Rapid Advance of Spring Arrival Dates in Long-Distance Migratory Birds

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Several bird species have advanced the timing of their spring migration in response to recent climate change. European short-distance migrants, wintering in temperate areas, have been assumed to be more affected by change in the European climate than long-distance migrants wintering in the tropics. However, we show that long-distance migrants have advanced their spring arrival in Scandinavia more than short-distance migrants. By analyzing a long-term data set from southern Italy, we show that long-distance migrants also pass through the Mediterranean region earlier. We argue that this may reflect a climate-driven evolutionary change in the timing of spring migration.

Many biological processes are affected by climate, and in temperate areas the increasing spring temperature over the past 20 to 30 years has caused an advancement of phenological events in plants and invertebrates (*1*, *2*). The earlier onset of spring has consequences for the timing of breeding in birds, which has evolved to match peak food availability (*3*, *4*). We may therefore expect the timing of breeding to track any temporal shift in food availability caused by a trend in spring temperature (*5*). Most passerine birds breeding in temperate areas of the Northern Hemisphere are seasonal migrants, and the timing of migration ultimately constrains when breeding can start (*6*, *7*). Short-distance migrants, spending the winter close to the breeding grounds, may be able to adjust the timing of migration in response to local climate change, which will be correlated to the conditions on the breeding grounds. In tropical-wintering long-distance migrants, the timing of migration is under endogenous control (*8*, *9*), and the cues needed to trigger the onset of migration are unlikely to be linked to the climate on their breeding grounds. Therefore, it has been assumed that short-distance migrants are more likely than long-distance migrants to vary migration timing in response to climate change (*10*). Here we show that such an assumption is not empirically justified.

We estimated trends in arrival time for the early, middle, and late phases of migration (that is, the species- and site-specific 10th, 50th, and 90th percentiles of the spring arrival distribution) in short- and long-distance passerine migrants, based on long-term banding and observational data (from 1980 to 2004) from four bird observatories in Scandinavia and a site in southern Italy (*11*, Fig. 1). We also investigated whether year-to-year variation in arrival time can be explained by short-term climate variability as measured by the North Atlantic Oscillation (NAO) (*12*). As explanatory variables we used the calendar year (TIME) and the deviations from linear regression of the winter NAO index on year [dNAO; the trend in NAO was weakly negative over this time period (*11*)]. Spring migration might advance for two distinct reasons. First, there can be a microevolutionary (genetic) response to the selection pressures for earlier breeding. Second, the migrants can show a phenotypically plastic response to trends in weather or climatic patterns on the wintering ground and/or along the migration route, whereby if spring arrives early on the wintering grounds, spring migration will also start early. Thus, a response to TIME may reflect either microevolutionary change or phenotypic plasticity, whereas a response to dNAO indicates exclusively phenotypic plasticity in the migratory behavior.

Long-distance migrants have advanced their arrival in northern Europe in all phases of migration (Fig. 2 and tables S1 to S3). The advancement in long-distance migrants is strongest in the early phase of migration, and there is limited variation between species. Furthermore, the analysis of the data set from Italy (from the island of Capri) showed that long-distance migrants wintering south of the Sahara desert are actually arriving in southern Europe progressively earlier. In fact, all of the nine species analyzed show a trend for earlier spring arrival at Capri in most phases of migration (Fig. 2 and table S4). The long-term trend on Capri is at least as strong as that observed in Scandinavia (Fig. 3). In short-distance migrants, instead, we find only a weak trend toward earlier arrival, and there is considerable variation between species (Fig. 2 and tables S1 to S3).

In accordance with previous findings (*13*–*15*), a high NAO index is associated with the early arrival of short-distance migrants in Scandinavia, but only in the early phase of migration (Fig. 2).

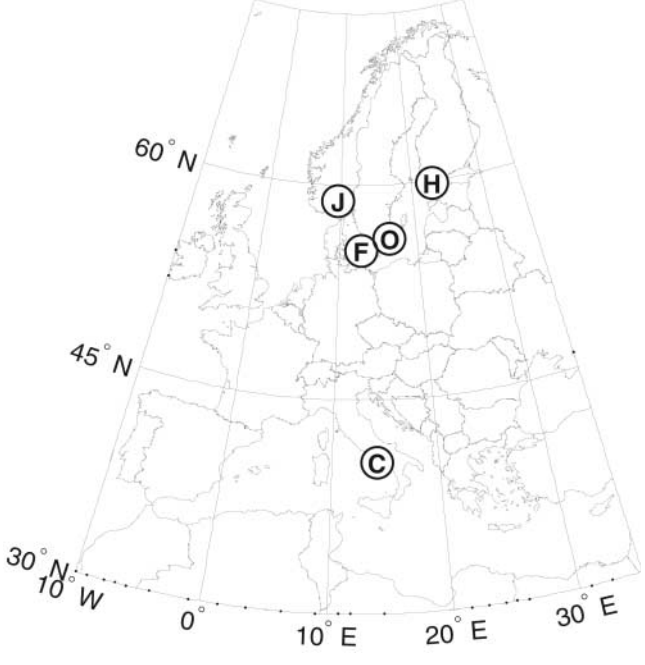

Fig. 1. The locations of the four bird observatories (F, Falsterbo, 55°23′N, 12°49′E; O, Ottenby, 56°12′N, 16°24′E; J, Jomfruland, 58°53′N, 9°37′E; H, Hanko, 59°48′N, 22°53′E) and of the banding site on Capri (C, 40°33′N, 14°15′E).

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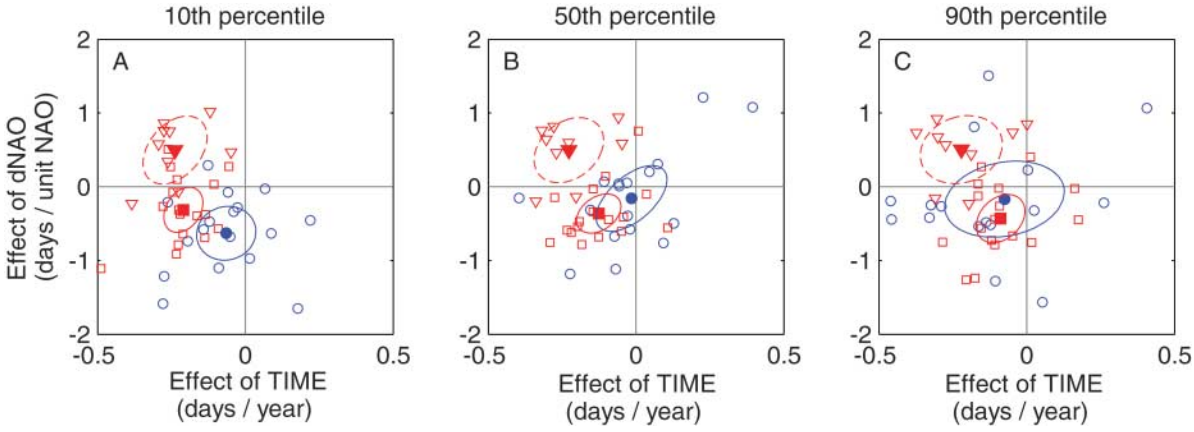

Fig. 2. Long-term trend (TIME) and the effect of short-term climatic fluctuations (dNAO) on the early [(**A**), 10th percentile], middle [(**B**), 50th percentile], and late [(**C**), 90th percentile] phases of the spring arrival distribution in short-distance migrants (blue circles) and long-distance migrants (red squares) in Scandinavia and on Capri (red triangles). The solid symbols are sample averages, and the ellipses delimit their 95% confidence regions (*11*). Estimates for each species are given in tables S1 to S4. The differences in effect size for early-phase arrival of short-distance migrants versus long-distance migrants was 0.15 [95% confidence interval (CI): 0.06 to 0.23] days per year for the effect of TIME.

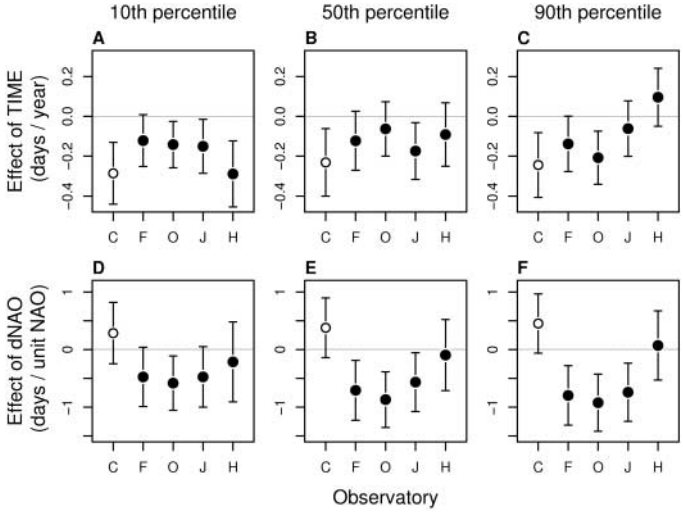

Fig. 3. Mean slopes and 95% CIs of the relationships between arrival dates and TIME (**A** to **C**) or dNAO (**D** to **F**) in the early (10th percentile), middle (50th percentile), and late (90th percentile) phases of migration for six species of long-distance migrants for which time series were available at each observatory (solid symbols; F, Falsterbo; O, Ottenby; J, Jomfruland; H, Hanko) and at the banding site on Capri (open symbol; C). The sites are sorted from south to north. Species-specific slopes were estimated with a mixed-effect linear model (*11*). The correlation between species, when species- and observatory-specific effects of both TIME and dNAO were accounted for, was estimated at 0.48 (95% CI: 0.17 to 0.75), 0.25 (95% CI: 0.04 to 0.51), and 0.34 (95% CI: 0.03 to 0.68) for the 10th, 50th, and 90th percentiles, respectively.

On the other hand, most long-distance migrants tend to arrive earlier in Scandinavia during years of high NAO in all phases of migration (Figs. 2 and 3 and tables S1 to S3). The opposite pattern is observed at Capri, where high NAO tends to delay arrival times (Figs. 2 and 3 and data in table S4). The underlying reason for this may be found south of the Sahara desert, because a high NAO index harms productivity over vast areas of northwestern and southeastern Africa (*16*), which may delay the spring departure of migrants from sub-Saharan wintering areas.

By showing that long-distance migrants have advanced their migration more than short-distance migrants, we have challenged the conventional wisdom that species wintering in temperate Europe should respond more strongly to climate change than trans-Saharan migrants (*10*). Furthermore, the earlier arrival of trans-Saharan migrants at Capri shows that the temporal trend for earlier arrival in Scandinavia cannot be explained simply by faster migration through Europe in response to a concomitant trend of increasing temperatures taking place within continental Europe (*17*). Instead it suggests that (i) the onset of migration has advanced, or (ii) the speed of migration through Africa has increased. Both alternatives could be seen as phenotypic responses to trends in the African climate patterns having a positive effect on the foraging conditions (*18*), thereby improving the birds' physical conditions, which in turn affects their timing of migration (*19*) and makes the migration (including flight and stopover) more efficient. A positive trend in African temperatures (*20*) has previously been suggested as a reason why long-distance migrants arrive earlier in northern Europe (*21*). However, increasing African temperatures should decrease productivity (*22*), thereby delaying long-distance migrants' departure from the wintering ground. Hence, the earlier arrival is probably not a phenotypic response to improved foraging conditions. More likely, the rapid advance in arrival dates of long-distance migrants in Europe is due to climate-driven evolutionary changes in the timing of spring migration. Even though migratory activity is under endogenous control, experiments have demonstrated individual variation in the response to the photoperiodic cues needed to trigger the mechanisms underlying the onset of migration (*23*). The passerine birds investigated here reproduce at just 1 year of age and thus have the potential for a rapid evolutionary response to environmental changes. Given the considerable heritable genetic variation in the timing of migration (*24*, *25*) and the selection pressure to breed earlier in Europe (*6*, *7*), a change toward earlier arrival is indeed to be expected.

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Supporting Online Material
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Tables S1 to S5
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Intron Removal Requires Proofreading of U2AF/3′ Splice Site Recognition by DEK

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Discrimination between splice sites and similar, nonsplice sequences is essential for correct intron removal and messenger RNA formation in eukaryotes. The 65- and 35-kD subunits of the splicing factor U2AF, U2AF^{65} and U2AF^{35}, recognize, respectively, the pyrimidine-rich tract and the conserved terminal AG present at metazoan 3′ splice sites. We report that DEK, a chromatin- and RNA-associated protein mutated or overexpressed in certain cancers, enforces 3′ splice site discrimination by U2AF. DEK phosphorylated at serines 19 and 32 associates with U2AF^{35}, facilitates the U2AF^{35}-AG interaction and prevents binding of U2AF^{65} to pyrimidine tracts not followed by AG. DEK and its phosphorylation are required for intron removal, but not for splicing complex assembly, which indicates that proofreading of early 3′ splice site recognition influences catalytic activation of the spliceosome.

A minimal U2AF heterodimer consisting of RNA recognition motifs (RRM) 1 and 2 of U2AF^{65} (*1*) and the U2AF homology motif (UHM or ΨRRM) of U2AF^{35} (*2*) was analyzed by nuclear magnetic resonance (NMR) spectroscopy in the absence or presence of an RNA containing a pyrimidine tract followed by a consensus 3′ splice site (3′ss) [5′ $(U)_{13}$ACAGG 3′]. As expected from the affinity of U2AF^{65} for uridine-rich sequences (*1*), the presence of the RNA caused extensive changes in the NMR spectrum of the U2AF^{65} RRM 1+2 subunit (Fig. 1A, left). In contrast, small perturbations concerning few residues were observed in the U2AF^{35} ΨRRM spectrum (right). The latter was unexpected, because previous observations suggested that U2AF^{35} specifically recognizes the 3′ss-AG (*3*–*5*). Gel retardation assays using ^{32}P-uridine–labeled RNAs [5′ GGG$(U)_{13}$AC-AG/CG-GUAAAAUAACUCA 3′] showed that, although U2AF^{35} ΨRRM increases the affinity of the complex threefold, the effect is similar for AG-, CG-, UG- or AA-3′ss, strong or weaker pyrimidine tracts (Fig. 1B and figs. S1 and S2). Lack of AG discrimination was also observed when different assays and recombinant full-length U2AF heterodimer or U2AF purified from HeLa cells were utilized (Figs. 1, C and D). In contrast, both endogenous U2AF and the minimal heterodimer showed preferential ultraviolet (UV) light–induced photo–cross-linking of U2AF^{65} to AG-3′ss RNAs in nuclear extracts (Fig. 1E). Reconstitution of U2AF-depleted extracts with recombinant U2AF subunits indicated that U2AF^{35} is required for AG discrimination (Fig. 1E, bottom). The presence of U2AF^{35} and other components of the nuclear extract decreased cross-linking of U2AF^{65} to the nonconsensus CG-3′ss, which suggests the existence of a proofreading activity that enforces specific association of U2AF with pyrimidine tracts followed by consensus AG-3′ss.

This activity cofractionated with U2AF during the two first chromatographic steps of U2AF purification (*6*) (fig. S3). In fig. 2A, compare lanes 3 and 4 with 7 and 8 for the U2AF-containing complex (identified in lane 2 by supershift with antibodies against U2AF^{65}). The activity was, however, separated from U2AF on the next chromatographic step [poly(U)-Sepharose]; whereas U2AF was retained in the column (*6*), the flow-through fraction provided AG versus CG discrimination to the truncated heterodimer in both UV-mediated cross-linking (Fig. 2B) and gel-retardation assays (fig. S4). The activity present in this fraction was retained on an affinity column containing the truncated U2AF heterodimer (Fig. 2D, lanes 1 to 4). Comparison of the protein profiles of the input and flow-through fractions revealed that a 50-kD protein was retained in the U2AF column (Fig. 2C, lower component of the 50-kD doublet). Mass spectrometry analyses identified this protein as DEK, a chromatin-, pre–mRNA- and mRNA-associated protein overexpressed or mutated in certain cancers (*7*, *8*). Consistent with a role for DEK in providing AG discrimination to U2AF, depletion of DEK from HeLa nuclear extracts (fig. S5) resulted in reduced AG versus CG discrimination by endogenous U2AF^{65} (Fig. 2E, lanes 1 to 4), an effect that was reversed when recombinant purified DEK was added to the depleted extracts (lane 5). Cross-linking between U2AF^{35} and an RNA radioactively labeled at the 3′ss dinucleotide (A-[^{32}P]-G or C-[^{32}P]-G) was reduced in DEK-depleted extracts, which indicated that DEK is required for 3′ss recognition by U2AF^{35} (Fig. 2F). Collectively, the results described above indicate that DEK provides a proofreading function that allows U2AF to discriminate between bona fide AG-containing and nonconsensus 3′ss regions.

DEK retention in U2AF affinity columns suggested the possibility of an interaction between these factors. Pull-down experiments using in vitro translated, ^{35}S-labeled U2AF^{65} or U2AF^{35} and recombinant purified glutathione *S*-transferase (GST)-DEK revealed formation of a complex between DEK and U2AF^{35}, which was, at least in part, RNA-independent and involved the 100 amino-terminal residues of DEK (Fig. 3, A and B). Interestingly, the interaction was disrupted by phosphatase treatment (Fig. 3B, lanes 3 versus 4 and 11 versus 12), which suggests the requirement for protein phosphorylation. Indeed, DEK is a phospho-

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