# Assortative Mating as a Mechanism for Rapid Evolution of a Migratory Divide

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**There have been numerous recent observations of changes in the behavior and dynamics of migratory bird populations, but the plasticity of the migratory trait and our inability to track small animals over large distances have hindered investigation of the mechanisms behind migratory change. We used habitat-specific stable isotope signatures to show that recently evolved allopatric wintering populations of European blackcaps *Sylvia atricapilla* pair assortatively on their sympatric breeding grounds. Birds wintering further north also produce larger clutches and fledge more young. These findings describe an important process in the evolution of migratory divides, new migration routes, and wintering quarters. Temporal segregation of breeding is a way in which subpopulations of vertebrates may become isolated in sympatry.**

There have been numerous observations of changes in the behavior and dynamics of migratory bird populations (*1*–*7*). Migration is naturally a plastic trait, and tracking migratory birds is problematic because of the distances and areas involved (*2*, *7*). Thus it has proved almost impossible to investigate the mechanisms behind, and the consequences of, migratory change. However, the recent formation of a migratory divide in a breeding population of European passerines (*1*), together with developments in stable isotope techniques, mean that we can now gain insights into these processes.

Fifty years ago, the blackcap *Sylvia atricapilla* was considered a summer visitor to northern Europe and the British Isles. However, an increasing number of birds began overwintering in Britain and Ireland, in numbers growing from a few individuals in the early 1960s to the point where a recent survey of birdwatchers found that 31% of their gardens had blackcaps visiting bird tables from October 2003 to March 2004 (*8*). Work in the early 1990s showed that the birds overwintering in Britain and Ireland actually bred in south central Europe and that this newly evolved migratory behavior had a genetic basis (*1*, *9*). The normal wintering areas associated with blackcaps breeding in south central Europe (and therefore the ancestral wintering areas for the British and Irish birds) are southern Iberia and North Africa. The new wintering area is disjunct from the original, and birds wintering in Britain and Ireland display a completely different migratory orientation (*1*).

Berthold *et al.* (*1*) argued that the rate of increase in the wintering population suggests that birds wintering in Britain and Ireland gain some fitness benefits by adopting this strategy. They also suggested that because the critical photoperiods that trigger migratory urges and the onset of gonadal development are reached around 10 days earlier at the more northerly wintering latitudes, assortative mating among early arrivals may be an important factor driving the increases in the British and Irish wintering populations (*1*, *2*).

Stable hydrogen isotope ratios $\{\delta^2H = ([^2H/^1H_{sample} \div {}^2H/^1H_{reference}] - 1) \times 1000\}$ and stable carbon isotope ratios ($\delta^{13}C$) in the tissues of migrant birds can be used to infer their breeding or wintering origins (*4*, *10*–*12*). Given the patterns of $\delta^2H$ in European rainfall (Fig. 1) and those measured in the feathers of several bird species (*13*), we hypothesized that measurements of $\delta^2H$ in tissues grown in the two wintering areas (Britain and Ireland or Iberia) may be sufficiently distinct isotopically to infer the wintering origins of blackcaps in the breeding grounds. We measured $\delta^2H$ (*14*–*16*) in proximal sections of claw tips (most recently synthesized) from wintering blackcaps in the two wintering areas (Britain and Ireland or Iberia), as well as from resident birds from Britain and Ireland (blue tits *Parus*

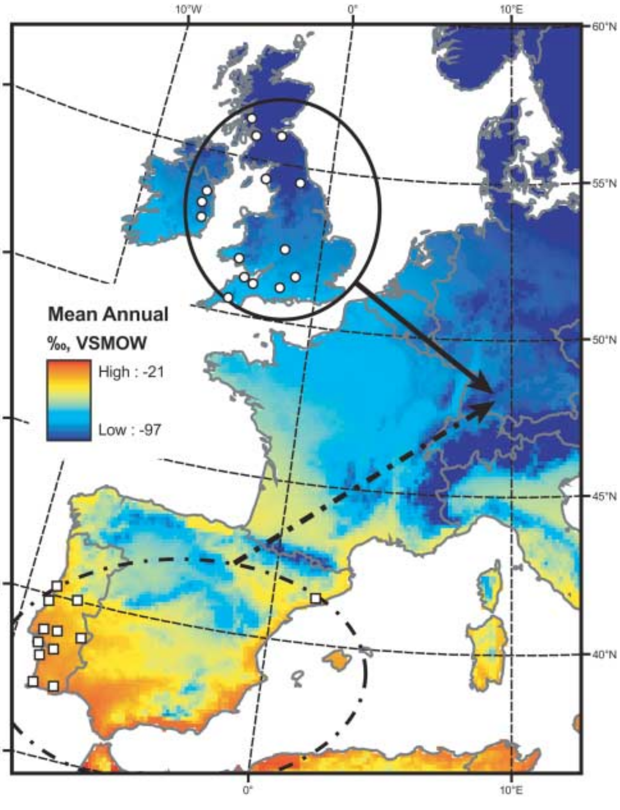

**Fig. 1.** Mean annual $\delta^2H$ in Western European rainfall and locations of blackcap winter sampling sites (white circles and squares). Map values were derived using a detrended interpolation model (*26*) and the data of the Global Network for Isotopes in Precipitation (*27*). A clear separation of $\delta^2H$ values over northern (British Isles) and southern (Iberia) blackcap wintering regions provides the basis for distinguishing the wintering area of birds sampled at summer breeding grounds. The international standard for $\delta^2H$ is Vienna standard mean ocean water (VSMOW). $\delta^2H$ values at more northerly latitudes (including the British Isles) are considerably lower than those in the south. Ellipses indicate the approximate wintering ranges of migratory blackcaps from the German breeding areas in Britain and Ireland (solid circle) and Spain and Portugal (dashed circle). Although blackcaps do occur in France during the winter, they are not thought to come from the central European breeding population. The arrows represent approximate spring migration directions.

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*caeruleus* and great tits *P. major*) and from resident birds from Portugal and Spain (Sardinian warblers *S. melanocaphala*) to provide two independent measures of over-winter isotope signatures.

Having sampled birds at multiple sites (Fig. 1) over two winters (December to March 2001–2002 and 2002–2003), we found that the $\delta^2$H values measured in the claws of residents from Britain and Ireland were significantly lower than those in resident birds from Portugal and Spain (Fig. 2A). $\delta^2$H signatures in the claws of overwintering blackcaps showed a similar pattern (Fig. 2B), with northerly wintering blackcaps having lower $\delta^2$H signatures than those from much further south (*14*). $\delta^2$H signatures in the tissues of birds are primarily a function of the signature in local rainfall (assimilated through the diet), with little or no additional species-level effects (*13*), and the patterns in both wintering blackcaps and residents correspond to those in precipitation (Figs. 1 and 2). Indeed, there was almost no overlap in values (Fig. 2A) among residents, meaning that this isotope ratio provided an excellent marker for discriminating between birds from these two regions (*14*). Because claws are metabolically inert and grow very slowly (*17*), measurement of $\delta^2$H in the oldest claw material (the distal portion of the claw tip) collected from birds soon after they return to the breeding grounds (*17*) should allow us to infer the relative wintering latitudes of individuals.

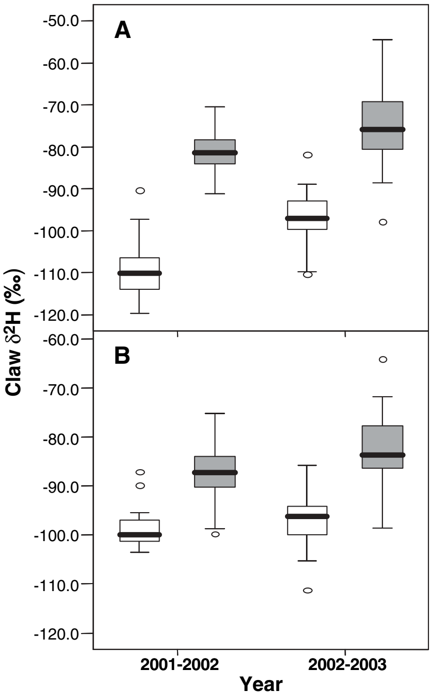

**Fig. 2.** Box plots (median, interquartile ranges, and outliers) for claws of birds captured during the winters of 2001–2002 and 2002–2003 at sites throughout Portugal (solid boxes) and the British Isles (open boxes). Data for resident birds are shown in (A) and for blackcaps in (B). The results of general linear models (GLMs) in which claw $\delta^2$H was the dependent variable, with year and status [four levels: Portuguese resident ($n$ = 63 birds), Portuguese blackcap ($n$ = 120), British Isles resident ($n$ = 43), and British Isles blackcap ($n$ = 42)] as factors, demonstrated that there was significant annual variation (residents only: $F_{1,264}$ = 52.6, $P$ < 0.001). This model was well supported (adjusted $r^2$ = 0.63); however, most of the variation is explained by status ($F_{3,264}$ = 147.4, $P$ < 0.001). (**A**) Scheffe tests indicated highly significant differences between the signatures of resident birds from Britain and Ireland and those in Portugal [mean difference = –23 per mil (‰), $P$ < 0.001]. (**B**) Scheffe tests also demonstrated that $\delta^2$H signatures in the claws of wintering blackcaps differ significantly from those of residents in the same country in 2001–2002 (Portugal mean difference –5.4‰, $P$ = 0.002; Britain and Ireland mean difference –6.0‰, $P$ = 0.001), This is probably due to a small amount of the claw sample being synthesized away from the wintering areas. However, the difference between blackcaps wintering in either region was over double this (mean difference –12.7‰, $P$ < 0.001).

During the summers of 2002 and 2003, breeding blackcaps were captured at eight sites in southern Germany and Austria (*14*). Birds were caught as soon as possible after establishment of their breeding territories. Where possible, both birds of a pair were captured, and in all cases the subsequent breeding attempt was followed. We found a highly significant relation between $\delta^2$H signatures in the claws of males and those in the females they were paired with (Fig. 3). To provide a direct estimate of the incidence of assortative mating, wintering area–specific reciprocal-normal probability density functions were modeled by smoothing the empirical distributions from winter populations, and these were used to assign a probability of origin to each breeding bird (*14*). We found that observed values for assortative pairings were significantly higher than expected ($\chi^2$ = 40.1, $P \ll 0.001$, Yates correction for 1 degree of freedom applied). Birds were 2.5 times more likely to pair assortatively than randomly (*14*), with "relative risks" for assortative pairing being 2.63 [95% confidence interval (CI) 1.3 to 28.5] for Iberian pairs and 2.63 (95% CI 1.2 to 5.8) for British and Irish pairs. This provides the strongest evidence yet for assortative mating (*1*, *9*).

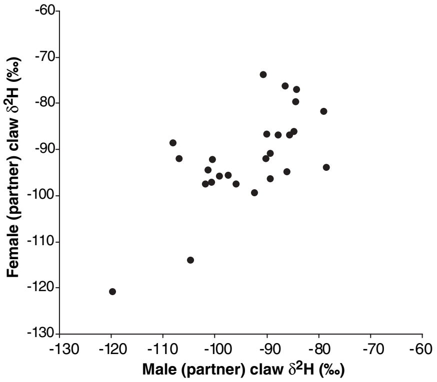

**Fig. 3.** There is a highly significant relation between $\delta^2$H signatures in the claws of male blackcaps and in those of the female they are paired with (GLM: $F_{1,30}$ = 28.719, $P$ < 0.001, $r^2$ = 0.5, year NS). This relation remains significant even when the two points with the lowest $y$ values are ignored ($F_{1,28}$ =12.2, $P$ = 0.002, $r^2$ = 0.3).

In 2003, the time of arrival on the breeding grounds was estimated for a subsample of blackcap males, and this was positively correlated with relative wintering latitude inferred from claw $\delta^2$H signatures (Fig. 4), indicating that British and Irish males tend to arrive earlier than their southern European counterparts (Fig. 4). This is consistent with the hypothesis that earlier onset of the critical photoperiods required to stimulate migratory restlessness and gonadal development at higher wintering latitudes drives assortative mating with respect to wintering area (*1*, *2*). Assortative mating in this blackcap population could be further favored by strong selection against the offspring of hybrid pairings, because they inherit migration directions and distances intermediate to those of their parents, which would lead them on flights that would cross out over the Bay of Biscay: routes that tend not to be observed in orientation experiments with adults (*1*, *9*).

Finally, we investigated whether we could detect any potential fitness benefits associated with the new migration route. We found that females paired with males having lower $\delta^2$H signatures, indicating higher wintering latitudes, produced significantly larger clutches [multiple logistic regression: Wald = 7.02, $P$ = 0.008, egg-laying date not significant (NS), $n$ = 38 males] (*14*). These results seem likely to be a consequence of male territory quality. Because differences in arrival times (Fig. 4) mean that males from more northerly wintering areas have more opportunity to settle in high-quality territories, the effect could arise in one of two ways: First, females on high-quality territories may enhance their body condition because of better foraging opportunities. It has been noted in a number of territorial passerine species that one of the most important aspects of territory selection is food availability (*18*), and provisioning experiments have shown that females can respond to high levels of food availability by producing larger clutches (*19*). Second, high-quality females may choose to settle in high-quality territories. Although there are studies demonstrating that male territory quality can influence clutch size (*18*), it is often extremely difficult to distinguish the effect of intrinsic female quality from the extrinsic quality of the territory she has selected (*18*).

We also found that females with lower $\delta^2$H signatures, (indicating more northerly wintering areas) were more likely to have a success-

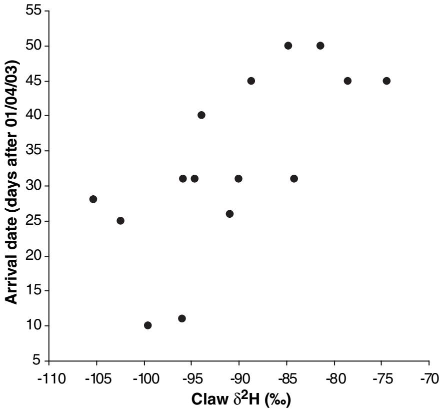

**Fig. 4.** There is a significant relation between estimated arrival dates (in 2003 only) of males and the $\delta^2H$ signatures in their claws ($F_{1,14}$ = 11.2, $P$ = 0.005, $r^2$ = 0.44), suggesting that birds from further north arrive earlier. Three birds that were first detected more than 60 days after the first of April were excluded from these analyses because we could not be certain that these observations represented their first breeding attempt.

ful breeding attempt than those from further south (multiple logistic regression: Wald = 5.88, $P$ = 0.015, clutch size and lay date NS, $n$ = 48 females) (*14*). Although incubation and brood-rearing duties are shared, this finding could also be an effect of female body condition or intrinsic quality.

There are several other ways in which birds wintering at higher latitudes may benefit over southern conspecifics. Blackcaps breeding in Central Europe that migrate to the Iberian Peninsula have much further to travel than those wintering in Britain and Ireland. Thus, birds that come from the northern wintering areas may have more resources to devote to their reproductive attempt (*1*), which can enhance offspring viability (*20*, *21*). Changes in urbanization patterns in Britain and Ireland have led to good feeding opportunities for several bird species in the form of garden feeders and planting of exotic plants that fruit year round (*1*). Although blackcaps do use these food resources heavily during winter months, their impact on migratory condition is yet to be quantified. It has also been suggested that the harsher conditions experienced by blackcaps at high wintering latitudes (when compared to Iberia) may make them better able to survive early-season conditions on the breeding grounds (*2*).

It is becoming increasingly clear that events outside of the breeding season have major impacts on the fitness of migratory birds (*7*, *12*, *22*), and our results provide further evidence of this. However, in contrast to other studies which do not distinguish among the roles of genetic variability or phenotypic plasticity, our results are primarily a consequence of a genetically controlled change in behavior (*1*). In addition, this blackcap population provides a rare example of assortative mating being driven by changes in the timing of breeding, and the stable isotope techniques used here are probably the only way in which this could be estimated at this point in time.

Speciation through temporal segregation of breeding populations has been proposed as a mechanism for differences in Madeiran storm petrel *Oceanodroma castro* populations (*23*). Although we have no evidence for morphometric or genetic differences between the two types of migratory blackcaps in our study, it has been shown that there are genetic differences among different migratory blackcap populations (*24*). Thus, our findings provide compelling support for a mechanism by which sympatric vertebrate populations may become isolated and thus how divergence and ultimately sympatric speciation could occur. Our findings also have implications for our understanding of migratory connectivity, indicating that higher levels of connectivity may influence adaptation to the breeding areas and lead to speciation (*25*).

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Materials and Methods
References

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