that we observed.

Changes in old age

We did not find evidence for regression in old age in any of the four characters we studied. Previous suggestions of decreasing plumage darkness (Winkel et al. 1970, Potti & Montalvo 1991) were not supported by our data. In a previous study, however, we found a tendency for the white forehead patch of male pied flycatchers to decrease in size in old age (Dale et al. 1999), although that character is not sexually selected in our population. Regression of sexually selected characters in birds has been reported only in the barn swallow where both the length of the outermost tail feathers and their degree of symmetry decreased among males at least five years old (Møller & de Lope 1999). Both barn

swallows and pied flycatchers are small tropical migrants with approximately the same life span, so a similar pattern might be expected.

Furthermore, wing length did not decrease at old age as found in the barn swallow by Møller and de Lope (1999). If anything, the wing length of old pied flycatchers was longer than that of younger birds (Figs. 2 and 4). Similarly, a study of reed warblers *Acrocephalus scirpaceus*, which is also a short-lived tropical migrant, did not find evidence for a decrease in wing length at old age (Merom et al. 1999). The tarsus length of pied flycatchers did not show any sign of decreasing in old age either. On the other hand, body mass dropped slightly at ages six and seven years, but the changes were far from significance. Thus, pair-wise tests did not indicate any evidence for regression of morphological characters (Fig. 2), and analyses of long-lived flycatchers likewise did not reveal decreases in old age in any of the characters (Fig. 4). One might object that our sample sizes at very old age were small so that we would be unable to detect changes. It is true that the statistical power of tests of changes at very old age was low, but we claim that the biological power of the analyses was high. We followed a population with a mean of 108 males each year over a period of 15 years, totalling 1080 different individuals. When no signs of regression in the four morphological characters could be detected in such a large sample, we argue that such changes, if they occur at all, are ecologically unimportant. Furthermore, had there been effects of the magnitude found in the study by Møller and de Lope (1999), this would have been easily detected with our sample size. Thus, there seems to be clear differences in patterns of morphological change in old age between the pied flycatcher and the barn swallow.

Decreased survival rates in old age have been found in many species of birds (Botkin & Miller 1974, Holmes & Austad 1995, Martin 1995). We did not find a significant decrease in survival for old birds although the rates for five- and six-year-old birds were lower than for younger birds (Fig. 5). Compared to other passerines the absolute values of survival rates for old birds were still relatively high in our population of pied flycatchers. However, sharper decreases in survival at old age have been found in other populations of this species (Sternberg 1989).
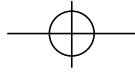
It is possible that most pied flycatchers die from extrinsic causes of mortality (e.g. predation) before they become old enough for intrinsic causes of mortality (such as senescence) to become significant (Lack 1954, Ricklefs 1998, 2000). Nevertheless, predation may still be the proximate cause of death, even though a reduced ability to escape predators due to senescence may be its ultimate cause. Provided that senescence is an important correlate of regressions of morphological characters at old age, we suggest that the short life span of pied flycatchers may be a reason for the lack of regression of the morphological characters we studied. This explanation does not explain, however, why effects of senescence were found in several characters (including morphological ones) in the study of Møller and de Lope (1999). Further studies are needed to assess whether pied flycatchers or barn swallows are more representative for short-lived birds in general regarding morphological change at old age.
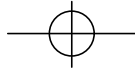
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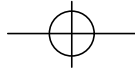
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The influence of hatching order on the thermo-regulatory behaviour of barn owl *Tyto alba* nestlings

Joël M. Durant

The behavioural responses of barn owl *Tyto alba* chicks to variation of ambient temperature were investigated in relation to hatching order. Four broods of wild barn owls were video-monitored during their postnatal growth. During the foraging trips of the adults, nest temperature and the behaviour of the chicks were recorded. The results show an influence of hatching order on the individual behavioural responses to variation in ambient temperature.

   The average temperatures at which the chicks showed specific thermal behavioural responses depend on the position in the size hierarchy. This result may indicate a difference in development among siblings in relation to the hatching order, possibly due to a difference in growth or tissue maturation associated with differences in food availability. That late-hatched chicks showed behavioural responses against cold and heat stress respectively at higher and lower temperature than their older siblings may be, in addition to being a consequence of food restriction, a way to divert part of their energy intake from muscle maturation to growth.
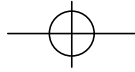
Key words: Nestling growth, thermoregulation, hatching asynchrony, raptor, behaviour.

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Adult birds maintain high body temperature in cold environments by the insulating properties of their plumage, particular behaviours such as huddling and communal roosting (Ancel et al. 1997), and a high capacity for thermogenesis. In nestlings, the capacity for thermoregulation is limited by constraints specific to young organisms. For example, compared to adults, chicks have a less favourable surface-to-volume ratio (Visser 1998) and their immature and underdeveloped muscles contribute little to thermogenesis (Hohtola & Visser 1998).

   In altricial birds, newborn chicks are poikilothermic, physically helpless and therefore dependent on parental care for heat, food, and protection. Normal development, shown by age at fledging and body mass, is achieved when these three factors are optimal. Heat transferred by brooding adults is necessary during incubation and the initial period of post-hatching growth, until homeothermy is achieved. Thereafter, homeothermy is maintained by thermogenesis and thus is dependent on parental food provisioning. However, heat transfer by brooding parents may alleviate the energetic costs of chick homeothermy. Chicks from large broods can benefit from the thermal inertia of their siblings (Westerterp et al. 1982), which decreases the amount of food required for thermoregulation, permitting parents to forage for longer periods (Clark 1982). The energetics of altricial chicks are difficult to characterise and such studies have therefore been done mainly in the laboratory. Hatching asynchrony complicates the issue by creating a size hierarchy amongst siblings (Newton 1979). A size hierarchy often results in

a slower growth or death of the last-hatched chick(s) due to sibling competition for food (Stoleson & Beissinger 1997).

In raptors, parents share the task of caring for the chicks. For some days after the eggs have hatched, generally asynchronously as most raptors begin incubation before they complete their clutches, the female broods the chicks and then starts hunting leaving the brood alone (Newton 1979, Taylor 1994). Because of their varying age, the consequence is that chicks are left unattended at different stages of development (Durant & Handrich 1998). The youngest chick is left unattended more often during its growth compared to its older siblings. Consequently, the younger and less developed chicks of a brood are confronted with more constraints, increased thermoregulatory cost and lower food intake, compared to their older siblings at the same age. In order to achieve normal growth and to fledge successfully, the younger chicks must be able to allocate resources differently from their older siblings (Nilsson & Svensson 1996). One hypothesis could be that the younger chicks allocate fewer resources to the maturation of thermogenic tissues than do their siblings. If this hypothesis is correct, there must be a difference in the behavioural responses to ambient temperature, in relation to hatching rank, between siblings at the same age.

To explore this problem, I measured nest temperature and recorded chick behaviour in broods of European barn owl *Tyto alba*. Barn owls have large broods, on average 4–5 chicks depending on food availability (Taylor 1994). The eggs hatch asynchronously at an average interval of 2.3 days (Durant & Handrich 1998), which creates a size hierarchy among the chicks (Roulin 1998). Body growth and plumage development are relatively slow for a bird of this size (Durant & Handrich 1998). The acquisition of homeothermy is dependent on the amount of energy available to the chicks and thus on food supply (Roulin 1998). Consequently, the hatching order and size hierarchy in a brood may influence the chicks' thermoregulatory responses. During the brooding period, nestlings experience little variation in body temperature except when both parents leave the nest for extended periods. I studied the behavioural responses of wild broods to natural temperature fluctuations inside the nest when adults were absent. The objective was to investigate under natural conditions the influence of hatching order on the chicks' behavioural responses to variation in temperature.

Methods

The study was conducted in eastern France (48°20'N, 7°45'E) on four barn owl broods each with 4 chicks. The nest chamber ($73 \times 42 \times 43$ cm), a wooden nest box regularly used by breeding barn owls, was equipped with an infrared video camera linked to a video recorder (Durant et al. 1996). Ambient temperature inside the chamber was monitored to the nearest 0.5°C every second at two points on opposite walls of the nestbox. The average of the two temperatures was recorded on a computer (there was no significant difference in temperature between the two spots). The computer and video-recorder clocks were synchronised, making it possible to associate temperature and behaviour. Both recordings were run continuously during the rearing period. The amount of time the chicks were left unattended was calculated each day from video analysis.

Nestlings were identified by video at hatching and followed individually throughout their development thanks to differences of development (size, feather growth). The chicks and their parents were not manipulated nor were they aware of human presence. Chick behaviour was recorded during the periods when both parents were outside the nest box. Recording was done over 2 min periods starting 2 min after the adult left, which was assumed to be long enough to observe any behavioural responses to the change in the chick's thermal environment. Five behavioural responses to temperature were considered, classified in a rank order according to their intensity, from cold to warm:
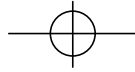
(1) Huddling and/or shivering: In huddling, the chick tries to put its head under the wing or belly of an older sibling and pushes its body against that of the other. Shivering is a tremor of the body that is usually observed when huddling is not possible.

(2) Leaning together: The first stage of cold stress behaviour: the chick leans against the body of one or more other siblings. In contrast to the previous behaviour, the chick does not actively try to put itself under its sibling.

(3) Staying alone: The chick is alone and shows no tremor. Its wings are kept against the body and its beak is closed. It is indifferent to ambient temperature.

(4) Wings spread and/or fluttering: The first stage of heat stress behaviour: the chick increases its body surface by opening its wings and/or fluttering them, and lying on the floor.

Table 1. Average ambient temperature (± s.e.) at which different thermal behaviours of barn owl chicks are observed in relation to their rank order of hatching. Behaviour categories are: 1 = huddling/shivering; 2 = leaning together; 3 = staying alone; 4 = wings spread/fluttering; 5 = panting.

Brood	Hatching order	Thermal behaviour 1	2	3	4	5
A	1	13.0 ± 0.5	15.5 ± 0.7	25.1 ± 0.5	31.6 ± 1.2	37.6 ± 1.5
	2	13.7 ± 0.7	16.6 ± 0.8	24.6 ± 0.7	29.4 ± 1.3	35.2 ± 1.5
	3	14.2 ± 0.8	17.6 ± 0.7	25.5 ± 0.6	29.5 ± 1.7	34.5 ± 1.4
	4	15.1 ± 0.8	17.9 ± 0.8	25.4 ± 0.6	30.4 ± 1.8	35.7 ± 1.6
B	1	19.9 ± 0.7	21.0 ± 0.4	22.7 ± 1.1	29.9 ± 1.3	33.6 ± 1.0
	2	20.4 ± 0.3	21.9 ± 0.7	21.7 ± 1.1	27.4 ± 1.0	30.8 ± 1.0
	3	20.7 ± 0.5	21.8 ± 0.7	25.2 ± 0.7	26.8 ± 1.9	30.0 ± 1.1
	4	20.6 ± 0.3	22.0 ± 0.8	25.9 ± 0.3	26.8 ± 1.9	29.3 ± 1.0
C	1	20.5 ± 0.4	22.4 ± 0.7	25.0 ± 0.8	30.0 ± 0.4	34.3 ± 2.7
	2	20.5 ± 0.4	22.5 ± 0.7	24.7 ± 0.9	28.4 ± 0.7	30.7 ± 2.6
	3	20.9 ± 0.4	22.6 ± 0.7	25.9 ± 0.8	28.4 ± 1.0	33.4 ± 2.0
	4	20.7 ± 0.4	23.1 ± 0.8	25.9 ± 0.9	27.7 ± 1.0	30.0 ± 4.5
D	1	18.0 ± 0.6	20.2 ± 0.8	27.8 ± 1.2	30.4 ± 2.4	32.6 ± 2.6
	2	17.9 ± 0.5	19.9 ± 0.8	27.5 ± 1.6	28.8 ± 0.5	31.1 ± 2.1
	3	18.4 ± 0.5	21.1 ± 1.4	27.7 ± 1.1	29.4 ± 1.7	30.0 ± 2.3
	4	19.4 ± 0.7	21.9 ± 0.7	27.1 ± 0.6	27.6 ± 0.9	29.3 ± 1.5

(5) Panting: The chick exhibits a high respiratory frequency visible by movements of the belly, the beak is open and the tongue protruding.

Each behaviour recorded was associated with the corresponding ambient temperature measured during the 2 min period. Thus, for each chick two columns of data (of c. 100 lines) were obtained relating ambient temperature (°C) and response behaviour index (1–5). The average temperature at which each behaviour was shown was calculated for each chick (Table 1). Comparisons were made using one-way repeated measures ANOVA.

Results

Nest attendance and temperature

In barn owls, only the female broods the eggs and young chicks. The first significant break in continuous brooding occurs when the female makes her first hunting trip. In this study, this happened on average 15 ± 1 days after the hatching of the first egg, which was on average 8 ± 1 days after the hatching of the fourth and last egg (Fig. 1). Before this 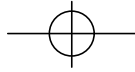date females rarely left the nest box. On average chicks were first left alone for the whole night 20 days after hatching of the first egg.

Nest temperature during the study ranged from 8° to 43°C and outside temperatures from 4° to 37°C.

Behavioural responses to ambient temperature

The average temperatures at which different thermal behaviours were observed are presented in Table 1. The pooled values for chicks of the same hatching order are presented in Figure 2. Average temperatures were significantly different between behaviour categories for every chick (Brood A, $F_{3,12} = 538.93$; Brood B, $F_{3,12} = 91.696$; Brood C, $F_{3,12} = 98.466$; Brood D, $F_{3,12} = 143.368$; all $P < 0.001$).

The cold stress responses (Behaviours 1 and 2) were observed at a significantly higher ambient temperature in the younger chicks of a brood compared to their elder siblings (Behaviour 1, $F_{3,9} = 6.157$, $P = 0.015$; Behaviour 2, $F_{3,9} = 6.643$, $P = 0.012$, Table 1 and Fig. 2). On the other hand, the heat stress responses (Behaviours 4 and 5) were observed at significantly lower ambient temperature in the younger chicks compared to their elder siblings (Behaviour 4, $F_{3,9} = 12.929$, $P = 0.001$; Behaviour 5, $F_{3,9} = 9.588$, $P = 0.004$, Table 1 and
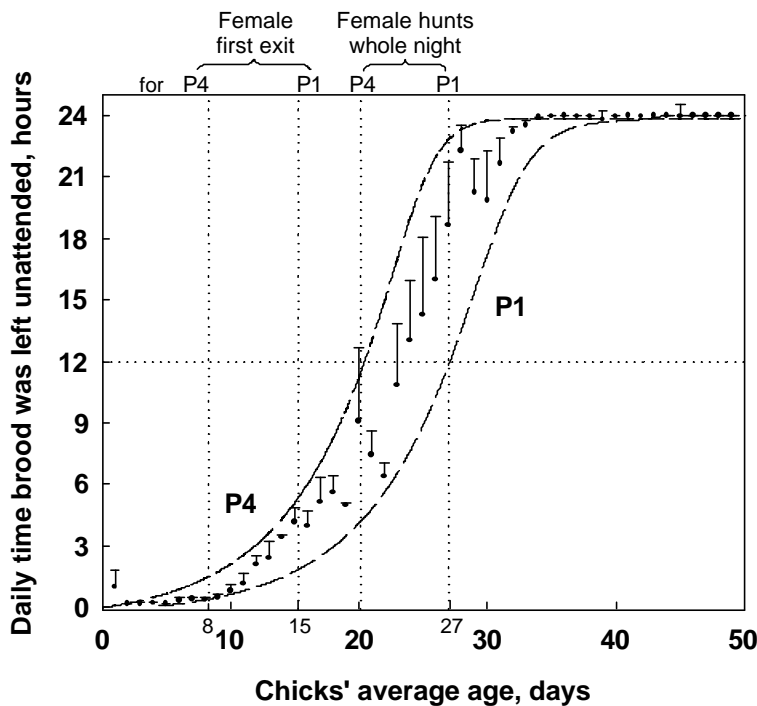
Figure 1. Number of hours (± s.e.) for which barn owl chicks were left unattended in relation to their age and hatching order (n = 4 nests). Smooth dashed curves are the averages for the first-hatched (P1) and for the last-hatched (P4) chicks of the pooled 4 nests. The females' first exit for hunting occurred around 15 days after the hatching of the first egg corresponding to 8 days after the hatching of the fourth egg. When a female stayed outside the nest for more than 12 hours it means that she spent the whole night period outside.

Fig. 2). No difference was found between siblings in the temperatures at which they showed Behaviour 3 ($F_{3,9}$ = 2.509, ns).
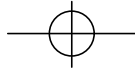
Discussion

Hatching order in barn owl chicks influences their individual behavioural responses to ambient temperature variation. The younger chicks of a brood showed heat and cold stress responses at less extreme temperatures than did their older siblings (Fig. 2). This result suggests a difference in thermoregulatory capacity depending on hatching position. As food intake directly influences development, differences in food availability between siblings may affect the acquisition of thermoregulatory capacity (Mertens 1977).

Responses to ambient temperature changes

The zone of thermal comfort (Mercer 2001) is the range of ambient temperatures within which birds do not show specific behaviours to cope with the thermal environment. It is slightly different from the thermoneutral zone, which is the range of ambient temperature

at which temperature regulation is achieved without changes in metabolic heat production or evaporative heat loss (Mercer 2001) and which cannot therefore be determined by behavioural observations. In this study, the chicks were in their zone of thermal comfort when they showed the Behaviour 3. The zone is delimited by the lowest temperature that did not induce a cold stress response (Behaviour 2) and the highest temperature that did not induce a heat stress response (Behaviour 4). Using these behavioural responses to temperature variations, the zone of thermal indifference then ranges between 20 and 31°C for the first born chick and 21 to 28°C for the last one (Fig. 2). These values differ from those found for the thermoneutral zone in fasting adults (23 to 32°C, Thouzeau et al. 1999). This indicates, taking into account the poorer isolation and smaller size of chicks, that under 23°C the chicks should use metabolic heat production to maintain their core temperature. The significant differences between the limits of the zone of thermal comfort for the various age ranks indicated that, in addition to behavioural differences, there might also be a difference in metabolic response to temperature changes. However, in the present study it was not possible to evaluate metabolic responses to temperature fluctuations.

Influence of hatching order

Hatching order is well documented to have an influence on growth (O'Connor 1977, Lesage & Gauthier 1998), as has also been suggested for barn owl chicks (Durant & Handrich 1998, Roulin 1998). In the present study, the influence of the hatching order appeared in the differences in the behavioural response to ambient temperature variations (Fig. 2). At the same age, late-hatched chicks relied more on behavioural responses to temperature variation than older ones did, as shown by the significant differences between them in their zones of thermal comfort (Fig. 2). Resorting to a behavioural response could be either the result of an inability to thermoregulate, e.g., because of poor tissue maturation, or a strategy for saving energy. Why do such differences in behaviour between chicks of different hatching order occur?
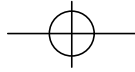
Due to the size hierarchy, access to food differs among chicks of an asynchronous brood (Newton 1979). Usually the younger chicks have poorer access to food than their older siblings and have on average a lower energy intake (Stoleson & Beissinger 1997, Roulin 1998). Such food restriction could entail a slower maturation of tissues, i.e., structural and functional changes associated with accumulation of materials such as contractile proteins in growing muscles (Visser 1998), preventing the chick from thermoregulating through heat production. On the other hand, a slower

tissue maturation is a means to achieve faster growth (Hohtola & Visser 1998). Indeed in barn owls, the youngest chick of a brood has two growth constraints. During the first period of growth, the last-hatched chick must attain sufficient size to be able to eat entire prey by itself and thus be able to feed when the female is away from the nest. In the present study, females started hunting on average eight days after the hatching of the last egg, although the capacity to eat entire prey is not attained until the age of 20 days (Taylor 1994). As a consequence, the youngest chick was partially deprived of food for 12 days. This partial food deprivation of the youngest chicks is common in barn owls (Taylor 1994) and may lead to death (brood reduction, Lack 1968). However, it has been shown in the laboratory that there exists a margin in which food deprivation has no effect on the linear growth and fledging time (Durant & Handrich 1998). Consequently, partial food deprivation may trigger an increase in growth rate at the expense of tissue maturation.

Another imperative for later hatched chicks is to be able to fledge at the same time as their older siblings, a period when adults begin to give prey to the young owls outside the nest (around 60 days of age, Taylor 1994). At this stage, only the male will bring food directly to the nest. The female, on the other hand, will give the prey to an owlet only if it follows her back to the nest. Thus, unfledged chicks remaining inside the nest get a smaller share of the food supplied. To be treated equal-

Figure 2. Average ambient nest temperatures (± s.e.) at which different thermoregulatory behaviours of barn owl chicks were observed in relation to hatching order. Data are pooled over 4 broods. Behavioural categories are: 1 = huddling/shivering; 2 = leaning together; 3 = staying alone; 4 = wings spread/fluttering; 5 = panting. * indicates a significant difference (P < 0.05) in mean temperature between the first and last hatched chick for the corresponding behaviour index.
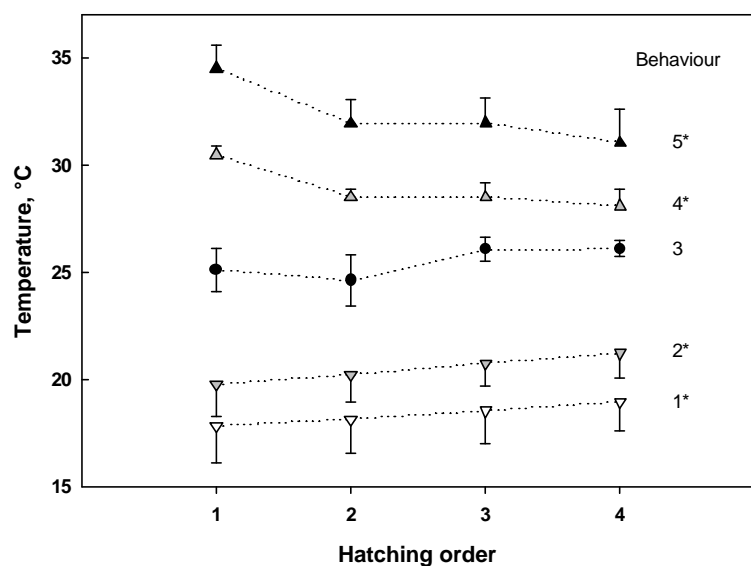
ly, the last-hatched chicks need to be able to fly, that is, to reach adult size and flight feather length at the same time as the first-hatched chicks, i.e., on average 10 days younger. Both of these two constraints require that the last-hatched chicks have a faster growth than their older siblings (Nilsson & Svensson 1996). This could be achieved by channelling part of the energy for thermo-regulation to growth, assuming that thermoregulation by modification of behaviour is less expensive than metabolic heat production. To test this hypothesis, it would be necessary to obtain data on tissue maturation and thermogenesis in relation to hatching order.

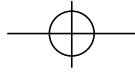## Importance of the timing of the female first foraging trip

During their first days of life, barn owl chicks need to be brooded in order for them to maintain their body temperature. In this species, homeothermy in achieved when chicks are 15–20 days old (Taylor 1994, Durant et al. 1996). As a consequence, the female could not leave the nest before this date without adverse con-sequences for the chicks. Indeed the first hunting trip of the female during our study began on average 15 days after hatching (Fig. 1). The first time she spent the whole night outside the nest was only 27 days after the first chick hatched, when the youngest was only 20 days old.

What could trigger the female's exit? As in many birds, growing barn owl chicks become more and more aggressive towards their parents and are calmed only when satiated. The increasing difficulty encountered by the male to supply enough food may accentuate this ag-gressive behaviour and chase the female from the nest (Durant pers. obs.). Indeed, the food requirement for a brood of 4 chicks is around 8 prey animals of vole size (c. 20 g), when the female makes her first foraging trip, calculated using the average food requirement measu-red on captive chicks (Durant & Handrich 1998). To this value must be added the food required by both adults, which is also provided by the male. The exist-ence metabolism (i.e. daily metabolised energy at con-stant body mass) calculated for Strigiformes of 300 g (allometric coefficient 0.58, Wijnandts 1984) is 233 kJ $24\ h^{-1}$, which corresponds to 42 g or two prey items of vole size (assuming a caloric value of 7.7 kJ g^{-1} and an energy assimilation efficiency of 72.3 %; Durant et al. 2000). As a consequence, the male should hunt c.

12 prey $(8 + 2 + 2)$ every night to fulfil the needs of the brood, his female and himself. The time available to hunt such a quantity of prey could be a threshold for the male. Moreover, male hunting effort may be fixed to a level where his survival is not jeopardised (Wiehn & Korpimäki 1997, Roulin et al. 1999). Consequently, the normal increase in the brood's requirements will make it impossible for the female to feed on the male's nest supply. The timing of the female's first hunting trip may then be adjusted in response to the shortfall between brood requirements and the supply of food to the nest by the male (Whittingham & Robertson 1993, Taylor 1994, Durant & Handrich 1998). Indeed, it was ob-served that female kestrels *Falco tinnunculus* decreased their hunting effort and prey delivery rate to the nest in response to supplementary feeding (Wiehn & Korpi-mäki 1997).

This study unravels two important points that should be taken into account for future research. First, intra-clutch competition for access to food imposes a strong selection pressure on the last hatched chicks, which leads to a different growth strategy. Second, the timing of the female's first exit appears to be a major threshold for the last chicks of a brood as it creates a different ther-mal and food environment from the one encountered by their older siblings at the same age.
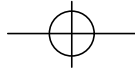
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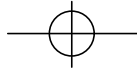
Fat-soluble antioxidants in the eggs of great tits *Parus major* in relation to breeding habitat and laying sequence

Peeter Hõrak[1], Peter F. Surai[2] and Anders P. Møller[3]

Incubation in many species of altricial birds starts before completion of the clutch, resulting in asynchronous hatching that may increase the probability of at least some nestlings dying during periods of food scarcity. Females may interfere with this process by enhancing or suppressing the survival probabilities of last-hatched nestlings by differential investment in egg quality with respect to laying order. We investigated the concentrations of fat-soluble antioxidants in the yolks of eggs in an urban and a rural population of great tits *Parus major*. Urban females deposited consistently smaller amounts of carotenoids and vitamin E in their eggs despite laying fewer eggs than rural females. Eggs in both populations showed a 40 % decrease in vitamin E concentration with laying sequence. Last-laid eggs of the rural great tits had 37 % lower concentration of carotenoids than first-laid eggs while no such difference emerged in the city. These results are consistent with the idea of reduced maternal investment in last-laid eggs, as predicted by models of adaptive brood reduction.
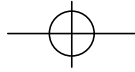Key words: carotenoids; hatching asynchrony; maternal effects; vitamin A; vitamin E.

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Among many altricial bird species, nestlings within a brood do not hatch simultaneously but over a period of several days (e.g. Clark & Wilson 1981, Ricklefs 1993, Stoleson & Beissinger 1995). Hatching asynchrony is mainly determined by incubation beginning before clutch completion, resulting in first-laid eggs hatching earlier than last-laid eggs (Lack 1968, O'Connor 1984) and consequent size hierarchies among nestlings that may cause death or slow development of late-hatched, small nestlings (Hahn 1981). The existence of hatching asynchrony has been considered a paradox since it can negatively affect reproductive success, due to death or poor condition at fledging of disadvantaged offspring (Stoleson & Beissinger 1995). At least seventeen different hypotheses have

been proposed to explain this phenomenon (reviews in Ricklefs 1993, Stoleson & Beissinger 1995, Stenning 1996).

Asynchronous hatching is usually considered adaptive because of the potential benefits accruing to parents by starting incubation before clutch completion, establishing a hierarchy in reproductive value among their offspring. Alternatively, asynchronous hatching (and accompanying partial brood mortality) may result from parental tactics to obtain benefits from an early start to incubation. In both cases, one might expect that it is in the interest of parents to modify the quality of their eggs with respect to laying sequence. In the case of adaptive brood reduction, parents would benefit from enhancement of the quality of first-laid eggs by

supplying these with greater quantities of micro- and macro-nutrients. On the other hand, if asynchronous hatching is an undesirable by-product of parental tactics accruing from benefits of early onset of incubation, then one might predict that it is in the interest of parents to enhance the quality of late-hatching young by supplying last-laid eggs with resources that would aid the youngest hatchling to catch up in competition with older siblings. Mothers can differentiate their investment in egg contents by various mechanisms, such as modifying egg size (e.g. Parsons 1975, Meathrel & Ryder 1987, Lamey 1990, Jover et al. 1993), macronutrient content (e.g. Bryant 1978, Bryant & Tatner 1990, Royle et al. 1999), concentrations of fat-soluble antioxidants (Royle et al. 1999, 2001, Blount et al. 2002), immunoglobulins (Blount et al. 2002, Saino et al. 2002), or androgens (Schwabl 1993, 1997, Gil et al. 1999, Royle et al. 2001) in relation to laying sequence.
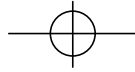
The aim of this study is to describe variation in egg antioxidant concentration within the laying sequence in great tits *Parus major*. Nestlings hatch usually within one or two days of each other (e.g. Orell 1983, Lebedeva 1994) but may take up to five or more days (Slagsvold & Amundsen 1992). The question of whether hatching asynchrony in the great tit serves an adaptive purpose to facilitate flexible brood reduction is unclear. Hõrak (1995) showed that partial brood mortality was accompanied by a decrease in fledgling weight and recruitment rate (but with increased female survival). On the other hand, great tit parents have been shown to participate actively in brood reduction by expelling the youngest, under-nourished young from the nest (Lebedeva 1994). Furthermore, Amundsen & Slagsvold (1998) suggested that high-quality parent great tits may benefit from hatching synchrony, while low-quality parents benefit from asynchrony.

We describe patterns over the laying sequence in the concentrations in eggs of three antioxidants that are expected to play an essential role in growth and development of nestlings. Because animals cannot synthesise carotenoids and vitamin E, these have been hypothesised to be a limiting resource, such that their availability can constrain the expression of antioxidant activity and immune function (Lozano 1994, von Schantz et al. 1999). Such antioxidant limitation is especially likely to occur in the avian embryo – a closed system which during its development completely relies on maternally deposited resources. Indeed, it has been shown repeatedly that maternal diet and subsequently the yolk composition play a crucial role in the development and efficiency of the antioxidant systems of avian embryos (reviewed in Surai & Sparks 2001). Furthermore, it has been demonstrated that dietary fat-soluble antioxidants such as carotenoids and vitamins A and E may have important consequences for the maintenance of a functional phenotype in rapidly growing nestlings (reviews in Surai 1999, Møller et al. 2000). Rapid growth is associated with the production of large amounts of free radicals that, if not neutralised, can cause permanent damage to DNA, proteins and lipids, including cell membranes, tissues and organs. Since last-hatched nestlings grow faster, beg more frequently, and show higher levels of activity than early hatched nestlings (Lebedeva 1994, Saino et al. 2001), we would expect last-laid eggs to contain more antioxidants if nestling growth requirements were the factor determining allocation patterns of antioxidants to eggs. Alternatively, if great tit mothers practice adaptive brood reduction, we would predict that first-laid eggs should contain more macro-nutrients and antioxidants than last-laid eggs. In this scenario, first-laid eggs are more likely to produce viable offspring due to hatching asynchrony, and it would be adaptive to enhance their quality by supplying them with extra resources. We test these predictions in two great tit populations, breeding under contrasting environmental conditions in high-quality rural and low-quality urban habitat. Based on our previous findings that carotenoid-based plumage coloration is paler among urban great tits (Hõrak et al. 2001), we hypothesise that dietary carotenoids appear more limiting in our urban study site. Hence, we expect the eggs of urban great tits to contain less carotenoids than these of their rural conspecifics.

To elucidate the possible relationship between egg content and hatching asynchrony, we compare the patterns of hatching asynchrony and brood reduction between these populations on the basis of the prevalence and extent of early nestling mortality and the magnitude of nestling size hierarchies. We predict that if egg antioxidant concentrations decrease in laying sequence then such a decline should be more prominent in the population with higher degree of hatching asynchrony.

Methods

Data were collected in April–May 2000 in two neighbouring (urban and rural) great tit populations breeding in nest boxes in and around Tartu (58°22' N, 26°43' E), south-eastern Estonia. The study areas, which are 8 km apart, were described by Hõrak & Lebreton (1998). Only data from first clutches were used. Reproductive parameters of breeding great tits were recorded by regular inspection of nestboxes. Clutches (randomly) chosen for measurement of egg variables in relation to laying sequence were visited on the day of laying of the first egg and on days of expected clutch completion. Eggs were marked individually to enable collection of the first and last eggs on the day when laying ceased. After collection, eggs were photographed for measurement of volumes as described by Hõrak et al. (1997) and stored at –20 ºC until analyses. Data were collected under licence from the Estonian Ministry of the Environment. Seven clutches from the rural population (clutch size = 11–12 eggs) and 8 clutches from the urban study area (clutch size = 8–10 eggs) were used for comparison of parameters of the first and last egg. Their respective differences were analysed with repeated measures ANOVA with habitat as a factor and laying sequence as a repeated measure. Values are reported as means ± s.d.. All tests are two-tailed with a 5 % significance level.

Because we were unable to measure directly the extent of hatching asynchrony and brood reduction between populations, we used indirect estimates such a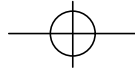s the magnitude of nestling size hierarchies and the prevalence and extent of early nestling mortality. The benefits of brood reduction in terms of reducing food competition among nestlings are clearly greater if nestling mortality occurs early. We therefore assumed that adaptive brood reduction is likely to be more prevalent in populations with a higher frequency and extent of early nestling mortality (occurring before the 8th day of the nestling period). Likewise, we assumed adaptive brood reduction to be associated with greater magnitude of nestling size hierarchies. To estimate the latter, we used the intra-brood coefficient of variation of nestling body mass on the 8th day of the nestling period (see Orell 1993). Since nestling mortality and growth are sensitive to experimental brood size manipulations, only non-manipulated broods were used for the above-mentioned analyses.

Vitamins E (α-tocopherol) and A (retinol) were determined by High Precision Liquid Chromatography (HPLC) as previously described by Surai et al. (2001). In brief, the egg yolk (100–200 mg) was mixed with a 5 % solution of NaCl (0.7 ml) and ethanol (1 ml) was added for protein precipitation. Hexane (5 ml) was then added and the mixture was homogenised for 1 min. The hexane phase, containing vitamins A and E and carotenoids, was separated by centrifugation and collected. The extraction was repeated twice more with 5 ml hexane. Hexane extracts were combined, evaporated and redissolved in a mixture of methanol/dichloromethane (1:1, vol/vol). Samples were injected into HPLC system (Shimadzu Liquid Chromatograph, LC-10AD, Japan Spectroscopic Co. Ltd. with JASCO Intelligent Spectrofluorometer 821-FP) fitted with a Spherisorb, type

Table 1. Comparison of reproductive parameters of urban and rural great tits in 2000. Nestling mortality is the number of nestlings that died before day 8 of the nestling period. All data (except for clutch size) are for non-manipulated broods.

Variable	Urban			Rural			Statistic	P
	Mean	(s.d.)	n	Mean	(s.d.)	n		
Clutch size	8.89	(1.58)	74	11.43	(1.00)	40	10.40[1]	<0.0001
% of broods with early mortality	18		22	30		10	0.56[2]	0.45
Nestling mortality	0.18	(0.39)	22	0.70	(1.60)	10	1.04[3]	0.30
CV of nestling mass	0.08	(0.06)	23	0.17	(1.16)	9	2.81[3]	0.005
% of hatchlings fledged	81	(28)	21	82	(18)	9	0.66[3]	0.50
Fledgling mass (g)	16.25	(1.69)	20	16.74	(1.27)	10	0.80[4]	0.43

[1] t-test for separate variances; [2] χ^2-test; [3] Wilcoxon test; [4] t-test

S30DS2, 3μ C_{18} reverse phase HPLC column, 15 cm $\times$ 4.6 mm (Phase Separations Ltd., UK). Chromatography was performed using a mobile phase of methanol/water (97:3, v/v) at a flow rate of 1.05 ml/min. Fluorescence detection of retinol involved excitation and emission wavelengths of 325 and 480 nm and detection of vitamin E used excitation at 295 nm and emission at 330 nm. Standard solutions of α-tocopherol and retinol in methanol were used for instrument calibration and tocol was used as an internal standard.

Carotenoids were determined from the same extract using the same HPLC system, but fitted with a Spherisorb, type S5NH2 5μ C_{18} reverse phase HPLC column, 25 cm $\times$ 4.6 mm (Phase Separations Ltd., UK). Chromatography was performed using a mobile phase of methanol/water (97:3, v/v) at a flow rate of 1.5 ml/min. Total carotenoids were detected at 445 nm as a single peak using lutein as a standard.
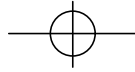
Results

Rural great tits laid clutches more than two eggs larger than their urban conspecifics but the proportion of hatchlings that fledged and fledgling weights did not differ between the study areas (Table 1). Neither did we find any significant differences in the extent or prevalence of early nestling mortality (Table 1). An indirect estimate of hatching asynchrony, coefficient of variation of nestling mass on day 8, was two times higher in the rural population. In general, 2000 appeared to be a favourable year for breeding great tits. Both rural and urban birds fledged more hatchlings in 2000 (81 % and 82 %; Table 1) compared to the average of the four previous years (rural 1996–99: 69 $\pm$ 27 %, n = 70 broods; urban 1996–99: 72 $\pm$ 28 %, n = 166 broods, respectively). This difference was significant in the city ($Z_{21,166}$ = 2.02, P = 0.043), but not in the rural population ($Z_{9,70}$ = 1.44, P = 0.147).

Rural great tits had about twice the concentrations of carotenoids in their eggs than their urban conspecifics (47.9 $\pm$ 20.7 μg/g v. 21.3 $\pm$ 6.0 μg/g; Table 2, Fig. 1). Rural birds also had significantly higher vitamin E concentrations in their eggs than urban birds (154.9 $\pm$ 51.1 μg/g v. 119.2 $\pm$ 46.7 μg/g; Table 2, Fig. 1). We found no significant between-habitat differences in egg vitamin A content, yolk mass or egg volume (Table 2).

Table 2. Effect of breeding habitat (urban v. rural) and laying sequence (first v. last egg) on egg parameters of great tits in repeated measures ANOVA. A significant habitat $\times$ sequence term means that variation of trait values in laying sequence differed between habitats. For direction of the effects, see Figure 1.

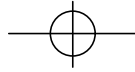| Variable	Effect	F_{df}	P
Carotenoids	Habitat	$23.8_{1,13}$	0.0003
	Sequence	$7.4_{1,13}$	0.017
	Habitat $\times$ Sequence	$9.3_{1,13}$	0.009
Vitamin E	Habitat	$15.0_{1,12}$	0.002
	Sequence	$19.1_{1,12}$	0.0009
	Habitat $\times$ Sequence	$0.3_{1,12}$	0.6
Vitamin A	Habitat	$0.1_{1,12}$	0.8
	Sequence	$2.3_{1,12}$	0.5
	Habitat $\times$ Sequence	$0.4_{1,12}$	0.5
Yolk mass	Habitat	$2.5_{2,12}$	0.143
	Sequence	$4.6_{1,12}$	0.056
	Habitat $\times$ Sequence	$0.6_{1,12}$	0.5
Egg volume	Habitat	$1.8_{2,13}$	0.2
	Sequence	$0.0_{1,13}$	1
	Habitat $\times$ Sequence	$0.5_{1,13}$	0.5
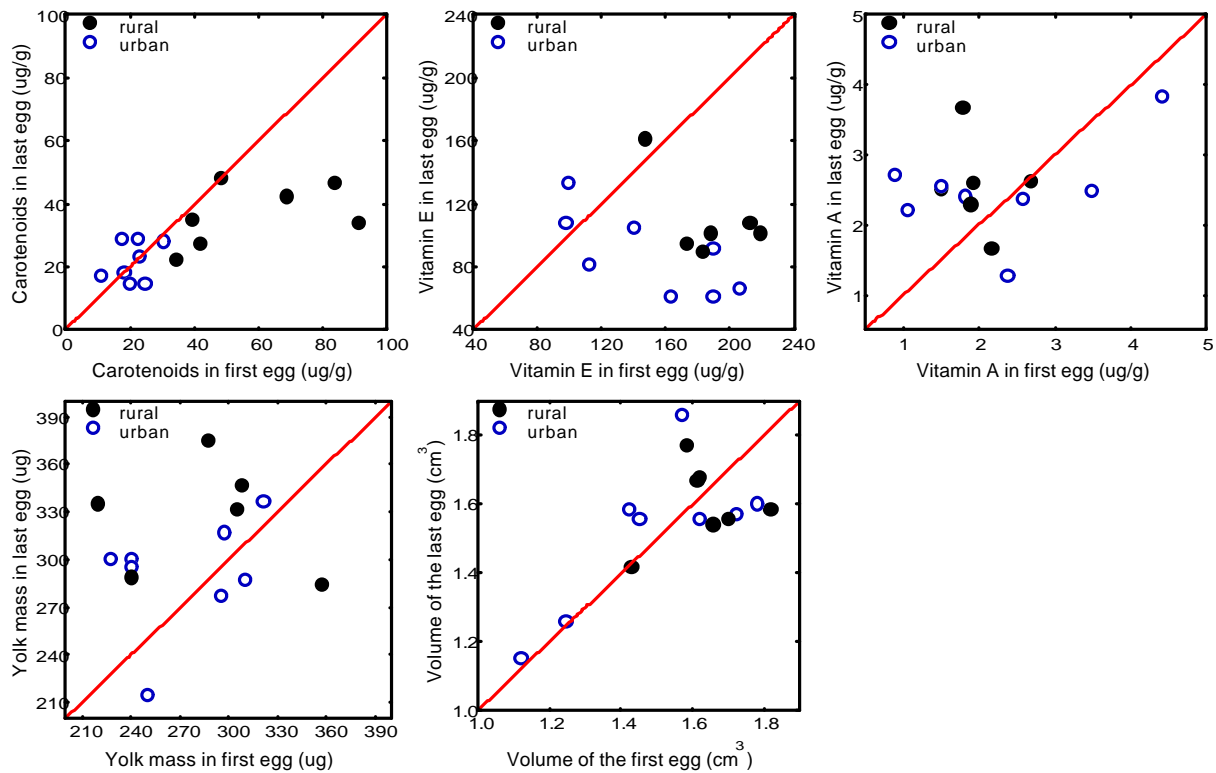
Figure 1. Comparison of egg variables in first-laid v. last-laid eggs in urban and rural great tits. Diagonal lines denote identical values.

Last-laid eggs contained about 40 % less vitamin E than first eggs (Fig. 1). These differences were of similar magnitude in both urban and rural populations, as indicated by a non-significant interaction term in Table 2 (Urban: difference $= 60.5 \pm 64.8$ µg/g; Rural: difference $= 77.0 \pm 47.4$ µg/g). In rural great tits, egg carotenoid concentration decreased by 37 % from first to last egg (difference $= 21.7 \pm 20.0$ µg/g; Fig. 1), while no decline in egg carotenoid concentration occurred in the town (difference $= 0.2 \pm 1.2$ µg/g). The habitat-specific pattern in intraclutch variation of egg carotenoid content was confirmed by the significant Habitat $\times$ Sequence interaction term in Table 2. Egg yolk mass was generally about 9 % larger in last-laid than in first-laid eggs (difference $= 28.5 \pm 51.8$ µg). This difference, however, was only marginally significant (P $= 0.056$; Table 2). We found no significant differences in vitamin A concentration or egg volume between first and last eggs in either population (Table 2).

Discussion

The coefficient of variation in nestling body mass was twice as large in rural great tits compared to urban birds. If this higher variation in nestling body masses was caused by greater hatching spread then our rural great tits appear more likely to adopt an adaptive brood reduction strategy than their urban conspecifics. However, at present we cannot exclude the possibility that larger intrabrood variation in nestling body mass of rural birds could have arisen because rural parents had a harder task of rearing a larger brood (independently of any influence of hatching asynchrony) or because of greater intrabrood variation in important egg constituents. Nevertheless, hatching asynchrony would probably be the easiest way to generate sibling size hierarchies and offers most parsimonious explanation for the high intrabrood variation in nestling mass. Notably, although the prevalence and extent of early nestling mortality did not differ significantly between popu-

lations, the direction of these differences was consistent with the idea of adaptive brood reduction being more prevalent in the rural population (Table 1).

Compared to urban birds in Tartu, eggs of rural great tits had twice the carotenoid concentration and a 23 % higher vitamin E concentration. This difference was found despite the fact that rural birds on average laid 2.5 more eggs than urban birds (Table 1). These differences are also reflected by parallel differences in the hue of carotenoid-based plumage of female great tits from the two populations (Hõrak et al. 2001). One possible explanation for the differences in egg content would be that female great tits had better access to carotenoid- and vitamin E rich food sources in our rural study area, as compared to the city. Although the conversion of carotenoids from folivorous lepidopteran larvae to carotenoid-based plumage of breeding great tits is well documented (Partali et al. 1987), we do not currently know to what extent caterpillars comprise a significant part of the diet during this period. Thus, we can at best only hypothesise that the food of female great tits in our rural study area contained more carotenoids and vitamins. On the other hand, great tits breeding in Tartu are probably exposed to considerably higher heavy metal (notably cadmium and lead) pollution from traffic exhaust fumes, than their rural conspecifics. Both of these metals are known as strong inducers of oxidative stress (e.g. Ercal et al. 2000, Congiu et al. 2000). Vitamin E is known to be protective against cadmium-induced oxidative damage (Sarkar et al. 1997) and a prophylactic action of β-carotene on Cd toxicity has been shown recently (El-Missiry & Shalaby 2000). Administration of β-carotene to rats, concurrent with cadmium, ameliorated Cd-induced lipid peroxidation in brain and testes and prevented decrease in antioxidant (superoxide dismutase, glutathione S-transferase and reduced glutathione) concentrations. Concentration of α-carotene in mouse kidney and testis was significantly decreased as a result of cadmium administration (Massanyi et al. 1999). Therefore, we cannot exclude the possibility that urban great tits laid eggs of lower antioxidant concentration due to maternal depletion of carotenoids and vitamin E for the scavenging of free radicals.
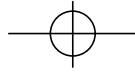
Our observation that concentrations in eggs of vitamin E and carotenoids declined significantly with laying order is consistent with the idea of great tits being adaptive brood reductionists. Under this scenario, parents would benefit by enhancement of the quality of first-laid eggs by supplying these differentially with greater quantities of micro- and macro-nutrients, because first-laid eggs are more likely to produce viable offspring than last-laid eggs. Notably, carotenoid concentration declined with laying order only in the rural population which, according to the results of the present study, seems to show a greater extent of brood reduction than our urban great tit population. Our observations therefore clearly reject the hypothesis that mothers supply last-laid eggs with higher concentration of antioxidants as to compensate for the higher oxidative stress burden accompanying faster growth and greater activity levels of last-hatched offspring.

The physiological and molecular mechanisms by which such differential transfer of antioxidants to egg yolk occurs are not known and need further investigation. For example, additional supplementation of lesser black-backed gulls *Larus fuscus* with carotenoids did not change the pattern of decline in yolk carotenoid and vitamin E concentration in relation to laying sequence (Blount et al. 2002). When lesser black-backed gulls were induced to lay additional eggs, carotenoid concentrations in the egg yolk started to recover from the third egg up to the sixth or eighth eggs (A. Fidgett and P. F. Surai, unpubl. data).

Despite prominent differences in carotenoid and vitamin E concentrations, last-laid eggs of great tits were not inferior compared to first-laid eggs in all aspects of quality. Egg size (an indicator of an egg's total energy content in the great tit; Ojanen 1981) did not vary with laying sequence (Fig. 1) while yolk size (an indicator of egg lipid content) even tended to increase in last-laid eggs (Fig. 1, Table 2). The latter result also suggests that the decline of carotenoid and vitamin E content in relation to laying sequence could hardly be ascribed to the deterioration of food supplies in the course of laying. If that were the case, we would expect last-laid eggs to be smaller and contain smaller yolks than first-laid eggs. Moreover, such a situation seems unlikely because the availability of nutritious and carotenoid-rich lepidopteran larvae is usually higher at the end of the laying period (T. Tammaru pers. comm.). It is thus possible that a tendency of yolk mass to increase in last-laid eggs reflects the generally improved food situation during the formation of these eggs.

In conclusion, we have shown that egg carotenoid concentrations in rural great tits was double that of their
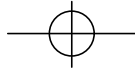
conspecifics in the neighbouring urban population, and that concentration of carotenoids and vitamin E decreased with laying sequence. The latter finding is consistent with the idea of reduced maternal investment in last-laid eggs, predicted from an hypothesis of adaptive brood reduction.
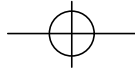
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Testes size and asymmetry in the tree swallow *Tachycineta bicolor*: a test of the compensation hypothesis

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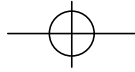
Gonad size in vertebrates typically shows directional asymmetry. In birds, the left testis is usually larger than the right, but the functional significance of this asymmetry is poorly understood. Møller (1994) showed that directional asymmetry in testes size was positively related to the expression of a secondary sexual character in two songbirds and he concluded that deviations from directional asymmetry can be seen as a measure of developmental homeostatis. Møller also suggested that the right testis only develops to compensate for a reduced function of the left one. We tested the compensation hypothesis in the tree swallow *Tachycineta bicolor*. In a sample of 36 males, we found directional asymmetry in testes shape, but not in size (mass or volume). We found no relationship between the size of the left testis and relative testes asymmetry. Furthermore, despite the fact that seminal glomerus mass was strongly correlated with testis size (for both sides), we found no difference in the efficiency of sperm production between the left and right testis. Hence, this study does not support the idea that the right testis compensates for a malfunctioning left one. Testes size, but not asymmetry, was related to wing length (reflecting age). Neither testes size nor asymmetry were related to body mass or condition, nor to a measure of ectoparasite load. In conclusion, we found no evidence that testes asymmetry in the tree swallow is adaptive or reflects male quality.

Key words: testes size, directional asymmetry, sperm production, tree swallow, *Tachycineta bicolor.*

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The significance of asymmetry in bilaterally symmetrical characters has received much attention in recent years (Møller & Swaddle 1997, Simmons et al. 1999). Most bilaterally symmetrical characters show fluctuating asymmetry (i.e. small, random deviations from perfect symmetry) and this is widely used as a measure of developmental stability (Palmer & Strobeck 1986). The degree of fluctuating asymmetry might be a reliable indicator of individual quality and thus play a role in sexual selection (Møller & Swaddle 1997, Thornhill & Møller 1998), but this hypothesis remains controversial (Bjorksten et al. 2000a, b, Møller 2000, Van Dongen & Lens 2000). Some characters show directional asymmetry, i.e. one side is consistently larger than the other, which might have an adaptive function (e.g. Alvarez 1995, Seligmann 1998, Windig & Nylin 1999).

Directional asymmetry is typically found in gonad size in vertebrates (Yu 1998). For example, in birds the left testis is generally larger than the right (Lake 1981). However, the adaptive significance of directional asymmetry in testes size is poorly understood.

Møller (1994) proposed that directional asymmetry in avian testes size is adaptive and he argues as follows. Assuming that it is costly to have two large testes, it might be advantageous to have mainly the left one functioning with the right one playing a compensatory role. Thus, the right testis would only increase in size to compensate for a reduced function of the left one. Assuming that the functional ability of the testis is reflected by its size, the compensation hypothesis thus predicts a positive relationship between the level of asymmetry and the size of the left testis (Birkhead et al. 1998). Alternatively, the reduced functional ability of the left testis might not be reflected in reduced size, but only in reduced sperm production. The compensation hypothesis would then predict that the degree of asymmetry is related to the number of sperm produced by the left testis.

However, the rationale behind Møller's (1994) compensation hypothesis is not clear. While it is obvious that it may be costly to develop two large testes, it is not clear why it would be better to have only one big testis (the left one) instead of two medium sized ones. If the left testis is more efficient in producing sperm than the right one, i.e. if it produced more sperm than a right testis of similar size, then this would provide an adaptive explanation for asymmetry. A higher efficiency in sperm production of the left testis can be expected based on the embryonic development of the gonads (Witschi 1935, Kimball et al. 1997; see Discussion), but this has not been further investigated.

Møller's (1994) study further suggested that testes asymmetry reflects individual male quality. This is based on the finding that in two species of songbirds the amount of directional asymmetry in testes size was positively related to the size of a secondary sexual character. Møller (1994) suggested that poor quality individuals might be unable to develop a fully functional left testis in combination with a small right testis. Other studies did not support the compensation hypothesis, nor did they find a correlation between directional asymmetry in testes size and the size of secondary sexual traits (Birkhead et al. 1997, 1998, Kimball et al. 1997; Merilä & Sheldon 1999). However, several studies found that testes asymmetry is age-related (e.g. Kimball et al. 1997, Merilä & Sheldon 1999).

The main aim of this study is to test and discuss the compensation hypothesis by investigating the relationships between testes size and asymmetry and between testes size and the amount of sperm stored in the seminal glomera in a small songbird, the tree swallow *Tachycineta bicolor*. An underlying assumption of this study is that the number of sperm found in the seminal glomera is related to the functioning of the corresponding testis. This is a reasonable assumption, because the sperm stored in the seminal glomera are quickly used up when males are copulating (Birkhead 1991). Given that male tree swallows copulate frequently (Venier & Robertson 1991), the seminal glomera have to be constantly refilled with sperm from the testes. We also investigate the relationship between combined testes size and testes asymmetry, and male characteristics that may reveal age, condition or quality.
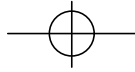
Materials and methods

Study species

The North American tree swallow is a secondary cavity breeder. In our study area in Canada individuals compete for nest cavities (nest boxes) and the population consisted of residents and a substantial number of floaters. We considered an individual to be a resident when it was observed with a partner entering the nestbox or sitting on the box on at least three consecutive days. We defined a floater as an individual that was caught in the study area but did not occupy a nestbox (for a detailed discussion of social status see Peer et al. 2000 and Kempenaers et al. 2001). Tree swallows show high levels of sperm competition (Kempenaers et al. 1999) and floaters and residents compete for fertilisations (Kempenaers et al. 2001). Thus, males will be under strong selection to produce large amounts of sperm. Indeed, tree swallows have relatively large testes and floaters and residents have similarly sized reproductive organs and sperm stores (Peer et al. 2000).

Study area and field methods

We collected tree swallows present on a nest box grid (Bracken Field) near the Queen's University Biological

Station (44°34´ N, 76°19´ W), Chaffeys Locks, Ontario, Canada. Details of the study area and general field procedures are described in Peer et al. (2000).

We collected 13 resident and 11 floater males between 7 and 23 May 1997 and 10 resident and 10 replacement males (presumably floaters that took over the female after her mate was removed) between 8 May and 5 June 1999. All birds were caught during the period when females were sexually receptive. Birds were either shot or captured with mist nets or trapped inside the box, and killed by thoracic compression and cervical dislocation. All birds were collected under licence.

Immediately upon death, we measured wing and tarsus length using calipers, and we estimated muscle score (index from 0 to 3 depending on the amount of muscle tissue covering the sternum) and fat score (index from 0 to 8 depending on the amount of visible subcutaneous fat; Vogelwarte Radolfzell, unpubl.). We also weighed each bird using a Pesola spring balance and counted the holes in wing and tail feathers caused by feather mites (Acariformes) as a measure of parasite load (see Dunn et al. 1994). We then dissected the males and cut out the part of the body wall with the reproductive organs (testes, vas deferens and the cloacal area including the seminal glomera) and stored it in 10 % buffered formalin. Formalin tends to shrink tissues, so measurements reported here could be slightly smaller than those from fresh material. Moreover, tissue shrinkage might not be equal in all dimensions. In 1997, however, we measured the testes (length and width) in the field before storing them in formalin and the measurements are highly correlated with those performed later and do not show a significant decline (data not shown).

Measurements of the reproductive organs

We examined the reproductive organs in the laboratory as described in Peer et al. (2000). In brief, we weighed the left and right testis and seminal glomerus to the nearest 0.0001 g on a Mettler AE160 electronic balance. In 1997, we also measured the maximum length, width and height of the left and right testis using digital calipers (to the nearest 0.01 mm). The testes are round to bean-shaped and we defined the height as the distance from the middle of the concave side (or the place where the testis was connected to the epididymis)
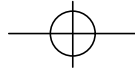
to the middle of the opposite (convex) side. The measurements were done independently by BK and KP and were highly repeatable (all $r > 0.90$ and $P < 0.001$). Average values were used in analyses. We estimated the total number of sperm in the left and right seminal glomerus using an Improved Neubauer and a Bürker-Türk counting chamber, following standard cell counting techniques (see Birkhead et al. 1991).

Data analyses

In this paper, we use only data from birds for which both testes and seminal glomera were intact after collection. All testis measurements (length, width, and height) were highly correlated with testis mass (all $P < 0.001$; Peer et al. 2000). To compare our data with Møller's (1994), we also estimated testis volume from the equation of an ellipsoid as $4/3\pi abc$, where a = testis length/2, b = testis height/2, and c = testis width/2. Testis volume correlated highly with testis mass (left: $r = 0.915$, n = 20, $P < 0.0001$; right: $r = 0.916$, n = 20, $P < 0.0001$). Seminal glomerus mass was highly correlated with sperm numbers (left: $r = 0.80$, n = 35, $P < 0.0001$; right: $r = 0.74$, n = 35, $P < 0.0001$). Thus, in most analyses we used only testis mass and seminal glomerus mass. Using testis volume rather than mass did not change the conclusions. Muscle and fat scores were treated as factors with three classes. Fat scores varied between 0 and 4 and we combined scores 0–1 and 3–4. We used the residuals of a linear regression of body mass on tarsus length ($r = 0.45$, n = 21, $P < 0.05$) as a measure of body condition.

We defined absolute asymmetry as the signed difference between the mass of the left and the right testis or seminal glomerus (Møller & Swaddle 1997). Combined mass was the sum of the left and right mass. We also calculated relative asymmetry as absolute asymmetry divided by half of the combined mass (Møller & Swaddle 1997, Birkhead et al. 1998). Relative asymmetry was used because asymmetry might increase with increasing trait size. We used both absolute and relative asymmetry for all analyses but, because the results were similar, we have not always reported the results for both.

Data were analyzed using SPSS 9.0. Parametric tests were used when frequency distributions did not deviate significantly from normality (all $P > 0.10$). All tests are two-tailed and data shown are mean ± s.e.

Table 1. Differences in left and right testis size, seminal glomerus size and sperm numbers in tree swallows (data shown are mean ± s.e.).

| Variable	Left			Right			t	df	P
Testis length (mm)	10.31	±	0.14	9.54	±	0.16	5.084	19	<0.0001
Testis width (mm)	8.10	±	0.15	8.12	±	0.12	−0.165	19	0.87
Testis height (mm)	6.65	±	0.10	7.15	±	0.08	−5.461	19	<0.0001
Testis volume (mm³)	292.40	±	10.81	291.36	±	9.78	0.156	19	0.88
Testis mass (g)	0.3255	±	0.0098	0.3275	±	0.0118	−0.263	35	0.79
Seminal glomerus mass (g)	0.0656	±	0.0033	0.0658	±	0.0034	−0.213	33	0.83
Sperm numbers ($\times 10^6$)	29.9	±	3.0	31.9	±	3.8	−1.519	33	0.14

Results

Testes asymmetry and the compensation hypothesis

The pattern of directional testes asymmetry was investigated using a repeated-measures ANCOVA with the left and right testis treated as repeated measures of the same subject (see Merilä & Sheldon 1999). To account for possible differences in age or body size, we included the factor status (floater or resident) and the covariate body mass in the model. None of the effects was significant, including side ($F_{1,28} = 0.47$, $P = 0.50$). Thus, there is no directional asymmetry in testes mass in the tree swallow. Twenty-two of 36 males (61 %) had a heavier left than right testis, which is not different from 50 % (binomial test, $P = 0.24$). On average, the left and right testis did not differ significantly in mass, nor in volume, but they were significantly different in shape (Table 1) with the left testis being more elongated compared to the right one.
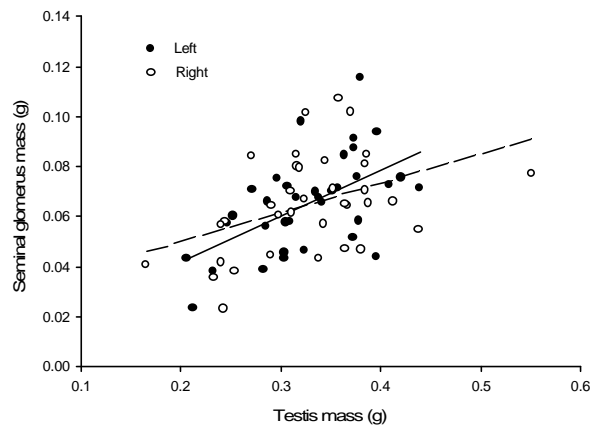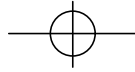
Relative testes asymmetry was not significantly correlated with the mass of the left testis, nor with combined testes mass ($r = –0.28$, $n = 36$, $P = 0.10$) but it was strongly negatively correlated with the mass of the right testis (Fig. 1). This negative correlation remains significant if the two extreme values are removed from the analysis ($r = –0.42$, $n = 34$, $P = 0.013$).

The mass of the left and right seminal glomerus was positively related to the mass of the left and right testis (Fig. 2). However, an analysis of covariance showed that there was no difference between the slope of the two regressions ($F_{1,66} = 1.05$, $P = 0.31$), nor was there a difference between left and right seminal glomerus mass when controlling for the corresponding testis mass ($F_{1,66} = 0.98$, $P = 0.33$). The residuals of the regressions of seminal glomerus mass on testis mass (for left and right side) were strongly correlated (Fig. 3), as one would expect if individuals differed in copulation history and/or testes function. However, there was no indication that one testis compensated for the other.
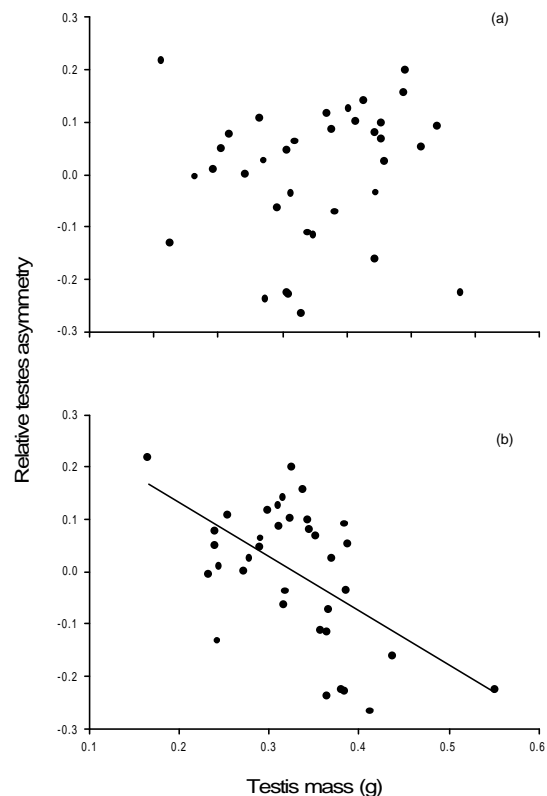
Figure 1. Relative testes asymmetry in tree swallows in relation to the mass of (a) the left testis ($r = 0.10$, $n = 36$, $P = 0.57$) and (b) the right testis ($r = –0.56$, $n = 36$, $P < 0.0001$).
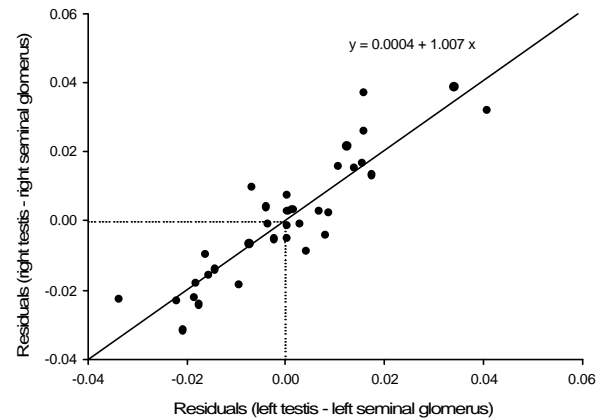
Figure 2. Testis mass in relation to seminal glomerus mass in tree swallows for (a) the left side (solid line: r = 0.56, n = 35, P < 0.0001) and (b) the right side (dashed line: r = 0.42, n = 35, P = 0.011).

Figure 3. Relationship between the residuals of the regression of seminal glomerus mass on testis mass for the left and right side (r^2 = 0.81, n = 34, P < 0.0001) in tree swallows. The intercept does not differ from zero (t = 0.25, P = 0.81).

Relative testes asymmetry was not significantly correlated with the mass of the left or right seminal glomerus, nor with combined seminal glomera mass (all P > 0.15).

Testes size and asymmetry and male characteristics

Combined testes mass was positively related to wing length but not to any other male characteristic (Table 2). Neither relative nor absolute asymmetry in testes mass was related to any of the male characteristics (Table 2). In floaters the left testis was on average larger than the

right, while the opposite was true in the residents. However, the difference was not significant (absolute asymmetry; residents: –0.011 ± 0.010, n = 20, floaters: 0.010 ± 0.011, n = 16, t = –1.38, P = 0.18). Combined testes mass and testes asymmetry were not related to muscle and fat scores (data not shown, ANOVAs, all P > 0.20).
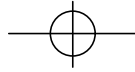
Discussion

This study provides little evidence for Møller's (1994) compensation hypothesis, nor do we find evidence for the hypothesis that testes asymmetry is related to male

Table 2. Correlations between testes size and asymmetry and male characteristics in tree swallows. Values given are correlation coefficients (Pearson for wing and tarsus length and body mass, Spearman rank for number of mite holes and capture date).

Variable	Combined testes size	Absolute asymmetry	Relative asymmetry
Wing length (n = 24)	0.50[1]	–0.02	0.01
Tarsus length (n = 23)	0.20	0.08	0.06
Number of mite holes (n = 33)	0.04	0.24	0.20
Body mass (n = 31)	0.31	0.15	0.16
Body condition[2] (n = 21)	0.33	0.02	0.02
Capture date (n = 36)	–0.19	0.23	0.28

[1]P = 0.013 (before sequential Bonferroni correction). All other P > 0.05.
[2]Residuals of a regression of body mass on tarsus length.

Table 3. Review of studies on directional asymmetry in testes size of birds.

Species		Variable	Asymmetry	n	Reference
Sedge warbler	*Acrocephalus schoenobaenus*	Testis mass	L > R ***	15	Birkhead et al. (1997)
Greenfinch	*Carduelis chloris*	Testis length	L < R ***	141	Merilä & Sheldon (1999)
Madagascar coucal	*Centropus toulou*	Testis size	L << R [c]	45	Ligon (1997) and refs therein
Stock pigeon	*Columba oenas*	Testis mass	L < R ***	10	Yu (1998)
Japanese quail	*Coturnix japonica*	Testis mass	L > R ns	10	Yu (1998)
Domestic chicken	*Gallus domesticus*	Testis mass	L ≈ R ns	378	Hocking (1992)
Red junglefowl	*Gallus gallus*	Testis mass[a]	L < R *	16	Kimball et al. (1997)
		Testis mass[b]	L > R ns	19	Kimball et al. (1997)
Red junglefowl	*Gallus gallus*	Testis mass	L > R ***	12	Yu (1998)
Barn swallow	*Hirundo rustica*	Testis volume	L > R ***	47	Møller (1994)
Domestic turkey	*Meleagris* sp.	Testis mass	L > R ***	35	Burke (1973)
House sparrow	*Passer domesticus*	Testis volume	L > R ***	177	Møller (1994)
Tree sparrow	*Passer montanus*	Testis mass	L ≈ R ns	7	Yu (1998)
Zebra finch	*Taeniopygia guttata*	Testis mass	L > R ***	31	Birkhead et al. (1998)
Tree swallow	*Tachycineta bicolor*	Testis mass	L ≈ R ns	35	This study
		Testis length	L > R ***	19	This study

* = P < 0.05, *** = P < 0.001, ns = P > 0.5
[a] group of 10 months old individuals
[b] group of 12 months old individuals
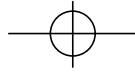[c] left testis is either absent or rudimentary (not functional)

quality or condition. Our study confirms the results of several previous studies (Birkhead et al. 1997, 1998, Kimball et al. 1997, Merilä & Sheldon 1999) except for Møller's (1994) study, which showed a strong correlation between testes asymmetry and (a) tail length in the barn swallow *Hirundo rustica* and (b) throat badge size in the house sparrow *Passer domesticus.*

Despite the general pattern in birds that the left testis is the larger one (Lake 1981), testes asymmetry clearly varies among and even within species (Witschi 1935; review in Table 3). In five species, the left testis was significantly larger than the right, but in three species the reverse was shown (Table 3). The most extreme asymmetry is found in the Madagascar coucal *Centropus toulou* (and probably in other coucal species), where the left testis is absent or non-functional (Ligon 1997). In several species, no asymmetry is found, despite adequate sample sizes (Table 3). We found that in the tree swallow the left and right testis differed in shape (the left was significantly longer, while the right was significantly broader), but not in mass or volume (Table 1). This suggests that one should be careful when measuring only testes length and using this as an overall measure of size or mass (as in Merilä & Sheldon 1999).

Testes asymmetry may be selected for to reduce overall body mass, which might be important for flight performance. Kimball et al. (1997) thus proposed that testicular asymmetry should be more pronounced in aerial species than in primarily terrestrial ones. Our study clearly refutes this hypothesis. Tree swallows are migratory and rely on flight for foraging, so following Kimball et al.'s (1997) suggestion, they should show high levels of directional asymmetry in testis size.

We did find substantial variation among individuals in the difference between left and right testis mass. Testes asymmetry was related to the size of the right testis, but not to the size of the left one, or to combined testes mass. This provides weak support for the compensation hypothesis, because one would expect a small right testis when the left one is big (and thus a higher level of asymmetry) and vice versa. The asymmetry in testes mass was not related to the mass of the seminal glomera (left, right or combined). Hence these results do not support the idea that the right testis compensates for a malfunctioning left testis (Møller 1994).

If asymmetric testes are selected for in birds because it is too costly to develop two large testes (e.g. because of flight constraints), then there should be an advantage

of having one large testis instead of two medium sized ones. This advantage might be the higher efficiency in sperm production of the left testis due to constraints during embryonic development. Primordial germ cells immigrate into the gonads before differentiation of the sexes. In most female birds, only the left ovary is present, while the right ovary is reduced at a very early stage in development as an adaptation to flight and the production of large eggs (Jones et al. 1993). This results in decreased attraction of primordial germs cells in the right gonad (Witschi 1935). Because this process occurs before gonad differentiation, one could conclude that the right male gonad contains fewer germ cells and is thus less efficient in spermatogenesis (Kimball et al. 1997). We investigated this by comparing the amounts of stored sperm (measured as seminal glomerus mass) in relation to testis size (mass). For both sides the amount of stored sperm were positively related to testis mass. However, we found no evidence for a difference in sperm production efficiency between the left and right testis (Figs. 2 and 3), although we do not have information about the rate of sperm production by the left and right testes, nor about the rate of sperm use from the left and right seminal glomerus. Nevertheless, the available data suggest that there is no advantage in developing a larger left testis, at least for tree swallows. It would be interesting to investigate this in a species that shows strong directional asymmetry in testes size.

Testes asymmetry was not related to our measurements of male quality or condition and it was not different for males of different social status. It remains possible that we did not measure the most relevant characteristics indicating male quality or condition. Although we do not know the age of the males (first year and older male tree swallows cannot be distinguished on plumage colour), there was no suggestion that testes asymmetry is related to age. This is based on the assumption that age is related to wing length and social status. In tree swallows, first year females have shorter wings and are more likely to belong to the floater population than older females (own unpubl. data, Stutchbury & Robertson 1987). We did not find any relationship between social status or wing length and testes asymmetry. We did find, however, that combined testes mass was correlated with wing length. Although this result would not be significant after sequential Bonferroni correction, we do not believe it is a type I error. Despite a small sample size, wing length was also high-

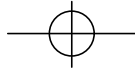ly correlated with the length of the left testis (r = 0.88, n = 9, P = 0.002).

Perhaps testes asymmetry is a by-product of selection for asymmetry in the female reproductive tract in birds, which could be an adaptation to flight (Witschi 1935; see discussion in Birkhead et al. 1998). On the other hand, asymmetry in testes size could also be linked to directional asymmetry and position of other internal organs in the body cavity (Witschi 1935, Kraak 1997). However, in species with high levels of sperm competition (such as the tree swallow) one would expect selection on sperm production to be so intense that both testes become fully developed. The presence of other asymmetric or asymmetrically positioned organs could then lead to asymmetry in testes shape, but not in overall size, as we found for the tree swallow.

Based on the current evidence, we conclude that directional asymmetry in testes size is not a general phenomenon in birds, and that its adaptive significance remains unclear.

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The cost of avian winter stores: intra-individual variation in basal metabolic rate of a wintering passerine, the greenfinch *Carduelis chloris*
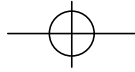
Åke Lindström[1,2] and Mikael Rosén[1]

Several factors may influence the optimal size of fuel stores of small passerines in winter. A relatively unknown factor is how basal energy costs may depend on the size of fuel stores. We measured how the basal metabolic rate (BMR) of wintering greenfinches *Carduelis chloris* varied within individuals in relation to body mass (m). Birds depleted of fat stores were allowed to increase in mass over a two-day period. Whereas body mass increased by 9 % on average, BMR increased by 23 %. Intra-individually, BMR was proportional to body mass as $m^{2.8}$. That BMR increases with body mass within individuals is in accordance with studies of other birds, most of which were conducted during migration. The change in BMR parallel to changes in body mass indicates that protein is stored along with fat. A basal energy cost of winter stores, as found in greenfinches, should be taken into account in estimates of energy budgets.

Key words: BMR, *Carduelis chloris*, energy, greenfinch.

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Much attention has been paid to the strategies evolved by small songbirds to survive the cold and dark temperate-zone winters. Particular focus has been put on the optimal size of fat stores, especially in the light of the trade-off between starvation and predation risk (e.g. Lima 1986, Lehikoinen 1987, Houston & McNamara 1993, Witter & Cuthill 1993). In energy terms, winter stores have both costs and benefits. Energy stores help the bird to survive long cold nights and act as an insurance against periods of harsh weather when foraging is restricted. Various energy costs associated with carrying winter stores have been postulated, but have only rarely been measured (Witter & Cuthill 1993). Most attention has been paid to mass-dependent costs of locomotion (flight power is higher for a fat than a lean bird; Pennycuick 1975, Kvist et al. 2001), and therefore, heavy birds have to spend more time foraging to maintain their mass (Witter & Cuthill 1993, Houston et al. 1997).

In their review of the cost of avian fat storage, Witter & Cuthill (1993) suggested that there may already be a cost of energy stores at the basal level, but they noted the lack of experimental evidence for this. They suggested that one reason for an increased basal cost could be due to protein deposition in the form of muscular hypertrophy. Indeed, the deposition of protein has been shown to be a substantial part of the mass gain of passerine birds during winter (Newton 1969, Jenni & Jenni-Eiermann 1987). We measured the basal metabolic rate (BMR) during winter in greenfinches *Carduelis chloris*, a 25 g passerine that regularly winters in northern Europe, to investigate how basal energy costs may vary with the size of winter stores.

Methods

The greenfinches were trapped at a bird feeder in Torna Hällestad (61°12'N, 13°50'E), 16 km east of Lund, Sweden, on 3 December 1996. Many greenfinches winter in Sweden and those that do migrate leave south Sweden mainly in October and early November (Enquist & Pettersson 1986). Hence, the birds we studied were most likely in a non-migratory wintering phase. To avoid potentially confounding effects of age and sex, only first-year females were used for the experiment (most birds trapped were first-year females).

The birds were kept indoors in separate cages under the simulated natural light regime of Lund, 8L: 16D, at an ambient temperature of about +20° C. Between the time of capture and the start of the experiment we let the finches get accustomed to the food (peeled sunflower seeds) and the special food containers required for the metabolic chambers. Birds that did not eat within two days from the date of capture were released. At the time of capture the birds were fat, with fat scores of 4–6 (Pettersson & Hasselquist 1985). For eight of these birds we restricted the food intake so that they reached an evening fat score of 2–3 at the start of the measurements. The average food deprivation process lasted 2–3 days depending on initial fat score.

Two rounds of experiments were carried out, starting on 9 and 12 December, respectively, each with four birds measured in parallel. During the experiment the birds were kept in metabolic chambers for three nights and the two days in between. Oxygen consumption was measured throughout. On the morning after the first night the birds were weighed to the nearest 0.01 g, within 30 min of lights-on. Fat scores were also recorded. The birds were then put back into the chambers and offered water and peeled sunflower seeds *ad libitum*. At lights-off, the food container was removed and the birds no longer had access to food. The same procedure was repeated on the second and third mornings.

Oxygen consumption was measured in an open-circuit system. The birds were kept in individual 22 l metabolic chambers, housed in a climate cupboard at a light regime of 8L:16D and constant temperature of +25° C. The lower critical temperature of greenfinches has been measured as +16° C (Gavrilov 1985), and hence our measurements were carried out in the thermoneutral zone. Air was sucked through the system at

a flow of $25 \, \mathrm{l \, h^{-1}}$ as measured by mass flow controllers (for further details of the system, see Lindström et al. 1999). The oxygen consumption of a given bird was measured for 25 min in sets of two birds, interrupted by 15-min measurements of reference air. This meant that the oxygen consumption of each bird was measured for 40 % of the time it was in the chamber.
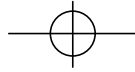
We used large volume chambers for the benefit of the animals, as they stayed for a long period in them. A potential problem is that large volume chambers can leave rapid changes in oxygen consumption undetected, resulting in BMR values that are too high. However, we registered changes in oxygen consumption of up to 9 % in 7 minutes during continuous measurements. Further, the short-term variation of oxygen consumption was very different between day and night. Daytime measurements were variable, indicating various activities, whereas night-time readings were smooth and constant, indicating that the birds were inactive at night, their normal rest phase. We therefore believe that our measurements did give representative values of BMR. Further, because the oxygen consumption patterns were very similar for the birds when light and heavy, the intra-individual comparisons that are the focus of this study should not be influenced to any important degree.

BMR (in Watts) was estimated using the lowest 10 min average rate of oxygen consumption at night. We assumed an RQ of 0.70 and an energy equivalent of 19.8 $\mathrm{kJ \, l^{-1}}$ oxygen consumed (Gessaman & Nagy 1988). The lowest energy consumption rate always occurred between midnight and 07.00 hrs, i.e. at least 8 h after food was witheld. The average time to digest sunflower seeds completely is less than 4 h for chaffinches *Fringilla coelebs* (Dolnik 1982), and it is therefore likely that our greenfinches were post-absorptive during the BMR estimates. Variations around averages are given as s.d.

Results

The increase in individual body mass averaged 9.1 % over the two days, with fat scores increasing in parallel from 1.3 to 4.4. The corresponding increase in BMR was 22.8 %. BMR was positively correlated with body mass for each of the eight birds (Table 1). As least squares regression underestimates the true functional relationship between body mass and BMR, we calcu-

Table 1. Body mass and basal metabolic rate (BMR) of eight juvenile female greenfinches during three consecutive nights. The slope (v) and r^2 of the reduced major axis of log BMR against log body mass are given for each bird.

Ind. #	Body mass in morning #				BMR in night #				RMA	
	Day 1 g	Day 2 g	Day 3 g	Increase %	Day 1 W	Day 2 W	Day 3 W	Increase %	v	r^2
528	25.2	26.5	27.3	8.3	0.378	0.411	0.407	7.7	1.12	0.79
529	23.0	24.4	24.1	4.8	0.349	0.456	0.441	26.4	4.67	0.99
533	22.9	25.0	25.8	12.7	0.401	0.451	0.441	10.0	1.01	0.82
545	21.2	23.2	24.1	13.7	0.307	0.348	0.374	21.8	1.50	0.99
534	22.8	23.9	24.3	6.6	0.344	0.408	0.426	23.8	3.44	1.00
544	21.9	22.8	23.5	7.3	0.293	0.400	0.408	39.2	5.27	0.86
539	22.8	24.2	25.2	10.5	0.343	0.380	0.445	29.7	2.61	0.95
530	22.6	23.8	24.6	8.8	0.353	0.410	0.436	23.5	2.54	0.99
Mean	22.8	24.2	24.9	9.1	0.346	0.408	0.422	22.8	2.77	
(s.d.)	(1.15)	(1.14)	(1.22)	(3.04)	(0.035)	(0.035)	(0.024)	(10.19)	(1.60)	

lated the reduced major axis, RMA (Pagel & Harvey 1988, McArdle 1988), for each individual separately on log-transformed data for body mass and BMR. The average RMA slope was 2.8 ± 1.60 (range 1.0–5.3, n = 8, 95 % confidence interval 1.4–4.1, Table 1). The average slope of 2.8 is significantly different from zero (one-sample t-test, $t_7 = 4.91$, P = 0.002). The RMA slopes were on average 4 % higher than the corresponding least square regression slopes (not presented). There was a negative correlation between the RMA slope and the overall mass increase of individual birds (r = –0.75, P = 0.033). Thus, the more a bird increased in mass, the relatively less its BMR increased.
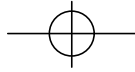
Discussion

Basal metabolic rate varied rapidly and substantially, and was positively correlated to body mass within individual greenfinches. The average mass-dependent exponent of 2.8 is comparatively high (see below), although the increase was on average less (slope of 1–1.5) for the birds increasing most in mass. In other studies, where intra-individual variation in BMR of raptors, shorebirds and passerines was measured during migration and in winter, mass-specific slopes (RMA) varied between 0.9 and 2.2 (Daan et al. 1989, Piersma et al. 1995, Scott et al. 1996, Lindström et al. 1999, Williams & Tieleman 2000, Kvist & Lindström 2001, Battley et

al. 2001). Hence BMR generally changes substantially and rapidly in individual birds along with changes in body mass.

We believe our study is the first to establish a mass-dependent basal cost of winter stores in songbirds (Witter & Cuthill 1993). An increase in BMR of 23 % almost certainly affects the energy budget of a small songbird to a significant degree. Our greenfinches did not fully increase body stores over the two days of measurement to the level they had when initially captured, and it is possible that if the birds had had the opportunity to lay down more stores, the increase in BMR would have been even higher. Mass-dependent basal metabolic cost should clearly be incorporated into models of avian winter survival strategies, in addition to the already well-recognised mass-dependent cost of locomotion (Pennycuick 1975, Kvist et al. 2001), which is often taken into account in studies of winter strategies (Houston & McNamara 1993, Houston et al. 1997).

Energy storage in wintering songbirds is normally referred to as 'fattening' (Houston et al. 1997 and references therein) but the potential inclusion of protein is not often acknowledged (Biebach 1996). However, Newton (1969) and Jenni & Jenni-Eiermann (1987) concluded that a major part of the 'winter fattening' in small temperate-zone passerines was due to protein, of similar magnitude to the 30–50 % protein fraction of mass increases in migrating birds (reviewed by Lind-

ström & Piersma 1993). The positive correlation between body mass and BMR in the greenfinches is in line with such winter protein deposition. The correlation occurs because protein is only deposited as metabolically active tissues such as muscles and digestive organs. In contrast, fat and carbohydrates are stored as metabolically low-active tissue and hence should have little direct impact on BMR (Martin & Fuhrman 1955, Scott & Evans 1992). That the slope of BMR against body mass was generally lower in the birds increasing most in mass indicates that proportionally more protein is stored at the beginning of a fuelling bout (cf. Jenni & Jenni-Eiermann 1998).

It is not clear how the protein is deposited in greenfinches, but most likely rapid changes in the size of pectoral muscles are involved, as has been shown for birds during reproduction (Jones & Ward 1976), moult (Lind & Jakobsson 2001) and migration (Lindström et al. 2000).

Protein (with its associated water) has an energy density of 5.4 kJ g^{-1}, which is considerably lower than the 38.1 kJ g^{-1} of stored fat (Jenni & Jenni-Eiermann 1998). The inclusion of protein in parallel to fat will therefore reduce the energy density of the stores, which should be taken into account in detailed energy budgets for wintering passerines.
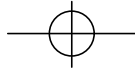
Klaassen & Lindström (1996) showed that the extra resting metabolic costs of fuel stores in migrating birds can alter the optimal size of migratory fuel stores. Houston et al. (1997) showed the need for a mass-dependent cost of winter stores to explain the fact that optimal winter stores are often kept at physiologically submaximal levels. They suggested that this cost may either be reduced agility, and an accompanying increased predation risk, or a (non-specified) mass-dependent effect on metabolism. We suggest that basal energy costs of winter stores, at rest and at night, is such a mass-dependent cost that may influence strategic decisions on the size of energy stores.
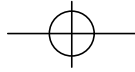
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Sexual selection, colour perception and coloured leg rings in grouse (Tetraonidae)

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Studies on birds have suggested that coloured leg rings, resembling ornamental male feathers or bare parts, may increase male attractiveness to females or be important in intrasexual interactions between males. Most of these studies, however, have not considered that the physiology of avian vision is different from that of humans. In consequence, colours that are perceived as similar by a person, may be seen as different by a bird. The effect of leg rings on various aspects of the reproductive behaviour of male black grouse *Tetrao tetrix* was studied using data obtained from central Finland in 1987–1989. Black grouse males have distinct reddish supra-orbital eye-combs, which have presumably evolved through sexual selection. We used a retinal physiological model of a chicken to compare reflectance spectrograms of orange and red leg rings and eye-combs. The model proposes that, unlike man, grouse are likely to discriminate between spectral reflectance of a red leg ring and eye-comb, but not between an orange leg ring and eye-comb. Black grouse males equipped with orange or red leg rings did not achieve more copulations than males with other colour combinations. Furthermore, we found no significant evidence that males with orange or red leg rings fought more than other males, which would be predicted if ornament-resembling leg rings elicit aggression from other males. Results from meta-analyses on the relationship between coloured leg rings and female choice in grouse proved consistent with our findings in black grouse, and suggest that ornament-resembling coloured leg rings do not reinforce mate choice of grouse females.
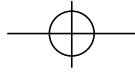Key words: Sexual selection, colour perception, leg rings, grouse.

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Several studies on birds have documented that females use conspicuous male colours in their mate choice and favour males that express the most elaborated plumage or ornamental bare parts (e.g. Andersson 1994). Additionally, ornaments may be of primary importance in male-male signalling (e.g. Peek 1972, Smith 1972, Metz & Weatherhead 1991).

In the context of sexual selection it has been suggested that coloured leg rings originally used for individual identification in field studies may attract prospecting females, as well as leading to escalated fights between males (Burley et al. 1982, Holder & Montgomerie 1993, Metz & Weatherhead 1993, Cuthill et al. 1997). These observations have induced a suite of empirical and experimental studies that have demonstrated that leg rings of an ornament-resembling colour may increase male attractiveness (e.g. Burley et al. 1982, Brodsky 1988, but see Ratcliffe & Boag 1987,

Holder & Montgomerie 1993, Zann 1994, Jennions 1998) and change the fighting behaviour of territorial males (Holder & Montgomerie 1993, Metz & Weatherhead 1993). A plausible explanation for these results is that leg rings reinforce the effect of the ornament. Rintamäki et al. (2000) have recently verified that a larger red eye-comb predicts higher mating success among successful males in black grouse *Tetrao tetrix*. We thus have a reason to suspect that leg rings resembling the eye-comb in colour may alter sexual behaviour in grouse.
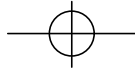
In most studies on bird coloration human perception has been used to define colours. However, colour vision in humans and birds is different in several respects. The eyes of most birds are tetrachromatic, which means that the retina contains four distinct classes of cones, whereas in humans there are three (Bowmaker et al. 1997). The sensitivity maxima of the corresponding cone classes occur at different wavelengths in humans and birds and among different bird taxa. Most noticeably, the sensitivity maximum of the fourth cone class in birds is positioned in or near the ultra-violet part of the spectrum (UV: 355–426 nm, Hart 2001), to which humans are blind. Avian cones contain a coloured oil droplet, which acts as a cut-off filter to sharpen spectral tuning (Bowmaker 1980, Hart 2001). Thus, because of their retinal characteristics, colours may be perceived differently by humans and birds. Birds may see more colours and with greater precision than humans, and what is a bright colour to a human is not necessarily as bright to a bird. Hence, the human eye is unsuitable for assessing the colours perceived by birds (Bennett et al. 1994).

The first part of this study quantifies the colour difference between leg rings and the eye-combs of black grouse as perceived by birds and humans. Second, we investigate the possible effect of orange and red leg rings on copulation frequencies and fighting behaviour of black grouse males. Finally, we use meta-analyses to unravel general patterns from studies that have looked for effects of coloured leg rings on female choice in grouse species.

Methods

Spectral reflectance measurements

To compare eye-comb and leg ring reflectance over the visual spectrum we performed reflectance spectrometry on an orange leg ring, a red leg ring and the supra-orbital eye-comb of seven black grouse males. The males were shot in October 1999 in Hillsand, 40 km north of Strömsund, central Sweden. No specimen showed any visible post-mortem change in eye-comb coloration and comparable measurements from living birds were identical to ours (R. V. Alatalo, pers. comm.). Reflected radiance was measured with an Ocean Optics S-2000 diode-array spectrometer, a fibre optic probe and a combined deuterium-halogen light source (TOP Sensor Systems DH-2000). The probe had one optical fibre connected to the spectrometer and six surrounding fibres connected to the light source. To standardise the distance from the illuminating fibres to the object, the probe was mounted inside a tube with a black inner surface. The tube had an inner diameter of 3.5 mm and the probe was mounted so that the tip was 3.5 mm from the open end, which had been cut perpendicular to its long axis. The open end was held against the eye-comb perpendicular to its surface to measure a 2 mm wide illuminated spot. Three measurements were taken from each bird (the anterior, middle and posterior part of the eye-comb on one side) and the mean was used in the analysis. To avoid direct reflectance from the more glossy leg rings we used a similar tube, but with the end cut at an angle of 60° to the long axis, to measure an illuminated oval, 2×3 mm in size, from an angle 60° from the surface. The reason for not using the standard angle of 45° was to avoid re-reflection of the object from the inner surface of the tube. Test measurements using a similar 45° tube, where the inner surface was at right angles to the light source, showed a 10 to 20 percent stronger reflection compared to the measurements with the 60° tube. Reflectance measurements were standardised against a white reference (TOP Sensor systems WS-2) and the inside of a dark box. The standards were scanned immediately before and from the same angle as the eye-combs and the orange and red leg rings, respectively. Reflectance data were recorded in 2048 consecutive wavelengths from 175.586–880.581 nm, but only every fifth nanometre between 300 and 750 nm were included in the analyses. This

range more than covers the commonly observed perceptible range for many birds (Bennett & Cuthill 1994). This reduction in data density did not affect our results because no narrow reflectance peaks were omitted.

Quantification of colour perception

The perceived difference between the colours for black grouse and humans was calculated from the spectral reflectance data using the model in Maddocks et al. (2001). This model is based on the model presented in Vorobyev et al. (1998) and describes the perceived difference between two colours, given as a one-dimensional distance. It is calculated from the reflectance spectrum of the respective colour surfaces, the spectral sensitivity of each single cone receptor mechanism, and the spectrum of incident light. All colour measurements in this model are scaled such that the maximum possible distance between two colours (two primary colours) is one.

Distances were calculated for all combinations of eye-combs of individual black grouse and combinations of eye-combs and orange and red leg rings. To calculate the black grouse values we used the eye design of the chicken *Gallus gallus*. As the chicken belongs to the same order (Galliformes) as the black grouse we assumed that the chicken eye design also applies to black grouse. All eye physiological parameters were kindly provided by Daniel Osorio. Spectrometric data of incident light was taken from ISO 9845-1: 1992(E).

Behavioural data on black grouse

Our behavioural data come from four black grouse leks in central Finland during 1987–1989 (n = 58 observation mornings when female visits peaked in late April and early May) and includes a total of 41 males. We included each male only once in the data set if he was present in more than one year (we used the data from the first year). Most birds (n = 30, 73 %) came from our largest lek (Lek 1: 10 ± s.d. 2.6 males/year; smaller leks, 2.2 ± 0.8 males). Birds were captured at winter feeding sites before the mating season. Males were marked with two or three coloured rings plus one aluminium ring on their legs. We used the following permanent colours: red, orange, yellow, white, black, green and light and dark blue. Colour-ringing was ran-

dom with respect to male attractiveness as most of the males were marked before their first mating season (i.e. at the age of less than one year). Details on the study area, population and general methods may be found in Rintamäki et al. (2001).
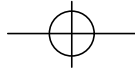
As in previous studies of coloured leg rings in rock and willow ptarmigan *Lagopus mutus* and *L. lagopus* (Brodsky 1988, Holder & Montgomerie 1993, Hannon & Eason 1995), we classified our males as having either orange leg rings (denoted as O), red (denoted as R), or one or the other of these colours (denoted as O/R), or wearing other colour rings described above (denoted as NO). We analysed each of these ornament-resembling colours against other colours separately. The analyses presented in Table 1 include at maximum 16 O/R, 12 O, 6 R and 35 NO males. Each O- or R-ringed male wore one O or R leg ring. The vertical size of the leg ring (ca. 1.0 cm) is somewhat smaller than the height of the male eye-comb during peak lekking activity (1.5 ± s.d. 1.6 cm, n = 17 males).

Black grouse copulations are easy to observe, and a male seen copulating is in most cases the true father of all chicks (Alatalo et al. 1996). Since we had particularly reliable information on the number of copulations of each black grouse male, we used copulations (denoted as Copulation) in all our analyses.

An orange or red leg ring might be expected to elicit territorial disputes among adjacent males on the lek arena. To study this, we measured each male's fighting behaviour (denoted as Fight) as the proportion of scans in which fighting was observed during repeated 1-min scan samples. Median total time budget values for each male in each year were 38 min in 1987, 17 min in 1988 and 32 min in 1989. We expected that if coloured leg rings altered (increased or decreased) a male's probability of being involved in territorial disputes, O/R males would have different scores than non-O/R-ringed males.

Meta-analyses

Meta-analysis was developed for obtaining generalisations (estimates) over several studies investigating the same problem (e.g. Hedges & Olkin 1985, Hunter & Schmidt 1990, Arnqvist & Wooster 1995). We searched the Biological Abstracts database (1980–2001) for grouse studies using colour rings and leg rings as keywords. The Pearson correlation coefficient (r) was

Table 1. Differences in sexual behaviour between male black grouse equipped with orange (O), red (R), orange or red (O/R) and other (NO) leg rings. Copulation is the mean no. of copulations for each category. The variable Fight is the proportion of time spent fighting (%). Values are means (± s.d.). The data were analysed using the two-tailed Mann-Whitney U-test.

Trait	orange or red ring	other leg-rings	Z	n_1	n_2	P
	orange (O)	*NO*				
Copulation	1.8 ± 2.2	1.2 ± 1.7	1.00	12	29	0.32
Fight	21.4 ± 14.2	17.5 ± 11.9	0.71	10	25	0.47
	red (R)	*NO*				
Copulation	0.2 ± 0.4	1.5 ± 1.9	1.70	6	35	0.09
Fight	26.0 ± 17.4	19.1 ± 12.6	0.90	6	29	0.37
	orange or red (O/R)	*NO*				
Copulation	1.2 ± 1.9	1.4 ± 1.8	0.28	18	23	0.78
Fight	20.8 ± 14.3	19.9 ± 13.2	0.02	16	19	0.99

used as a measure of effect size. If effect sizes were unavailable in the original publications, we transformed them from the test information given in the papers (Table 3). The program used to transform test statistics and calculate meta-analysis statistics was developed by Ralf Schwarzer (Internet address: http://userpage.fu-berlin.de/~health/meta_e.htm). In the analyses we used Fisher's Z-transformation (Hedges & Olkin 1985) and population effect sizes were calculated from the weighted mean r by the Schmidt-Hunter method (Hunter & Schmidt 1990). This option adjusts effect sizes for sample size so that studies based on larger samples have larger impact than studies based on small samples.
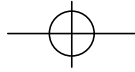
For analyses of copulation success and fighting behaviour (Table 1) we used all data, including males from smaller leks. In the meta-analysis we used black grouse data from Lek 1 only, because the number of males from smaller leks was inadequate to make the relevant analyses. In studies on rock and willow ptarmigan, males were divided into non-successful, monogamous and polygynous males (Brodsky 1988, Hannon & Eason 1995) or monogamous and polygynous males (Holder & Montgomerie 1993).

Results

Differences in spectral reflectance between eye-combs and leg rings

The average perceived colour difference between eye-combs was 0.041 (s.d. 0.023) if perceived by a chicken and 0.036 (s.d. 0.018) if perceived by man. The average distance between a red leg ring and eye-comb was 0.074 (s.d. 0.037) for the chicken and 0.068 (s.d. 0.018) for man. The average distance between an orange leg ring and the eye-comb was 0.039 (s.d. 0.028) for the chicken and 0.025 (s.d. 0.012) for man.

Using resampling with replacement (10 000 repeats; source code and data available from the authors) from a pool of all spectral reflectance measurements of eye-comb–eye-comb and eyecomb–colour-ring values, we tested the probability of producing a mean distance equal to or greater than the observed means by chance. For man, the mean distances from the randomisations did not deviate significantly from the observed mean among eye-combs (P = 0.35 for red leg rings and P = 0.13 for orange leg rings). This roughly coincides with visual observations by the authors, for whom both orange and red leg rings were perceived as being rather close in colour to the combs, even though the colour of the orange rings looked somewhat too pale. For the black grouse, the randomised means differed significantly between red leg ring and eye-comb (P = 0.012) but not in the case of orange leg ring and eye-comb (P = 0.80). This implies that a black grouse is not likely to

perceive the colour of the red leg ring as that of an eye-comb, but it may confuse the colour of the orange leg ring with that of an eye-comb.

Copulation success and fighting behaviour

The copulation success and fighting behaviour of male black grouse with O or R rings did not differ significantly from males that wore rings of other colours (Table 1). Our sample sizes admittedly were small, and hence the test power (Erdfelder et al. 1996) was relatively low; at best $(1–\beta) = 0.34$ (two-tailed) using the mean effect size $r = 0.24$ obtained for extravagant traits in the meta-analysis of lekking species conducted by Fiske et al. (1998). Most of the studied variables, however, did not show any direction whatsoever that could be argued to become significant with a larger sample size. In the comparison of red ring v. other rings and copulation success, however, we find that red-ringed males tended to have lower copulation success than males equipped with other colour rings. This result may imply that eye-comb-resembling leg rings may rather impede than strengthen copulation success.

By analysing the data on a yearly basis we could not refute the null hypothesis that coloured leg rings have no effect on mating success (Table 2). The same applied when only successful males were included in the analyses (Copulation: Z-values between 0.09–1.41, P-values between 0.16–0.93, Fight: Z: 0.04–1.04, P: 0.30–0.97, respectively, Mann-Whitney U- test).

Meta-analyses

In our literature search (including our own data), we found a total of 11 grouse studies or data sets comprising 343 individuals (Table 3). Some males and females were likely to have been included more than once in the analysis but, because published datasets did not always contain information on this, we were unable to correct for it. As far as possible we analysed the data on a yearly basis to present a picture of annual differences of the relationship between ornament-resembling coloured leg rings and copulation success. In lekking species the effect size (r) was negative $(r = –0.17)$ and close to significance $(P = 0.07,$ Table 2). This suggests that leg rings matching eye-comb colour may be detrimental rather than beneficial to males. In non-lekking grouse, the effect size was close to zero $(r = 0.04)$. Meta-analyses on available studies of all grouse species combined suggest that coloured leg rings did not affect male copulation success $(r = –0.00,$ Table 2). All meta-analyses showed preferred homogeneity (non-significant χ^2-P-values, Table 2). This implies low a standard deviation of effect sizes within different studies and data sets.
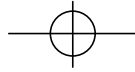
Discussion

The model based on the spectral reflectance data suggests that grouse perceive colours differently from humans. Grouse but not humans may discriminate between the red colour of the supra-orbital eye-comb and

Table 2. Meta-analyses by the Schmidt-Hunter-method (weighted effect size) of studies and data sets of male copulation frequency and colour rings in Tetraonidae. Lekking and mono- or polygamous non-lekking species are analysed separately and together (all species). See Table 3 for detailed results and references. The table includes effect size r, the standard deviation (s.d.) estimate of effect size, Z-test to determine whether the effect size is significantly different from zero, diffuse test for homogeneity of the data set (χ^2; significant P-value indicates non-desirable heterogeneous effect sizes among studies), confidence interval (95 % CI; an estimate of 95 %-credibility interval gives an impression of the possible variation of effect sizes after accounting for sampling error), number of different studies or data sets and total number of individuals on which the estimates are based. 'Hidden studies' (the 'file safe number') refers to the number of studies needed with a zero mean effect size to make the results nonsignificant at $\alpha = 0.05$.

Group	r	s.d.	Z	P	χ^2	P	95 % CI	n studies	n individuals	Hidden studies
Lekking	–0.17	0.30	–1.46	0.07	7.13	0.07	–0.39 – 0.07	4	77	0
Non-lekking	0.04	0.16	0.69	0.33	6.91	0.33	–0.08 – 0.17	7	266	0
All species	–0.00	0.21	–0.09	0.46	15.20	0.13	–0.12 – 0.10	11	343	0

Table 3. Species, sample sizes (n) and effect size (r) for studies of lekking and non-lekking grouse species providing information on coloured leg rings and copulation frequency. A negative coefficient means that males with orange or red colour rings resembling the eye-comb ornament have lower copulation success.
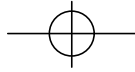
Group	n	r	P	Reference	Comments
Lekking					
Tetrao tetrix	14	−0.391	0.17	This study	Lek 1 1987
(black grouse)	8	−0.832	0.01	This study	Lek 1 1988
	8	−0.053	0.90	This study	Lek 1 1989
Tympanuchus phasianellus	47	0.058	0.70	Gratson et al. 1991;	Both studies
(sharp-tailed Grouse)				Gratson 1993	contained the same data
Non-lekking					
Lagopus mutus	16	0.470	0.07	Brodsky 1988*	Sarcpa Lake, NWT 1985
(rock ptarmigan)	11	−0.389	0.24	Holder and Montgomerie 1993	Sarcpa Lake, NWT 1987
	16	−0.033	0.90	Holder and Montgomerie 1993	Sarcpa Lake, NWT 1988
	8	−0.267	0.52	Holder and Montgomerie 1993	Sarcpa Lake, NWT 1989
	5	0.500	0.39	Holder and Montgomerie 1993	Sarcpa Lake, NWT 1990
Lagopus lagopus	15	0.045	0.87	Hannon and Eason 1995	Removal experiment
(willow ptarmigan)	195	0.036	0.62	Hannon and Eason 1995	Whole data set

* Data from O/R and non-O/R ringed males from year 1984 were not included into analysis because males were colour ringed after pair formation.

red leg rings, whereas neither grouse nor humans distinguish orange rings from eye-comb colour. Coloured leg rings corresponding to ornament colour did not improve male mating success in the black grouse but instead might reduce it, a result supported by meta-analysis of lekking grouse species. The fighting behaviour of black grouse males equipped with reddish or other leg rings did not vary significantly.

To our knowledge, there have been only two studies that paid attention to the effects of leg rings and ornaments in relation to avian colour vision. McGraw et al. (1999), using a spectrophotometer to examine the reflectance properties of plastic leg rings, showed that their reflectance in the UV-range is too low to interfere with signalling. In that study the plastic leg rings were measured perpendicular to the glossy surface with considerable direct reflection as a result. Although, as the authors pointed out, this does not change the overall shape of the spectrographic curves, it may change the ratios between the signal strength of different wavelengths, which is a determining factor for the colour perceived. Furthermore, concentrating on UV might underestimate the importance of differences in colour perception between man and birds in 'normal' light that

are due to differently positioned tetrachromacy absorbance maxima of the cone pigments and the spectral tuning of oil droplets (see Bennett et al. 1994, Hunt et al. 2001). In another study, Hunt and co-workers (1997) suggested that differences in methodology might be a reason for the different outcomes in studies of zebra finches *Taeniopygia guttata*. In some of these, females viewed males through UV-blocking filters, and these females were unable to show any preference for red-ringed males. As a consequence, studies of leg ring effects (including their symmetry) on sexual selection on the same species have not always reached the same conclusions (zebra finch, Burley et al. 1982, Burley 1986, Zann 1994, Swaddle 1996, Jennions 1998), even when studying birds from the same population (rock ptarmigan, Brodsky 1988, Holder & Montgomerie 1993). In addition to methodological differences among different studies, one plausible reason for these contradictory results may be that leg rings affect the behaviour of an individual wearing them. For example, Hjorth (1970) noticed that red leg rings altered male behaviour in black grouse and we have observed two territorial males trying to peck at their own leg ring (in both cases these rings were orange) during lekking.
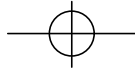
Differences in the visual perception of colour between birds and humans are difficult to interpret because of the morphological differences in eye design. While the model of Maddocks et al. (2001) provides a useful tool for quantifying estimates of colour perception, the model and/or measuring accuracy do not describe colour perception exactly. For example, the spectral difference (the one-dimensional distance ΔS) between orange leg rings and eye-comb colour did not differ significantly for either humans or chicken although human observers perceive a difference. Nevertheless, we propose that future studies of mate choice and sexual selection should focus on the perception of colour by animals rather than perception by humans.

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