

Spring arrival along a migratory divide of sympatric blackcaps (*Sylvia atricapilla*)

Gregor Rolshausen · Keith A. Hobson ·
H. Martin Schaefer

Received: 24 February 2009 / Accepted: 27 July 2009 / Published online: 27 August 2009
© Springer-Verlag 2009

Abstract The recent formation of a migratory divide in the blackcap (*Sylvia atricapilla*) involves sympatrically breeding birds migrating to different overwintering quarters. Within the last 50 years, a novel migratory strategy has evolved resulting in an increasing proportion of birds now wintering in Britain instead of migrating to the traditional sites in the Mediterranean area. This rapid microevolution has been attributed to allochronic spring arrival of migrants from the respective wintering quarters leading to assortative mating. Moreover, blackcaps wintering in Britain may experience fitness advantages owing to improved local wintering conditions. We used stable hydrogen isotope signatures (δD) to scrutinize the degree of temporal segregation of blackcaps upon spring arrival and to test for carry-over effects in body condition associated with the disparate wintering environments. Although we found that migrants from Britain arrive significantly earlier on German breeding grounds than migrants from the Mediterranean region, we also found a considerable overlap in arrival times. In a resampling model, the mean probability of assortative mating of birds wintering in Britain is $\leq 28\%$ in both years. These results suggest that allochrony alone is not a strong isolating barrier between the two subpopulations. Migrants from both wintering

locations did not differ in terms of body mass, mass-tarsus residuals or mass-tarsus ratio and arrived in a similar reproductive disposition. Thus, blackcaps wintering in Britain do not gain an apparent fitness advantage on spring migration due to carry-over effects in body condition. Future studies should explore additional factors such as differences in song quality and habitat that might contribute to the rapid microevolution of the blackcap.

Keywords Microevolution · Allochrony · Migratory divide · Carry-over effects · Deuterium

Introduction

Adaptive divergence and speciation in sympatry are among the most contentious topics in evolutionary biology (Dieckmann et al. 2004; Price 2007). The fundamental question concerns the nature of barriers to the homogenization of gene flow between diverging sub-groups. In the absence of substantial spatial segregation, reproductive isolation can only evolve and be maintained through postzygotic incompatibilities, polyploidization (restricted to flowering plants) or assortative mating (Coyne and Orr 2004). The latter is a form of nonrandom pairing, where mating phenotypes are, on average, more similar than expected with random pairing (Hartl and Clark 2007). Prezygotic barriers mediating the assortment of mates in sympatry include behavioural traits under disruptive sexual selection, distinct resource phenology in host races and temporal isolation (Bolnick and Fitzpatrick 2007; Coyne and Orr 2004; Gavrilets 2003).

Temporal restriction to gene flow occurs because different members of a population reproduce at different times. This divergence in timing can lead to complete

Communicated by Carlos Martinez del Rio.

G. Rolshausen (✉) · H. M. Schaefer
Department of Evolutionary Biology and Animal Ecology,
Faculty of Biology, University of Freiburg,
Hauptstrasse 1a, 79104 Freiburg, Germany
e-mail: gregor.rolshausen@biologie.uni-freiburg.de

K. A. Hobson
Environment Canada, 11 Innovation Blvd,
Saskatoon, SK S7N 3H5, Canada

reproductive isolation, or allochrony, if populations isolated by time are outside each other's range during the formation of pair bonds (Coyne and Orr 2004; Dieckmann et al. 2004). Empirical evidence for allochronic speciation in sympatry includes the well-documented studies from 13- and 17-year periodical cicadas (Cooley et al. 2003), spawning allochrony in pink salmon (Gharrett et al. 1988) and divergent breeding phenology in seabirds (Friesen et al. 2007; Monteiro and Furness 1998). Moreover, although veritable allochronic speciation in sympatry is undoubtedly rare, there are numerous examples where temporal restriction to gene flow is important as one of several barriers reinforcing population divergence (Hendry and Day 2005).

Central European blackcaps (*Sylvia atricapilla*) represent a model system for studying how allochrony might be associated with differences in migratory behaviour. Assortative mating attributed to temporal segregation has been reported in sympatric subpopulations that differ in overwintering areas (Bearhop et al. 2005). Since the 1960s, blackcaps breeding in southern Germany and Austria have established a novel migratory direction towards the British Isles (Berthold et al. 1992). Using stable isotope analyses as a marker of origin, Bearhop et al. (2005) documented that birds migrating along this newly established north-western route are more likely to mate with each other than with migrants from the traditional south-western route with winter quarters in the Mediterranean region. The lag in mean arrival time from the two distinct wintering grounds may be sufficient to diminish gene flow (Bearhop et al. 2005; Berthold et al. 1992; Terrill and Berthold 1990). However, the delay in spring arrival has only been estimated indirectly based upon flight distance and average migration rate (Terrill and Berthold 1990) rather than based upon observed arrival dates in spring.

Owing to the pronounced climatic and geographic differences between the north-western and south-western winter quarters, the arrival of sympatric blackcaps also provides an ideal study system with which to scrutinize carry-over effects in physical condition. First, the migration distance from the British Isles is considerably shorter (~1,090 km) than that from the Mediterranean region (~1,640 km). Second, it has been suggested that artificial feeding opportunities in Britain during winter may result in significant carry-over effects that enable birds wintering in Britain to devote more resources to reproduction (Berthold et al. 1992; Berthold and Terrill 1988). Although blackcaps use the food resources provided by humans heavily (Chamberlain et al. 2005), this hypothesis has not yet been tested. Short migratory distance and improved winter feeding opportunities have both been attributed to the rise of the subpopulation wintering in the British Isles (see Hewson et al. 2007). Feasibly, carry-over effects may also

reinforce the efficacy of the allochronic barrier (Norris et al. 2004; Saino et al. 2004).

Our objectives were to investigate: (1) temporal isolation as a segregation mechanism mediating the incipient population divide in Central European blackcaps, and (2) the strength of carry-over effects that may affect population dynamics. To this end, we scrutinized individual spring arrival of south-west German blackcaps during 2 years based on assigning birds to their respective wintering quarters by stable hydrogen isotope (δD) analysis consistent with the approach used by Bearhop et al. (2005). Specifically, we tested whether north-western migrants from the British Isles arrive earlier than conspecifics that wintered in the Mediterranean. To investigate carry-over effects in physical condition we compared morphological and physiological (haematocrit) indicators as condition indices between the two subpopulations. To assess mating disposition of arriving males, we calculated the cloacal protuberance (CP), a parameter that reflects the progress in spermatogenesis (Gill 2007; Lombardo 2001). Finally, we included a resampling model based on observed arrival dates to assess the probability of assortative pairings based solely on temporal segregation.

Materials and methods

Field procedures

We caught blackcaps upon spring arrival in Radolfzell (47°45'N, 08°59'E) and Freiburg (48°00'N, 07°51'E) in 2006 and 2007, respectively. We were not able to collect data at the same field site in consecutive years because the umbrella project in Radolfzell was discontinued in 2007.

We started capture by mid-March, when the first birds arrived from their wintering quarters, and caught birds every day from early morning to noon until the end of April. Blackcaps were caught along edges of forest or copse within an area of 50 ha with mist nets using tape recordings of their song as a decoy. Each morning we patrolled the entire area for singing activity and captured all newly singing males as well as non-singing individuals at standardised sites that were dominated by ivy (*Hedera helix*) which provided both food and cover. In total we obtained data for 126 birds in 2006 and 139 birds in 2007. Each individual was marked with a standard aluminium ring, sexed, aged (Shirihai et al. 2001), and weighed (digital balance ± 0.1 g precision) before morphological measurements were taken (calliper with 0.1-mm precision and ruler with 0.5-mm precision). Both sexes and age classes (yearlings and adults) were evenly distributed in our sample (χ^2 -test for deviation from balanced data; sex, $P = 0.366$; age, $P = 0.302$).

We caught birds in well-defined area where we recorded daily territory establishment and presence at the main food resources. We therefore considered the first day a bird was caught as a proxy for arrival date and we calculated a day score as the difference in arrival relative to the start of the field season for statistical analysis (days from 15 March). Given the number of non-significant tests in our study, we used this large dataset to increase statistical power for studying the relationship between spring arrival and migratory direction. However, our results do not change qualitatively as we restricted tests to birds of known breeding status in the area that were re-sighted or re-trapped either in the same year ($n = 75$) or in subsequent years (four in 2006, six in 2008 which is comparable to the published data on the weak year-to-year site fidelity in south-west German blackcaps: $\sim 7\%$; Berthold and Bairlein 1984). Given that both analyses yielded consistent results which we report in the results, we trust that our conclusions are not biased by confusing migrants on passage with resident breeders.

Body condition and reproductive disposition

The condition of blackcaps upon arrival was assessed using body mass and two additional morphological indices as indicators: body mass divided by tarsus length (Matthysen 1989; Moreno 1989), and the residuals of the body mass \times tarsus regression (2006, $F_{1,104} = 19.83$, $R^2 = 0.160$, $P < 0.001$; 2007, $F_{1,107} = 4.13$, $R^2 = 0.04$, $P < 0.05$). Residuals from the regression, that is observed minus fitted values, were taken as a proxy for body condition (Bize et al. 2006; Hochachka and Smith 1991). The usage of body mass in condition estimates is contentious because of fluctuations in weight during daytime due to food intake or thermoregulation (Berthold 1971; Clark 1979). We therefore include the time of day a bird was caught as a covariate in our models to control for daytime fluctuations in weight. Moreover, we analyse haematocrit as a proxy for condition to obtain an estimation of condition independent of morphological measurements (Cuervo et al. 2007; Dawson et al. 2001; Potti 2007; Potti et al. 1999; Svensson and Merila 1996). To determine haematocrit, we obtained a blood sample ($\pm 75 \mu\text{l}$) from the wing vein of each bird in 2006 using heparinised capillaries. We centrifuged blood immediately and separated plasma from the cells. Birds were released directly after blood sampling. The haematocrit was assessed as the proportion of capillary length of packed red blood cells in relation to all blood components (0.1-mm-precision ruler).

To assess the reproductive state of males, we calculated the CP volume (mm^3) for each male in 2006 as: $\text{CP}_{\text{Volume}} = \pi \times \text{height} \times (\text{diameter}/2)^2$ assuming a cylindrical shape (Kempnaers et al. 1999; Lombardo 2001).

Tarsus length and haematocrit did not differ significantly from a normal distribution (Shapiro–Wilk's W -test, $P > 0.15$); however, body mass and CP were square-root transformed to achieve normality before further analysis. Residuals from the linear regression of (transformed) body mass on tarsus length were calculated and the absolute value of the smallest residual was added to all other residuals to avoid negative results. We tested for differences in body condition indices using linear analysis of covariance (ANCOVA) models entering sex and wintering area as factors and arrival date (relative to the 15th of March) as a covariate, as well as the interactions among them. Comparisons of arrival time based on the hypothesis that migrants from Britain arrived earlier on the breeding grounds (Berthold et al. 1992) were tested with one-sided Wilcoxon exact rank tests.

Stable isotope analyses

We obtained two to four distal claw sections from each individual ($\leq 1\text{mm}$, to avoid keratin synthesized between departure from the wintering area and sampling). Claws constitute a reliable source of dietary and habitat isotope signatures and their growth rates provide evidence that the information is integrated over a medium time scale such as weeks to months (Bearhop et al. 2003; Carleton et al. 2008). Hence, claw tips of spring-arriving birds appropriately reflect the isotopic signature of their wintering grounds (Bearhop et al. 2005; Mazerolle and Hobson 2005).

All claw tips were cleaned of surface oils in a 2:1 chloroform:methanol solvent rinse and prepared for stable hydrogen isotope analysis at the Environment Canada stable isotope laboratory in Saskatoon, Canada. Stable hydrogen isotope analyses of claw tips were completed using the comparative equilibration method described in detail by (Wassenaar and Hobson 2003) and through the use of calibrated keratin isotope reference materials. Stable hydrogen isotope measurements were performed on H_2 derived from high-temperature flash pyrolysis of nails using continuous-flow isotope-ratio mass spectrometry. All $^2\text{H}/^1\text{H}$ measurements are expressed in the typical delta notation (δD), in units of per mil (‰), and normalized on the Vienna standard mean ocean water-standard light Antarctic precipitation standard scale (Fig. 1). Repeated analyses of hydrogen isotope inter-comparison material IAEA-CH-7 (-100‰) and keratin references yielded an (within run) external repeatability $\pm 2.0\text{‰}$.

Statistical analysis and individual assignment

To assign individuals to their wintering quarters, we modelled distinct density functions for the distributions of

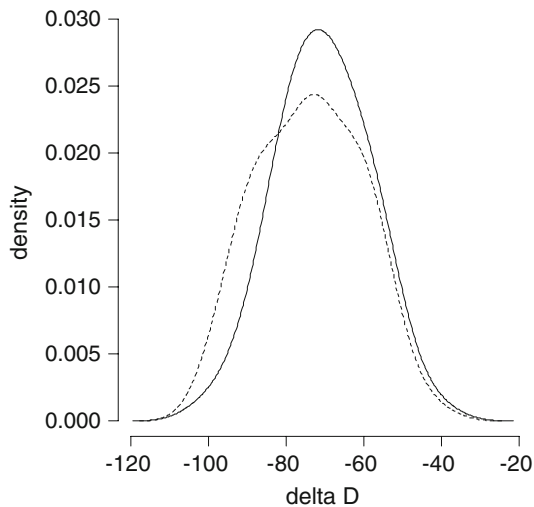


Fig. 1 Density plot of $^2\text{H}/^1\text{H}$ measurements from blackcap claw tips in delta notation (δD). Dashed line corresponds to the 2006 δD distribution (mean: -73.92 ± 1.24 SE), solid line corresponds to the 2007 δD distribution (mean: -71.42 ± 1.11 SE)

δD from resident birds and wintering blackcaps, respectively (Royle and Rubenstein 2004; Wunder et al. 2005). Data were obtained from Bearhop et al. (2005), who measured δD in claw tips from wintering blackcaps in the two wintering areas (Britain or Iberia), as well as from resident birds from Britain (blue tits *Parus caeruleus* and great tits *Parus major*) and from Spain (Sardinian warbler *Sylvia melanocapala*). Population assignment did not differ if we used δD data from wintering blackcaps in Spain and blue tits and great tits in Britain from 2007 and 2008 (G. Rolshausen et al., unpublished data). Assuming the pools of isotope values from residents and migrants to be normally distributed, we modelled the probability density functions according to the parameters estimated from the Bearhop et al. (2005) data. Normal probability density of a continuous variable is given by:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2\right),$$

where σ is the SD and μ the expected value of the distribution. With these specifications we calculated the ratio of probabilities [$p_{(r)}$] for each individual to belong to either of the modelled distributions. The $p_{(r)}$ is given by:

$$p(r) = \frac{f(x)}{g(x) + f(x)},$$

where $f(x)$ and $g(x)$ are the modelled probability density functions for British and Mediterranean isotope signatures respectively, calculated for the δD ‰ values from our claw tip analysis. Thus we were able to assign >80% of the birds caught in 2006 and 2007 to either one of the wintering areas with a confidence of $p_{(r)} > 0.6$ (2006 north-western

migrants, $n = 18$; south-western migrants, $n = 85$; 2007 north-western migrants, $n = 9$; south-western migrants, $n = 100$; Fig. 2). We obtained the same individual assignment regardless of which reference distribution from Bearhop et al. (2005) was used to model the probability density (i.e. data from wintering blackcaps or data from resident birds from the respective wintering area). Moreover, we applied a Bayesian likelihood-based approach to assign birds with marginal signatures [$p_{(r)} < 0.6$] using relative abundance data for the two subpopulations based on posterior probabilities of origin (Royle and Rubenstein 2004). The overall proportion of assigned birds overwintering in the British Isles was 17.5% in 2006 and 8.3% in 2007, which is nearly consistent with estimations of local populations between Belgium and central Germany where the percentage of north-west migrants among blackcaps has increased from almost zero before 1960 to 7–11% in 1992 (Berthold et al. 1992; Helbig 1992). The source of the between-year variation, which was not the focus of our study, is unknown as it might be related to spatial variation in the size of the north-western wintering population, between-year variation in isotope distributions at the wintering sites or to temporal variation in population sizes (we obtained a similar estimate of 10% of north-western migrants in Freiburg in 2008; G. Rolshausen et al., unpublished data). The accuracy of our assignment procedure might be lowered because the isotope ratio

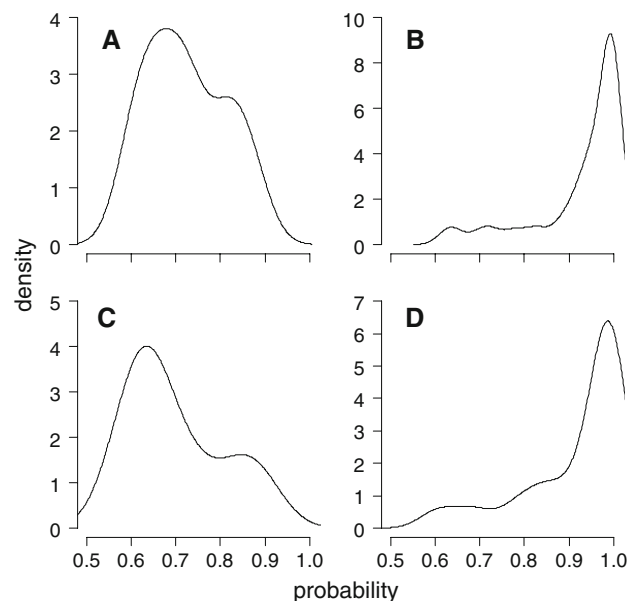


Fig. 2 Distributions of posterior probabilities of assignment for all birds by wintering area. **a** North-western (NW) migrants 2006 [$n = 18$, mean ratio of probabilities [$p_{(r)}$] = 0.72 ± 0.02 SE], **b** south-western (SW) migrants 2006 [$n = 85$, mean $p_{(r)}$ = 0.92 ± 0.01 SE], **c** NW migrants 2007 [$n = 9$, mean $p_{(r)}$ = 0.70 ± 0.04 SE], **d** SW migrants 2007 [$n = 100$, mean $p_{(r)}$ = 0.91 ± 0.01 SE]

reference data from Bearhop et al. (2005) were collected several years before our study was conducted. However, we did not expect pronounced shifts in claw δD values between wintering locations among years due to the relatively strong isotopic gradient between Britain and Spain (Bowen et al. 2005; Hobson 2003; Hobson et al. 2004) but controlled for this possibility by repeating our analyses using only the extremes of both wintering ground δD claw distributions (i.e. 10th and 90th quantiles of the overall distribution). Independently from the likelihood-based assignment we correlated individual arrival time with claw δD value (as a continuous variable) to account for birds that might winter further south or north than expected.

Resampling model

We based our model on the simplified assumption that allochryony upon arrival alone influences mating. In our model, we assume that each female mates once and will choose its mate randomly from a group of males that had all arrived at least 1 day prior to the female. The results did not change qualitatively if we changed the time interval between males' arrival and females' mate choice. The model does not account for competition since females and males that remain unpaired are retained in the subsequent pool of males available to later arriving females. Mating was assumed to continue until all females were mated or until there were no males left. We calculated the proportion of assortative mating (according to migratory direction) from this set of matings based upon temporal segregation separately for 2006 and 2007. To average the probability of assortment we ran the model 10,000 times. The aim of the model was to incorporate the considerable differences in the size of the two sub-populations into the analysis of the strength of the temporal isolating barrier. All statistical procedures were conducted using the free software package R (R Development Core Team 2009).

Results

Arrival times

Arrival occurred from the middle of March until the end of April. Altogether we found no effect of sex ($P = 0.32$) or age on arrival times ($P = 0.11$) but there was an effect of year or site ($P = 0.04$, ANOVA) on arrival times for the 2 years combined. Therefore we calculated arrival dates separately for each year (and consequently for each site). In both years, arrival dates were not related to age (Wilcoxon rank sum test: in 2006, $W = 2,196.5$, $P = 0.27$; in 2007, $W = 2,477.5$, $P = 0.43$). Birds isotopically assigned to north-western wintering quarters in Britain and Ireland

(hereafter NW) arrived significantly earlier on breeding grounds than birds from south-western Mediterranean areas (hereafter SW; in 2006, $n = 18$ vs. $n = 85$; $W = 451$, $P = 0.01$; in 2007, $n = 9$ vs. $n = 100$; $W = 182.5$, $P = 0.003$; analysis with resighted birds only, $P < 0.01$; Fig. 3a). Only the 2006 sample allowed for testing differences in arrival times separately for the sexes. Males from both subpopulations tended to arrive earlier than the respective females, although there was considerable overlap between subpopulations and between sexes within a subpopulation (Fig. 3b). NW males considerably preceded SW females ($n = 7$ vs. $n = 48$; $W = 73.5$, $P = 0.03$; resighted birds, $P < 0.01$) but not NW females ($n = 7$ vs. $n = 11$; $W = 26$, $P = 0.26$; resighted birds, $P = 0.10$), whereas there was only a marginal effect for NW males preceding SW males ($n = 7$ vs. $n = 37$; $W = 69.5$, $P = 0.07$; resighted birds, $P < 0.05$). SW males did overlap with both NW females ($n = 37$ vs. $n = 11$; $W = 152$, $P = 0.11$; resighted birds, $P = 0.58$) and SW

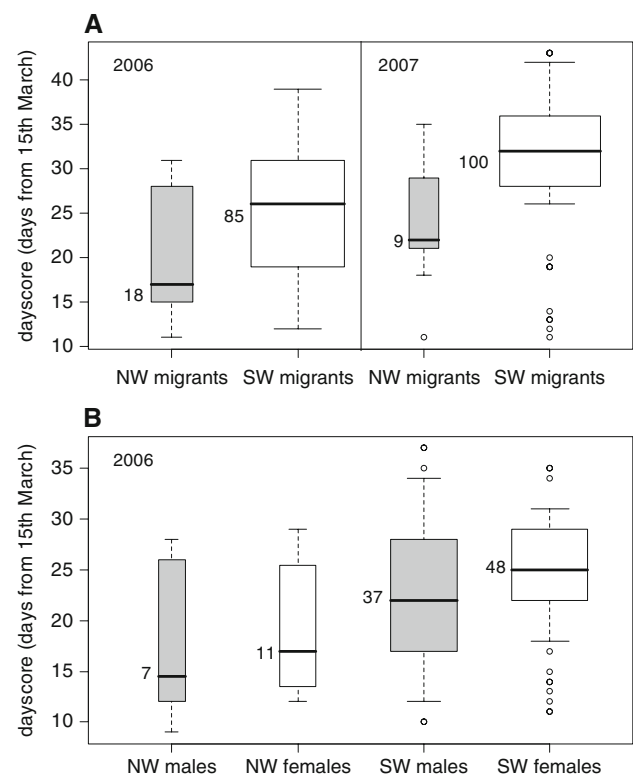


Fig. 3 Comparison of spring arrival times of blackcaps with different migratory strategies assigned via density functions of stable isotope ratios. **a** Overall arrival times. NW migrants precede SW migrants in both years ($P \leq 0.01$). **b** Sex-dependent arrival on the breeding grounds in 2006. NW males tended to precede SW males ($P = 0.07$) and SW females ($P = 0.03$). All other comparisons were non-significant (see text). The widths of the boxes correspond to the respective sample size (boxplots show median, 1st and 3rd quartiles, 10th and 90th quantiles and outliers; sample sizes are shown next to the boxes)

females ($n = 37$ vs. $n = 48$; $W = 746$, $P = 0.11$; resighted birds, $P = 0.27$; Fig. 3b).

Our results remained unchanged when we restricted this analysis to the 10th and 90th quantiles of the isotope distribution. Again, birds migrating from the north-west arrived earlier (Wilcoxon rank sum test in 2006, $n = 11$ vs. $n = 11$, $W = 37.5$, $P = 0.05$; in 2007, $n = 11$ vs. $n = 11$, $W = 16.5$, $P = 0.02$). Due to insufficient sample sizes for the reduced quantiles, we did not run discrete tests for sexes. Correlation analysis also revealed a strong relationship between winter area and spring arrival, as in both years there was a positive correlation between the day score and the individual isotope ratio (Pearson product-moment correlation in 2006, $t = 2.91$, $P < 0.01$, $r = 0.28$; in 2007, $t = 4.04$, $P < 0.001$, $r = 0.36$) providing evidence that birds wintering farther north tended to arrive earlier.

Carry-over effects

Neither time of arrival nor wintering origin affected the body condition of blackcaps upon arrival at the breeding grounds. In both years, we did not find an effect of arrival date (all $P > 0.4$) or wintering origin (all $P > 0.15$) on body mass, body mass divided by tarsus or body mass residuals. Females were constantly heavier upon arrival than males: body mass (ANCOVA; means, female₂₀₀₆ = 17.2 ± 0.16 g SE vs. male₂₀₀₆ = 16.7 ± 0.19 g SE; female₂₀₀₇ = 17.3 ± 0.21 g SE vs. male₂₀₀₇ = 16.5 ± 0.15 g SE, $P < 0.03$) and body mass residuals (means, female₂₀₀₆ = 0.46 ± 0.02 SE vs. male₂₀₀₆ = 0.41 ± 0.02 SE, $P < 0.1$; female₂₀₀₇ = 0.28 ± 0.02 SE vs. male₂₀₀₇ = 0.20 ± 0.02 SE, $P < 0.02$). However, there was no interaction effect for sex \times arrival date or sex \times wintering area, suggesting that birds from the two subpopulations had altogether a similar physical condition upon arrival on the breeding grounds. We corrected for daily fluctuations in body mass by including the time of day a bird was caught in the models. There was a marginal effect of daytime on body mass and body mass residuals in 2007 ($0.08 < P < 0.1$) but no significant interaction of daytime with sex or wintering area, respectively (ANCOVA, daytime \times sex, $P > 0.4$; daytime \times wintering area, $P > 0.6$).

Haematocrit, as an additional index for body condition in 2006, did not differ between birds from different wintering areas either, and was not affected by time (ANCOVA; winter area effect, $F = 0.89$, $P = 0.35$; effect of arrival time, $F = 1.59$, $P = 0.21$; sex effect, $F = 6.90$, $P = 0.01$). Overall, females had higher haematocrit values (mean, 48.61 ± 0.63 SE) than males (mean, 46.07 ± 0.70 SE). Our results remained unchanged when we only analysed the 10th and 90th quantiles of the isotope distribution (ANCOVA all model factors, $P > 0.14$).

Reproductive disposition

There was no difference in the reproductive disposition, measured as CP on arrival, for males coming from distinct winter quarters (ANCOVA; winter area effect, $F = 1.16$, $P = 0.29$). Although there was an overall effect of arrival time on CP ($F = 7.67$, $P = 0.01$), there was no significant interaction effect for winter area and arrival time ($F = 0.07$, $P = 0.79$), suggesting that the reproductive condition of NW males was comparable to that of SW males. In addition, there was no difference in reproductive disposition for the birds from both ends of the distribution (winter area effect, $F = 0.01$, $P = 0.92$; winter area \times arrival time, $F = 2.14$, $P = 0.19$).

Resampling model

In both years, the resampling model yielded a low probability of assortative mating (P) in NW migrants, mainly because they were diluted in the much larger SW migrating population (mean $P_{NW-2006} = 0.28 \pm 1e-03$ SE; mean $P_{NW-2007} = 0.24 \pm 2e-03$ SE; 10,000 resampling runs; Fig. 4). However, the probability for assortative mating in migrants from the south-west was relatively high (mean $P_{SW-2006} = 0.86 \pm 3e-04$ SE; mean $P_{SW-2007} = 0.95 \pm 2e-04$ SE; 10,000 resampling runs; Fig. 4). Consequently there was a high overall probability of assortment (including both SW–SW and NW–NW pairings; mean $P_{overall-2006} = 0.68 \pm 4e-04$ SE; mean $P_{overall-2007} = 0.84 \pm 3e-04$ SE; 10,000 resampling runs).

Discussion

Within a few decades, a fraction of the central European blackcap population has evolved a new migration route to the British Isles, a strategy that is apparently maintained by assortative mating, while most central European blackcaps still winter in the Mediterranean (Bearhop et al. 2005). The rapid evolution of this new migration strategy has been attributed to temporal segregation in arrival times as well as to carry-over effects in physical condition (Berthold et al. 1992). Below we discuss these factors separately.

Spring arrival

We found evidence that central European blackcaps differed significantly in spring arrival depending on the migratory route they followed. This result was obtained in both years and for both sites and was independent of whether we analysed the isotope data as a continuous or discrete variable. This finding was also supported when analysis was restricted to birds originating from both ends

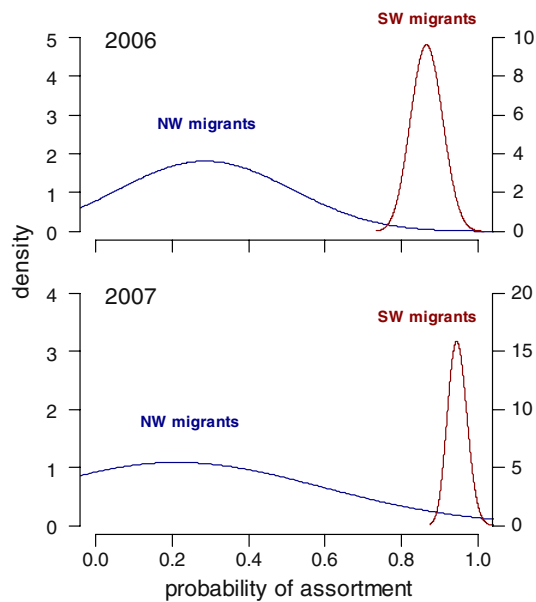


Fig. 4 Probability of assortative pairings on the strength of temporal segregation upon spring arrival of blackcaps. Smoothed density functions (over 10,000 resampling runs) for the proportions of assortative mating for each subpopulation in both years. *Left y-axes* refer to the density of NW migrants, *right y-axes* to the density of SW migrants

of the overall distribution of claw δD values (10th and 90th quantiles, respectively). In all analyses, NW migrants (birds from the lower end of the distribution) preceded SW migrants (birds from the upper end).

NW migrants arriving from the British Isles returned, on average, 10 days earlier to the breeding grounds in southern Germany than their conspecifics arriving from the Mediterranean region. There was also a trend for males preceding females within both the NW and the SW migrants. Our results therefore suggest that wintering areas affect arrival on the breeding grounds, emphasizing the connectivity between both seasonal habitats (Bearhop et al. 2005; Berthold et al. 1992). Our population assignment is based upon isotope analysis of claws, although we did not measure the growth rates of the claws. Interestingly, however, our observed time delay in the arrival pattern of birds from the two subpopulations corresponds to the estimated time lag of 14 days that Terrill and Berthold (1990) calculated. Their estimation was based on the observed shifted start of migratory activity in captive blackcaps combined with the average migration rate for covering the distinct flight distances between groups. However, although our field data corroborate the staggered arrival pattern there was considerable overlap in the arrival time of birds from both subpopulations, as NW males only marginally preceded the SW males, and there was no time difference between NW females and SW males (Fig. 3b).

Allochrony and assortative mating

Given the overlap in arrival times observed in our study, we asked whether allochrony alone is a strong isolating barrier that results in assortative mating. Our mate choice model revealed that assortative mating occurs in over 85% of the SW migrants and in more than two-thirds of the overall population, but assortment among NW migrants is much rarer (probability < 0.3). This discrepancy can be explained by a dilution effect where the relatively small proportion of NW migrants is diluted within the overall population. In other words, even though the birds from the Mediterranean region arrive on average later, some birds from that area returned relatively early. These birds outnumber NW migrating birds and lower the probability of assortative mating, based upon temporal segregation, in NW migrants notably.

Given that the novel migratory direction towards the British Isles has a genetic basis, it must have evolved through rapid microevolution (Berthold et al. 1992). Studies on the mode of inheritance of this behaviour are inconsistent with the spread of a dominant allele or sex-linked inheritance (Helbig 1996; Helbig et al. 1994). Assortative mating appears to be a better explanation for the rapid rise of the new migratory behaviour in blackcaps. Based upon stable isotope analysis, Bearhop et al. (2005) concluded that assortative pairings between NW migrants and SW migrants were 2.5 times more likely than random pairings. So far, the prevailing hypothesis explaining the spread of the novel migratory route has been that temporal segregation upon arrival is sufficient to maintain assortative mating and therefore might constitute an incipient isolating barrier to gene flow (Bearhop et al. 2005; Berthold et al. 1992). Moreover, theory predicts that assortative mating following group formation (i.e. formed in time or space) is more conducive to the evolution of complete assortative mating than assortment based on female preference (Otto et al. 2008). Since the level of selection against the offspring of non-assortative mating is unknown, it should thus be kept in mind that the actual minimal threshold of assortative mating to maintain distinct migratory strategies in sympatry is unknown. Further, although we found a low probability of assortment it might still contribute to ongoing divergence. Based on our arrival time data, however, we suggest that allochrony alone is not a strong isolating barrier and that further factors may contribute to assortative mating.

Carry-over effects

Males wintering in Britain and in the Mediterranean area did not differ in CP, suggesting that both NW and SW males arrived in a similar reproductive state (see Kempenaers et al. 1999; Lombardo 2001). This can be explained, in part,

by common triggering mechanisms because the onset of migratory activity, and therefore arrival times, and gonadal development are triggered by the vernal shift in photoperiod (Gwinner 1996; Terrill and Berthold 1990). Consistent with this conjecture, we found an overall time effect on CP, independent of winter quarters.

Individual blackcaps from both winter quarters arrived on German breeding grounds in equal body condition in terms of body mass, mass-tarsus residuals, mass-tarsus ratio or haematocrit. We point out that these results rely chiefly on condition proxies that include body mass, a parameter subject to short-term fluctuations due to food intake and thermoregulation (Berthold 1971; Clark 1979). However, we controlled to some extent for diurnal fluctuations by integrating the time of day a bird was caught and did not find significant interactions effects challenging our conclusions. Still we found a marginal effect of daytime on condition, suggesting the use of alternative condition estimates in future studies. Again the results remained unaltered even if we tested only 10th and 90th percentiles of the claw δD distribution.

Blackcaps wintering in Britain are known to use feeder resources heavily (Chamberlain et al. 2005) and effects of supplemental winter feeding can be carried over to the subsequent breeding season (Brown and Sherry 2006; Robb et al. 2008). Yet, supplemental winter feeding in Britain and the shorter flight distance did not result in a better condition upon arrival. Yet, migrants from Britain arrived earlier with the same condition on the breeding grounds. If early arrival leads consistently to fitness benefits, migrants from Britain might still gain a selective advantage.

Sex-dependent differences

Females arrived heavier and with higher haematocrit values than males independent of their wintering origin. We found these sex-dependent differences only when all birds were analysed and not for birds pertaining to the 10th and 90th percentile of the δD distribution. This difference may be explained by the smaller sample size involved when only the extremes were considered.

The sex-dependent differences in body condition are consistent with protandrous migration. Males are likely selected for early arrival, whereas females are primarily selected for arriving in good condition (the insurance hypothesis; Sandberg and Moore 1996). Since females invest more in the production of gametes than males, they may derive more benefits than males from arriving in good condition for breeding (Drent et al. 2003; Newton 2006; Smith and Moore 2005).

To conclude, our data confirm the predicted temporal segregation upon spring arrival in blackcaps. However, we

suggest that allochrony alone might not be a strong isolating barrier due to temporal overlap of the two subpopulations. Moreover, we did not observe significant carry-over effects that could be related to ecologically disparate winter habitats. We emphasize that additional proximate factors accounting for the establishment and maintenance of the new migratory divide, such as differences in microhabitat use or sexually selected traits, may provide alternative and stronger isolating mechanisms in the blackcap.

Acknowledgments The authors thank Carlo Catoni, Rebecca Bloch and Miriam Liedvogel for their help in collecting the field data. The discussions with Martin Woywod and Prof. Dr Juergen Schulte-Moenting significantly improved the statistical analyses. We also thank Carlos Martinez del Rio, Mike Wunder and an anonymous reviewer for helpful comments on the manuscript. G. R. and H. M. S. were supported by DFG grants. Stable isotope analyses were supported by Environment Canada.

References

- Bearhop S, Furness RW, Hilton GM, Votier SC, Waldron S (2003) A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material. *Funct Ecol* 17:270–275
- Bearhop S, et al. (2005) Assortative mating as a mechanism for rapid evolution of a migratory divide. *Science* 310:502–504
- Berthold P (1971) Seasonal rhythm of *Sylvia*. *Experientia* 27:399
- Berthold P, Bairlein F (1984) On the return and breeding of a hand raised and released blackcap. *J Ornithol* 125:485–486
- Berthold P, Terrill SB (1988) Migratory behaviour and population growth of blackcaps wintering in Britain and Ireland: some hypotheses. *Ringing Migr* 9:153–159
- Berthold P, Helbig AJ, Mohr G, Querner U (1992) Rapid microevolution of migratory behavior in a wild bird species. *Nature* 360:668–670
- Bize P, Piau R, Moureau B, Heeb P (2006) A UV signal of offspring condition mediates context-dependent parental favouritism. *Proc R Soc B Biol Sci* 273:2063–2068
- Bolnick DI, Fitzpatrick BM (2007) Sympatric speciation: models and empirical evidence. *Annu Rev Ecol Syst* 38:459–487
- Bowen GJ, Wassenaar LI, Hobson KA (2005) Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* 143:337–348
- Brown DR, Sherry TW (2006) Food supply controls the body condition of a migrant bird wintering in the tropics. *Oecologia* 149:22–32
- Carleton SA, Kelly L, Anderson-Sprecher R, del Rio CM (2008) Should we use one-, or multi-compartment models to describe C-13 incorporation into animal tissues? *Rapid Commun Mass Spectrom* 22:3008–3014
- Chamberlain DE et al (2005) Annual and seasonal trends in the use of garden feeders by birds in winter. *Ibis* 147:563–575
- Clark GA (1979) Body weights of birds—review. *Condor* 81:193–202
- Cooley JR, Simon C, Marshall DC (2003) Temporal separation and speciation in periodical cicadas. *Bioscience* 53:151–157
- Coyne JA, Orr HA (2004) *Speciation*. Sinauer, Sunderland
- Cuervo JJ, Moller AP, De Lope F (2007) Haematocrit is weakly related to condition in nestling barn swallows *Hirundo rustica*. *Ibis* 149:128–134
- Dawson A, King VM, Bentley GE, Ball GF (2001) Photoperiodic control of seasonality in birds. *J Biol Rhythms* 16:365–380

- Dieckmann U, Doebeli M, Metz JAJ, Tautz D (2004) Adaptive Speciation. Cambridge University Press, Cambridge
- Drent R, Both C, Green M, Madsen J, Piersma T (2003) Pay-offs and penalties of competing migratory schedules. *Oikos* 103:274–292
- Friesen VL et al (2007) Sympatric speciation by allochrony in a seabird. *Proc Natl Acad Sci USA* 104:18589–18594
- Gavrilets S (2003) Perspective: models of speciation: what have we learned in 40 years? *Evolution* 57:2197–2215
- Gharrett AJ, Smoot C, McGregor AJ, Holmes PB (1988) Genetic-relationships of even-year northwestern Alaskan pink salmon. *Trans Am Fish Soc* 117:536–545
- Gill FB (2007) Bird sex, chapter 14. In: Ornithology, 3rd edn. Freeman, New York
- Gwinner E (1996) Circannual clocks in avian reproduction and migration. *Ibis* 138:47–63
- Hartl DL, Clark AG (2007) Principles of population genetics. Sinauer, Sunderland
- Helbig AJ (1992) Population differentiation of migratory directions in birds—comparison between ringing results and orientation behavior of hand-raised migrants. *Oecologia* 90:483–488
- Helbig AJ (1996) Genetic basis, mode of inheritance and evolutionary changes of migratory directions in palearctic warblers (Aves: Sylviidae). *J Exp Biol* 199:49–55
- Helbig AJ, Berthold P, Mohr G, Querner U (1994) Inheritance of a novel migratory direction in central-European blackcaps. *Naturwissenschaften* 81:184–186
- Hendry AP, Day T (2005) Population structure attributable to reproductive time: isolation by time and adaptation by time. *Mol Ecol* 14:901–916
- Hewson CM et al (2007) Recent changes in bird populations in British broadleaved woodland. *Ibis* 149:14–28
- Hobson KA (2003) Making migratory connections with stable isotopes. In: Berthold P, Gwinner E, Sonnenschein E (eds) Avian migration. Springer, Berlin
- Hobson KA, Bowen GJ, Wassenaar LI, Ferrand Y, Lormee H (2004) Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. *Oecologia* 141:477–488
- Hochachka W, Smith JNM (1991) Determinants and consequences of nestling condition in song sparrows. *J Anim Ecol* 60:995–1008
- Kempnaers B, Congdon B, Boag P, Robertson RJ (1999) Extrapair paternity and egg hatchability in tree swallows: evidence for the genetic compatibility hypothesis? *Behav Ecol* 10:304–311
- Lombardo MP (2001) Individual and seasonal variation in external genitalia of male tree swallows. *Auk* 118:789–795
- Matthysen E (1989) Territorial and nonterritorial settling in juvenile Eurasian nuthatches (*Sitta europaea* L.) in summer. *Auk* 106:560–567
- Mazerolle DF, Hobson KA (2005) Estimating origins of short-distance migrant songbirds in North America: contrasting inferences from hydrogen isotope measurements of feathers, claws, and blood. *Condor* 107:280–288
- Monteiro LR, Furness RW (1998) Speciation through temporal segregation of Madeiran storm petrel (*Oceanodroma castro*) populations in the Azores? *Philos Trans R Soc Lond B Biol Sci* 353:945–953
- Moreno J (1989) Body-mass variation in breeding northern wheatears—a field experiment with supplementary food. *Condor* 91:178–186
- Newton I (2006) Can conditions experienced during migration limit the population levels of birds? *J Ornithol* 147:146–166
- Norris DR, Marra PP, Kyser TK, Sherry TW, Ratcliffe LM (2004) Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. *Proc R Soc Lond B Biol Sci* 271:59–64
- Otto SP, Servedio MR, Nuismer SL (2008) Frequency-dependent selection and the evolution of assortative mating. *Genetics* 179:2091–2112
- Potti J (2007) Variation in the hematocrit of a passerine bird across life stages is mainly of environmental origin. *J Avian Biol* 38:726–730
- Potti J, Moreno J, Merino S, Frias O, Rodriguez R (1999) Environmental and genetic variation in the haematocrit of fledgling pied flycatchers *Ficedula hypoleuca*. *Oecologia* 120:1–8
- Price T (2007) Speciation in birds, 1st edn. Roberts, Greenwood
- Robb GN, McDonald RA, Chamberlain DE, Reynolds SJ, Harrison TJE, Bearhop S (2008) Winter feeding of birds increases productivity in the subsequent breeding season. *Biol Lett* 4:220–223
- Royle JA, Rubenstein DR (2004) The role of species abundance in determining breeding origins of migratory birds with stable isotopes. *Ecol Appl* 14:1780–1788
- Saino N, Szep T, Ambrosini R, Romano M, Moller AP (2004) Ecological conditions during winter affect sexual selection and breeding in a migratory bird. *Proc R Soc Lond B Biol Sci* 271:681–686
- Sandberg R, Moore FR (1996) Fat stores and arrival on the breeding grounds: reproductive consequences for passerine migrants. *Oikos* 77:577–581
- Shirihai H, Gargallo G, Helbig AJ (2001) *Sylvia warblers*. Identification, taxonomy and phylogeography of the genus *Sylvia*. In: Kirwan G, Svensson L (eds) Black, London
- Smith RJ, Moore FR (2005) Fat stores of American redstarts *Setophaga ruticilla* arriving at northerly breeding grounds. *J Avian Biol* 36:117–126
- Svensson E, Merila J (1996) Molt and migratory condition in blue tits: a serological study. *Condor* 98:825–831
- R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Terrill SB, Berthold P (1990) Ecophysiological aspects of rapid population-growth in a novel migratory blackcap (*Sylvia atricapilla*) population—an experimental approach. *Oecologia* 85:266–270
- Wassenaar LI, Hobson KA (2003) Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes Environ Health Stud* 39:211–217
- Wunder MB, Kester CL, Knopf FL, Rye RO (2005) A test of geographic assignment using isotope tracers in feathers of known origin. *Oecologia* 144:607–617