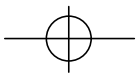



# Parentage determination in kin-structured populations: microsatellite analyses in the Siberian jay *Perisoreus infaustus* during a 25-year population study

**Bo-Göran Lillandt[1, 2], Staffan Bensch[2] and Torbjörn von Schantz[2]**

We used nine polymorphic microsatellite markers for parentage determination in the Siberian jay *Perisoreus infaustus*, and performed analyses on 419 individuals, using feather or blood samples collected during a 25-year population study in western Finland. In strongly kin-structured populations, as in the Siberian jay, parentage determination is difficult because putative parents might be closely related and hence genetically similar. In order to increase the power of the parentage determination system we developed a new method, in which juveniles were tested against observed parent pairs rather than each parent separately. This method was based on the assumption of genetic monogamy in the study population, which was supported by several lines of evidence. The power of the parentage determination system was examined by extensive calculations of within-population and between-population mismatch frequencies. Parentage was examined for 298 juveniles, most of which were sampled within small groups of jays in the autumn. Altogether, we identified the parents of 89 % of all the juveniles sampled during the study period; 19 % of them had left their natal territory before sampling. The study shows that a priori information on the identity of the mother, or information on parental pair-bonds, may be crucial for successful parentage determination in kin-structured populations.

Key words: Parentage determination, Siberian jay, *Perisoreus infaustus*, microsatellite analyses, kin-structured populations.

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When microsatellites were introduced as a tool for parentage determination, they were considered to be the ideal markers for this purpose (Queller et al. 1993). Their main advantages are their large numbers in the genome, high levels of polymorphism, and the fact that they can be scored locus by locus with PCR. Analyses can thus be performed on tiny and even partially degraded DNA samples. Unfortunately microsatellites must be cloned separately for every studied species.

Later studies using microsatellites for parentage determination have revealed new weaknesses. If the number of candidate parents is large (Coltman et al. 1998) or the candidate parents are mutually close relatives (Double et al. 1997), the efficiency of microsatellite analysis may be much weaker than initially calculated

on theoretical grounds. Furthermore, if the studied population is incompletely sampled or the allele data contain typing errors, the reliability of parentage determinations suffers (Marshall et al. 1998) and the power of analysis may be insufficient to make any conclusions at all about parentage (Taylor et al. 1997). Two basic problems may occur: (1) more than one female or male may be identified as a potential parent, or (2) no matching parent may be found. Success in parentage determinations is thus not only dependent on the exclusion power of the microsatellite loci used, but also on the mating system and population structure of the studied species, and on the proportion of individuals sampled for DNA.

In this study we investigate the problems of parentage determination in the Siberian jay *Perisoreus infaustus*, a species that lives in small territorial groups around an apparently monogamous adult pair (Ekman et al. 1994). The Siberian jay is strongly philopatric, whereby candidate parents are often closely related. We applied a set of nine microsatellite markers on a data set of feathers or blood samples collected during a long-term population study in Finland. Because of the strong kin-structure of the population we developed a new method, in which juveniles were tested against observed parent pairs rather than each parent separately. This method is based on the assumption of total monogamy in the population, and this assumption was tested in several ways. The aim of this paper is (1) to confirm the assumption of total monogamy in the study species, and thereby validate our new method for parentage assignment; (2) to check parentage within observed groups of jays; and (3) to trace the origin of juveniles that had left their natal territory before sampling. This important information will be used in later studies of the family structure and dispersal behaviour of this social bird species.

## Materials and methods

### Species and study area

The Siberian jay (also called 'jay' in this paper) is a long-lived (<20 years), resident bird species living in mature coniferous forests of the Eurasian taiga (Helle & Lillandt 1997). The jays form life-long, monogamous pair-bonds and live in permanent territories. Divorces are rare, but widowed birds can establish a new territory and pair-bond. Established pairs are commonly accompanied by retained offspring and non-offspring juveniles, forming small flocks of 3–5 individuals. Juveniles may disperse at any time of the year and dispersal distances are mostly short, especially among males, and thus neighbouring males are often close relatives (pers. obs.). However, long-distance dispersal does occur, as confirmed by ringing recoveries.

This study was conducted from 1974 to 1998 in the forests around Kristinestad and Närpes in western Finland (62° 22`N, 21° 30`E), close to the Gulf of Bothnia. Jays were monitored mainly in three neighbouring forest areas (120, 70 and 155 $km^2$, respectively), separated from each other by 100–1500 m wide agricultural fields or peatlands. The study started in the first mentioned area in 1974, and was successively extended into the neighbouring areas from 1985 to 1992. The areas maintained jay populations of 7–17, 3–5 and 15–32 jay territories, respectively. These areas are referred to as different 'populations' in this paper. More details about the study area will be given elsewhere.

### DNA sampling and data collection

DNA samples were collected during annual monitoring and capture of the birds, which was performed in summer and autumn (July–October) when food-hoarding jays could easily be attracted to feeding stations put in trees in their territories. Juvenile birds were distinguished from adults by the shape of their outermost tail-feathers (Svensson 1992). With the original intention of checking their age later, one tail-feather (generally the left outermost) was collected from most individuals since 1976. These feathers made the extensive genetic analyses possible, spanning the whole 25-year study period. During 1997–98 a 25–50 µl blood sample was taken from every captured individual (n = 158). Altogether, we have DNA samples from 419 of the total number of 542 jays ringed in this study. Of these 419 sampled birds 298 were observed as juveniles, and a large number of them were later found as adults. Feather samples were not collected from every individual during the years 1974–75 and 1989–91, and were not taken from nestlings. However, DNA samples were later collected from 28 individuals that had been ringed previously as nestlings. Feather samples were stored in paper envelopes at room temperature, while blood sam-

ples were stored frozen in 500 µl SET-buffer (0.15 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH 8.0).

All birds were ringed with unique combinations of colour rings. They were sexed by morphological measurements (males are slightly bigger), and sex determination based on morphology has been found to be almost completely correct in adult birds when checked against sexing by molecular techniques (unpublished data). Our goal was to check every individual bird at each feeding station annually during the years 1974–98. To make sure that every surviving individual was observed, every territory was visited several (3–5) times during July–October. The maximum number of unringed birds observed that escaped capture was not more than 23 individuals during the whole period of study, but the number of birds that escaped observation altogether is probably higher. There were gaps in the monitoring efforts in some years (1978, 1980 and 1983), and a few pairs escaped attention because of insufficient coverage of the study area in other years, especially 1984–87 (details given by Lillandt 1993). When artificial feeding started in new study areas, it took more than a season to locate all the jays, because some birds needed a long time to find and learn to visit feeding stations. Altogether, information was obtained from 456 group-years.

## Genetic analyses

*Microsatellite loci*

We used a set of nine polymorphic microsatellite loci (Table 1), one of which was cloned from the Siberian jay genomic library. Eight loci were found by amplification of loci isolated in other species; in three of these the primer sequences needed modification before successful amplification in the Siberian jay. The new or modified primer sequences as well as details about amplification conditions will be published elsewhere (see also Hansson et al. 2000).

*DNA extraction*

DNA from 158 blood samples was extracted according to a standard protocol including proteinase K, phenol-chloroform and ethanol precipitation (Sambrook et al. 1989). In feathers collected from 261 individuals, approximately 5 mm of the base of the shaft was cut into strips with a paper knife. This was put into 400 µl lysis buffer (0.1 M Tris-HCL pH 8.5, 0.005 M EDTA, 0.2 % SDS, 0.2 M NaCl; Laird et al. 1991) with 12 µl proteinase K (10 mg/ml) for digestion at 56 °C overnight, followed by standard phenol-chloroform treatment and ethanol precipitation. The edge part of the paper knife was renewed between feather samples to avoid DNA contamination across samples. DNA extracted from blood was stored in 1xTE buffer. DNA concentration was estimated by spectrophotometry and samples were

**Table 1.** Microsatellite loci used for parentage determination in the Siberian jay. New primer sequences and other details on amplification conditions will be published elsewhere.

| Locus | source | No. of alleles | $H_O$ | $H_E$ | Parentage exclusion probability | |
|---|---|---|---|---|---|---|
| | | | | | First parent | Second parent |
| Ck.1B5D | Tarr & Fleischer 1998 | 2 | 0.552 | 0.497 | 0.123 | 0.187 |
| Ck.2A5A | Tarr & Fleischer 1998 | 16 | 0.754 | 0.751 | 0.375 | 0.554 |
| CkL5 | mod. fr. Tarr & Fleischer 1998 | 11 | 0.854 | 0.820 | 0.476 | 0.649 |
| LTML7 | mod. fr. McDonald & Potts, unpubl. | 2 | 0.411 | 0.403 | 0.081 | 0.161 |
| LTML8 | mod. fr. McDonald & Potts 1994 | 14 | 0.864 | 0.844 | 0.525 | 0.691 |
| MJG1 | Li et al. 1997 | 2 | 0.461 | 0.431 | 0.093 | 0.169 |
| Per1 | Siberian jay, Lillandt et al., unpubl. | 6 | 0.547 | 0.537 | 0.159 | 0.322 |
| Ppi1 | Martinez et al. 1999 | 4 | 0.595 | 0.542 | 0.150 | 0.298 |
| Ppi2 | Martinez et al. 1999 | 5 | 0.768 | 0.737 | 0.317 | 0.492 |

$H_O$ = observed heterozygosity, $H_E$ = expected heterozygosity.

diluted to 25 ng/µl for PCR reactions. DNA from feathers was stored in 25 µl $H_2O$ (without quantification), because of some amplification problems apparently caused by the TE-buffer (Jackson et al. 1991).

PCR reactions of 10 µl included 25 ng genomic DNA from blood samples or 1–3 µl from the total amount of 25 µl DNA dilution from one tail feather.

## Data analysis

Total allele frequencies, observed heterozygosities and expected heterozygosities for the pooled data set from all 419 individuals were calculated with the software 'Cervus' (Marshall et al. 1998). With the same computer program we also calculated the probability of excluding a randomly chosen individual from parentage both in cases where no parent is known ('first parent' test) and cases where one parent is known ('second parent' test). Despite a reasonable theoretical exclusion power of the marker system (based on the assumption of panmictic populations the exclusion probabilities were 0.945 and 0.993 for 'first' and 'second' parent, respectively), the large genetic similarities between close relatives in the population prevented us from a successful search for mothers and fathers separately from the whole population of established birds. A test with the software 'Cervus', presuming that neither parent is known (as is normally the case with juvenile jays sampled in summer–autumn), gave several or even many potential parental candidates. The available microsatellite markers were insufficiently polymorphic to allow parentage to be assigned with a satisfactory level of significance with this method. The parameters used for simulation and the outcome of the analysis are summarised in Tables 2 and 3.

**Table 2.** Parameters used in the simulation for maternity inference in Siberian jays with the software 'Cervus'.

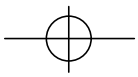
| Parameter | Value used |
|---|---|
| Number of candidate females | 35 |
| Proportion of candidate females sampled | 0.9 |
| Proportion of loci typed | 0.97 |
| Rate of typing error | 0.005 |
| Number of tests | 10,000 |
| Relaxed confidence level | 80 % |
| Strict confidence level | 95 % |

**Table 3.** The success of maternity inference using 'Cervus' for 298 Siberian jay juveniles sampled in the study populations in western Finland 1976–98. The analysis was performed as a 'first parent' test, with only females included as parental candidates. The observed number of obtained maternity with 80 % and 95 % confidence are shown along with the predicted numbers from the simulation, and the critical value of D.

| Number of maternities | 80 % | 95 % |
|---|---|---|
| Observed | 160 (54%) | 44 (15%) |
| Predicted | 181 (61%) | 68 (23%) |
| Critical value of D | 0.78 | 2.23 |

As an alternative strategy, we first tested genetic monogamy in cases where the female attending the nest was found to be the mother of the nestlings (20 birds, from 15 clutches, ringed as nestlings and DNA-sampled as juveniles, for which both parents were sampled). The problem that potential extra-pair fertilisations could pass undetected, especially if the extra-pair male is a close relative of the male attending the nest (Double et al. 1997), was checked by testing 15 real cases. For each nestling we combined the nest attending mother with every sampled male living in the same population during the year the nestling was ringed (2–16 potential extra-pair males/nestling, totalling 90 combinations), and counted the number of 'novel bands' in the nestling, i.e. the number of alleles that could not have been inherited from that particular male or from the nest attending mother. The proportion of total matches with 'wrong' males describes the power of the paternity exclusion system for this 'second parent' test. As a comparison we also combined the nest attending mothers with males sampled in the neighbouring populations during the same year and counted the number of 'novel bands' in nestlings in this situation (243 combinations). Only birds for which we had complete microsatellite data (nine loci) were included in these tests.

To check further the possibility that the nest attending female was not the mother of the nestlings because of egg-dumping, we performed another test for females, in which no information concerning potential fathers was included. In this test we compared every one of the same 15 nestlings with all other females within the same population (104 comparisons) and between po-





pulations (263 comparisons), and counted the frequency of mismatches in the nestlings (i.e. the number of loci in which the nestlings had no allele that could have been inherited from the tested female). The proportion of total matches with 'wrong' females describes the power of the testing system for females as 'first parent' in these kin-structured populations.

After confirming monogamy we examined each one of the 298 sampled juvenile birds by performing a search for their potential parents among all the established pairs that were found or could have been found in the same population during their hatching year. On territories where one or both parental birds had been replaced between monitoring in subsequent autumns, every possible combination of parents was tested as a potential parental pair. Pairs not observed in a particular year, but that could have been undetected in the territory where they were found later, were also included as potential parents in the comparisons. If no parents were found in the same population, juveniles were also checked against established pairs in the neighbouring populations during the same year. In families for which we had incomplete allelic information, parentage determination was performed only if the level of variation on scored loci allowed this to be done, i.e. if one parental pair could be unambiguously found.

As it has been shown that theoretical exclusion probabilities based on assumptions of panmictic populations are severely overestimated if the studied population is in fact kin-structured (Double et al. 1997), we also investigated the reliability of our parentage determinations by examining overall allelic mismatch frequencies within and between populations. By comparing every juvenile bird (with complete microsatellite data, n = 261) with every potential, completely scored parental pair in the same population during the same year (1–36 pairs, altogether 4,040 comparisons), we obtained an estimate of the proportion of total matches 'by chance' (i.e. the proportion of juveniles with an allelic composition matching more than one parental pair) and information about the distribution of mismatch frequencies. A smaller set of 489 comparisons was also made between 84 juveniles and parental pairs from different populations, to investigate the distribution of mismatch frequencies in a situation where the problem of relatedness should be smaller.

To test the assumption of total genetic monogamy further we analysed the distribution of mismatch frequencies among those juveniles that did not match any parental pair (30 individuals, out of which 24 were completely scored) and checked how many mismatching alleles these birds had at a minimum. If these young were the results of extra-pair fertilisations the mismatch frequency should be similar to the situation where the fathers of nestlings with known mothers were 'replaced'; if the mismatches were caused by mutations or typing errors the minimum number of mismatches should be low. But if their parents were not sampled, or if the juveniles were immigrants from other populations, the mismatch frequency should be similar to the outcome of the comparisons between juveniles and non-parental pairs within or between populations. We also checked the 49 juveniles found not to be offspring of their 'social parents' (i.e. the established pair with which they were associated) analysed as a pair, for the possibility that one parent could have been a genetic parent while the other one had been cuckolded or replaced. Note that this group of 49 birds is not exactly the same as the later mentioned group of 50 juveniles that had left their natal territory before sampling, because some of the 50 birds were not found together with an established pair.

## Results

### Microsatellite typing and inheritance patterns

In total we successfully scored 96.8 % of all loci (including sex determination, unpublished data) in the 419 individuals, despite the fact that we had collected only one tail-feather from 130 of them. In the 261 individuals from which we had only feather samples we successfully amplified at least three microsatellite loci in all cases, and in 87.7 % of them at least eight loci were successfully scored. Samples from feathers more than 10 years old resulted in fewer amplified loci (Fig. 1.) and weaker amplified fragments than samples from feathers collected in the 1990s, which mostly gave amplification products indistinguishable from results based on blood samples.

Mendelian inheritance was confirmed in 10 families (15 different clutches), where 20 sampled juveniles had been ringed as nestlings. The presence of a null allele was obvious in the Ck.2A5A locus; a female that seemed homozygous for a rare allele did not transfer this allele to two juveniles (not ringed as nestlings), that

**Figure 1.** Amplification results from old Siberian jay feather samples. Number of successfully amplified loci (nine microsatellites and sex determination, altogether 10 tests), based on one tail-feather from each bird (n = 130), collected during the field study 1976–96. Circles indicate one individual, increasingly complex stars indicate 2, 3, 4 and 5–21 individuals, respectively.

otherwise perfectly matched both social parents. The concordance between observed and expected heterozygosity (Table 1), and the fact that the observed heterozygosity was in every case larger than expected, suggests no significant frequencies of null alleles at any of the nine scored loci. The frequency of null alleles calculated by 'Cervus' was negative at every locus. Except for the apparent null alleles in the two mentioned juveniles, no aberration from perfect matching was 'allowed' in the parentage determinations.

## Genetic monogamy

All of 20 sampled birds ringed as nestlings perfectly matched both parents attending the nest. Thus there were no indications of extra-pair paternity or female egg-dumping in this species. Despite the high theoretical exclusion probability for the 'second parent' (0.993), the actual paternity exclusion probability was lower because of close relatedness between neighbouring males. The 'second parent' test in which the social male was replaced by other males from the same population demonstrated that in nine cases out of 90 replacements, another male could have fathered the nestlings without transferring any mismatching alleles to the offspring (paternity exclusion probability 0.90, Fig. 2). If candidate fathers were taken instead from the neighbouring population only two extra-pair fertilisations could have passed undetected in 243 replacements (paternity exclusion probability 0.992, Fig. 2). The

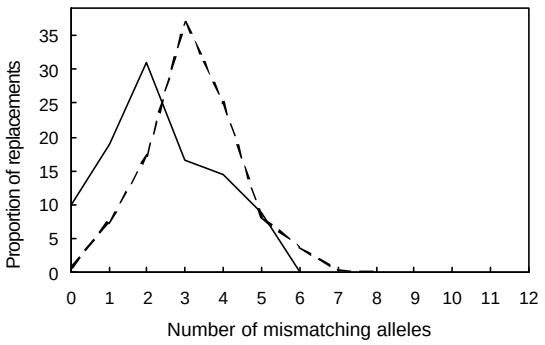



**Figure 2.** Numbers of mismatching alleles in Siberian jay nestlings with known mothers, when 'replacing' their fathers with other males ('second parent' test). The lines indicate the proportion of cases with different numbers of mismatching alleles when candidate males were taken from the same population (90 replacements, solid line) or from neighbouring populations (243 replacements, broken line). Zero mismatching alleles indicate that another male could have fathered the nestling without being detected by our parentage testing system. Only completely scored (9 loci) individuals were included.

mean number of mismatching alleles in the offspring when fathers were replaced within a population was 2.3 and if the candidate fathers were taken from a neighbouring population it was 3.2. In females the 'first parent' test showed that in 12 cases out of 104 within-population comparisons another female totally matched the nestlings when no information about the fathers was included (exclusion probability 0.885, Fig. 3). In comparisons between populations the corresponding value was 15 out of 263 (0.943, Fig. 3, close to the theoretically calculated value 0.945). The mean number of mismatching alleles in female-nestling comparisons within populations was 1.9 and between populations 2.1.

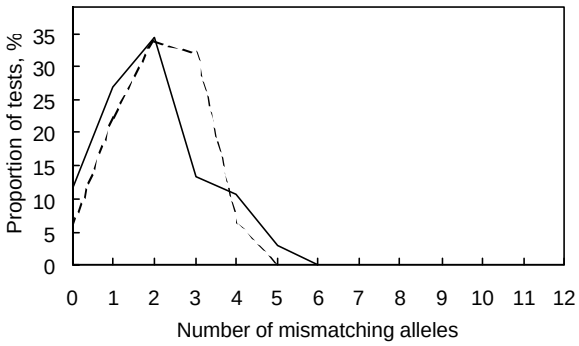



**Figure 3.** Numbers of mismatching alleles in Siberian jay nestlings, when tested against females other than the nest attending female, assuming that the father is unknown ('first parent' test). The lines indicate the proportion of cases with different numbers of mismatching alleles when candidate females were taken from the same population (104 comparisons, solid line) or from neighbouring populations (263 comparisons, broken line). Zero mismatching alleles indicate that another female could have been the mother of the nestling without being detected by our parentage testing system. Only completely scored (9 loci) individuals were included.

## Finding parental pairs

The results of parentage determinations are summarised in Table 4. Altogether, we found totally matching parental pairs for 268 of the 298 sampled juveniles (89.9 %). Among these 268 juveniles there were 30 whose parents had been incompletely sampled. In 21 cases we lacked DNA samples from one of the social parents. However, these juveniles perfectly matched the other social parent and no other sampled pair in the population during the same year. In nine cases we lacked DNA samples from both social parents, but four of these were ringed as nestlings. None of the nine juveniles was found to match any other sampled parental pair, hence it is likely that their social parents were also their genetic parents. Twelve juveniles (of which seven

**Table 4.** Results of parentage determinations in 298 Siberian jay juveniles, when their allelic profile was compared to observed parental pairs in the populations studied in western Finland 1974–98. Parentage assignment presumed that no allelic mismatches between the juvenile and the parental pair occurred. Juveniles were divided into two groups: (a) juveniles that totally matched their social parents in the autumn (if they were sampled); and (b) juveniles that did not match their social parents or that had no potential parents in the same territory.

| | Matched no sampled pair | Matched only one pair | Matched two pairs | Total |
|---|---|---|---|---|
| (a) Juveniles observed with matching parents | | | | |
| Both social parents sampled | | 176 | 7+5 [1] | 188 |
| Only one social parent sampled | 21 [2] | | | 21 |
| None of the social parents sampled | 9 [3] | | | 9 |
| (b) Juv. obs. after leaving natal territory | 30 | 47 | 2+1 [4] | 80 |

[1] In seven cases the allelic data were complete, the rest were based upon 3-8 compared loci.
[2] All 21 birds matched the only sampled social parent; four of them were ringed as nestlings.
[3] Four of these nine were ringed as nestlings.
[4] In two cases the allelic data were complete; one was typed on only four loci.

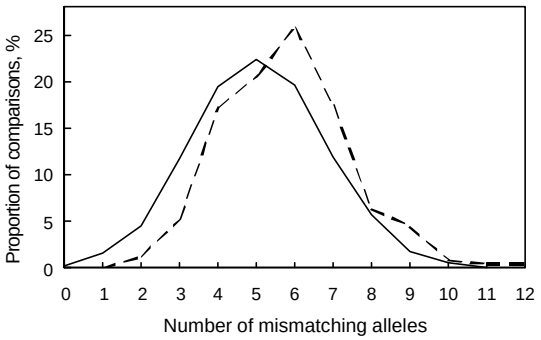



**Figure 4.** Numbers of mismatching alleles in completely scored Siberian jay juveniles sampled in the autumn, when comparing them with every potential parental pair except their presumed parents. The lines indicate the proportion of cases with different numbers of mismatching alleles when juveniles were compared to pairs within the same population (261 juveniles, 4,040 comparisons, solid line) or from the neighbouring populations (84 juveniles, 489 comparisons, broken line). Zero mismatching alleles (in within-population comparisons, n = 9) indicate that the juvenile perfectly matched an additional pair besides the presumed parents.

were completely scored) that totally matched their social parents did also match another pair. Among the 268 juveniles for which matching parental pairs were found, there were 50 individuals (18.7 %) that had left their natal territory before sampling. In 47 of these one totally matching parental pair living in another territory could be identified, while in only three cases (two of which were completely scored) two matching parental pairs were found. In these three cases it is not possible to determine which one of the two matching parental pairs included the true genetic parents. For the remaining 30 juveniles (10.1 %), no matching parental pair could be found in the whole study area.

In 4,040 comparisons between 261 completely scored juveniles and potential parental pairs within the same population, we found 9 cases where the allelic composition of all nine loci perfectly matched two different parental pairs; i.e. a perfect match with one pair that could not have been the genetic parents (Fig. 4). All of these cases were found in the northern study area during the years 1994–98, and, according to pedigree data, in five cases one or both pair-mates were closely related with the other perfectly matching pair-mates. The average number of mismatching alleles between a juvenile and all breeding pairs present during its hatching year was 5.1, while the proportion of cases with only one mismatched allele was 1.7 % and with two mismatches 4.6 %. When comparing 84 juveniles to parental pairs in the neighbouring population (489 comparisons) the average number of mismatching alleles was higher, 5.8, and no total matches with 'wrong' parents or any one-allele mismatches occurred, while the proportion of cases with two mismatches was 1.2 % (Fig. 4).

The analysis of the minimum number of mismatching alleles in all the 30 juveniles for which no parents were found (Fig. 5) showed that four birds had a mismatch in only one scored locus compared to at least one parental pair. Two of these four birds were scored on eight, one on five and one on all nine loci. Five more birds had at least two mismatching alleles, while 21 juveniles had a minimum number of 3–6 mismatching alleles in every comparison. The average number of mismatching alleles in the 24 completely scored juveniles that did not match any parental pair, when compared to potential parental pairs in the population where they were ringed, was 5.6 (Fig. 6), which is close to the value 5.8 obtained in the between-population 'simulation' above.

A more detailed analysis of the 49 juveniles observed with an adult pair that was not their genetic parents sup-

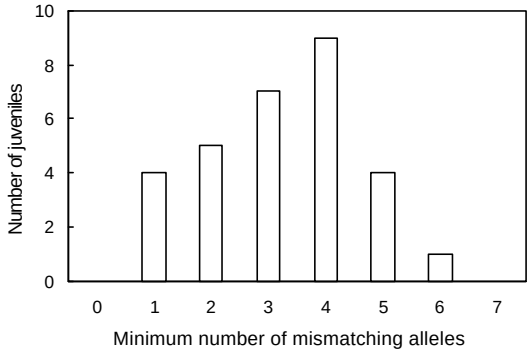



**Figure 5.** The minimum number of mismatching alleles found when comparing all the 30 juvenile Siberian jays for which no parents were found with every potential parental pair within the same population, regardless of the number of scored loci (4–9 loci, mean 8.1).

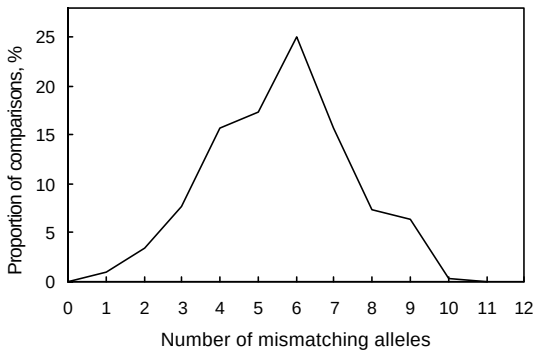



**Figure 6.** Numbers of mismatching alleles in completely scored Siberian jay juveniles, that did not match any parental pair (n = 24), when comparing them with every potential parental pair within the same population. The line indicates the proportion of cases with different numbers of mismatching alleles.

ported the view that they were non-offspring that had joined the group rather than young from extra-pair matings. Thirty-seven of these (75.5 %) did not match either of the two adults, while the remaining 12 could have been an offspring of one of the social parents. In seven cases the juvenile matched the male, but not the female, and in only four cases the female but not the male. In one case the juvenile matched both adults when compared to them separately, but did not match when they were combined.

## Discussion

### Microsatellite analysis

This study demonstrates the power of microsatellites as markers for parentage testing in complicated data sets. Because microsatellite analysis is PCR-based it was possible to make parentage determinations based on feather samples collected over a long period of time. However, we experienced increasing amplification problems in feathers more than 10 years old, suggesting a slow degradation of the DNA in feather samples when stored at room temperature. The microsatellites allowed us not only to check parentage within observed groups, but also to find the parents of juveniles that had moved away from their natal territory before sampling. This would not have been possible had we used minisatellite DNA fingerprinting, nor is it possible to run DNA fingerprinting using feather samples.

### Parentage determination

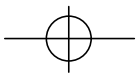
Nine polymorphic loci with a total theoretical exclusion probability of 0.945 for 'first parent' and 0.993 for 'second parent' were not enough to enable a search for mothers and fathers separately, the reason being insufficient allelic variation between close relatives living contemporaneously in the population. A similar situation has been found in an Australian marsupial, the northern hairy-nosed wombat *Lasiorhinus krefftii* (Taylor et al. 1997), where insufficient variation at nine polymorphic loci, combined with incomplete sampling and missing demographic information, prevented parentage determination. Double et al. (1997) examined the same kind of problem in the superb fairy-wren *Malurus cyaneus*, an Australian cooperatively breeding bird with several male relatives living in clusters. Their study showed that the 'paternity exclusion probability' calculated assuming panmictic populations will be severely overestimated if the population is kin-structured.

To circumvent these problems we applied another strategy, based on the assumption of genetic monogamy in the population. As we had quite complete information about the established pairs each year, we were able to search for parents among pairs instead of evaluating potential mothers and fathers separately. By doing so we greatly increased the power of the parentage testing system (Meagher and Thompson 1986), and were able to find matching parental pairs to 268 of the 298 sampled juvenile birds. For three juveniles (among these 268) that had left their natal territory before sampling, two matching pairs were found, and therefore the genetical parents could not be determined. We failed to find any matching parents for only 30 sampled juveniles (10.1 %) during the whole 25-year study period.

### Possible error sources in parentage determinations

*Extra-pair fertilisations*

We were able to confirm genetic monogamy in 15 clutches (20 nestlings), a sample size that is too limited to detect rare cases of extra-pair fertilisations. However,





similar results have been presented by Ekman et al. (1994), based on DNA-fingerprinting. Despite the high theoretical exclusion probability for 'second parent' (0.993) in our study, the estimated paternity exclusion probability was not more than 0.90 (see Double et al. 1997). According to our 'simulation' with replaced fathers in observed families, we found that extra-pair fertilisations would in most cases produce offspring with a low number (1–2) of mismatching alleles. There were only nine such juveniles among those for which we failed to identify the parents, and in only four of them there was a mismatch at only one locus. Furthermore, of the 49 birds found not to be offspring of their social parents in the autumn, only five matched the social mother, and for four of these we found another matching parental pair. Altogether, these results strongly suggest that extra-pair fertilisations could at most account for only a few cases of failed parentage determination among all the 298 juveniles evaluated in this study.

*Female egg-dumping*

All of the tested nestlings perfectly matched the female attending the nest, giving no indications of female egg-dumping in this species. The exclusion power of our analyses for females as 'first parent' was 0.885, according to our within-population 'simulation', and 0.943 for females from other populations. The later value is very close to the theoretically calculated exclusion probability (0.945) for 'first parent', assuming panmictic populations. Because female Siberian jays exhibit longer dispersal distances than males (pers. obs.), neighbouring females are not close relatives as frequently as neighbouring males. The risk that egg-dumping performed by neighbouring females would pass undetected is thus smaller than in the case of extra-pair fertilisations. Both on basis of the genetic analyses and our own field observations we find the occurrence of female egg-dumping to be very improbable in this population.

*Null alleles, mutations and typing errors*

In one family we observed a null allele at one locus. However, calculations of observed and expected heterozygosities suggest that null alleles are rare at all loci, despite the fact that most of the primers were developed for other species. Because our parentage determinations extended over as many as eight successive generations, many individuals were included first as offspring and later as parents. We therefore had repeated opportunities to detect occasional mismatches caused by null alleles, point mutations or typing errors in particular individuals. Nevertheless, only one apparent null allele was found. Because most juveniles were observed together with their potential parents, we were able to check for mismatches on occasional loci during the typing process, and thereby minimise the occurrence of typing errors. Our within-population 'simulation' showed that in only 1.6 % of the comparisons a juvenile mismatched a non-parental pair in only one allele, i.e. occasional mutations or typing errors are very unlikely to cause false parentage determinations.

*Incomplete information about pair-bonds during the breeding season*

In 32 group-years (7.0 % of the observed 456 group-years) we lacked DNA samples from both parents. There were also an undetermined, low number of unchecked groups as a result of gaps in the monitoring efforts during some autumns, and possibly there were cases where territory holders had been replaced twice between subsequent autumns, and thereby some breeding pairs escaped attention. These three sources of missing information certainly contributed to the number of individuals for which no parents were found (30 juveniles), but they should not affect parentage determinations in the other juveniles.

## Two statistical problems

In the analyses we were faced with two different statistical problems: (1) checking parentage within observed family groups, and (2) searching for the parents of juveniles that were not ringed on their natal territory.

(1) Our within-population 'simulation' showed that the probability that a randomly chosen juvenile from the same population would perfectly match a non-parental pair is very low; this happened in only one case out of 449 comparisons (0.22 %). Many, or possibly all, of the matched non-parents were close relatives to the correct parents, suggesting that the risk of multiple matches is connected to the occurrence of large kin-clusters in the population (unpublished data). The probability of multiple matches is therefore higher for individuals hatched within successful 'clans', while the risk of immigrants being mistakenly regarded as offspring is very low.

(2) Among the 50 juveniles for which matching parents were found in a territory other than that in which the juvenile was ringed, it is hard to evaluate the reliability of the parentage determinations. However, we found only three cases (two of which were completely scored) of total matches with two different pairs in these comparisons. The risk of mistakenly assigned parentage when presuming total matching between parents and offspring is therefore still small, even if it is considerably larger when systematically searching through the whole population than when testing only within observed groups.

Considering the potential error sources discussed above we expect that the risk of not finding the parents, due to mismatching alleles caused by mutations, null alleles, typing errors or extra-pair paternity, is larger than the risk of falsely assigning parentage. The fact that we found very few such slightly mismatching individuals, however, implies a low impact of these errors.

To conclude, we find the examined microsatellite typing system to be sufficiently reliable for making inferences about parentage in the Siberian jay populations studied here. Every line of evidence supports the assumption of genetic monogamy in this species, and the high success of parentage determinations that we achieved would not have been possible had extra-pair fertilisations been frequent. However, this study shows that reliable parentage determination in kin-structured populations demands a large number of polymorphic microsatellite loci, especially if there is no a priori information about who is the mother, or about parental pair-bonds.

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# A test for effects of radio-tagging on survival and movements of small birds

**Beat Naef-Daenzer, Fritz Widmer and Maria Nuber**

There is little experience and scant data about possible adverse effects of radio-tagging on birds of less than 20 g. To quantify possible effects, we analysed the survival rate and movements of 221 radio-tagged and 121 colour-marked great tit *Parus major* and coal tit *P. ater* fledglings. In these species, post-fledging survival is strongly related to the physical condition of juveniles, so that the additional load of a transmitter might have an adverse effect on the bird's energetics and body condition, and therefore affect the survival rate. Cormack-Jolly-Seber models confirmed the effect of fledging weight on juvenile mortality. In neither weight class, however, did the survival rate of birds equipped with a 0.5 g transmitter differ from the control-group of untagged individuals. Radio-tagged birds did not disappear earlier from tit families than untagged individuals, which indicates that tagged birds were not more vulnerable to predation or accidents than their untagged siblings. We found no effect of the number of transmitters deployed per family on the distances covered per 5-min interval. Although these results are limited to survival and selected parameters of behaviour, we conclude that the additional load of 2.4–3.3 % of the birds' body mass in great tits and 4.2–5.8 % in coal tits did not significantly affect the birds' body condition, manoeuvrability or range use.
Key words: Great tit *Parus major*, coal tit *Parus ater*, telemetry, tagging effects, survival.

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Extremely miniaturised tracking transmitters have been available for only a short period of time. Thus, there is little experience and scarce data about the possible effects of radio-tagging on birds of less than 20 g (e.g. Brigham 1989, Sykes et al. 1990, Naef-Daenzer 1993, 1994, Neudorf & Pitcher 1997). In small passerines such as great and coal tits, *Parus major* and *P. ater*, mortality rates are strongly related to body condition. This relationship is particularly pronounced during the first post-fledging weeks (overviews in Van Balen 1973, Perrins 1991, Naef-Daenzer et al. 2001). For example, 16 % of full grown (17 g) great tit fledglings in the Hoge Veluwe, Netherlands, were recaptured in the next breeding season, whereas only 5 % of birds with a fledging mass of 14 g were recaptured (Tinbergen & Boerlijst 1990, Verboven & Visser 1998). Although little is known of the physiological and ecological mechanisms that relate post-fledging survival to fledgling condition, this indicates that even small changes in the birds' physical condition may have a significant impact on survival. In the context of testing for increased energetic costs, predation risk or discomfort caused by transmitter loads, we used post-fledging survival rates as a sensitive estimate of possible adverse effects of radio-tagging. Since juveniles have never flown before, such effects might be even more pronounced than in adult birds. Here we present data from an investigation of range use and mortality of juvenile great and coal tits during the first 20 days after fledging, i.e. the period of post-fledging dependence. Since visual recording of the birds in dense vegetation is almost impossible, radio-tracking is an indispensable tool to observe the birds outside the nest. On the other hand, an analysis of fledgling behaviour

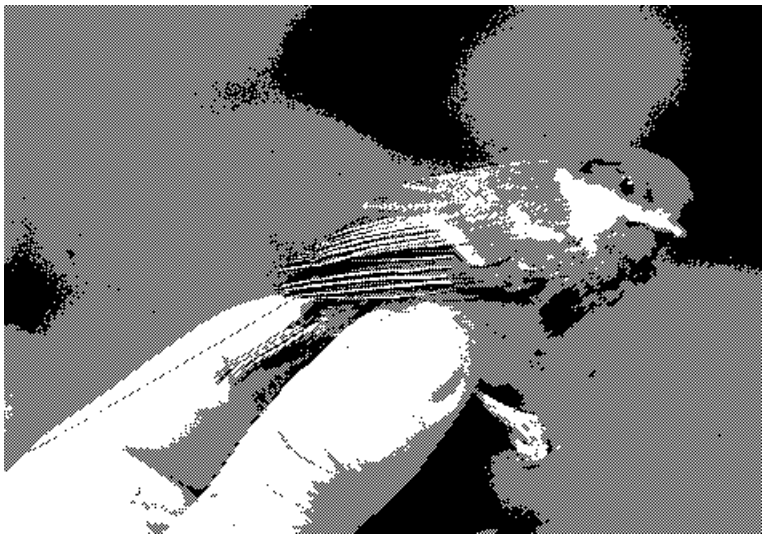

**Figure 1.** A coal tit fledgling equipped with radio-tag and colour-mark on the ventral feathers. The 0.5 g transmitter is fitted with a Rappole harness fitting around the thighs. The transmitters disappears completely in the plumage. Colour-marks were applied with waterproof pen. When dry, this results in a pastel staining sufficient to identify birds at distances of 20–30 m.

and survival is questionable if radio-tagging has significant effects on the birds' condition, flight ability and survival. Here we first compare mortality and movements of radio-tagged and untagged juveniles. Second, we analyse differentials in the failure rate of tagging attempts in relation to the time after fledging and body condition at the end of the nestling period. Because tagging success was affected by the condition of juvenile birds, there are implications for the optimisation of mounting techniques.

## Methods

The study was conducted from 1995 to 1997 in the 'Blauen' region, a mixed deciduous/coniferous forest of the north-eastern Jura near Basel, Switzerland, at altitudes of 300–600 m asl. Over an area of c. 3 $km^2$, about 350 nestboxes were placed along forest roads and footpaths. About 150 pairs produced 400–700 juveniles per year (c. 60 % great, 40 % coal tits).

### Radio-tagging

Radio-tracking bird fledglings requires small transmitters and extremely careful treatment. We used an improved version of the miniature transmitter described in Naef-Daenzer (1993, 1994). The device had a mass of 170 mg without batteries. The tags were powered by zinc-air cells of AC10 size (290 mg) giving a life of 25–35 days. The aerials consisted of 7 cm of a flexible 0.15 mm multistrain steel used originally for fishing. The tags were attached either by gluing them into the feathers of the back (n = 31 in 1995, Raim 1978, Naef-Daenzer 1993) or with a Rappole harness fitting around the thighs (n = 190 in 1996–97, Rappole & Tipton 1990). The harnesses were made from 0.5 mm cotton thread and had a mass of 18 mg. Harness size was not adjusted to individual body size, the span of the two loops was 46 mm for great tits and 39 mm for coal tits, respectively. The complete tags weighed 470–480 mg which is 2.4–3.3 % of a great tit's and 4.2–5.8 % of a coal tit's body mass, respectively. Great tits below 14 g and coal tits below 8 g were not radio-tagged.

Radio-tagging of juvenile tits was undertaken after several years' experience with adult titmice (Naef-Daenzer 1993, 1994, 2000, Naef-Daenzer & Keller 1999). Before tags were attached to free-living juveniles, tags and harnesses were tested on captive broods that had been brought to the Swiss Ornithological Institute for rearing. No effects on their skin or plumage, or changes in behaviour were observed in these birds.

A total of 221 birds were radio-tagged (178 great tits, 43 coal tits). To provide insurance against transmitter loss and failures, 3 to 5 transmitters were attached per brood, and all radio-tagged birds were individually colour-marked. Birds with lost or ‘dead’ transmitters could therefore be identified visually. In a small number of broods, one of the adult birds was also equipped

with a transmitter. A control group of 121 fledglings (88 great tits, 33 coal tits) were individually colour-marked with waterproof pen. The pen marks resulted in a rather pastel staining just sufficient to identify birds at distances of 20–30 m. Two spots of different colour were applied onto the ventral feathers. Combinations of two out of four colours (black, blue, red, none) allowed each bird of a family to be marked individually, but patterns were repeated among different families. Except for a few occasions, the families were well separated from each other, so that repeated patterns did not influence the identification of individuals. In the few cases where two families met in the same group of trees, the identification of individuals was postponed until the groups had separated again.

To prevent premature fledging, radio-tagging or colour-marking of juveniles was done immediately before they were expected to fledge. The age of nestlings and the expected fledging date were estimated by comparing the length of the developing feathers with standard tables derived from birds with exactly known hatching date. From nestling day 15 until fledging, broods were visited only for short visual inspections. We used the length of wings and tail feathers compared to the bird's body to estimate the expected fledging date. On nestling day 18, which is the average age at which tits fledge, tail and body length are similar, and the wing tips exceed the rump. Because of the large variance in individual development this estimate was imprecise, and several broods had already fledged when the field team arrived for marking (<10 % of the tagging attempts). Particularly in broods in low condition, fledging was delayed by up to 3 days beyond the expected fledging date, which indicates that radio-tagging or colouring the birds did not result in immediate and premature fledging.

Using a handheld 4-element Yagi antenna, the radio tags could be tracked over 200–300 m in dense vegetation. For birds sitting on the ground (and also for dead birds and lost transmitters) reception ranges were 50-150 m. Maximum ranges of up to 1.5 km were obtained from elevated sites such as some rocky parts of the slope of the Blauen area.

Data were collected in a period of 20 days from fledging, i.e. in the period of post-fledging dependence. No dispersal of juveniles out of the families was observed. Of the 115 radio-tagged juveniles surviving for longer than two weeks after fledging none was observed to disperse out of the study area within the observation period. In two out of 68 families the juveniles separated spatially fom their parents but all birds remained in the natal home-range (<200 m from the parents). Furthermore, all fledglings were fed by their parents throughout the observation period. This suggests that birds disappearing from their family had died (Naef-Daenzer et al. 2001).

Usually, all members of a family were present within a 50 m radius. Therefore, all juveniles could be observed and identified as long as one transmitter worked in the family.

## Data collection

Tit families were located and all birds identified at least twice a day. Observation sessions were 1 h. After locating the family groups by 'homing-in' (Kenward 2001), all birds were identified visually. To assess range use and to collect data on behaviour and resource selection, additional observations were carried out on families selected at random. The total observation time per family was therefore 2 to 6 h per day depending on the number of families that were tracked simultaneously. In most cases, mortality of juveniles was not observed directly, and carcasses of birds taken by predators were rarely found because their transmitters quickly ceased functioning. In 26 cases, visual observations provided information on the causes of mortality. In all other cases (n = 135) birds were denoted as dead according to radio-observations or when they had not been observed for 48 h (the reason for this is that 90 % of the 1426 time intervals between consecutive visual observations of great and coal tits were within two days).

Missing birds were sought throughout an area of c. 500 m around the last known location using a handheld aerial. In addition, a range of c. 10 $km^2$ was scanned periodically from a vehicle at night, when tits usually sit in the top of trees. It was very rare that a bird was recovered after being missed for 5 days or longer (n = 36 or 2 % of intervals between sightings). In all uncertain cases, in particular when the last transmitter in a family was lost, birds were denoted as surviving (n = 18 birds). This may have resulted in an underestimation of mortality rates by about 5 % if in fact all those 18 birds had died.

**Table 1.** Selection of Cormack-Jolly-Seber models for daily survival of great and coal tit fledglings. Models include fledging mass and date as covariates to survival (Φ) but not to observation probability (p). Models were built on the basis of 21 one-day intervals (time 0 = fledging day). The four groups comprised radio-tagged/colour-marked great and coal tits, respectively. AICc = Adjusted Akaike's Information Criterion.

| Model | AICc | AICc weight | n Parameters |
|---|---|---|---|
| Φ(time)p(group) | 3710.23 | 0.9992 | 27 |
| Φ(group+time)p(group) | 3724.55 | 0.0008 | 28 |
| Φ(group·time)p(group) | 3757.88 | <0.0001 | 68 |
| Φ(group·time)p(group·time) | 3760.74 | <0.0001 | 125 |

The percentage of survivors after the 20-day observation period was 54.3 % in great tits (n = 266 birds) and 47.4 % in coal tits (n = 76), respectively. We found no significant difference in the mortality rates of great and coal tit fledglings (ANCOVA, $F_{1,41} = 0.17$, $P = 0.68$, see also Table 1 and Naef-Daenzer et al. 2001). Therefore, data from great and coal tits were pooled for the present analysis. The fledging weights of individual birds were expressed as the percentage of the yearly average fledging weight for their species.

For statistical tests birds were grouped into two weight classes (below or equal / above the yearly average fledging mass). The software MARK was used to model fledgling mortality in relation to time after fledging and type of marking (White & Burnham 1999). Cormack-Jolly-Seber (CJS) models were built on a raster of one-day intervals between observations (Lebreton et al. 1992).

Because three tests were applied to the same data set, significance levels were adapted to $p^* = 3\alpha$, according to Sachs (1967). Mortality and behavioural parameters may be confounded by local or seasonal variance in habitat factors such as predation risk or food distribution. These factors were controlled for by the combination of both marking techniques in each of the families.

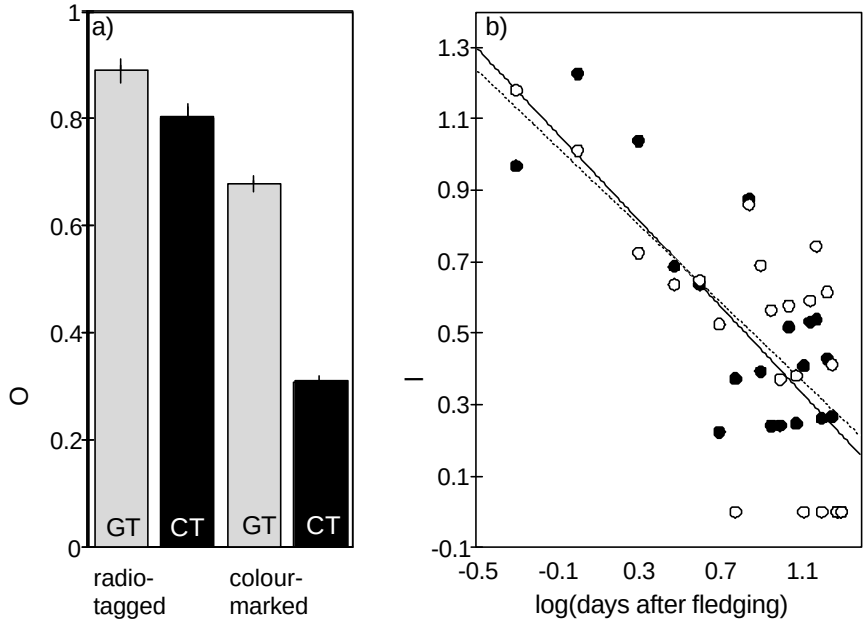


**Figure 2.** a) The observation probability of great and coal tits wearing radio-tags compared to that of colour-marked individuals. b) The daily mortality rates among 221 radio-tagged juvenile great and coal tits (dots) compared to that among 121 colour-marked juveniles (circles) in relation to time after fledging (log scale). Regression lines do not differ in slope or intercept, indicating that radio-tagging did not cause measurable effects on post-fledging mortality. Statistical analyses to both graphs are given in Table 1.

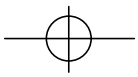



**Table 2.** Frequency distributions of the rank of tagged and untagged birds in the sequence of losses in 68 tit families. Rank 1 means that a bird was the first to disappear from a particular family. The frequency distributions for radio-tagged and colour-marked juveniles did not differ significantly. Thus, radio-tagged individuals were not more likely to be the first victim in a family than any other member.

| Mark | | Surviving >20 d | Rank 1 | Rank 2 | Rank 3 | Rank 4 | Rank 5 |
|---|---|---|---|---|---|---|---|
| Transmitter | obs. | 116 | 55 | 34 | 8 | 6 | 2 |
| | exp. | 116.9 | 54.3 | 33.0 | 8.4 | 6.5 | 1.9 |
| Colour | obs. | 65 | 29 | 17 | 5 | 4 | 1 |
| | exp. | 64.0 | 29.7 | 18.0 | 4.6 | 3.5 | 1.1 |
| Totals | | 181 | 84 | 51 | 13 | 10 | 3 |

Pearson $\chi^2 = 0.30$, df = 5, P = 0.99.

## Results

### Survival rates

Although family groups with radio-tagged chicks were easily located, there were significant differences in the probabilities of observing individuals visually. There was a small difference in the daily observation probability of radio-tagged great and coal tits ($p_{great\ tit} = 0.89 \pm 0.009$ (s.e.), $p_{coal\ tit} = 0.80 \pm 0.022$), whereas colour-marked great tits were markedly more likely to be observed than were colour-marked coal tits ($p_{great\ tit} = 0.68 \pm 0.023$, $p_{coal\ tit} = 0.31 \pm 0.028$, Fig. 2a). This indicates that radio-tagged birds were more likely to be observed visually than their colour-marked siblings, probably because the 'homing-in' to the radio-tags led the observers closer to the radio-tagged birds than to the untagged individuals. Colour-marked coal tits were particularly difficult to see because the species used mostly the tops of trees, whereas great tits mainly used the lower and middle layers of the canopy (B. Naef-Daenzer, unpublished data).

The best-fitting CJS-model was obtained with equal age effects for all four groups of birds (i.e. great/coal tit, transmitter/colour-marked, respectively), with different estimates of observation probability for each group and with fledging mass and fledging date as covariates to survival but not to observation probability (Table 1, adjusted Akaike's Information Criterion AICc weight = 0.999). According to this model, there was no significant difference in the survival between the two species or between radio-tagged and colour-marked individuals (Fig. 2b).

Radio-tagged fledglings might be handicapped in adapting to their new environment and suffer from increased vulnerability to predation or accidents when they learn to fly. We tested whether radio-tagged birds disappeared earlier from families than colour-marked individuals. For each individual denoted to have died we determined its rank in the sequence of losses within its family. We found no significant difference in the frequency distribution of ranks between radio-tagged and colour-marked birds (Table 2; $\chi^2 = 0.30$, df = 5, P = 0.9). Therefore, a radio-tagged individual was not more likely to disappear earlier in a series of losses than a colour-marked bird. The number of losses in a particular family can be described as a Poisson process. Although there were marked weight-related differentials in mortality at the level of the individual, the frequency distribution of losses at the level of families was not diffe-

**Table 3.** ANOVA results for the average distance per hour covered by great and coal tit families relation to age and number of transmitters per family. Average distances were log-transformed and are illustrated in Fig. 2.

| Effect | df Effect, Error | F | P |
|---|---|---|---|
| n of transmitters | 2,149 | 1.64 | 0.19 |
| Age (covariate) | 149 | 24.00 | <0.001 |

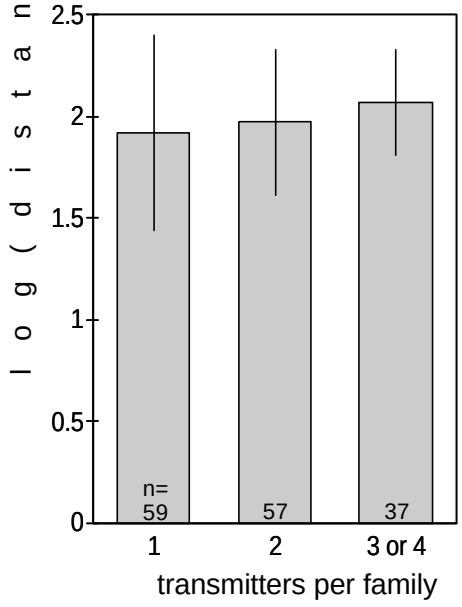


**Figure 3.** Average distances covered per hour by great and coal tit families in relation to the number of transmitters deployed per family. Columns give the log-transformed means adjusted to the effect of the covariate age (age = 0, ANCOVA statistics in Table 3). Error bars indicate standard deviations. Numbers refer to the number of observation days. No significant effect of the number of transmitters was detected.

rent from a random distribution ($\lambda = 2.39$, $\chi^2 = 3.95$, df = 6, P = 0.4 for deviations from Poisson distribution).

We next analysed whether the movements of tit families were affected by the tags. Since at least one radio-tag per family was required to locate the group in the forest, there were limited options to test this aspect. We compared the average distance covered per 5 min in relation to the number of birds carrying a transmitter. Time (days) after fledging was included in the analysis as a covariate to account for any ontogenetic changes in movement patterns. The average distance per 5 min interval (log-transformed) increased significantly with time after fledging ($F_{149} = 24.0$, $P < 0.001$, Table 3 and Fig. 3). However, we found no significant effect of the number of transmitters deployed per family ($F_{2,149} = 1.64$, P = 0.19). This indicates that the proportion of juveniles carrying a radio-tag did not significantly affect the short-term movements of the family groups.

## Transmitter failures

67 (30 %) out of 221 radio-tags stopped within 15 days after fledging, before the expected minimum life of 20 days. These failures were caused by two main factors. First, electronic failures, such as battery defects or damages to the transmitters by the birds occurred at a mean rate of 2.8 % per day. Over the first 12 days after fledging, we found no significant deviation of the observed failures from the average daily rate (Fig. 4. $\chi^2 = 4.21$, df = 6, P = 0.35). Second, transmitters were frequently lost due to improper fit of the harness, i.e. the tagged individual was observed alive but without its transmitter. Assuming that post-fledging growth in

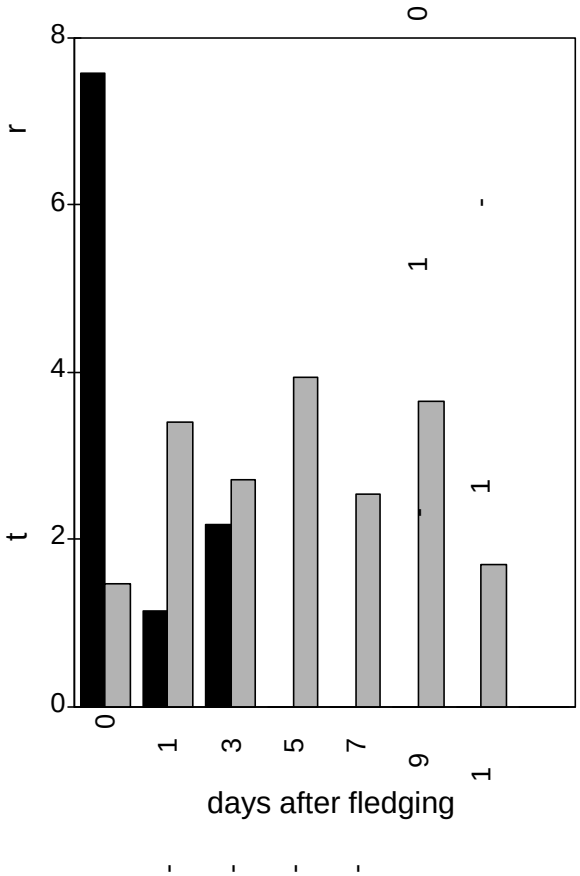


**Figure 4.** Frequency distribution of transmitter losses (black histograms) and technical failures (grey) in relation to time after fledging, as a percentage of the total number of radios present. Loss of the transmitter occurred most frequently on the fledging day due to poor fit of harnesses. The average rate of circuit failures was 2.8 % per day and did not change significantly with time.

light-weight fledglings is possible, we used standard harness sizes (see methods), and no adjustments were made for the bird's size. As a consequence, the fit of the harnesses was less perfect in those birds that fledged in poor condition. This resulted in a bias in transmitter losses in relation to weight class ($\chi^2 = 17.2$, df = 4, $P < 0.005$). Overall, 26 out of 38 losses due to improper fit occurred in the cohort of birds with low fledging mass, 10 of these within 6 h from fledging.

## Discussion

Although the direct causes of post-fledging mortality are largely unknown, our results indicate that attaching a 0.5 g radio transmitter (3–5 % of body mass) to tit fledglings did not have significant effects on their mortality or movements compared to colour-marked individuals. Furthermore, we found no evidence that radio-tagged birds behaved differently or were more vulnerable to predation than their untagged siblings. We conclude from this that radio-tagging did not impair the birds' physical condition nor their behavioural ability to adapt to their new environment.

Any increase in mass, e.g. fat, eggs or artificial loads may affect the take-off speed and ascent angle in response to a potential predator (Witter et al. 1994, Metcalfe & Ure 1995, Veasey et al. 2000). Even if there is no long-term effect of tagging on physical condition, a radio-tag may handicap the bird in escaping dangerous situations. Although our experience in the field suggests that predation is a main cause of fledgling mortality (Naef-Daenzer et al. 2001) we found no indication that the radio-tagged individuals were the first victims in a series of losses in a family. We conclude therefore that the additional load had no significant effect on a bird's ability to escape potential predators.

One general problem in testing for tagging impact is that usually the target species is not readily observable in the field, so that a control group of untagged individuals may not be recorded equally well. This may cause systematic errors confounding the effects to be tested. Because tit families keep close together for at least 20 days after fledging, this study provided a unique opportunity to observe untagged control individuals flying together with radio-tagged siblings, whereby any bias in the survival estimates for radio-tagged and colour-marked birds was small. Like many comparable investigations, this study was not primarily designed to quantify the effect of additional loads attached to an animal. As a result, our analysis is not free of drawbacks and more detailed information is desirable. In particular, we assume that colour-marks did not affect the birds' performance, whereas they might have had an equally strong effect on the birds' survival and movements as did transmitters. A counter argument is that Götmark & Olsson (1997) found no significant difference in predation rates of sparrowhawks *Accipiter nisus* between red-painted and untreated juvenile great tits. Furthermore, radio-tagging part of a brood may have an impact on the fate of the whole family which makes the comparison between tagged and untagged siblings meaningless. Against this interpretation are the results of earlier ringing-recapture studies (Dhondt 1979, Tinbergen & Boerlijst 1990, Perrins 1986) reporting similar mortality rates of juvenile birds in the period from fledging to the onset of autumn (for details see Naef-Daenzer et al. 2001).

### Transmitter failures and mounting technique

With a properly fitted Rappole harness, the radio-tags disappeared completely in the plumage, the aerial being positioned on top of the tail feathers. In contrast, glue-mounts caused some local damage to the plumage because feathers had to be clipped and a considerable number of feathers were torn off when tags were removed by the bird. We therefore favour the Rappole harness over the gluing procedure used in earlier studies (Naef-Daenzer 1994).

Poor fit of harnesses resulted in a considerable failure rate. In future studies, this problem may be solved by using slightly different sizes for large and small birds. Because it is impossible to recapture an improperly tagged bird and a harness that is too tight may impair the bird's movements (see Rappole & Tipton 1990), we preferred to start with relatively large loops, and to accept a number of failures.

### Implications for telemetry applications

Although few studies have been specifically designed to test possible effects of radio-tagging (e.g. Obrecht et al. 1988, Pennycuick et al. 1989, Gessaman et al. 1991) researchers are aware of the methodological and ethical problems involved. Although many papers report no ill

effects (for small birds Neudorf & Pitcher 1997, Hill & Talent 1990, Brigham 1989, Powell et al. 1998), others report disturbance to time budgets (Hooge 1991), foraging behaviour and nest attendance (Massey et al. 1988, Brigham 1989). Not surprisingly, the reported results do not allow general conclusions or rules to be drawn on the maximum acceptable load, or on optimal attachments because radio-tagging may affect different species in different ways. Moreover, details in the design of harnesses and in the handling of animals may make the difference between serious impact and negligible effect. Therefore, evidence that a particular technique did not alter the animals' natural behaviour provides strong support for the biological relevance of results. Particularly in developing methods and field techniques, any information (qualitative and quantitative) should be used to evaluate and eliminate transmitter effects. Minimising the impact on study animals should be preferred over maximisation of technical reliability.

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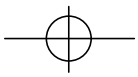



# Nestling growth and post-juvenile moult under a tight seasonal schedule in stonechats *Saxicola torquata maura* from Kazakhstan

**Barbara Helm and Eberhard Gwinner**

Juvenile growth and the timing of moult in bird populations have been related to their migratory behaviour and the length of the breeding season. We tested this relationship in a model species, the stonechat *Saxicola torquata*, held in captivity. We studied growth and timing of post-juvenile moult of the long-distance migrating subspecies *S. t. maura* in comparison with data from resident conspecifics from Africa and short-distance migrants of Central Europe. Stonechats from Kazakhstan had a faster wing growth than the other subspecies, in accordance with predictions for a long-distance migrating species with a short breeding season. Nestling growth of body mass was intermediate, faster than in European but similar to that in African stonechats. The post-juvenile moult of Kazakh stonechats was assessed under different photoperiods (European natural day; long-day hold: LD 17.33:6.66 h; short day: LD 12.25:11.75 h) to distinguish between environmental effects and different reaction norms to a timing cue. Under natural changes in daylength, *S. t. maura* moulted earlier and faster than its European and African conspecifics, as expected from its migratory behaviour. Constant long daylength did not change moult timing in *S. t. maura*. Under short daylength, moult started only slightly earlier than under natural daylength, but was completed more rapidly. In contrast, European and African stonechats advanced moult markedly under short photoperiods, but its duration was unchanged. Heritability estimates from full-sib analyses in *S. t. maura* were low for moult onset, but high for moult duration. The opposite pattern was observed in the other subspecies. The data suggest that the stonechat subspecies differed in the reaction norms of moult timing to photoperiodic conditions. These differences were paralleled by differences in additive genetic variance over the course of moult.
Key words: Nestling growth, post-juvenile moult, photoperiod, stonechat, *Saxicola torquata*.

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The timing of post-juvenile moult in bird populations has been related to their migratory behaviour and the length of their breeding season (e.g., Noskov & Rymkevich 1985, Jenni & Winkler 1994, Berthold 1996, Voelker & Rohwer 1998, Noskov et al. 1999). In general, pressure on a bird to finish post-juvenile moult early is thought to increase with a shorter breeding season and a longer migratory route. The rate of nestling growth has likewise been found to increase with both the migratory tendency of a population and the latitude of its breeding area, which in turn have effects on the length of the breeding season (e.g., Ricklefs 1976, Berthold 1988, 1996, Klaassen 1994, Dingle 1996). Such differences in the timing of juvenile (e.g., nestling growth) and post-juvenile development (e.g., post-juvenile moult) might be due to different environmental conditions, to different reaction norms (defined as the set of all phenotypes that a given genotype produ-

ces, depending on the environmental conditions; van Noordwijk 1990), or to a combination of the two. The sources of variation can be distinguished if distinct populations are compared under a variety of experimental conditions. Photoperiodic manipulations have been widely used as a tool for studying seasonal behaviour in organisms (e.g., Gwinner 1986, Dingle 1996). For birds at high latitudes, decreasing daylengths indicate the approaching end of the breeding season.

Among passerine birds, stonechats *Saxicola torquata* have the widest north-south breeding range and hence are exposed to the widest range of photoperiodic conditions (Underhill 1999, see also Moreau 1972, Cramp 1988). They have served as a model species for comparing life histories and timing responses in different environments. Stonechats from a sedentary population in East Africa and from a short-distance migrating European population differed in several life-history traits (e.g., Gwinner et al. 1983, 1995, König & Gwinner 1995, Starck et al. 1995, Helm & Gwinner 1999). Among nestlings of the two subspecies raised in the laboratory, African stonechats unexpectedly grew faster than their European conspecifics (Starck et al. 1995). Growth patterns in both subspecies showed flexibility related to rearing conditions and family effects in the growth of body mass. The subspecies differed genetically in the timing and duration of their post-juvenile moult, with European stonechats moulting earlier than African conspecifics (Gwinner et al. 1983). Both subspecies advanced moult in response to short daylengths, but the moult advance was much more pronounced in European than in African stonechats. We estimated high heritabilities ($h^2$) in both subspecies for the timing of moult onset and peak, indicating a great potential for evolutionary adjustment to local conditions (Helm & Gwinner 1999). From these results, moult timing, but not nestling growth, was clearly related to migratory behaviour and the length of the breeding season in stonechats.

In the present paper, we test the pertinence of these earlier results by studying stonechats from Kazakhstan with an extremely seasonal life cycle and a very short reproductive period. Table 1 summarizes important life-history differences among the three subspecies. Although the birds from Kazakhstan live at a similar latitude to Central European stonechats, the continental climate sets strict temporal limits to reproduction and development of the young (Johansen 1943). Kazakh stonechats are long-distance migrants that fly to India, southern continental China, and to northeastern Africa (Glutz & Bauer 1988, Cramp 1988). They arrive late on their breeding grounds, shortly after the May thaw, raise one brood, and subsequently initiate migration in August. Hence, stonechats from Kazakhstan spend only about half as much time on their breeding grounds as the short-distance migrants of Central Europe. Thus, we expected their growth and post-juvenile moult to give insights into timing mechanisms under an extremely tight seasonal schedule. We studied the timing of post-juvenile moult in Kazakh stonechats under three different photoperiodic conditions and compared nestling growth and moult timing to that of the other two stonechat populations.

**Table 1.** Life-history information on the three stonechat subspecies compared here (after Johansen 1954, Dementiev & Gladkov 1968, Glutz & Bauer 1988, Cramp 1988, Keith et al. 1992, & M. Raess, pers. comm.).

| | Kazakhstan<br>*S. t. maura* | Central Europe<br>*S. t. rubicola* | East Africa<br>*S. t. axillaris* |
|---|---|---|---|
| Geographical range | 40–70° N | 30–60° N | equatorial |
| Migratory status | long-distance | short-distance | sedentary |
| Present on breeding grounds | early May to August or early September | March to late September or mid October | throughout the year |
| Number of clutches | 1 clutch, occasionally relaying after nest loss | 2–3 | 1 (–2) |
| Mean clutch size | 6 | 5 | 3 |
| Time of hatching | late May, June | April to August | (related to rainy season) |

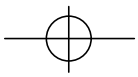



**Table 2.** Experimental groups and daylength conditions under which the three stonechat subspecies moulted.

| | short day (SD) | natural day (ND) | long day hold (LD) |
|---|---|---|---|
| Kazakhstan *S. t. maura* | 16 birds in ND until day 14–25; then constant equatorial daylength (LD 12.25:11:75 h) | 56 birds kept in European natural daylength of 47.5°N | 6 birds in ND until maximal daylength (age 16–21 days); then constant maximal daylength (LD 17.33:6.66 h) |
| Central Europe *S. t. rubicola* | 23 birds, treatment like *S. t. maura* | 57 birds, treatment like *S. t. maura* | |
| East Africa *S. t. axillaris* | 88 birds kept in SD from hatching | 15 birds hatched in SD; ND from ages 1–9 days | |

## Birds and methods

### Birds and holding conditions

The data in this paper were obtained between 1997 and 1999 from 78 stonechats of the subspecies *S. t. maura* (Pallas 1773). The median hatching date was 5 June (range: 30 days). Sixty eight birds were collected as nestlings in May and June of 1997 (cohort 1) and 1998 (cohort 2) in the vicinity of Naursum National Park (c. 51.5°N, 63°E), south of Kustanaj, Kazakhstan, at an altitude of c. 200 m a.s.l. They were transferred to Andechs, Germany, and hand-raised as described in Gwinner et al. (1987). Two breeding pairs were placed in outdoor aviaries where they produced 10 young from four clutches (cohort 3, four females and six males). The young were taken from their nests at an age of five days and treated like the birds collected in Kazakhstan. After they had gained independence, all birds were kept in individual cages and fed a standard diet. Light intensities were 400 lx (day) and 0.01 lx (night). Initially, all nestlings were kept in the natural photoperiod of 47.5°N (ND) to approximate their native daylight conditions (see Helm & Gwinner 1999 for details). Sibling groups of cohorts 1 and 2 were later divided into groups subjected to different light conditions (Table 2) in a split-plot design. Short daylength (SD) represented the native condition for African stonechats and indicated severe time pressure for high latitude populations. ND was the control condition for Kazakh and European stonechats. Long daylength (LD, only cohort 2 of 1998) simulated persistent midsummer. Since photoperiods in LD and SD were constant, these birds received no further photoperiodic time cues.

The data on European and African stonechats, *S. t. rubicola* and *S. t. axillaris*, used here for comparison are subsets of the data published by Starck et al. (1995) and Helm & Gwinner (1999). These stonechats were collected between 1982 and 1990 in Eastern Austria (48°14'N, 16°22'E; median hatching date 27 May) and in the Lake Nakuru region in Kenya (0°14'S, 36°0'E; median hatching date 24 April). Most were taken as nestlings and hand-reared in Andechs as described above. About one quarter of the stonechats had hatched in captivity. The light conditions for European stonechats were the same as those for the Kazakh subspecies. African stonechats were treated in an analogous way (Table 2). We included data from 23 African and 14 European stonechats in the comparative analysis of nestling growth, and from 103 African and 80 European stonechats in that of post-juvenile moult. For the calculation of reaction norms, we included additional data from Helm & Gwinner (1999).

*Analysis of growth:* Wing length and body mass of seven aviary-bred Kazakh stonechats were measured daily from the first or second day of life. For two birds measured from day 5 only wing growth was analysed. For each bird, we calculated the following individual growth parameters by fitting three-parameter logistic growth curves to their empirical data: growth rate constant K, asymptotic value A, and point of inflection of the curve $t_0$. Mean growth parameters ± s.d. were averaged over individual values. The procedure was identical to that of Starck et al (1995) in their growth analysis of European and African stonechats. From their data from hand-reared birds, we selected the subset of young that were fed by their natural parents for the first days after hatching and subsequently handfed. We excluded birds that were hand-reared from hatching be-

cause these birds behaved differently from all others. As a consequence, descriptive growth values for European and African stonechats differ slightly from those given by Starck et al. (1995), but all birds in our comparison were raised under identical conditions. We tested growth parameters in one-way ANOVAs and employed Tukey's Honestly Significant Difference Test (Tukey's HSD) for post hoc comparisons. If variables were slightly heteroscedastic between subspecies, we used Games-Howell's pairwise comparisons, which do not assume homoscedasticity (Sokal & Rohlf 1995).

*Post-juvenile body moult:* Post-juvenile moult corresponds to the first prebasic moult in the terminology of Humphrey & Parkes (1959). Every second day, the birds were checked for body moult in 19 plumage areas (Helm & Gwinner 1999). The moult process was visualised in mean moult curves of semi-weekly means ± s.e. against the age of birds. For statistical analyses, we derived four measures of moult timing: onset (age when first moulting at least five body parts), peak (mean age between the first and last recorded moult in 17 or more body parts), completion (age when last moulting at least five body parts), and duration (interval between onset and completion of post-juvenile moult). By defining a threshold of five simultaneously moulting body parts, our study focuses on the timing of the main portion of post-juvenile body moult, rather

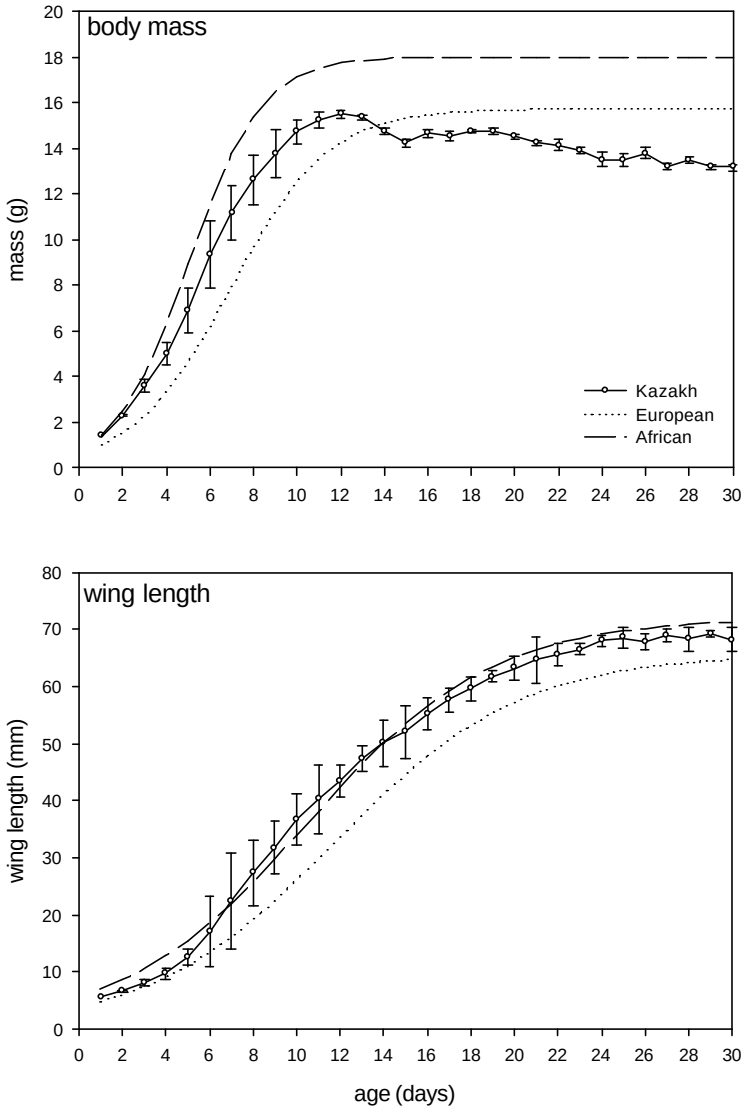


**Figure 1.** Growth curves for body mass (upper diagram) and wing length (lower diagram) of three stonechat subspecies. Growth curves of stonechats from Kazakhstan (solid) showing empirical means and standard deviations. Those of European (dotted) and African stonechats (dashed) were generated from the growth parameters given in Table 3.

**Table 3.** Growth parameters (mean ± s.d.) for the three subspecies of stonechats. The estimates for African and European stonechats are calculated from a subset of the data analysed by Starck et al. 1995.

| | Body mass | | | Wing length | | |
|---|---|---|---|---|---|---|
| Subspecies | Kazakhstan (n = 7) | Europe (n = 14) | Africa (n = 7) | Kazakhstan (n = 9) | Europe (n = 11) | Africa (n = 7) |
| Growth rate constant K ($day^{-1}$) | 0.600 ± 0.057 | 0.454 ± 0.073 | 0.603 ± 0.037 | 0.262 ± 0.011 | 0.235 ± 0.014 | 0.236 ± 0.016 |
| Age at inflection point $t_0$ | 5.18 ± 0.47 | 6.93 ± 1.10 | 5.04 ± 0.37 | 9.95 ± 0.64 | 11.78 ± 0.51 | 10.49 ± 0.56 |
| Final asymptotic size A | 15.01 ± 0.29 | 15.72 ± 1.28 | 18.0 ± 0.89 | 68.34 ± 1.39 | 65.56 ± 1.60 | 71.96 ± 1.45 |

than on its absolute onset, completion, and duration. Daylength on the date of each moult parameter was extracted for each bird from the original light protocols. No unique daylength could be assigned to moult duration.

Data were ln-transformed and analysed by Residual Maximum Likelihood Analysis (REML, Genstat 5 Committee 1993, see Helm & Gwinner 1999). Year, photoperiod, their interaction, and sex were tested as fixed factors by assessing the difference in model fit for each factor. The resulting Wald statistics are asymptotically distributed like $\chi^2$, and probabilities were calculated from a cumulative $\chi^2$ distribution. The factors sex and interaction between year and photoperiod were non-significant and dropped from the main model. Post hoc testing was carried out on the original data, using Mann-Whitney & Kolmogorov-Smirnov tests which differ in their assumptions. The more conservative results are included. In 1998, when birds were assigned to three groups, photoperiodic effects were tested in a Kruskal-Wallis analysis. In addition, we used linear regressions to test for effects of hatching date and effects of age differences during photoperiodic manipulation in the SD group (Table 2).

Heteroscedasticity among some groups complicated our analysis. For moult onset, the LD group had to be dropped from the main model. Variances were also very heterogeneous between subspecies. Therefore, we compared them in non-parametric Kruskal-Wallis analyses separately for the photoperiodic groups of SD and ND. We quantified and compared the photoperiodic responses of the subspecies in median regressions (median of age at moult against median of daylength for each cohort and photoperiodic group).

Family effects were tested over the residuals of the main analysis. We kept sample sizes relatively large but avoided inflating family effects by photoperiod and cohort differences. We excluded a few values gathered by a different investigator to avoid potential observer effects. The remaining data (16 families; number of birds: onset n = 72; peak n = 77; completion and duration n = 70) were analysed for significant family effects, and variance components were estimated. We calculated upper limits of heritability ($h^2$) as twice the sibling correlation t given by the ratio of the between-nest variance component over the sum of between-nest and within-nest variance components (Helm & Gwinner 1999). For comparison, we conducted a one-way ANOVA over families and calculated $h^2$ as described by Roff (1997) for unequal family sizes. Since $h^2$ estimates differed at most by 0.002, we publish only REML results. Standard errors were calculated as described by Roff (1997). In addition, we estimated $h^2$ separately for the photoperiodically unmanipulated birds kept under ND.

## Results

### Growth of body mass and wing length

Figure 1 shows the mean growth curves for body mass and wing length of Kazakh stonechats, in relation to growth of the other two subspecies. Both body mass and wing length of the Kazakh stonechats reached their

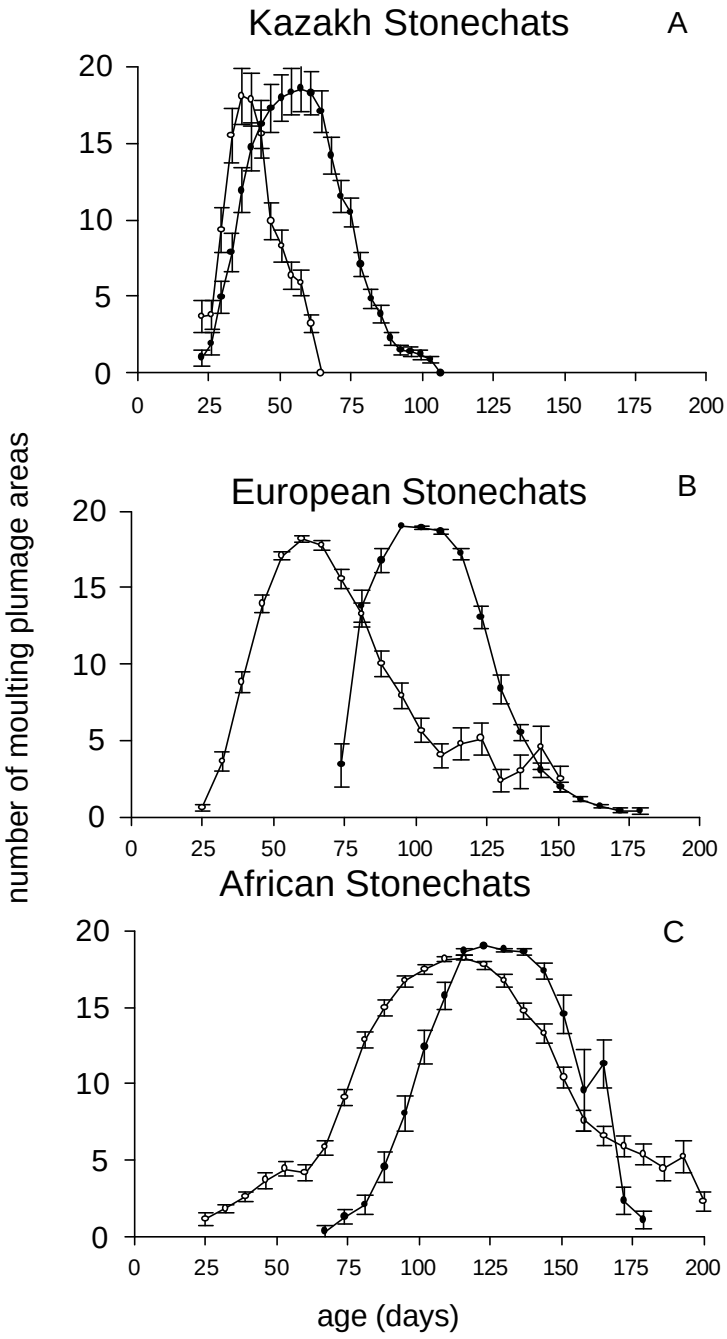


**Figure 2.** Moult curves of juvenile Kazakh, European, and African stonechats kept under short daylength (open circles) and natural daylength (filled circles). Curves show the mean number (± s.e.) out of a total of 19 plumage areas that were moulting at any given age. A: semi-weekly means of Kazakh stonechats. B + C: weekly means of European and African stonechats. Standard errors are smaller than those given in Helm and Gwinner (1999) owing to a computational error detected in the original values.

asymptotic size before those of the other two subspecies. Males were of slightly larger final sizes than females, but as sample sizes were small, statistical testing was not possible. Table 3 specifies mean growth parameters for the populations. In one-way ANOVAs, subspecies was a significant factor for all parameters ($P < 0.001$). Kazakh stonechats had lower final body masses than African (Games-Howell's: $P < 0.001$), but not European birds. The final wing length differed between all three subspecies. Kazakh stonechats had significantly longer wings than European stonechats (Tukey's HSD: European v. Kazakh $P < 0.01$), but shorter wings than African stonechats (Tukey's HSD: $P < 0.001$). Kazakh stonechats gained body mass at significantly higher

rates than European (Tukey's HSD, $P < 0.001$), but not African stonechats. They reached the age of fastest body mass growth ($t_0$) significantly earlier than European stonechats (Tukey's HSD, $P < 0.001$), but slightly later than their African conspecifics. For wing length, Kazakh stonechats had the highest growth rate constant of all three subspecies (Tukey's HSD, $P < 0.01$). The time of fastest growth ($t_0$) was earlier in Kazakh stonechats than in their European conspecifics (Tukey's HSD, $P < 0.001$), but similar to that of African stonechats.

## Post-juvenile moult

*Variation in moult timing*

Kazakh stonechats started moult early and finished it quickly. Their first moult was recorded at an age of 21 days, before growth of wing feathers was completed. Variation in moult timing increased during the course of moult (difference between earliest and latest moult age: onset 19, peak 37.5, completion 51, duration 43 days). The factors that were associated with differences in moult timing changed over the course of moult. At moult onset, the highest proportion of the variance between stonechats was associated with individual differences (variance component of 63.6 %). The variance components associated with cohort and photoperiodic group were smaller and similar in magnitude (17.4 % and 19.0 %, respectively). During the course of moult, the proportion of variance associated with photoperiod increased to 73.9 % (peak), 82.5 % (completion), and 87.6 % (duration). Thus, by the time of moult completion differences between birds were mostly related to photoperiodic treatment. Variance components for cohort and individual decreased to 11.5 % and 2.4 % respectively (peak), 9.1 % and 8.4 % (completion), and 3.8 % and 8.6 % (duration). The three cohorts differed significantly (n = 78; Wald statistics on df = 2: for onset = 13.6; $P < 0.01$; for peak = 24.4; $P < 0.001$; for end = 35.5; $P < 0.001$; for duration = 16.7; $P < 0.001$). Nestlings collected in Kazakhstan in 1998 started moult earliest, those from 1997 at intermediate ages, and stonechats raised in captivity in 1999 last. However, the cohorts responded in precisely the same manner to the photoperiodic manipulations.

*Effects of photoperiod*

Moult timing differed markedly between the photoperiodic groups (Fig. 2a; n = 78; peak: Wald = 227.4, df = 2, $P < 0.001$; end: Wald = 372.0, df = 2, $P < 0.001$; duration: Wald = 378.8, df = 2, $P < 0.001$; onset excluding the LD group: n = 72, Wald = 11.8, df = 1, $P < 0.001$). Kazakh stonechats initiated moult slightly earlier under SD than under ND and LD (Table 4) and progressively accelerated moult in SD so that the differences between the photoperiodic groups increased with time. Effects of all three photoperiodic treatments were compared within the cohort of 1998. Daylength affected all moult parameters except for onset (Kruskal-Wallis, onset: $\chi^2 = 4.3$, df = 2, n.s.; peak: $\chi^2 = 18.0$, df = 2, $P < 0.001$; completion: $\chi^2 = 18.1$, df = 2, $P < 0.001$; duration: $\chi^2 = 17.9$, df = 2, $P < 0.001$). Post-hoc analyses revealed that moult timing of birds kept in SD differed from that of the other groups, whereas stonechats kept in LD moulted at the same ages as those kept in ND (LD: median ± interquartile range (IR): onset 31 ± 1; peak 51.5 ± 4; completion 72 ± 16; duration 40 ± 18; n = 25; Mann-Whitney n.s.). Unexpectedly, we found no effect of hatching date on moult timing among Kazakh stonechats under ND. In the SD group, the age at the photoperiodic switch exerted a significant influence on all moult timing measures, except for moult duration (linear regression with cohorts as groups: onset slope 0.9 ± 0.11; constant 12.3; $r^2 = 81.9$; peak: slope 0.8 ± 0.13; constant 23.4; $r^2 = 79.2$; completion: slope 0.8 ± 0.24; constant 38.4; $r^2 = 64.2$; for all moult measures, n = 16; $P < 0.001$). The earlier a bird was placed under SD, the earlier it moulted. The effects of the age at first exposure to SD were identical in slope for the cohorts of 1997 and 1998.

*Family effects on the timing of moult*

There were significant family effects for all moult parameters, except for onset, after accounting for effects of photoperiod and year (onset: Wald = 22.7; n.s.; peak: Wald = 35.1; $P < 0.01$; end: Wald = 28.6; $P < 0.05$; duration: Wald = 81.9; $P < 0.001$; analyses based on 16 families; number of birds: onset n = 72; peak n = 77; completion and duration n = 72). We calculated the following $h^2$ values: onset $h^2 = 0.22 \pm 0.23$; peak $h^2 = 0.44 \pm 0.24$; completion $h^2 = 0.36 \pm 0.25$; duration $h^2 = 1.00 \pm 0.25$. Separate $h^2$ estimates for the birds kept in ND were only slightly elevated (n families = 15, n birds = 50 to 56): onset $h^2 = 0.25 \pm 0.28$; peak $h^2 = 0.64 \pm 0.30$; completion $h^2 = 0.55 \pm 0.32$; duration $h^2 = 1.09 \pm 0.28$.

*Comparison of moult timing among the three stonechat subspecies*

Kazakh stonechats initiated moult at earlier ages than the other subspecies. In ND, the three stonechat subspecies differed significantly in the timing and duration of their moult (Table 4, Fig. 2). Kazakh stonechats started moult much earlier than European stonechats and were quicker to finish it (Kolmogorov-Smirnov Test: onset $Z = 2.79$; $P < 0.001$; peak $Z = 4.04$; $P < 0.001$; completion $Z = 3.97$; $P < 0.001$; duration $Z = 1.73$; $P < 0.005$). European stonechats, in turn, moulted earlier and for a shorter time than African stonechats. The differences between the three stonechat subspecies in moult timing and duration were even more pronounced under SD. Kazakh stonechats moulted earlier and faster than European stonechats (Kolmogorov-Smirnov test: onset $Z = 2.45$, $P < 0.001$; peak $Z = 3.47$, $P < 0.001$; completion $Z = 3.54$, $P < 0.001$; duration $Z = 3.51$, $P < 0.001$), which, in turn, moulted earlier and more quickly than African stonechats.

In all subspecies, the timing of moult was linearly related to daylength (Fig. 3). The shorter the daylength, the earlier the moult occurred. We present the subspecies responses to daylength for peak moult as an example but we obtained similar results for moult onset and completion. The median regression with subspecies as groups was highly significant ($n = 18$, $r^2 = 96.7$; $P < 0.001$). Stonechats from Kazakhstan responded to short daylength with the smallest advance of their moult peak. Their response differed significantly in slope from that of their European conspecifics (slope of Kazakh + additional slope of European stonechats: 3.5 + 21.0 days per hour; $P < 0.001$), but not from that of African stonechats (3.5 + 4.3 days per hour; n.s.).

## Discussion

Body mass increased faster in stonechats from Kazakhstan than in European stonechats (Fig. 1). These results are in line with studies from other taxa that related faster growth to a shorter breeding season and to longer migratory routes (Ricklefs 1976, Klaassen 1994, Dingle 1996). However, in spite of even more pronounced differences in migratory behaviour and breeding range, Kazakh stonechats did not differ from African conspecifics in body mass growth. The fast growth of African stonechats may be related to other selective pressures, e.g. high nest predation rates (Scheuerlein 2000). Starck & Ricklefs (1998) calculated a body mass growth rate constant of 0.548 $day^{-1}$ from field data of the British stonechat *S. t. hibernans* (Greig-Smith 1985). This subspecies is partially migratory, and its breeding season is even longer than that of Central European stonechats (Cramp 1988). Its growth rate constant in the field is close to that of Kazakh and African stonechats bred in captivity. Thus, the relationship between body mass growth, breeding range, and migrato-

**Table 4.** Timing of moult under natural and short daylight for the three subspecies of stonechats. The table shows sample size, median, interquartile range (IR), and results from a Kruskal-Wallis analysis of the four moult parameters. Comparative data on European and African stonechats were taken from Helm & Gwinner (1999).

| | Kazakhstan | | | Europe | | | Africa | | | Kruskal-Wallis | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | median | IR | n | median | IR | n | median | IR | $\chi^2$ (2df) | P ≤ |
| *European natural daylength (ND)* | | | | | | | | | | | |
| Onset | 56 | 31 | 6 | 9 | 84 | 4 | 15 | 94 | 11 | 50.94 | 0.001 |
| Peak | 56 | 54 | 8.25 | 23 | 101.5 | 33 | 15 | 129 | 7.3 | 71.66 | 0.001 |
| Completion | 56 | 76.5 | 9.5 | 22 | 134 | 11 | 15 | 161 | 8.2 | 70.38 | 0.001 |
| Duration | 56 | 44 | 7.5 | 9 | 48 | 2.75 | 15 | 64 | 6.5 | 39.73 | 0.001 |
| *Short daylength (SD)* | | | | | | | | | | | |
| Onset | 16 | 29.5 | 5 | 53 | 38 | 8 | 73 | 72 | 16 | 103.56 | 0.001 |
| Peak | 16 | 39.5 | 4.5 | 57 | 63.5 | 13.5 | 88 | 111.5 | 24 | 126.43 | 0.001 |
| Completion | 16 | 49.5 | 7 | 57 | 91 | 20 | 86 | 159 | 24 | 121.97 | 0.001 |
| Duration | 16 | 20.5 | 5 | 53 | 55 | 21.25 | 71 | 91 | 27.25 | 87.90 | 0.001 |

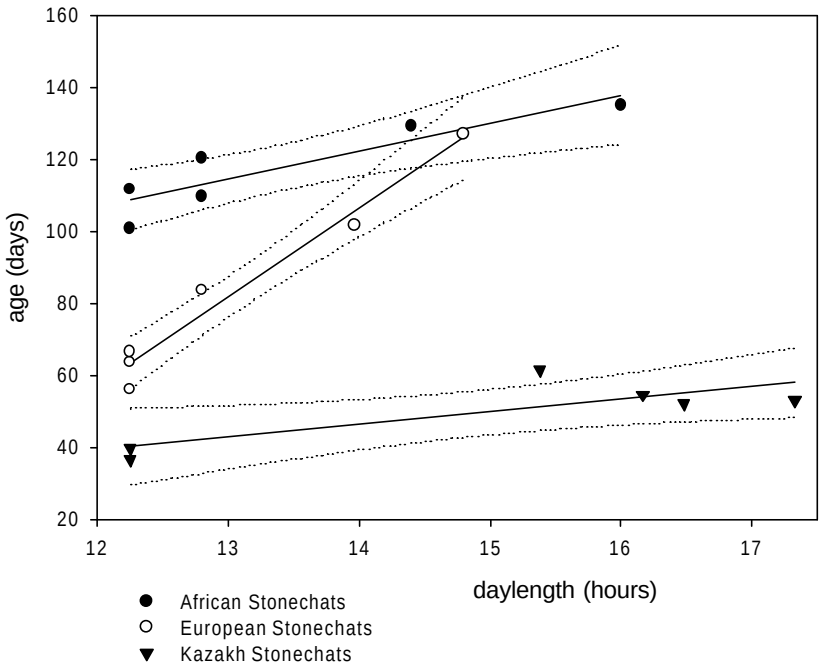


**Figure 3.** Mean reaction norms of moult timing in relation to photoperiod. For the three Stonechat subspecies, timing of peak post-juvenile moult is related to daylength by linear regression (regression slopes with confidence intervals). Data for Kazakh, European, and African Stonechats are the median ages at moult peak of several experimental groups in relation to the median daylength on the respective date.

ry behaviour in stonechats is ambiguous. Data on wing growth are in better accordance with predictions made about the influence of these factors. The wings of *S. t. maura* grew faster than those of European and African stonechats. However, the fast wing growth of *S. t. maura* from Kazakhstan might be related to several other factors affecting their juvenile development. Avian growth rates are considered to evolve as a compromise between limitations in food availability on the one hand, and acceleration through time-dependent mortality on the other (Lack 1968, Bosque & Bosque 1995; reviews of ongoing discussion in Gebhardt-Henrich & Richner 1998, Ricklefs et al. 1998). Mortality due to sibling competition has been shown to select for fast growth (Werschkul & Jackson 1979), and Greig-Smith (1985) found sibling competition to affect body mass growth in British stonechats. Given the large clutch size of Kazakh stonechats, this factor may have selected for their fast wing growth.

Post-juvenile body moult in *S. t. maura* started and ended at very young ages. Our results from the lab are comparable to the only field record from Kazakhstan that has come to our attention. Dementiev & Gladkov (1968) observed young stonechats that had almost finished moult in early August. In view of the fairly constant hatching time in early June (Johansen 1954, Dementiev & Gladkov 1968), the stonechats were presumably about two months old, in good accordance with the median age of 76.5 days at moult completion calculated here.

In our study, the differences between subspecies in the timing and duration of post-juvenile moult were pronounced. *S. t. maura*, under all photoperiodic conditions, moulted earlier and more quickly than European stonechats, which in turn moulted before African stonechats (Fig. 2). This sequence of moult timing is precisely as expected on the basis of migratory behaviour of the subspecies and length of their breeding seasons (Jenni & Winkler 1994, Noskov et al. 1999). All subspecies displayed plasticity of moult timing by advancing their moult under SD. However, they differed in the slopes of their estimated 'mean reaction norms' (Fig. 3; van Noordwijk 1990, Helm & Gwinner 1999). *S. t. maura* showed the smallest advance of moult peak under SD relative to ND. The regression slope was similar to that of African stonechats but much smaller than in European stonechats. One could argue that transfer to SD occurred too late to have its full effect on the onset of moult (Lindström et al. 1994). However, unpublished data from six stonechats that hatched under SD in 2000 make this interpretation unlikely. These birds moulted at a median age of 31 days, and

hence no earlier than the SD group in the present study (Helm & Gwinner, unpubl. data). Theoretically, physiological constraints may keep stonechats from advancing their moult below the age of about 30 days in *S. t. maura*. However, literature data from other passerine species indicate that post-juvenile moult can start at earlier ages. Widmer (1999) found a mean moult onset of 16 days (minimum: 14 days) for an Alpine population of garden warblers *Sylvia borin*. Out of 13 Russian passerine species studied by Noskov et al. (1999) 10 species started post-juvenile moult at younger ages than *S. t. maura*, with 19 days for the garden warbler as the lowest value. In view of such early moulting ages, the small SD-induced advance of moult onset in Kazakh stonechats does not appear to be due to physiological limitations.

While moult onset in Kazakh stonechats was only slightly affected by SD, later stages of moult were increasingly more advanced. As a consequence, moult duration was drastically reduced. Such a time-dependent increase of the photoperiodic response in *S. t. maura* was also indicated by the growing variance components associated with photoperiod. Apparently, stonechats from Kazakhstan achieve the timely completion of their moult by starting at a fairly inflexible early age, and by subsequently adjusting moult rate according to photoperiodic cues. Conversely, the other two subspecies responded most strongly to SD by advancing moult onset, whereas moult duration was hardly affected (Helm & Gwinner 1999). The stonechat subspecies thus differed in the plasticity patterns of post-juvenile moult timing. The overall effect – an earlier completion of post-juvenile moult in SD compared to ND – was negligible in resident equatorial stonechats. In contrast, the two migrant populations showed a considerable advancement of moult completion. Despite their different patterns of plasticity in the timing of moult, stonechats from Europe and Kazakhstan achieved very similar advances of moult completion under SD compared to ND (Europe: 67.9 %; Kazakhstan: 64.7 % of ND under SD).

The low photoperiodic responsiveness of moult onset in *S. t. maura* confirms previous studies of bird populations with short breeding seasons (Jenni & Winkler 1994, Lindström et al. 1994). In the two northern hemisphere stonechats, the different patterns of plasticity in the timing of moult may reflect different selection pressures. Given the short reproductive period of stonechats from Kazakhstan around midsummer, photoperiod may not be a useful cue for the timing of moult onset because nestlings are not exposed to daylengths that differ markedly from the maximal midsummer photoperiod. Unpredictable seasonal effects, such as a rapid deterioration of environmental conditions in some years, may select for an early start of post-juvenile moult (M. Raess, pers. obs.) as a low-risk strategy to avoid changing plumage under unfavourable circumstances. In contrast, young of the multi-brooded European stonechat experience a wide range of photoperiods from hatching and they differ distinctly in the ages at which they initiate post-juvenile moult (Flinks 1999). Photoperiod becomes more useful as a time cue for *S. t. maura* later during moult because the decrease in daylength accelerates in late summer. Hence, the range of daylengths experienced by birds from early versus late clutches at a given age widens as the season progresses. Small differences in hatching date can lead to large differences in daylength during the later stages of moult. In response to short daylengths, immatures reduce moult duration by increasing the moult rate, presumably incurring high costs. An increased speed of moult can raise energetic and life history expenses and reduce the quality of the replaced plumage (Hahn et al. 1992, Serra 1999, Hall & Fransson 2000, Lind 2001).

Estimates of heritabilities also indicate a low flexibility of moult onset in *S. t. maura*. At the beginning of moult family effects were not significant and additive genetic variance was low. Later during moult, however, family patterns were more clearly expressed, and the estimate of $h^2$ for moult duration was high. Heritability estimates from full-sib analyses represent upper limits of additive genetic variance since they are inflated by nest effects and dominance deviation (Roff 1997). Comparative data on the genetics of moult timing are scarce. Larsson (1996) published heritability values for the onset of wing moult in wild adult barnacle geese *Branta bernicla*. Estimates for $h^2$ from parent-offspring regressions ranged between 0.2 and 0.4, and estimates from full-sib analysis were similar except for one outlier. Widmer (1999) found altitudinal differences in moult timing between alpine and lowland populations of garden warblers, in which the values of $h^2$ for the onset, median, and end of post-juvenile moult ranged between 0.41 and 0.64 in both populations. High-altitude birds with a short breeding season showed a slight increase of $h^2$ values over the course of moult, whereas

in lowland birds, $h^2$ remained constant. In contrast to lowland birds, the moult of mountain birds was not responsive to short daylength and different hatching dates (M. Widmer, pers. comm.). The similarity of the results obtained by Widmer in his altitudinal study and our findings obtained from comparing breeding seasons of different lengths supports the idea that under high seasonal pressure, bird populations initiate their moult early and that family differences and responses to timing cues become evident later during moult.

It is interesting to note that in all subspecies the changes in additive genetic variance over the course of moult paralleled those of photoperiodic responsiveness. While in *S. t. maura* both $h^2$ and the plasticity of the photoperiodic response were initially low but increased over the course of moult, the opposite was true in European and African stonechats. Based on $h^2$ estimates, and thus on the genetic potential to respond to changing selection pressures, we would expect the three subspecies to evolve differently when exposed to new seasonal pressures. Kazakh stonechats should respond with successive timing adjustments over the course of moult, and specifically change their moult duration. The other two subspecies should advance or delay the onset and peak of moult but keep its duration approximately constant. This is exactly in line with the respective responses of the subspecies to different photoperiods.

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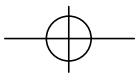



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# Kleptoparasitism in cormorants *Phalacrocorax carbo*

**Jesus Mari Lekuona[1] and Francisco Campos[2]**

Two hundred and seventy attempts of kleptoparasitism were recorded in cormorants *Phalacrocorax carbo*, most of which were intraspecific. For both young and adult birds, performing kleptoparasitism was more successful than fishing alone, suggesting that it could be used as a flexible feeding strategy to decrease the metabolic costs of diving. Cormorants attacked more frequently in groups than individually and group attacks were more successful. Cormorants also took larger prey items during group attacks than individually. Handling time increased with fish size, implying that large prey were avoided as being less profitable. There appeared to be a social dominance in kleptoparasitic behaviour since adult cormorants preferentially attacked younger birds. Age-related differences in foraging parameters may favour discrimination between victim age classes by kleptoparasites.
Keywords: Cormorant, *Phalacrocorax carbo*, kleptoparasitism, foraging success.

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Kleptoparasitism has been thoroughly studied in gulls, terns, skuas, waders and cattle egrets (Caldwell 1980, Burger & Gochfeld 1981, Thompson 1983, 1986, Hockey et al. 1989, Amat & Aguilera 1989, 1990, Caldow & Furness 1993, Oro 1996), but most research has focused on interspecific rather than intraspecific kleptoparasitism. Kleptoparasitism typically occurs when captured prey are clearly visible to an attacker. This is the case for aquatic birds which, although they may swallow small prey underwater, need to bring larger prey to the surface. Here the adoption of a kleptoparasitic strategy may be more profitable than self-feeding, especially for specialist and dominant individuals or for birds that have not yet mastered the skills necessary for underwater pursuit.

Several factors may influence the decision to steal food, including bird age, prey size and the number of kleptoparasites present. Since adults are more skilled at capturing prey (Orians 1969, Verbeek 1977a, Quinney & Smith 1980, Lekuona 1997), they should be the object of more attacks (Carroll & Cramer 1985, Tershy et al. 1990), but there is not always evidence for this (Verbeek 1977b). Conversely, older birds should be more successful kleptoparasites since they are more efficient hunters than younger birds (Burger & Gochfeld 1979, Heps & Barnard 1989). Birds with larger prey should also be the object of more attacks (Hulsman 1976, Brockmann & Barnard 1979), but Dunn (1973) observed the opposite, and Hulsman (1984) emphasised that prey width and shape are also important. Futhermore, Amat & Aguilera (1990) confirmed that group attacks are more successful than individual attacks since they increase the relative success for individual birds.

Kleptoparasitic behaviour in cormorants *Phalacrocorax carbo* was described in Johnsgard (1993), but no detailed data were provided. In this paper we describe a study in northern Spain, where cormorant numbers have increased over the past few years (Campos & Lekuona 1994, Lekuona & Campos 1996a). This has increased the number of foraging birds at feeding sites and the number of cases of kleptoparasitism. We pre-

sent data on the influence of bird age and prey size on the success of kleptoparasitic behaviour and discuss whether it is a profitable strategy for older birds or an opportunistic behaviour for subordinates.

## Methods

The study area included northern Spain (the provinces of Navarra, Guipúzcoa and Alava) and southwestern France (the Orx lagoon and the Atlantic coast at Arcachon and Biarritz). Visits were made to coastal bays, inland lakes, fish farms and reservoirs from October to March over four years (1992–1995). We observed foraging cormorants 2–3 days a week from dawn to dusk, using 20–60× telescopes at distances of up to 100 m. We recorded: (1) the ages of kleptoparasitic birds and their victims: adult birds (≥3 year old) were distinguished from young birds (≤2 year old) were identified by their plumage (Van Eerden & Munsterman 1995); (2) the results of kleptoparasitic attempts (successful if prey was stolen, failure if not, even if the victim lost the prey); (3) the number of birds participating in each attempt; and (4) the size of the prey captured, which was classified into five size categories in relation to bill-length (Koffijberg & Van Eerden 1995, Voslamber et al. 1995, Lekuona & Campos 1997): class 1 (<8 cm), class 2 (8–14 cm), class 3 (15–21 cm), class 4 (22–28 cm) or class 5 (>28 cm).

For each foraging bout we recorded: (1) foraging time $F_t$, the minutes spent feeding at the foraging site from arrival to the end of fishing; (2) number of feeding attempts and apparent result (success or failure); foraging success was determined by counting successful dives (prey seen or swallowing observed) and calculating the number of successful dives out of all the recorded dives; (3) size of the fish captured; (4) mean biomass (g wet weight) of each prey item calculated using standard equations (Arne 1938, Richner 1986, Lekuona & Campos 1996b); and (5) handling time of prey after surfacing until swallowed. Whenever possible we identified the prey species and estimated its size in order to calculate the food intake of individuals in the same feeding area and foraging period. Intake rates can be slightly underestimated because some small prey may be swallowed underwater (Voslamber et al. 1995, Carss 1997). A normal foraging bout was defined as the time that an individual stayed at the same feeding site (Voslamber et al. 1995, Lekuona & Campos 1997) without kleptoparasitic attacks. Individual birds were identified by age (Van Eerden & Munsterman 1995), coloured rings (n = 93), and plumage (yearling birds have a variable amount of white feathers on their breast and belly, Lekuona & Campos 1994).

All observations were grouped into a single dataset because the number of kleptoparasitic attacks recorded each year and at different specific feeding sites was low. Unless otherwise stated, the data on successful attempts, size of prey, bird age, number of attacks and number of kleptoparasites were compared using a G-test (Sokal & Rohlf 1979). Handling time and biomass intake were compared using a Mann-Whitney Z-test, and foraging time with Student's t-test.

## Results

### Description of attacks

Kleptoparasitic cormorants approached their victims by a rapid low-level flight ending in a quick dive towards the host's bill, or along the water surface by beating the water with their wings until the victim was reached. The choice of attack seemed to depend on the distance to the victim. Group attacks were generally from the air with the typical diving behaviour of individual attacks. Group attacks on lone victims were usually initiated by one bird and the rest followed. Usually only the leading cormorant obtained the prey but followers would sometimes make a secondary attempt on the leading bird. If a kleptoparasitic group attacked a dense flock of fishing cormorants, each bird attacked a different victim. The birds under attack defended themselves either by submerging, by flying away with the prey in their bills, or by facing up to the attacker. Some attackers were also unsuccessful members of a foraging flock that tried to steal prey from successful companions that emerged with fish. The cormorants observed made more intra- than interspecific attacks (grey heron *Ardea cinerea*, black-headed gull *Larus ridibundus*) (94.8 % in comparison to 5.2 %, n = 270, G = 15.8, df = 1, P < 0.001; Table 1).

Most attacks were made in groups and were more successful than individual attempts (G = 208.2, df = 1, P < 0.001; Table 1). The average group size (± s.d.) was 3.0 ± 1.3 birds (n = 199), but the fish was always eaten

**Table 1**. Number (n) of kleptoparasitic attacks and corresponding success rates made by cormorants individually and in groups.

| | Individuals | | Groups | | Total | |
|---|---|---|---|---|---|---|
| Victim | n | % success | n | % group success | n | % success |
| Cormorant | 52 | 36.5 | 204 | 42.2 | 256 | 41.0 |
| Grey heron | 4 | 75.0 | 9 | 33.3 | 13 | 53.8 |
| Other species | – | – | 1 | – | 1 | – |
| Total | 56 | 39.3 | 214 | 41.6 | 270 | 41.1 |

by one bird. Thus, the per capita success was less in group attacks. Two-thirds (67.3 %, n = 450) of the kleptoparasitic groups included young birds.

## Influence of fish size

Kleptoparasitic groups tended to take larger prey of size class 3 and 4, and individual birds of size class 2 ($G = 18.3$, $df = 2$, $P < 0.001$; Fig. 1). Handling time increased significantly with fish size, though differences between fish species could have contributed additional variation. Size classes 2 and 3 were robbed in the same proportion (39.2 %; Fig. 2), although their handling times were significantly different (Mann-Whitney Z-test, $Z = 7.75$, $P < 0.001$, $n_1 = 55$ and $n_2 = 76$). Larger fish of size class 4, with still greater handling time, were taken much less often (15 %), and fish of size class 5 not at all.

## Influence of age

Both adult and young cormorants made a similar number of attacks ($G = 1.13$, $df = 1$, n.s.) but with different success rates, according to age (corrected for relative numbers of adult and young birds in the population, $G = 35.9$, $df = 1$, $P < 0.001$; Table 2). Adults attacked young cormorants more frequently than other adults, whereas young birds attacked more adults than other young birds ($G = 14.7$, $df = 1$, $P < 0.001$). In the overall dataset, adult cormorants were the main victims but the percentage of food losses was significantly lower ($G = 12.5$, $df = 1$, $P < 0.001$; Table 2). In our study area, the population age structure was dominated by adults (Campos & Lekuona 1994; Lekuona & Campos 1996a). This suggests that adults selected for young victims, whereas young birds attacked victims in relation to their abundance. Large differences were found be-

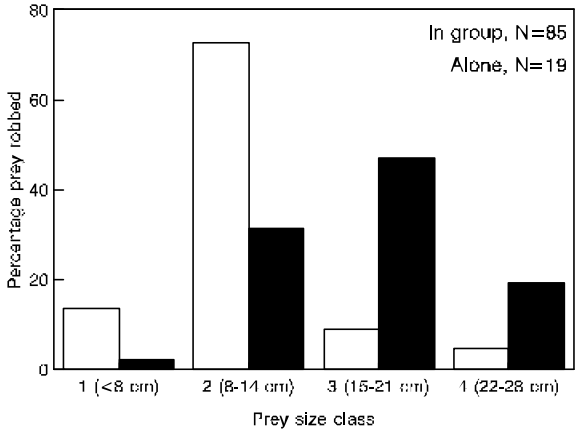


**Figure 1.** Percentage of prey items robbed by cormorants in groups (black bars) and by individuals (white bars), according to the size class of prey (cm).

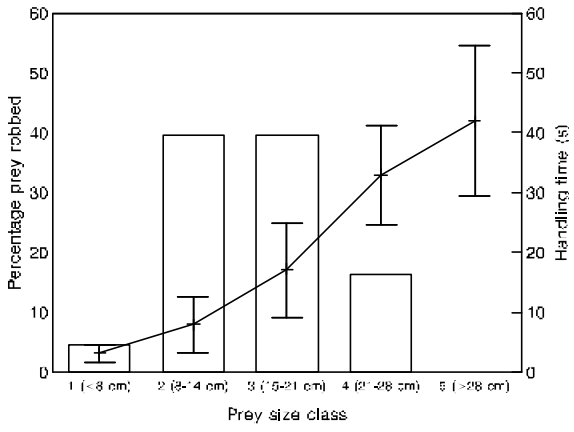


**Figure 2.** Percentage of prey items robbed (bars) by Cormorants according to size classes (cm), and mean handling time ± s.d. (line).

**Table 2**. Number (n) of kleptoparasitic attacks and corresponding success rates made by cormorants according to age.

| | Kleptoparasite | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Adult | | Young | | Mixed groups | | Total | |
| Victim | n | % success | n | % success | n | % success | n | % success |
| Adult cormorant | 42 | 30.9 | 68 | 20.6 | 10 | 20.0 | 120 | 24.2 |
| Young cormorant | 57 | 57.9 | 30 | 73.3 | 5 | 60.0 | 92 | 63.0 |
| Other species | 11 | 54.5 | 2 | – | 1 | – | 14 | 42.8 |
| Total | 110 | 47.3 | 100 | 36.0 | 16 | 31.3 | 226 | 41.2 |

tween age structure of attacking groups and the age structure of the observed wintering population ($G = 282.9$, $df = 1$, $P < 0.001$)

### Normal foraging bouts

Table 3 summarises the feeding measurements during normal foraging bouts in the areas where kleptoparasitic attacks occurred. In 364 normal foraging bouts where bird age was noted, adult cormorants spent the same time foraging as young birds ($t = 0.98$, n.s.). The frequency of successful attempts was also similar for adult and young birds ($G = 0.19$, $df = 1$, n.s.) but the estimated biomass intake of adult cormorants was higher (Mann-Whitney Z-test, $Z = 10.1$, $P < 0.001$).

## Discussion

In our study area, cormorants most frequently attempted to steal prey when foraging in groups, suggesting that the frequency of kleptoparasitism might be density-dependent, as proposed by Krebs & Barnard (1980). This hypothesis is supported by the fact that most attacks were intraspecific, probably because the number of cormorants in the area was greater than that of other potential victims (pers. obs.).

Although group attacks were more successful and obtained larger prey, kleptoparasites did not share the prey, so the benefit per bird diminished with increasing group size. The feeding success of young cormorants is lower than adults during normal foraging (Johnsgard 1993, Lekuona 1997), which probably favours the formation of groups to steal food, thus compensating for their lack of experience in the search and detection of prey.

The profitability of large prey (defined as the ratio between ingested biomass and manipulation time; Campos & Lekuona 2000) may be reduced because its handling time is significantly greater. Thus, kleptoparasites might be expected to direct their attacks against victims with more profitable (smaller) prey in order to maximise benefits, as observed here. In addition, attacks by individuals were more effective than group attacks because their success per capita was higher and they mainly captured small prey (8–14 cm) with reduced handling time.

The kleptoparasitic attacks occurred in winter, when the average monthly temperatures fell as low as 4 ºC (Lekuona 1999). The lower critical temperature in cormorants is estimated to be 11.3 ºC (Grémillet et al. 2000), so that cormorants must use additional energy to maintain body temperature under winter conditions. Other metabolic costs include, the time spent feeding at

**Table 3.** Foraging parameters of cormorants (adult and young birds) in winter.

| Age | Number of foraging bouts | Number of dives | % success | Foraging time (min) (mean ± s.d.) | Biomass taken (g/bout) (mean ± s.d.) |
|---|---|---|---|---|---|
| Adult | 163 | 5182 | 18.9 | 22.9 ± 7.3 | 315.7 ± 87.0 |
| Young | 201 | 5587 | 17.1 | 22.7 ± 7.6 | 203.4 ± 67.2 |

the foraging site (Schmid et al. 1995, Gremillet & Wilson 1999, Gremillet et al. 2000), performing dives (Schmid et al. 1995), heating of ectothermic prey in the stomach (Wilson & Culik 1991), flying (Pennycuick 1989) and wing-spreading behaviour (Hennemann 1985). Decreasing some of these daily expenditures may be advantageous with respect to conspecifics. In our study area, adult cormorants fed closer to the roost, and young birds farther away, which increased the energy costs for flight (Lekuona 2000). In addition, adult cormorants obtained more biomass per feeding bout than younger birds. Low biomass intake in young cormorants might therefore influence their survival rate by forcing them to perform more fishing sequences per day and increasing their energy costs. In this situation, young birds could opt to decrease energy costs by stealing food from more experienced adults. In both adult and young cormorants, however, the success of kleptoparasitism was greater than fishing success (Tables 2 and 3). Thus, kleptoparasitism should be considered as part of a flexible feeding strategy to decrease the metabolic cost associated with diving and maximise feeding efficiency.

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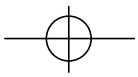



# Which factors influence the abundance and range size of Central European birds?

**Katrin Böhning-Gaese* and Reik Oberrath**

The abundance and range size of 151 Central European bird species were correlated with 15 of the most prevalent morphological, life-history and ecological species attributes. The study was conducted at two different spatial scales, at the scale of the Lake Constance region and at the scale of Germany as a whole. However, the results were robust with regard to the spatial scale of the analysis. The most significant predictor of both abundance and range size was breeding habitat with urban and forest species being more abundant and widespread than wetland and farmland species. Other important variables were body mass and variables correlated with body mass, especially incubation time. Abundance and range size were influenced by the position in the geographic range with species in the centre of their range being more abundant and widespread than species towards the edge or at the margin of their range. Furthermore, migratory status was a significant predictor of abundance and range size (after controlling for body mass). Long-distance migrants were less abundant and widespread than short-distance migrants and residents. Controlling for phylogenetic relatedness among the species using Signed Mantel tests did not change any of the results. The results demonstrate that 36–48 % of interspecific variation in abundance and range size can be explained by only four species attributes.
Key words: European birds, abundance, geographic range size, morphology, life-history.

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One of the core problems in ecology is the identification of factors that determine the abundance and range size of species. This question has gained particular importance in conservation biology. Rare species are more threatened by extinction than abundant species. Therefore, it is important to know which factors cause species to be rare (Gaston 1994, Lawton 1995). Unfortunately, the answer to these questions is extremely complex.

One possible approach is to identify factors correlated with abundance and range size across a large number of species (Lawton 1989, Pomeroy & Ssekabiira 1990, Damuth 1991, Blackburn et al. 1996). Birds are particularly suited for such a macroecological approach because comparatively reliable data exist about the abundance and range size of many species (Brown 1995, Gaston & Blackburn 2000). The abundance of bird species has been shown to be correlated with body size (Juanes 1986, Cotgreave & Harvey 1992, Gregory & Blackburn 1995), life-history variables (Blackburn et al. 1996, Duncan et al. 1999), and habitat type (Pomeroy & Ssekabiira 1990, Thiollay 1994, Duncan et al. 1999, Gregory & Gaston 2000). Furthermore, migratory birds have been demonstrated to be less abundant than resident birds (Cotgreave 1994).

However, it is difficult to judge the relative importance of these factors because most of them have been tested individually. Correlations among the factors, e.g. between migratory status and life-history variables (Kipp 1943, Whitcomb et al. 1981, Mönkkönen 1992)

or between migratory status and nest type (von Haartman 1968) have not been considered. Thus, a possible explanation for the low abundance of migrants compared to residents is that migrants produce fewer eggs per year than residents. Migrants have both smaller clutches (Whitcomb et al. 1981, Mönkkönen 1992, Böhning-Gaese et al. 2000) and fewer clutches per year than residents (Kipp 1943, Mönkkönen 1992, Böhning-Gaese et al. 2000). A second explanation for the low abundance of migrants is that long-distance migrants might be excluded from good territories or safe nest sites by short-distance migrants and residents because of their late arrival on the breeding grounds (von Haartman 1968, Whitcomb et al. 1981, O'Connor 1990).

The objective of this study was to correlate the abundance and range size of bird species with the most prevalent morphological, life-history and ecological species attributes, both by testing each species attribute individually and by using combined models of multiple species attributes. Besides body size, life-history traits, breeding habitat, and migratory status, additional factors such as diet, nest type, and position in the geographic range were tested. To account for possible scale effects, the study was conducted at two spatial scales, at the landscape and at the regional scale. Analyses were conducted both without and with controlling for phylogenetic relatedness among the species.

## Methods

### Data sets

*Landscape scale*

At the landscape scale we used the breeding bird atlas 'Lake Constance'. The atlas data were collected in 1980–81 and in 1990–92 by the 'Ornithologische Arbeitsgemeinschaft Bodensee' around Lake Constance (Germany, Switzerland and Austria) and consist of 303 grid squares of 2 × 2 km, covering 1212 km$^2$ in total (Schuster et al. 1983, Bauer & Heine 1992, Böhning-Gaese & Bauer 1996, Böhning-Gaese 1997). The landscape around Lake Constance is composed of a fine-grained mosaic of forests, meadows, fields, natural orchards, fruit plantations, reedbeds, lakes, and urban areas and covers an altitudinal range from 396 m (Lake Constance) to 980 m (foothills of the Alps).

Censuses were standardised across squares and between the two census periods (Schuster et al. 1983). For each square the presence and abundance of each breeding bird species was recorded by trained observers along line transects (Schuster et al. 1983). Each square was visited in the early morning five times between the end of March and the middle of June, avoiding adverse weather conditions that might reduce singing activity. During each visit the observer sampled a 4 km line transect extending to 50 m on each side, thus sampling 4 km x 100 m = 40 ha. Over five visits 200 ha, i.e. 50 % of the square area, were sampled. Whenever possible, transects were chosen to cover the different habitat types of a square in proportion to their occurrence. The observers were asked to ensure that species that are difficult to record with standard censuses, e.g. colonial species, waterfowl, or owls, were adequately counted. These extra censuses were conducted outside the five visits described above. From the numbers of recorded individuals observers were asked to classify the abundance of each species in one of six abundance classes (1–3, >3–10, >10–30, >30–100, >100–300, >300–1000 breeding pairs). For subsequent analyses the abundance of each species in each square was substituted by the geometric mean of the abundance class boundaries (1.7, 5.5, 17.3, 54.8, 173.2, 547.7 breeding pairs, respectively).

Data quality was tested by several methods: standard line transect data were compared with those obtained by territory mapping of the whole square, the same squares were sampled independently by two observers, and counts of colonial and waterfowl species were compared between independent observers (Schuster et al. 1983, Bauer & Heine 1992). Abundance estimates obtained by transect counts were comparatively robust: after sampling two squares, two independent observers were found to have classified 58 species to the same abundance class. For 60 species the deviation was one abundance class and for seven species more than one abundance class. Most deviations between observers were found for rare species in the abundance classes >1–3 and >3–10 breeding pairs (Schuster et al. 1983).

Total bird species richness for the two census periods was 151 species. Additionally, observers were asked to estimate for each square the total area covered by forest, meadow, field, natural orchard, fruit plantation, water, reed, and urban areas. Habitat data were available for 294 of the 303 squares.

*Regional scale*

At the regional scale we used the German breeding bird atlas (Rheinwald 1993). The atlas consists of 625 squares of 25 x 25 km, covering 390,625 km$^2$ in total. The atlas extends over all of former West and East Germany and ranges in altitude from sea level along the North and the Baltic Sea coasts to the Zugspitze in the Alps (2962 m). Most central European biome types such as coastal plains, large river valleys, urban regions, highlands, and Alpine tundra are represented.

Originally, the German bird atlas was to be compiled using the same sampling protocol as for the European bird atlas (Hagemeijer & Blair 1997), though the latter was sampled at a scale of 50 x 50 km squares. In Germany bird monitoring is organised at the basis of the Federal States (Bundesländer). Some of the Bundesländer followed the sampling protocol of the European atlas. However, some Bundesländer already had their own atlas data collected at an earlier time period, at a different spatial scale and using a different sampling protocol. These Bundesländer usually did not have the resources to sample the same area again. In the end, the German atlas was compiled using the data from the different Bundesländer in a manner similar to that in which the European atlas was compiled using the data of the different European countries (Rheinwald 1993, Hagemeijer & Blair 1997). In the German data set the abundance of each species in each square was classified in one of five abundance classes (1–10, >10–100, >100–1000, >1000–10 000 and >10 000–100 000 breeding pairs). Again, for analysis these classes were substituted by the geometric mean of the abundance class boundaries (3.2, 31.6, 316.2, 3162.3, 31 622.8 breeding pairs, respectively). Data quality of the German data set was not tested rigorously but is very probably lower than that of the Lake Constance atlas (Rheinwald 1993).

Total bird species richness for the German bird atlas was 262 species. However, data were compiled only for the 151 species that were recorded in the Lake Constance atlas. Habitat distributions at the German scale were taken from Statistisches Bundesamt (1995).

## Population abundance and range size

As a measure of abundance we used the mean abundance in occupied squares and as a measure of range size we used the number of occupied squares. For the Lake Constance data set, two values for abundance and range size were available for each species because the Lake Constance census was performed twice (1980-81 and 1990-92). Therefore, a year term was included in the statistical models to prevent an inflation of the degrees of freedom. For the German data set, only one value for abundance and range size was available for each species. Abundance values were log transformed.

## Functional classification of species

As species attributes we considered breeding habitat, migratory status, diet, nest type, nest site, position in the geographic range, body mass, egg mass, clutch size, clutches per year, eggs per year, seasonal start of the breeding period, age at maturity, incubation time, and fledging time. We did not include data about juvenile or adult survivorship because reliable data are available from only a limited number of species. We did not include data about maximum age because the available data are biased by the number of ringed individuals (Blackburn et al. 1996).

In order to test the influence of breeding habitat, migratory status, diet, nest type, nest site, and position in the geographic range on abundance and range size, the species were assigned to the following categories.

– *Breeding habitat:* The 151 species were classified by one of four breeding habitat categories independently by three ornithologists: primarily wetland (open water, reedbed, water edge), farmland (fields, dry and damp meadows, pasture, natural orchard, hedgerows), forest (forest, forest clearings), or urban (city, suburbs, cemeteries, parks, farm houses). If at least two of the ornithologists agreed in their classification the respective assignment was used for analyses. Five species could not be classified and were omitted from analyses including breeding habitat.
– *Migratory status:* The species were assigned to one of three categories by the same ornithologists using the same procedure as for habitat: primarily long-distance migrant (wintering south of the Sahara or east of the Pakistan-Indian border), short-distance migrant (wintering in the Mediterranean region or west of the Pakistan-Indian border), or resident. We classified partial migrants according to the behaviour of the larger part of the population. All species could be classified.
– *Diet:* The species were assigned to one of three categories using diet data from Bezzel (1985, 1993):

primarily vertebrates (small mammals, birds, reptiles, amphibians, fish, or carrion), invertebrates (land or water invertebrates, such as insects, spiders, molluscs, earthworms), or plants (land or aquatic plants, fruits, berries, seeds).

- *Nest type:* The species were assigned to one of three categories using data from Bezzel (1985, 1993): open (scrape, cup, saucer, platform, pendant, or sphere nests), half-open (niches, crevices), or closed (cavities, burrows).
- *Nest site:* The species were assigned to one of three categories using data from Bezzel (1985, 1993): primarily low (ground, embankments), intermediate (bushes, shrubs), or high (canopy, buildings, bridges, cliffs).
- *Position of the Lake Constance region relative to the geographic range of the species in Europe:* The species were assigned to one of three categories using range maps in Peterson et al. (1993): centre, towards the edge, or at the margin of their geographic range in Europe. When interpreting the results it is important to keep in mind that position in the geographic range is not independent of geographic range size which itself is correlated with abundance (Bock & Ricklefs 1983, Brown 1984). Rare species with small geographic ranges are more likely to be classified as being at the margin of their geographic range. However, this effect is not large because the Lake Constance region is very small compared to the whole geographic range of the species in Europe.

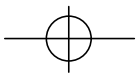
For body mass, egg mass, clutch size, number of clutches per year, number of eggs per year, seasonal start of the breeding period, age at maturity, incubation time, and fledging time we used data from Bezzel (1985, 1993). The start of the breeding season was classified as time intervals of 10 days (beginning, middle, or end of March, April, May, etc.).

## Statistical analysis

Not taking into account the phylogenetic relatedness among the species, the relationship between abundance or range size and the attributes of the species was analysed, first, by testing each species attribute individually, and second, by combining multiple species attributes in multivariate models. For the test of individual species attributes, we applied regression analysis or ANOVA (depending on the attribute). For the Lake Constance analysis this was modified to ANCOVA or two-factor ANOVA because the Lake Constance analysis included an additional year term. For the combined test of multiple species attributes, in order to find the best set of independent variables, we used backward elimination and stepwise forward-selection techniques (SAS/STAT 1987). The backward elimination technique began by including all of the independent variables in an ANCOVA model. Then the variables were deleted from the model one by one. At each step, the variable that was least significant was deleted. The process was stopped when all the variables remaining in the model were significant at the $P = 0.05$ level. The stepwise forward-selection technique began with no variables in the model. Then, variables were added one by one. At each step, the variable that was most significant was included to the model. The process was stopped when no remaining variable, if added to the model, was significant at the $P = 0.05$ level. Variables already in the model that lost their significance during the process were deleted. For analyses all continuous variables except seasonal start of the breeding period were log transformed.

## Control for phylogenetic effects

When analysing statistical patterns across species one has to keep in mind that species might not represent independent data points because some of them are more closely related than others (Felsenstein 1985, Harvey & Pagel 1991). To control for these possible phylogenetic effects we used an extension of the Mantel test (Mantel 1967, Smouse et al. 1986, Lapointe & Legendre 1990, 1991, 1992, Legendre et al. 1994), the Signed Mantel test (Böhning-Gaese & Oberrath 1999, Böhning-Gaese et al. 2000, Oberrath & Böhning-Gaese 2001). In this method the dissimilarity in abundance or range size for each pair of species is compared both with their dissimilarity in a particular species attribute and with their phylogenetic distance. Thus, for each test the bird community is characterised by three matrices. The Y-matrix describes the dissimilarity in abundance or range size, the $X_1$-matrix the dissimilarity in the attribute, and the $X_2$-matrix the phylogenetic distance among the species. The Y-matrix is then regressed on the $X_1$ and $X_2$-matrices and tested for significance using Mantel tests (Mantel 1967, Smouse et al. 1986, Oberrath & Böhning-Gaese 2001). In Mantel tests the regression of the





individual values in the matrices yield the partial regression coefficients $b_1$ and $b_2$, and the respective t-values (Smouse et al. 1986). The significance of the t-values is tested against a null distribution of t-values constructed by Monte Carlo randomisations, whereby the $X_1$ and $X_2$-matrices are held constant and the species in the Y-matrix are randomly permutated (Smouse et al. 1986). We used 2000 randomisations to construct the null distribution of t-values in the present study. A computer program to conduct these simulations written in IDL (Version 4.0, Research Systems, Inc.) is available from the authors.

To construct the dissimilarity matrix for the abundance and range size values and for body mass, egg mass, clutch size, clutches per year, eggs per year, age at maturity, incubation time, and fledging time we calculated a trait dissimilarity index d by dividing the species with the higher value by the species with the lower value. This procedure is based on the assumption that the similarity between two species, e.g. weighing 100 g and 10 g is the same as between two species weighing 1000 g and 100 g. The dissimilarity values were log transformed to improve fit to the linear regression. For the start of the breeding season, the trait dissimilarity index d between two species was calculated as the difference in timing in number of 10-day periods. With respect to breeding habitat, migratory status, diet, nest type, nest site, and position in the geographic range, the dissimilarity index d was ranged between 0 and 1. Species with the same attribute were given the dissimilarity index 0, species with different attributes the dissimilarity index 1.

The phylogenetic distance between each pair of species was defined as their genetic distance $\Delta T_{50}H$ according to the molecular phylogeny of Sibley & Ahlquist (1990). Sibley & Ahlquist's phylogeny is a resolved phylogeny based on DNA-DNA hybridisation with $\Delta T_{50}H$ being the temperature when 50 % of hybridizable DNA has melted. Although Sibley & Ahlquist's phylogeny has been very controversial, several new studies support it and suggest that it is generally valid especially when conducting large scale analyses (Mooers & Cotgreave 1994). Some of the species and genera we used in the present study are not represented in the phylogeny of Sibley & Ahlquist (1990). We estimated the genetic distance values for these species by calculating the average distance value of the other species or genera in the same genus or tribe, respectively.

The influence on the results of phylogenetic relatedness among species was tested by comparing the results

**Table 1.** Influence of 15 species attributes on the abundance and range size of birds in the Lake Constance region and in Germany. For the German data, regression analysis or ANOVA was applied. For the Lake Constance data, ANCOVA or two factor ANOVA was used because the Lake Constance analysis included an additional year term. Numbers in table are $R^2$-values [%]. * P < 0.05, ** P < 0.01, *** P < 0.001.

| | | Lake Constance | | Germany | |
|---|---|---|---|---|---|
| | n | Abundance | Range size | Abundance | Range size |
| Breeding habitat | 146 | 16.3 *** | 28.3 *** | 23.6 *** | 25.4 *** |
| Migratory status | 151 | 1.0 | 1.7 | 1.8 | 4.9 * |
| Diet | 151 | 9.2 *** | 7.0 *** | 11.8 *** | 3.9 |
| Nest type | 150 | 0.8 | 3.6 ** | 2.0 | 4.3 * |
| Nest site | 150 | 4.6 *** | 16.4 *** | 9.2 *** | 13.3 *** |
| Position in geographic range | 151 | 3.9 ** | 11.2 *** | 5.4 * | 14.3 *** |
| Body mass | 151 | 15.7 *** | 13.8 *** | 19.5 *** | 10.8 *** |
| Egg mass | 150 | 15.8 *** | 16.3 *** | 20.8 *** | 12.5 *** |
| Clutch size | 149 | 0.0 | 0.5 | 0.0 | 0.7 |
| Clutches per year | 150 | 10.9 *** | 4.8 *** | 9.8 *** | 3.1 * |
| Eggs per year | 149 | 5.2 *** | 3.8 *** | 3.9 * | 3.5 * |
| Seasonal start breeding | 150 | 0.2 | 0.0 | 0.1 | 0.0 |
| Age at maturity | 150 | 8.0 *** | 8.1 *** | 7.6 *** | 12.9 *** |
| Incubation time | 150 | 16.9 *** | 16.2 *** | 23.2 *** | 8.5 *** |
| Fledging time | 147 | 11.4 *** | 11.8 *** | 19.0 *** | 10.5 *** |

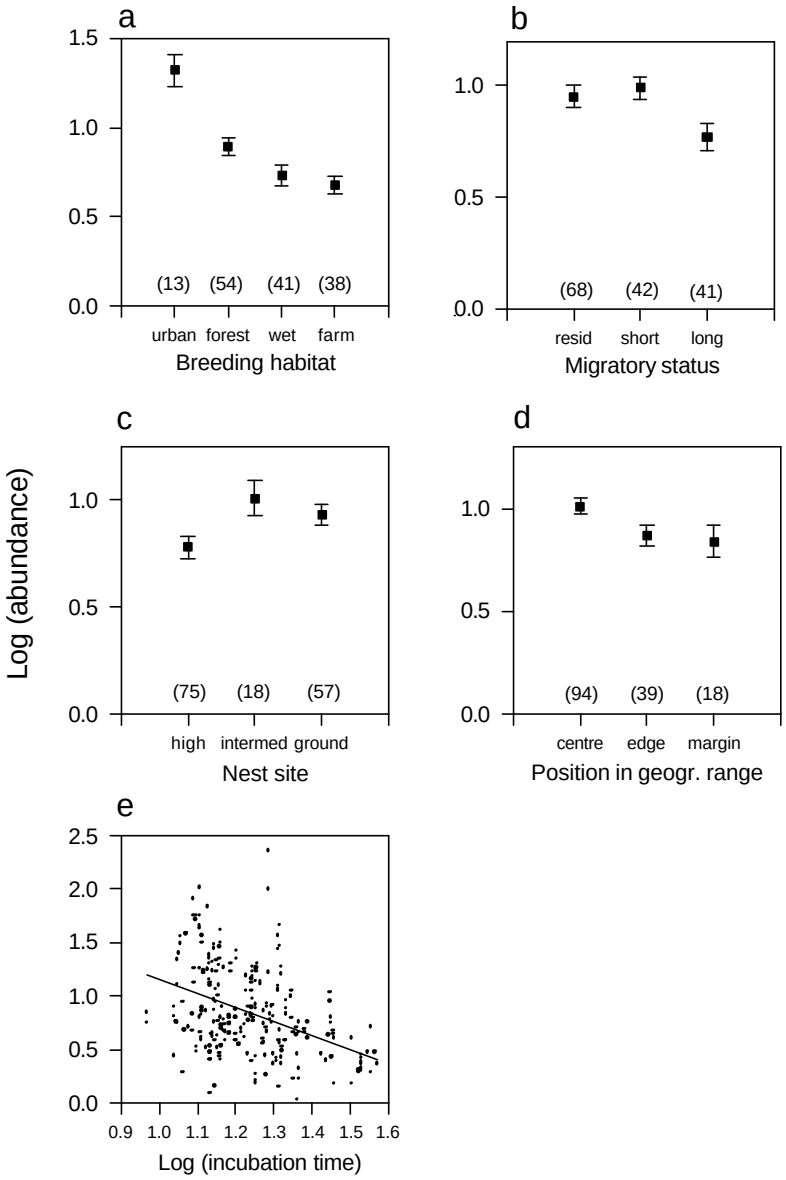



**Figure 1.** Relationships between the abundance of birds in the Lake Constance region and five species attributes. Displayed values are least squares means (± s.e.) taken from the analysis conducted in Table 2. a: breeding habitat; wet = wetland, farm = farmland; b: migratory status; resid = residents; short = short-distance migrants; long = long-distance migrants; c: nest site; intermed = intermediate; d: position in the geographic range; edge = towards the edge, margin = at the margin of the range; and e: incubation period. The relationships were similar for range size in the Lake Constance region and for abundance and range size in Germany as a whole.

obtained by including both the species attribute and the phylogenetic distance matrix in the model with those obtained by including only the species attribute matrix.

## Results

### Test of individual species attributes

*Excluding phylogeny*

The abundance and range size of the species at both spatial scales were correlated with similar species attributes (Table 1). Breeding habitat was the variable explaining the highest amount of variation in abundance and range size (on average 23.4 %) in three of the four analyses. The most abundant and widespread birds were urban species, followed by forest, wetland, and farmland species (Fig. 1a). This order did not correspond to the available amount of habitat types. In the Lake Constance region, the most widespread habitat type was farmland, followed by forest, urban areas, and wetland (Fig. 2a). In Germany, land use patterns were very similar. Again, the most widespread habitat type was

farmland, followed by forest, urban areas, and wetland (Fig. 2b; Statistisches Bundesamt 1995).

Among the morphological and life-history variables in Table 1, the highest amounts of variation were explained by egg mass (on average 16.4 %), incubation time (16.2 %), body mass (15.0 %), and fledging time (13.2 %). Egg mass was always more significant than body mass and incubation time was more significant in three of the four cases. Fledging time was never more significant than body mass. The relationship between these variables and abundance or range size was negative; see, for example, the correlation between incubation time and abundance at the Lake Constance scale (Fig. 1e).

Furthermore, consistently significant amounts of variation in abundance and range size were explained by nest site (on average 10.9 %), age at maturity (9.2 %), position in the geographic range (8.7 %), diet (8.0 %), clutches per year (7.2 %), and eggs per year (4.1 %; Table 1). Species nesting at intermediate heights were more abundant and widespread than species nesting on the ground or high up (Fig. 1c). Species in the centre of their range had higher abundance and larger range sizes than species towards the edge or at the margin of their range (Fig. 1d). Invertebrate- and plant-eating species were more abundant and widespread than vertebrate-eating species. Age at maturity was negatively correlated with abundance and range size, whereas numbers of clutches and eggs per year were positively correlated with abundance and range size.

*Including phylogeny*

Taking into account the phylogenetic relatedness among the species hardly changed any of the results. Phylogenetic distance had a significant influence on the abundance or range size of the species in 18 of the 60 tests conducted in Table 1. However, including phylogeny in the model changed the influence of the species attribute on the abundance or range size of the species in only two of the 60 tests in Table 1. The presence of phylogeny altered the effect of migratory status on range size in Germany from not significant ($P = 0.074$) to significant ($P = 0.046$), and the effect of incubation time on range size in Germany from significant ($P = 0.045$) to not significant ($P = 0.087$). Note that these significance values are Mantel P-values that do not correspond to the significance values in Table 1.

## Combined test of multiple species attributes

*Excluding phylogeny*

Using multivariate statistics, the backward elimination and the stepwise forward-selection techniques converged in three analyses on the same set of variables. In the remaining case the backward elimination technique provided a model that explained a higher amount of variance in abundance and range size than the model using stepwise forward-selection. Therefore, only the results of the backward elimination technique are presented (Table 2). All four models in Table 2 contained breeding habitat, position in the geographic range, and a life-history variable highly correlated with body mass (Pearson correlation coefficient body mass and incubation time: $r = 0.83$ ($n = 150$), body mass and age at maturity: $r = 0.57$ ($n = 149$), body mass and number of eggs per year: $r = –0.35$ ($n = 148$), $P < 0.0001$ in all cases). Replacing the life-history variable with body mass always gave a significant effect of body mass. Other va-

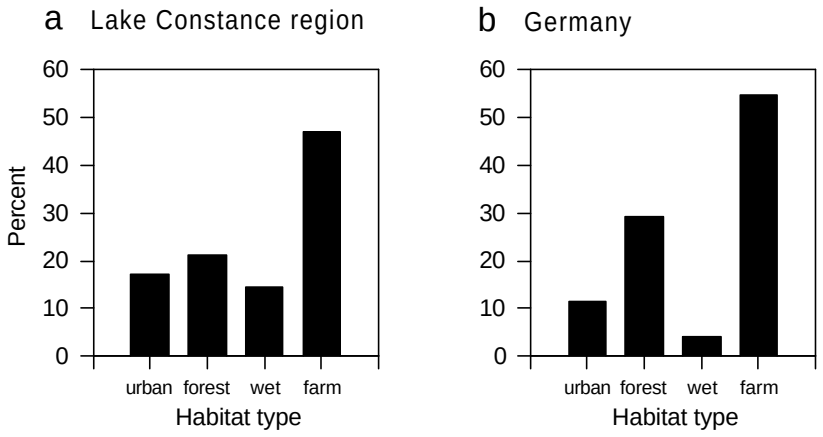



**Figure 2.** Availability of different habitat types as a percentage of land cover for a: the Lake Constance region and b: Germany as a whole. The pattern in the percentages does not correspond to the pattern in the abundances of species (Fig. 1a).

**Table 2.** Result of the backward elimination technique looking for the best set of independent variables that explain the abundance and range size of birds in the Lake Constance region and in Germany.

| Variable | model d.f. | error d.f. | F | P | $R^2$ [%] |
|---|---|---|---|---|---|
| *Lake Constance* | | | | | |
| Abundance: | | | | | |
| Model | 11 | 280 | 14.5 | 0.0001 | 36.3 |
| Breeding habitat | 3 | | 14.7 | 0.0001 | |
| Migratory status | 2 | | 6.4 | 0.002 | |
| Nest site | 2 | | 4.4 | 0.01 | |
| Position in geogr. range | 2 | | 4.2 | 0.02 | |
| Incubation time | 1 | | 55.3 | 0.0001 | |
| Year | 1 | | 0.0 | n.s. | |
| Range size: | | | | | |
| Model | 13 | 274 | 18.2 | 0.0001 | 46.3 |
| Breeding habitat | 3 | | 12.6 | 0.0001 | |
| Migratory status | 2 | | 3.1 | 0.048 | |
| Diet | 2 | | 9.0 | 0.0002 | |
| Nest site | 2 | | 6.2 | 0.002 | |
| Position in geogr. range | 2 | | 11.5 | 0.0001 | |
| Eggs per year | 1 | | 8.4 | 0.004 | |
| Year | 1 | | 0.0 | n.s. | |
| *Germany* | | | | | |
| Abundance: | | | | | |
| Model | 10 | 135 | 12.5 | 0.0001 | 48.1 |
| Breeding habitat | 3 | | 7.8 | 0.0001 | |
| Migratory status | 2 | | 7.8 | 0.0006 | |
| Nest site | 2 | | 3.4 | 0.04 | |
| Position in geogr. range | 2 | | 3.4 | 0.04 | |
| Incubation time | 1 | | 36.8 | 0.0001 | |
| Range size: | | | | | |
| Model | 6 | 137 | 14.3 | 0.0001 | 38.4 |
| Breeding habitat | 3 | | 9.2 | 0.0001 | |
| Position in geogr. range | 2 | | 6.5 | 0.002 | |
| Age at maturity | 1 | | 15.5 | 0.0001 | |

riables that entered the models were migratory status (three cases), nest site (three cases), and diet (one case). In contrast to the test of individual species attributes, migratory status was an important predictor of abundance and range size once body mass was controlled for. Residents and short-distance migrants were more abundant and widespread than long-distance migrants at both spatial scales (Fig. 1b).

*Including phylogeny*

Taking into account the phylogenetic relatedness among the species did not change any of the results. In the multivariate models phylogenetic distance never had a significant influence on the abundance or range size of the species. Furthermore, including phylogeny in the model never changed the influence of the species attributes on the abundance or range size of the species.

## Test of long-distance migrants

*Excluding phylogeny*

Testing whether the low abundance and small range size of long-distance migrants was caused by their low annual egg production revealed no significant effect. In a model that included migratory status and number of eggs per year, only migratory status was a significant predictor of abundance (Table 3). Testing to see whether the low abundance and small range size of long-distance migrants was caused by their late arrival or by their choice of open nest types was also not significant for either arrival date or nest type. In a model that included migratory status, timing of the start of the breeding season and nest type, migratory status was the only significant variable (Table 4). Again, these relationships were similar at both spatial scales.

**Table 3.** Relative importance of migratory status, number of eggs per year, and body mass in determining the abundance of 148 bird species in the Lake Constance region. Similar results were obtained for range size in the Lake Constance region and for abundance and range size in Germany as a whole.

| Variable | model d.f. | error d.f. | F | P | $R^2$ [%] |
|---|---|---|---|---|---|
| Model | 5 | 290 | 13.7 | 0.0001 | 19.1 |
| Migratory status | 2 | | 5.8 | 0.0035 | |
| Eggs per year | 1 | | 0.8 | n.s. | |
| Body mass | 1 | | 46.9 | 0.0001 | |
| Year | 1 | | 0.0 | n.s. | |

**Table 4.** Relative importance of migratory status, seasonal start of the breeding period, nest type, and body mass in determining the abundance of 149 bird species in the Lake Constance region. Similar results were obtained for range size in the Lake Constance region and for abundance and range size in Germany as a whole.

| Variable | model d.f. | error d.f. | F | P | $R^2$ [%] |
|---|---|---|---|---|---|
| Model | 7 | 290 | 9.7 | 0.0001 | 19.0 |
| Migratory status | 2 | | 4.6 | 0.010 | |
| Start breeding | 1 | | 0.0 | n.s. | |
| Nest type | 2 | | 0.0 | n.s. | |
| Body mass | 1 | | 55.7 | 0.0001 | |
| Year | 1 | | 0.0 | n.s. | |

*Including phylogeny*

Taking into account the phylogenetic relatedness among the species did not change any of the results. Again, phylogenetic distance never had a significant influence on the abundance or range size of the species. Including phylogeny in the model never changed the influence of the species attributes on the abundance or range size of the species.

## Discussion

Multivariate analysis of possible factors determining the abundance and range size of 151 Central European bird species revealed that breeding habitat, body mass (or variables highly correlated with body mass), position in the geographic range, and migratory status were important independent predictors of bird abundance and range size. The results were comparatively robust with regard to the spatial scale of the analysis. Similar results were obtained for abundance and range size at the Lake Constance and at the German scale. Controlling for phylogeny did not change any of the results.

The little influence that phylogenetic relatedness among species had on the relationship between abundance or range size and species attributes might be explained by the fact that the abundance and range size values of the bird species used in the present study (Y-variables) had only small phylogenetic effects. Testing the influence that the phylogenetic distance matrix alone had on the abundance and range size matrix of the species using Mantel tests revealed that only two of variables showed significant phylogenetic effects (range size, Lake Constance: $t = 9.6$, $P = 0.021$, $R^2 = 0.8$ %; abundance, Germany: $t = 8.2$, $P = 0.046$, $R^2 = 0.6$ %; Böhning-Gaese & Oberrath 1999).

Breeding habitat appeared to be the most important factor influencing the abundance and range size of Central European birds. This is consistent with studies that have demonstrated the prevalent importance of land use patterns determining the abundance of birds (Gregory & Gaston 2000), mice (Glazier 1980), butterflies (Hodgson 1993), and plants (Hodgson 1986). The abundance and distribution of British birds were correlated with niche position, such that birds using typical resources of the environment were common and widely distributed (Gregory & Gaston 2000). The most abundant and widespread species of North American *Peromyscus* mice were found in 'new, disturbed, sparsely vegetated areas and islands' (Glazier 1980). The abundance of British butterflies and plants were correlated with their 'capacity to exploit the artificial, disturbed and productive habitats which have been created by modern land-use and now occupy much of the landscape' (Hodgson 1986, 1993). In contrast to these studies, however, Central European bird species breeding in farmland were comparatively rare, although farmland was the most widespread habitat type in the Lake Constance region as well as in Germany as a whole (compare Fig. 1a with Fig. 2). The most abundant and widespread Central European birds were found in urban areas although those are relatively rare 'habitat types'. Thus, the abundance and range size of birds appear not to be influenced exclusively by the amount of habitat available.

Additionally, birds might be affected by the amount of resources available in these habitat types. Modern, intensively cultivated farmland is possibly an unproductive habitat type for birds because both food and nest sites are lacking. In Germany farmland practices have greatly intensified over the past few decades. Pesticide usage has increased, hedgerows and field boundaries have been destroyed, and pasture has been turned into arable land (Hölzinger 1987). Farmland species that were more abundant in the past have declined, as shown by significant decreases in farmland species in the Lake Constance region (Böhning-Gaese & Bauer 1996) as well as in other regions in Europe (Gibbons et al. 1993, Fuller et al. 1995, Siriwardena et al. 1998,

Chamberlain et al. 2000, Gates & Donald 2000, Donald et al. 2001).

The importance of body mass in predicting the abundance and range size of bird species is well known (Juanes 1986, Cotgreave & Harvey 1992, Gregory & Blackburn 1995). This pattern has recently been challenged by Blackburn et al. (1996), who demonstrated that other life-history variables, particularly incubation and fledging time, were better predictors of abundance than body mass. In the present study, egg mass and incubation time were better correlates of abundance than body mass, but fledging time was not. The strong correlation of these variables with body mass poses severe statistical problems. In the multivariate analyses, body mass and the life-history variables were exchangeable. Replacing the life-history variables with body mass always gave a significant effect of body mass. It is possible that egg mass and incubation time appear to be better predictors of abundance and range size because they can be measured more precisely than body mass, which shows – at least in migrants – profound annual fluctuations (Berthold 1975, 1993).

The reason why long-distance migrants are less abundant and widespread than residents is rather difficult to understand (Cotgreave 1994). The hypothesis that long-distance migrants are rare because they produce comparatively few eggs per year was not confirmed in the present study (Table 3). Also the hypothesis that long-distance migrants are rare because of their late arrival and choice of open nest types could not be supported (Table 4). Presumably long-distance migrants are limited by factors on their migratory pathways or on their wintering grounds in the tropics (Hjort and Lindholm 1978, Svensson 1985, Baillie & Peach 1992, Kaiser 1992). Again, it is possible that the environmental conditions for long-distance migrants have deteriorated in the last decades, as indicated by their recent declines in the Lake Constance region (Böhning-Gaese & Bauer 1996).

Although the macroecological approach is simplistic it is encouraging that we were able to explain between 36 % and 48 % of the variance in the abundance and range size of Central European bird species. Additionally, the influence of the above factors on abundance and range size was strong and predictable across both spatial scales. It is surprising, however, how little we understand about factors determining abundance and range size even for well studied organisms such as European birds. Why have farmland species low abundance? Why are long-distance migrants less abundant than short-distance migrants and residents? These questions might be a challenge to further and more detailed studies of the factors determining the abundance and range size of birds.

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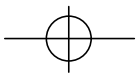



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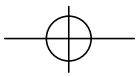



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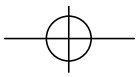



# Plasma metabolite levels indicate change in body mass in reed warblers *Acrocephalus scirpaceus*

**Lukas Jenni**[1, 4] **and Regine Schwilch**[1, 2, 3]

Information about changes in energy stores is most easily obtained from changes in body mass, measured by catching and weighing a bird twice. However, this method has several drawbacks. The aim of this study was to evaluate a method for estimating body mass change from plasma metabolite concentrations measured in birds caught only once. We examined how plasma concentrations of triglycerides and β-hydroxy-butyrate correlated with change in body mass and time of day. In an experiment, change in body mass of 18 reed warblers *Acrocephalus scirpaceus* was manipulated by providing different amounts of food, so that body mass varied from increasing to decreasing. Blood samples were obtained at different times of day. Triglyceride levels were positively related to change in body mass and time of day, while β-hydroxy-butyrate levels were negatively related to change in body mass and time of day. Other parameters such as the individual, various measures of diurnal and nocturnal locomotor activity and absolute body mass were only marginally correlated with plasma metabolite levels. Hence, plasma levels of triglycerides and β-hydroxy-butyrate can be used to estimate change in body mass during the hours prior to catching in birds caught only once.
Key words: *Acrocephalus scirpaceus,* body mass change, plasma metabolites, triglycerides, β-hydroxy-butyrate.

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In birds, energy stores fluctuate widely in anticipation of and in response to periods of negative energy balance and risk of starvation. Changes in energy stores are generally reflected in changes in body mass, although water content, growth processes (growth of the individual, egg formation) and variation in organ size may also influence body mass. Moreover, the composition, and consequently the energy density, of stores may vary (Lindström & Piersma 1993). Hence, change in body mass is a crude, but widely used, estimate of changes in energy stores and has the advantage that it is an easy and non-invasive measure.

In field studies, change in body mass is usually measured by catching a bird at least twice. However, this has drawbacks, such as: (1) sample size may become very small, because only birds trapped at least twice can be used (Winker et al. 1992); (2) retraps may not be representative of the entire population (Winker et al. 1992); (3) capture may have an adverse effect on subsequent body-mass changes (Schwilch & Jenni 2001); (4) change in body mass is usually measured over several days (Schaub & Jenni 2000b) which may be advantageous for some studies, but not for those dealing with short-term changes in energy stores (e.g. as in a reaction to weather).

In an earlier study on garden warblers *Sylvia borin,* we demonstrated that plasma concentrations of two metabolites estimated the change in body mass that had occurred during the hours prior to catching (Jenni-Eiermann & Jenni 1994). Similar findings were obtained from a wader species (Williams et al. 1999). By measuring plasma metabolite concentrations it seems the-

refore possible to estimate body-mass change of birds caught only once. However, neither of these studies took into account changes in plasma metabolite levels with time of day, as they occur in free-living birds (e.g. Jenni & Jenni-Eiermann 1996), and consequently the application of this method was limited.

In the present study on reed warblers *Acrocephalus scirpaceus*, we examined experimentally the dependence of the plasma concentration of two metabolites on change in body mass and also on time of day. We examined the two metabolites found earlier to be the most suitable: triglycerides and β-hydroxy-butyrate (Jenni-Eiermann & Jenni 1994). Plasma triglyceride levels are well known to increase with food intake. Dietary lipids and lipids originating from the conversion of carbohydrates and proteins into lipids in the liver are transported in the blood as triglycerides either to adipose tissues or to the energy consuming organs (Robinson 1970). Hence, plasma triglyceride levels indicate the deposition of lipids which are the main reason for a change in energy stores. In contrast, β-hydroxy-butyrate is synthesized from free fatty acids and replaces glucose, especially in the brain (Robinson & Williamson 1980). Hence, an increase in β-hydroxy-butyrate indicates that food intake does not meet the energy requirements and that stored fat is catabolized.

As a long-distance migrant breeding over much of Europe and wintering in sub-Saharan Africa, the reed warbler naturally undergoes large changes in body mass.

## Material and methods

### Animals

Eighteen first-year reed warblers were caught in the Wauwilermoos, Switzerland, on 8 August 1997. They were kept singly in cages in a room with artificial light simulating the natural photoperiod shifted backward by one hour. The two perches in each cage were mounted on microswitches and a computer counted the hops in 15 min intervals. The birds were accustomed to the cages for 10 days with mealworms and small crickets provided ad libitum. After the experiments, birds were released between 10 and 20 September.

### Experiments

In order to obtain data from birds with stable, increasing and decreasing body mass, body mass changes were induced experimentally by varying the amount of food given. Birds were induced to go through two or three cycles of increasing and decreasing body mass. Increasing body mass was achieved by giving food ad libitum. Stable and decreasing body mass was induced by providing an individually restricted amount of food. When a stable body mass was aimed at and a relatively large total amount of food was given, one-half was provided in the early morning, the other half at midday. When a decreasing body mass was induced, the little total amount of food was given in 4–6 small portions over the entire day.

One of nine predetermined times of day (at lights on = time 0 and at intervals of 1.25 h from lights-on onwards) was assigned to the samples, so that each bird was sampled at different times of day and that samples from birds with approximately stable, increasing and decreasing body mass were evenly distributed over the day.

Birds were weighed daily at 0700 (3–30 min after lights on) and at 1630 (4–5.5 h before lights off), as well as at the time of blood sampling. This allowed us to determine the change in body mass over each day and from early morning to the time of blood sampling.

Blood samples were taken when the desired body-mass change had been achieved for at least one previous day. Most blood samples were taken 3–6 days apart (2 samples each 2 days apart and 3 samples each 7 days apart).

On average each bird was sampled six times. In total 108 blood samples were collected which, due to varying amounts of plasma obtained, provided material for 96 triglyceride and 96 β-hydroxy-butyrate measurements, both being measured in 84 samples.

Blood (10–60 µl) was sampled by puncturing the wing vein and collected with a capillary system (Microvette CB 300 Fluore, Sarstedt). The blood was centrifuged and the plasma stored at –20 ºC until analysis. Metabolite concentrations in plasma were determined using standard test combinations for β-hydroxy-butyrate and triglycerides (Sigma Diagnostics No 310 and 337).

We calculated diurnal and nocturnal perch hopping activity for each day and each night (total number of re-

corded hops). The variation in registered hopping activity between individuals was partly caused by differences in the sensitivity of the microswitches. Therefore, we expressed hopping activity as the deviation from the overall mean of the individual (= cage) and used hopping activity during the day of sampling, during the night before sampling, during the day before sampling and during the previous night (D-ACTIV, N-ACTIV, Y-D-ACTIV, Y-N-ACTIV) in the analysis.

## Data analysis

We modeled the concentration of either triglycerides or β-hydroxy-butyrate as a function of time of day and change in body mass, taking into account body mass and the four measures of activity. In our experiments, three different changes in body mass could be used.

(1) Change in body mass from morning to blood sampling (ΔMASS-B). This variable could not be used for all samples, because the difference in time was non-existent (time 0) or too small (time 1.25) in samples from the early morning. Therefore, when using ΔMASS-B, we omitted the first two blood sampling times (0 and 1.25) from analysis.

(2) Change in body mass from morning to evening of the day of sampling (ΔMASS-D). This variable included some body-mass change after blood sampling had occurred and might have been affected by the blood sampling procedure itself, since evening body mass was taken after or at blood sampling. Therefore, we examined whether blood sampling affected body-mass development in the following way. We estimated, for positive body-mass changes, the increase in body mass before blood sampling from a regression of the body-mass change with time, thus predicting mean body-mass increase over 10 h before blood sampling (0.935 g). The same was done for body mass changes after blood sampling (0.995 g). Hence, predicted change in body mass according to the data after blood sampling was slightly higher than according to the data before blood sampling, the opposite of what we suspected. Because change in body mass may be poorly measured over only a short time interval of 1 or 2 h, we applied various restrictions, i.e. omitting the first two or the last two (or the first and last two) sampling times, but this did not change the result. Therefore, we concluded that blood sampling had no adverse effect on subsequent body-mass development under the conditions of this study. Hence, we also used ΔMASS-D in our analyses.

(3) Change in body mass from early morning to evening of the day before blood sampling (ΔMASS-Y). This variable was correlated with ΔMASS-D ($R^2$ = 0.43, n= 108), because we tried to induce a similar change in body mass over at least two days.

The dependence of either triglyceride or β-hydroxy-butyrate levels on one of the three measures of change in body mass, time of day, four measures of activity, body mass and the individual was evaluated by nested analysis of covariance (Steel & Torrie 1980). Because several measurements were taken from the same individual, all factors and covariates except individuals and individuals×ΔMASS-D (or individuals×ΔMASS-B) were tested against the variation among individuals (thus accounting for repeated measures from the same individual); the effects of individuals and individuals×ΔMASS-D (or individuals×ΔMASS-B) were tested against the residual variation. The full model (see Appendix) was subsequently reduced by backward elimination of non-significant terms (results given in Tables 1 and 2).

Because the residuals of the models deviated from a normal distribution, β-hydroxy-butyrate and triglyceride levels were transformed into ln(β-hydroxy-butyrate + 0.5) and ln(triglycerides) which normalised them.

We evaluated whether restricted food given twice per day (17 samples) produced a different diurnal pattern of the two metabolites from when restricted food was given in 4–6 portions over the day (31 samples). When the manner in which food was given (twice versus 4–6 times a day) was introduced into the full model as an additional factor and as interactions with time, $time^2$ and $time^3$ (to allow for two peaks or lows), these terms were not significant in both metabolites ($P > 0.3$). Visual inspection of the data also did not indicate a different diurnal metabolite pattern. It seemed that birds given food twice a day did not experience temporal fasting, but fed over most of the two feeding intervals, probably because food given twice a day was comparatively abundant (aimed at inducing a stable, not a decreasing, body mass).

## Results

### Body mass and changes in body mass

Early morning body mass on the day of blood sampling varied between 9.6 and 16.4 g (mean 11.9 g ± 1.47 s.d., n = 108) and evening body mass between 9.8 and 17.0 g (12.6 ± 1.53). This falls in the natural range of mean body mass found at 22 ringing sites between Finland and Morocco during autumn migration (11.0–12.8g; Schaub & Jenni 2000a). However, it did not include the maximum body weights (up to 22.3 g) achieved by individual birds before crossing the Sahara (Glutz von Blotzheim & Bauer 1991).

The induced hourly change in body mass from morning to blood sampling (ΔMASS-B) varied between –0.08 and 0.40 $gh^{-1}$ (mean 0.10 ± 0.09 s.d., n = 96, excluding samples taken at time 0 and 1.25). Change in body mass induced from morning to evening of the day of sampling (ΔMASS-D) varied between –0.40 and 2.10 g (mean 0.66 ± 0.59, n = 108), while change in body mass over 24 h of the day of blood sampling and the day before varied between –1.50 and 1.60 g. This encompassed the range of the mean rate of body mass change of –0.04 to 0.32 g over 24 h found at various stopover sites in autumn (Schaub & Jenni 2000b) as well as the maximum reported (0.95 $gd^{-1}$; Bairlein 1988). Although we tried to induce increasing, stable and decreasing body mass, there resulted a continuous range of body mass changes (rather than three clear categories), because it was difficult to keep body mass changes within narrow limits.

**Table 1.** Dependence of triglyceride levels (ln-transformed) of reed warblers on time of day since lights on (TIME in h), its square $(TIME)^2$, change in body mass and individuals. The models resulting from backward elimination of non-significant terms are presented (see Appendix for the full model). (a) and (b) include change in body mass from morning to evening of the day of blood sampling (ΔMASS-D in g), while (c) and (d) include the hourly change in body mass from morning to the time of blood sampling (ΔMASS-B in $gh^{-1}$). (b) and (d) give the models (a) and (c), respectively, without the effects of the individuals (in this case, the F-values cannot be calculated). For each variable, df is 1, except when indicated. * P < 0.05, ** P < 0.01, *** P < 0.001.

| | F | b | s.e. |
|---|---|---|---|
| ΔMASS-D | | | |
| (a) $R^2_{adj}$ = 0.61, n = 96 | | | |
| Intercept | 322.1*** | 0.403 | 0.157 |
| TIME | 17.4*** | 0.189 | 0.037 |
| $(TIME)^2$ | 6.1* | –0.017 | 0.004 |
| ΔMASS-D×TIME | 16.2*** | 0.073 | 0.010 |
| Individuals (df = 17) | 3.0*** | | |
| (b) without individuals; $R^2_{adj}$ = 0.47, n = 96 | | | |
| Intercept | | 0.349 | 0.090 |
| TIME | | 0.201 | 0.041 |
| $(TIME)^2$ | | –0.017 | 0.004 |
| ΔMASS-D×TIME | | 0.061 | 0.010 |
| ΔMASS-B | | | |
| (c) $R^2_{adj}$ = 0.61, n = 76 | | | |
| Intercept | 408.1*** | 0.840 | 0.161 |
| TIME | 1.2 | –0.014 | 0.014 |
| ΔMASS-B×TIME | 24.9*** | 0.701 | 0.077 |
| Individuals (df = 17) | 3.1*** | | |
| (d) without individuals; $R^2_{adj}$ = 0.41, n = 76 | | | |
| Intercept | | 0.794 | 0.103 |
| TIME | | –0.011 | 0.016 |
| ΔMASS-B×TIME | | 0.612 | 0.085 |

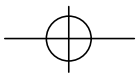




**Figure 1.** Dependence of triglyceride (A, C) and β-hydroxy-butyrate levels (B, D) in reed warblers on time (hours since lights on) and either change in body mass from morning to evening of the day of blood sampling (ΔMASS-D in g; A, B) or hourly change in body mass from morning to the time of blood sampling (ΔMASS-B in $gh^{-1}$; C, D). Note that in B and D, the axis of the change in body mass is reversed and that in C and D the scale of time since lights on starts at 2. The planes indicate the relationships given in Table 1b, 1d, 2a and 2b (the latter two calculated for a mean body mass of 11.9 g), respectively.

## Triglycerides

Plasma triglyceride levels varied significantly with time of day and change in body mass from morning to evening of the day of blood sampling (ΔMASS-D) and additionally with the individual (Table 1a, Fig. 1a).

Triglyceride levels increased curvilinearly with time of day (Fig. 1a). They increased steeply in the morning and levelled off in the afternoon. The relationship between triglyceride levels and ΔMASS-D (including time of day, its square and individuals in the model) was better when the interaction ΔMASS-D×time of day was included ($R^2_{adj}$ = 0.61, Table 1a) instead of ΔMASS-D alone ($R^2_{adj}$ = 0.57). Hence, triglyceride levels increased more steeply over the day the larger ΔMASS-D was (interaction ΔMASS-D×time of day significant). After accounting for this interaction term, ΔMASS-D (indicating different intercepts according to body-mass change) was not significant, indicating that triglyceride levels of all birds were at a similar level at time 0 (Fig. 1a). This is supported by the finding that the values at time 0 (or alternatively the values at time of day 0 and 1.25 taken together) did not depend on change in body mass during the day before blood sampling (correlation, $P > 0.5$) or from the morning of the day before blood sampling to the morning of the day of blood sampling ($P > 0.5$) or overnight ($P > 0.7$), or during the day of blood sampling ($P > 0.1$). This indicated that triglyce-

**Table 2.** As Table 1, but for the dependence of β-hydroxy-butyrate levels (ln-transformed+0.5).

| | F | b | s.e. |
|---|---|---|---|
| ΔMASS-D | | | |
| (a) $R^2_{adj}$ = 0.52, n = 96 | | | |
| Intercept | 133.5*** | 1.593 | 0.282 |
| TIME | 24.2*** | –0.247 | 0.037 |
| $(TIME)^2$ | 32.8*** | 0.024 | 0.004 |
| ΔMASS-D×TIME | 45.5*** | –0.061 | 0.009 |
| MASS-M | 5.0* | -0.050 | 0.022 |
| ΔMASS-B | | | |
| (b) $R^2_{adj}$ = 0.53, n = 77 | | | |
| Intercept | 75.2*** | 1.361 | 0.300 |
| ΔMASS-B | 73.2*** | –1.943 | 0.929 |
| TIME | 1.6 | 0.012 | 0.021 |
| ΔMASS-B×TIME | 4.6* | –0.309 | 0.151 |
| MASS-M | 10.4** | –0.066 | 0.021 |

rides were at a similar level early in the morning irrespective of the previous or incipient changes in body mass.

The interaction term between individual and ΔMASS-D was clearly not significant (see Appendix), indicating that the individuals did not differ in their relationship between triglyceride levels and ΔMASS-D, but they differed significantly in intercept. If the effect of the individuals was ignored, the parameter estimates for ΔMASS-D and time of day changed only little (Table 1b).

None of the four measurements of locomotor activity during the day or night had a significant relationship with triglyceride levels (see Appendix). Body mass on the morning of blood sampling or, alternatively, body mass on the morning or evening before sampling (not shown), was also not significantly related to triglyceride levels.

Because change in body mass was kept at approximately the same level during the day before blood sampling as during the day of sampling, ΔMASS-Y, or alternatively change in body mass from morning of the day before blood sampling to morning of the day of blood sampling, was almost as good an indicator of triglyceride levels as ΔMASS-D (not shown).

Similar relationships with plasma triglyceride levels as described above were obtained when the hourly body-mass change between early morning and blood sampling ΔMASS-B was used instead of ΔMASS-D (Table 1c). However, a linear relationship with time of day was obtained (Fig. 1c), because data from the early morning, when no reliable measurement of ΔMASS-B was possible, were omitted from this analysis. Again the slopes were similar whether the factor individual was included in the model or not (Table 1d).

### β-hydroxy-butyrate

Plasma β-hydroxy-butyrate levels decreased with time of day (Table 2, Fig. 1b). The significant interaction term ΔMASS-D×time of day indicated that this decrease was not uniform among birds differing in change in body mass. In birds increasing in body mass, β-hydroxy-butyrate levels decreased rapidly and remained at low levels throughout the afternoon. In birds with stable or decreasing body mass over the day, however, β-hydroxy-butyrate levels increased again, showing that these birds were food restricted. After accounting for this interaction term, ΔMASS-B was not significant, indicating that β-hydroxy-butyrate levels of all birds were at a similar level at time 0 (Fig. 1b). Again, this is supported by the finding that the values at time 0 (or alternatively the values at time of day 0 and 1.25 taken together) did not depend on change in body mass during the day before blood sampling ($P > 0.2$) or from morning of the day before blood sampling to the morning

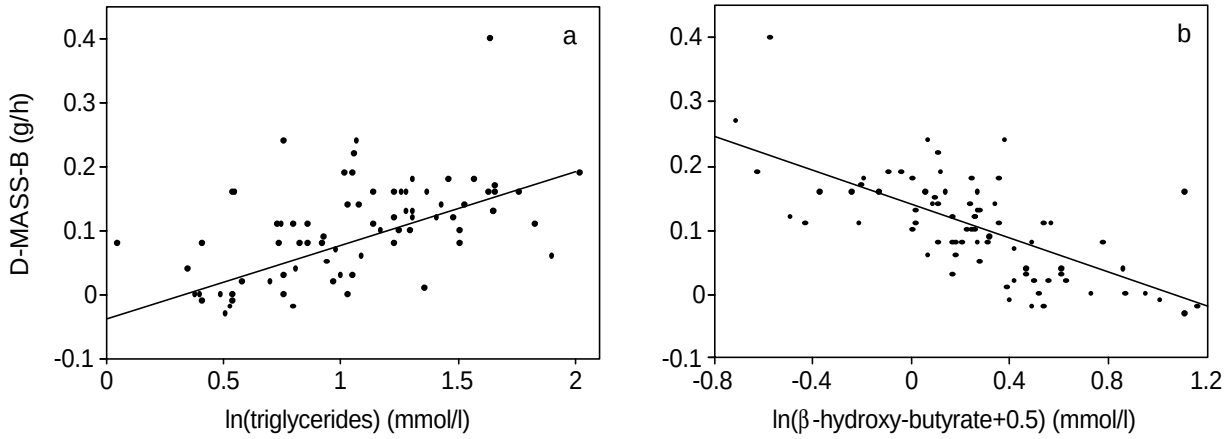



**Figure 2.** Relationship between hourly change in body mass of reed warblers since early morning (ΔMASS-B) and triglyceride (a) or β-hydroxy-butyrate levels (b) according to the models given in Table 3b and 3c, respectively. The metabolite values displayed have been standardised to 6 h after lights on according to the models.

of the day of blood sampling ($P > 0.4$) or overnight ($P > 0.2$) or during the day of blood sampling ($P > 0.5$). As with triglycerides, this indicated that β-hydroxy-butyrate levels were at a similar level early in the morning, irrespective of the previous or incipient change in body mass.

There was no effect of the individual on β-hydroxy-butyrate levels (see Appendix). As with triglyceride levels, none of the measures of activity was related to β-hydroxy-butyrate levels. However, β-hydroxy-butyrate levels were dependent on body mass in the early morning of the day of blood sampling (Table 2a).

Because change in body mass was kept at approximately the same level during the day before blood sampling as during the day of sampling, ΔMASS-Y or change in body mass from morning of the day before to morning of the day of blood sampling was almost as good a indicator of β-hydroxy-butyrate levels as ΔMASS-D.

When ΔMASS-B was used instead of ΔMASS-D, similar relationships resulted, but, as with triglycerides, the dependence of β-hydroxy-butyrate on time was linear, because the early morning values were omitted from analysis (Table 2b, Fig. 2d).

## Predicting body-mass change from metabolite levels

The relationships between triglyceride or β-hydroxy-butyrate levels and change in body mass may be used to predict change in body mass of a bird caught once, taking into account time of day and possibly other factors. The data of this study provide a calibration for such predictions. The aim of the following analysis was to estimate the parameters for predicting change in body mass from both metabolite levels and to find out which of the two metabolites was more suitable to predict body mass change.

When ΔMASS-B was predicted, either β-hydroxy-butyrate levels (together with time of day and body mass in the early morning; Table 3a) or triglyceride levels (together with time of day and its square; Table 3c) were significant predictor variables, β-hydroxy-butyrate and triglyceride levels being highly correlated. However, in field studies body mass in the early morning is unknown. If omitted, β-hydroxy-butyrate levels predicted hourly change in body mass just as well as triglyceride levels ($R^2_{adj} = 0.49$ versus 0.47; Table 3b, c; Fig. 2).

ΔMASS-D was dependent on both triglyceride and β-hydroxy-butyrate levels, as well as on a non-linear function of time (Table 3d). The variables that did not reveal significant effects were the individual, body mass of the morning of blood sampling, the various measures of activity and interactions between each of the two metabolites and time of day, its square or individuals. The two metabolite levels contributed about equally well and significantly to predict ΔMASS-D. However, only 34 % of the variation in ΔMASS-D was ex-

**Table 3.** Dependence of change in body mass of reed warblers (either change in body mass from early morning to evening ΔMASS-D or hourly change in body mass from morning to blood-sampling ΔMASS-B) on triglyceride levels, β-hydroxy-butyrate levels, time of day since lights on (TIME in h) and its square $(TIME)^2$. Only the model with the significant factors after backward elimination is given. * P < 0.05, ** P < 0.01, *** P < 0.001.

| | F | b | s.e. |
|---|---|---|---|
| (a) ΔMASS-B with β-hydroxy-butyrate ($R^2_{adj}$ = 0.55, n = 77) | | | |
| Intercept | 291.1*** | 0.347 | 0.053 |
| ln(β-hydroxy-butyrate+0.5) | 75.8*** | –0.140 | 0.016 |
| TIME | 8.3** | –0.0077 | 0.003 |
| MASS-M | 10.5** | -0.0133 | 0.004 |
| (b) ΔMASS-B with β-hydroxy-butyrate ($R^2_{adj}$ = 0.49, n = 77) | | | |
| Intercept | 258.1*** | 0.185 | 0.019 |
| ln(β-hydroxy-butyrate+0.5) | 67.2*** | –0.133 | 0.017 |
| TIME | 7.4** | –0.0074 | 0.003 |
| (c) ΔMASS-B with triglycerides ($R^2_{adj}$ = 0.47, n = 76) | | | |
| Intercept | 255.1*** | 0.146 | 0.044 |
| ln(triglycerides) | 44.8*** | 0.116 | 0.015 |
| TIME | 18.6*** | –0.049 | 0.015 |
| $(TIME)^2$ | 6.2* | 0.00302 | 0.00121 |
| (d) ΔMASS-D with triglycerides and β-hydroxy-butyrate ($R^2_{adj}$ = 0.34, n = 84) | | | |
| Intercept | 145.9*** | 0.955 | 0.252 |
| ln(triglycerides) | 28.6*** | 0.457 | 0.163 |
| ln(β-hydroxy-butyrate+0.5) | 4.6* | –0.533 | 0.177 |
| TIME | 3.6 | –0.255 | 0.070 |
| $(TIME)^2$ | 10.4** | 0.0205 | 0.006 |

plained ($R^2_{adj}$ = 0.34) against 45–55% for the variation in ΔMASS-B. This is due to the fact that the hourly change in body mass after blood sampling was not significantly correlated with either triglyceride or β-hydroxy-butyrate levels or both (P > 0.08).

## Discussion

### Metabolic responses to body-mass changes

This study confirmed for the reed warbler that the plasma concentrations of triglycerides and β-hydroxy-butyrate were correlated with change in body mass induced by experimental variation of food intake, as found earlier in two other species (Jenni-Eiermann & Jenni 1994, Williams et al. 1999). The study also showed that triglyceride and β-hydroxy-butyrate levels changed during the daylight hours, as demonstrated in free-living small passerines (Jenni & Jenni-Eiermann 1996).

Triglyceride levels increased with time of day and when body mass increased, confirming that triglyceride levels increase with food intake (Robinson 1970). In contrast, β-hydroxy-butyrate levels decreased with increasing body mass and with time of day, confirming that β-hydroxy-butyrate is indicating the catabolism of lipids. In the early morning, β-hydroxy-butyrate levels decreased particularly rapidly from overnight fasted levels (as also found in free-living birds; Jenni & Jenni-Eiermann 1996) to a level which depended on change in body mass. If body mass increased, β-hydroxy-butyrate levels remained low throughout the day, while they increased again after midday in food restricted birds as a response to food restriction (Fig. 1).

This study suggests that triglyceride and β-hydroxy-butyrate levels in the early morning (before substantial food intake) are similar in birds with decreasing, stable or increasing body mass during the previous day or with varying overnight mass losses. It seems that metabolites return to the same level at the end of a normal over-

night fast (the pattern may be different in birds fasting for several days). Hence, metabolite levels in the early morning (i.e. for about 1.5 h after the onset of feeding) cannot be used to predict change in body mass. The return to similar levels in the early morning also suggests that triglyceride and β-hydroxy-butyrate levels reflect change in body mass during the day of blood sampling rather than body-mass change over several days (this may be different in birds which are in a prolonged state of fasting, and for other metabolites). Although this conclusion was also reached earlier (Jenni-Eiermann & Jenni 1994), the experimentally induced correlation between body-mass change during the day of blood sampling and the previous day in both studies prevented a proper test.

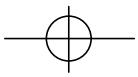
Body mass and activity were not correlated with triglyceride levels, further supporting earlier findings (Jenni-Eiermann & Jenni 1994). Hence, within the range of body masses examined (9.6–16.4 g), fat stores apparently did not influence triglyceride levels. In contrast, body mass in the early morning was negatively correlated with β-hydroxy-butyrate levels (Table 2). However, over a range of body mass of 6 g, β-hydroxy-butyrate levels varied only little (13–18 % of the range in ln(β-hydroxy-butyrate+0.5)). It is possible that heavy birds have energy substrates other than stored lipids available for overnight fasting and, consequently, rely less on the catabolism of lipids from adipose tissue, resulting in lower β-hydroxy-butyrate levels in the early morning. Similar findings were obtained from free-living birds fasted overnight during the migratory season which are heavier, rely less on lipids from adipose tissue and have lower β-hydroxy-butyrate levels than light birds before the migratory period (Jenni-Eiermann & Jenni 1996).

## Predicting change in body mass from metabolite levels

As discussed above, metabolite levels in the early morning were not correlated with change in body mass. This is one reason for models including early morning samples having a lower fit than models excluding these values (Tables 1 and 2). Another reason is that change in body mass after blood sampling, although it was not affected by blood sampling, was not significantly correlated with triglycerides and β-hydroxy-butyrate levels. Hence, models predicting change in body mass over the day of blood sampling (i.e. partially occurring after blood sampling) had a lower fit than models predicting change in body mass up to the time of blood sampling. Consequently, early morning metabolite levels (up to about 1.5 h after dawn) should not be used to predict change in body mass. The models presented in Tables 3b and 3c (Fig. 2) are to be preferred as a calibration.

The relationships between body-mass change and the two metabolites accounting for time of day (Table 3b and 3c) may serve to predict body-mass change of birds caught once in field studies. Still, several points need to be observed when applying this method (see also Jenni-Eiermann & Jenni 1994). The method should not be used at this stage for birds fasting for extended periods or for birds after a long flight, because this may alter the metabolism more profoundly (Jenni-Eiermann & Jenni 1991). Time of year and stage of moult may also affect their metabolism (Jenni-Eiermann & Jenni 1996, Jenni & Jenni-Eiermann 1996) and should be kept constant or included in an analysis. Diet may affect plasma metabolite levels, although we have no indication of such an effect for triglycerides and β-hydroxy-butyrate in birds. Although both metabolites measured in this study indicate change in body mass, triglycerides are likely to reflect change in fat stores more closely, because they are metabolically directly related to fat deposition, the focus of interest of most studies. β-hydroxy-butyrate levels seem to be associated also with transitions from one metabolic state to the other (Jenni-Eiermann & Jenni 1991). Hence, triglyceride levels may in fact more accurately predict changes in lipid stores than overall change in body mass (including change in protein and carbohydrates).

In conclusion, there is growing evidence (Jenni-Eiermann & Jenni 1994, Williams et al. 1999, this study) that plasma metabolite levels can be used to estimate short-term changes in body mass (or energy stores) of birds caught only once in the field during the natural feeding period of the day. Estimating changes in body mass from plasma metabolite levels in birds caught only once may help to assess feeding conditions based on the performance of the birds. For instance, the method may be applied to assess fattening rates of migratory birds, habitat quality and the influence of exogenous factors (e.g. weather, disturbance) on body condition.





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**Appendix**

Full model of the dependence of triglyceride levels (ln-transformed) or β-hydroxy-butyrate levels (ln-transformed+0.5) in reed warblers on change in body mass from morning to evening of the day of blood sampling (ΔMASS-D in g), time of day since lights on (TIME in h), its square $(TIME)^2$, body mass in the early morning (MASS-M in g), four measures of activity (see methods) and individuals, analysed by type I model of analysis of covariance. For each variable, df is 1, except when indicated. Through backward elimination, N-ACTIV becomes non-significant in the model for β-hydroxy-butyrate. * P < 0.05, ** P < 0.01, *** P < 0.001.

| | Triglycerides ($R^2_{adj}$ = 0.62, n = 96) | | | β-hydroxy-butyrate ($R^2_{adj}$ = 0.55, n= 96) | | |
|---|---|---|---|---|---|---|
| | F | b | s.e. | F | b | s.e. |
| Intercept | 250.1*** | –0.679 | 0.534 | 125.7*** | 2.135 | 0.533 |
| ΔMASS-D | 12.0** | 0.210 | 0.545 | 34.4*** | 0.030 | 0.552 |
| TIME | 14.2** | 0.230 | 0.053 | 16.9*** | –0.247 | 0.047 |
| $(TIME)^2$ | 8.0* | –0.019 | 0.005 | 29.9*** | 0.024 | 0.004 |
| ΔMASS-D×TIME | 0.9 | 0.060 | 0.023 | 5.9* | –0.048 | 0.023 |
| MASS-M | 0.9 | 0.080 | 0.042 | 6.2* | –0.102 | 0.042 |
| D-ACTIV | 0.7 | $-0.41.10^{-4}$ | $2.16.10^{-4}$ | 0.8 | $1.77.10^{-4}$ | $2.12.10^{-4}$ |
| N-ACTIV | 0.4 | $-2.40.10^{-4}$ | $4.37.10^{-4}$ | 4.8* | $8.55.10^{-4}$ | $3.61.10^{-4}$ |
| Y-D-ACTIV | 0.1 | $1.74.10^{-4}$ | $2.49.10^{-4}$ | 0.7 | $-0.45.10^{-4}$ | $2.02.10^{-4}$ |
| Y-N-ACTIV | 0.1 | $0.03.10^{-4}$ | $5.29.10^{-4}$ | 1.0 | $-5.34.10^{-4}$ | $4.58.10^{-4}$ |
| Individuals (df =17) | 3.2*** | | | 1.1 | | |
| Individuals×ΔMASS-D (df = 17) | 0.4 | | | 0.7 | | |

As above, but for the dependence on the hourly change in body mass from morning to the time of blood sampling (ΔMASS-B in $gh^{-1}$).

| | Triglycerides ($R^2_{adj}$ = 0.62, n = 96) | | | β-hydroxy-butyrate ($R^2_{adj}$ = 0.55, n = 96) | | |
|---|---|---|---|---|---|---|
| | F | b | s.e. | F | b | s.e. |
| Intercept | 351.4*** | –0.933 | 0.736 | 74.2*** | 1.181 | 0.586 |
| DMASS-B | 18.8*** | 1.369 | 3.992 | 59.6*** | -0.553 | 3.257 |
| TIME | 4.8* | 0.082 | 0.151 | 2.5 | 0.025 | 0.100 |
| $(TIME)^2$ | 3.6 | –0.050 | 0.012 | 4.8* | 0.0014 | 0.007 |
| ΔMASS-B×TIME | 1.0 | 0.451 | 0.292 | 3.5 | –0.498 | 0.222 |
| MASS-M | 0.6 | 0.108 | 0.051 | 9.2** | –0.086 | 0.037 |
| D-ACTIV | 0.6 | $-0.73.10^{-4}$ | $2.70.10^{-4}$ | 1.1 | $3.30.10^{-4}$ | $2.07.10^{-4}$ |
| N-ACTIV | 0.5 | $2.88.10^{-4}$ | $5.42.10^{-4}$ | 3.4 | $9.25.10^{-4}$ | $3.55.10^{-4}$ |
| Y-D-ACTIV | 0.2 | $1.84.10^{-4}$ | $2.63.10^{-4}$ | 0.4 | $-1.34.10^{-4}$ | $1.79.10^{-4}$ |
| Y-N-ACTIV | 0.1 | $-4.95.10^{-4}$ | $7.54.10^{-4}$ | 0.7 | $-7.43.10^{-4}$ | $5.22.10^{-4}$ |
| Individuals (df =17) | 2.6* | | | 1.2 | | |
| Individuals×ΔMASS-B (df = 17) | 0.4 | | | 0.8 | | |

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# Optimal climbing flight in migrating birds: predictions in a stochastic environment

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Key words: Climbing flight, migrant birds, flight mechanical theory, flight models.
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Hedenström & Alerstam (1994) presented a theoretical prediction for the optimal rate of climb in birds when setting out on a migratory flight. The prediction was based on flight mechanical theory (Pennycuick 1975, 1978, 1989, Hedenström & Alerstam 1992) and gives the optimal rate of climb in relation to the tail wind assistance at the cruising altitude. There is a trade-off so that if tailwinds are strong it pays to climb fast and lose forward speed, whereas if tailwinds are weak a lower rate of climb is optimal, thus giving the bird a faster forward speed during the climb. An example of the model's prediction of how rate of climb should depend on wind assistance is given by the solid line in Figure 1.

A provisional comparison against empirical data on two species of shorebird gave no reason to reject the prediction (Hedenström & Alerstam 1994). However, further data on climb rates of departing shorebirds in Mauritania generally showed lower rates of climb than predicted (Piersma et al. 1997). Seven out of nine species showed significantly lower climb rates than expected, one species did not differ significantly from the prediction, while one species showed a significantly higher climb rate than predicted. Although a number of problems which could possibly have confounded the observations were identified (Piersma et al. 1997), there remained the possibility that the theoretical prediction needed revision. Here we offer a realistic amendment to the original prediction.

The original model is deterministic, i.e. it is assumed that the birds have perfect knowledge about the winds aloft. However, a more realistic assumption is that, even if birds can estimate the wind situation at the cruising altitude, there is some degree of uncertainty about the magnitude of the wind assistance. Foraging animals are known to be sensitive to variability in their environment (e.g. food encounter rate), which is well established in foraging theory (McNamara & Houston 1992) and by empirical studies (Caraco et al. 1980, Moore & Simm 1986, but see Kacelnik & Bateson 1996).

Here, we modify the prediction on optimal climb rate presented by Hedenström & Alerstam (1994) by letting the wind assistance W be a discrete random variable with mean

$$E(W) = \sum_{i=1}^{n} p_i w_i \,, \qquad (1)$$

where $\{w_1, w_2, .., w_n\}$ is a discrete set of possible winds encountered at the cruising altitude and with wind $w_i$ occurring with probability $p_i$. The model is based on the assumption that the bird climbs to the height that gives the best wind assistance. In the case of a zero wind, all other heights would be worse (i.e. wind speed would be negative).

The currency used by Hedenström & Alerstam is the energy cost of a migratory flight of defined length, including the initial climbing phase and the cruising phase. The wind assistance W is encountered only at the cruising altitude z. The currency can be written as

$$R = \frac{P_{\max} z}{V_z} + \sum_i p_i \frac{P_{mr}(w_i)(x - x_1)}{V_{mr}(w_i) + w_i} \,, \qquad (2)$$

where $P_{max}$ is the maximum power available from the flight muscles, $V_z$ is rate of climb (the decision varia-

ble), $P_{mr}(w_i)$ is the power of flapping flight at the maximum range speed $V_{mr}(wi)$, x is the total flight distance and $x_1$ is the horizontal flight distance during the climbing phase (see Hedenström & Alerstam [1994] for a detailed specification of the model). The maximum range speed (and therefore also $P_{mr}$) should be adjusted in relation to tail and head winds (Pennycuick 1978), which is indicated by the functional dependence on $w_i$. The calculation was done in pure head and tail wind situations, but generally the angle between track and heading should also affect the wind speed adjustment (Liechti et al. 1994).

By differentiating equation (2) with respect to $V_z$ and setting the derivative equal to zero we can solve the optimal rate of climb yielding the minimum cost of transport of a migratory flight. Notice that $x_1$ can be replaced by $V \cdot z/V_z$, where V is forward airspeed during the climb, z is the cruising altitude and $V_z$ is the rate of climb. The derivative of equation (2) is

$$\frac{dR}{dV_z} = -\frac{P_{\max} z}{V_z^2} + \left(\frac{V}{V_z^2} - \frac{V'}{V_z}\right) z \sum_i p_i \frac{P_{mr}(w_i)}{V_{mr}(w_i) + w_i} \quad (3)$$

where V´ is the derivative of the horizontal airspeed with respect to $V_z$. After rearranging the terms equation (3) yields the optimal rate of climb ($V_z$*) as

$$V_z^* = \frac{V}{V'} - \frac{P_{\max}}{V'} \left( \frac{1}{\sum_i p_i \frac{P_{mr}(w_i)}{V_{mr}(w_i) + w_i}} \right) \quad (4)$$

The effect of this modified prediction in relation to the deterministic case is shown in Figure 1 for the knot *Calidris canutus*. Notice that the probability distribution of the wind represents a hypothetical case only. Because the optimal rate of climb is a decelerating function of wind assistance (as in Fig. 1, for example), it follows from Jensen's inequality that introducing variability will decrease the optimal climb speed. Clearly, the predicted optimal rate of climb is reduced in relation to the deterministic case, but the two predictions converge with increasing expected tail wind. This is because the reduction due to variability is decreasing as the curve relating $V_z$* to *E(W)* becomes flatter, as it does with increasing *E(W)*. For an expected zero wind assi-

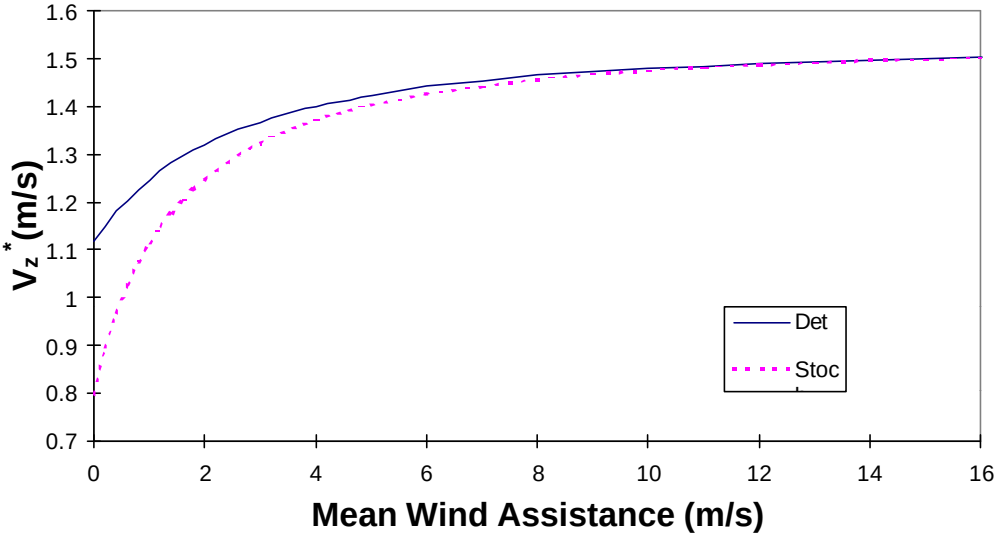


**Figure 1.** Optimal rate of climb ($V_z$*) in relation to wind assistance at the cruising altitude calculated for spring migrating knots *Calidris canutus* (body mass = 205 g, wing span = 0.52 m, wing-beat frequency = 9.3 Hz, muscle mass = 33 g; Hedenström & Alerstam 1994). In the deterministic case (solid curve), the wind assistance is E(W) with probability = 1. In the stochastic case (dotted curve) the mean wind assistance is E(W), but the assistance takes one of the three possible values: $w_1$ = E(W)–5, $w_2$ = E(W) and $w_3$ = E(W)+5 ($ms^{-1}$), with $p_1=p_2=p_3=1/3$. Notice that a negative w represents a headwind.

stance, the prediction is reduced from 1.12 m/s to 0.80 m/s, while at an expected wind assistance of 15 m/s the predictions are within 0.07 % of each other. Hence, it is only at relatively low expected tail wind that the prediction is significantly affected by introducing stochasticity. However, a low wind assistance is probably a rather typical situation (cf. Piersma & Van De Sant 1992, Gudmundsson 1993).

The present model assumes that the energy cost of transport is minimised during migration. An alternative currency often used in models of migration strategies is the overall migration speed (see Hedenström & Alerstam 1997 for a discussion of migration currencies). In the case of optimal rate of climb these alternative currencies will yield very similar results (Hedenström & Alerstam 1994).

In conclusion, the discrepancy between predicted and observed rates of climb in migrating birds found by Piersma et al. (1997) could be due to the deterministic nature of the model used. Introducing a probability distribution of encountered tail winds reduces the predicted optimal rate of climb, which is in line with observations. Hence, when testing this model accurately we also need information about the variance of the winds aloft.

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