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Programmed and flexible: long-term *Zugunruhe* data highlight the many axes of variation in avian migratory behaviour

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ABSTRACT

Studies of *Zugunruhe*—the "migratory restlessness" behaviour of captive birds—have been integral to our understanding of animal migration, revealing an inherited propensity to migrate and an endogenous timing and navigation system. However, differences between *Zugunruhe* in captivity and migration in the wild call for more data, in particular on variation within and among taxa with diverse migration strategies. Here, we characterise *Zugunruhe* in a long-term dataset of activity profiles from stonechats (genus *Saxicola*) with diverse migratory phenotypes (976 migration periods from 414 birds), using a flexible and consistent quantitative approach based on changepoint analysis. For East African, Austrian, Irish, and Siberian stonechats and hybrids, we report key inter-population differences in the occurrence, timing, and intensity of *Zugunruhe*. In line with expectations, we found the highest *Zugunruhe* intensity in the longest-distance migrants, more variable patterns in short-distance migrants, and intermediate characteristics of hybrids relative to their parental groups. Inter-population differences imply high evolutionary lability of *Zugunruhe* timing within a robustly structured annual cycle. However, counter to theory, Irish partial migrants showed no segregation between migrant and resident individuals, and previously reported nocturnal restlessness was confirmed for resident African stonechats. Further features of nocturnal restlessness that did not align with migratory behaviour of stonechats were juvenile nocturnal restlessness even prior to postjuvenile moult, and protandry in spring, although stonechats winter in heterosexual pairs. Importantly, *Zugunruhe* of all populations declined with age, and

the intensity of an individual bird's *Zugunruhe* was correlated with activity levels during other parts of the annual cycle. Our results confirm endogenous, population-specific migration programmes, but also reveal apparent discrepancies between *Zugunruhe* and migration in the wild. We thus highlight both the continued potential of *Zugunruhe* study and the need for circumspect interpretation when using migratory restlessness to make inferences about migration in the wild.

Keywords: migration, *Zugunruhe*, changepoint analysis, age, activity, annual cycle, stonechat

Main Text

Introduction

The phenomenon of bird migration, particularly regular biannual movements, has captured human interest throughout recorded history (Alerstam 1990, 2011). How do birds know when to depart, which direction to fly, when to stop, and when to return? Given that migration takes place in mid-air and often spans continents, answering these questions based solely on observations of free-living birds has been intrinsically difficult (Birkhead 2008). Although new tracking technologies are beginning to overcome this challenge, they are limited in their potential to answer fundamental questions because each bird's journey is a unique experience under a particular suite of environmental conditions (e.g. Delmore and Irwin 2014, Bäckman et al. 2016). Much of our understanding of the regulation of migration therefore continues to be based on studies of songbirds in captivity. These studies leverage the fact that many nocturnally migrating species spontaneously modify their activity patterns during the migration seasons of wild conspecifics, even when kept under constant conditions in captivity. Instead of their usual rest at night, captive migratory birds extend their activities after sunset, flying, hopping and whirring their wings often until the morning (e.g. Berthold 2001, Birkhead 2008). This behaviour, called migratory restlessness, or *Zugunruhe*, has been extensively used as a proxy for studying migration. In general, intensity and/or duration of *Zugunruhe* increases with the "migratoriness" of a population, measured for example by the distance covered by wild migrants or by the proportion of individuals that migrate. The behavioural phenomenon of migratory restlessness is not restricted to birds and has also been characterised in, for example, fish and insects (e.g., Leverton 1997, Mouritsen and Frost 2002, Sudo and Tsukamoto 2015).

However, the comparability between *Zugunruhe* and actual migration in the wild is not always clear (Farner 1955, Helms 1963, Berthold 1988ab, Newton 2008). For example, resident populations of otherwise migratory species can show apparent *Zugunruhe* (Chan 2005, Helm and Gwinner 2006), and juvenile birds of some migratory species display nocturnal restlessness well in advance of actual migration (Mukhin 1999). Migratory songbirds in captivity often extend *Zugunruhe* far beyond the seasonally appropriate migration time window (e.g. summer restlessness, Gwinner and Czeschlik 1978) and are often restless every night, whereas wild birds typically migrate for single nights and then refuel at stopover sites (Bäckman et al. 2016 compared to Gwinner and Biebach 1977). In light of these discrepancies, there is a need for more information on the relationship between *Zugunruhe* and diverse migratory phenotypes in the wild, and on the factors associated with variation in *Zugunruhe* within and between populations. Here, we investigate a long-term nocturnal activity dataset from several taxa of the stonechat complex (genus *Saxicola*), providing new insight into the variation and regulation of this migratory trait. Furthermore, comparison of *Zugunruhe* across studies and taxa has been hampered by heterogeneous and sometimes subjective quantifications. To address this issue, we propose a single quantitative approach to analyse nocturnal activity data in the phenotypically diverse stonechat system.

Stonechats are a well-studied taxon in avian biology (e.g., Gwinner et al. 1983, Wikelski et al. 2003, Illera et al. 2008, Helm 2009, Zink et al. 2009, Versteegh et al. 2014). These widespread palearctic songbirds breed across an unusually extensive latitudinal range, from c. 70º N in Siberia to 30º S in South Africa (Figure 1A, Urquhart 2002). Within this range, they show large variation in morphology, physiology, and life history, including a broad spectrum of migratory behaviours from resident to long-distance migrant. Therefore, studies on stonechats offer opportunities to examine variation in these traits within a single species complex, with the benefit of reduced cross-species comparative noise. Extensive studies in the field and laboratory have compared stonechats from various environments with different migratory phenotypes. The least migratory population in our dataset comprises residents from East Africa (Kenya), followed by partial migrants from the British Isles (Ireland), obligate short-distance migrants from Central Europe (Austria), and the most migratory population, long-distance migrants from West Siberia (Kazakhstan) (Helm 2009).

Stonechats included in this study were raised under common garden conditions in decades-long captive breeding studies, which collected data on a broad range of traits. To examine patterns of inheritance, birds were selectively bred within and crossbred between

populations (Helm et al. 2005, Helm 2009). Key findings demonstrated a high degree of inheritance of annual cycle organization and physiological traits (e.g., Wikelski et al. 2003, Helm 2009, Tieleman et al. 2009, Versteegh et al. 2014). Stonechats generally displayed distinct population-specific phenotypes, even under common-garden conditions, and F1 hybrids mostly exhibited intermediate trait values (Gwinner 1996, Helm et al. 2009, Tieleman et al. 2009, Versteegh et al. 2014). Variation in many traits was associated with migratoriness, including dimensions of wing (Baldwin et al. 2010) and brain (Fuchs et al. 2014).

Nonetheless, the strengths of the stonechat system for advancing migration research have hardly been fully played out (Zink 2011, Ketterson et al. 2015), and analyses of nocturnal activity profiles collected within the experimental breeding scheme have only been touched upon (Helm and Gwinner 2005, 2006, Helm et al. 2005, Helm 2006). One striking finding was that resident Kenyan stonechats display *Zugunruhe*: lower in intensity compared to short-distant migrants from Austria, but with similar timing characteristics (Figure 1B, Helm and Gwinner 2006). Here, we present a more complete analysis of *Zugunruhe* in stonechats. Our long-term data, which for many birds started soon after fledging, enable us to compare population-specific programmes and address open questions in *Zugunruhe* research: Do *Zugunruhe* patterns remain consistent as birds age? Are differences between spring and autumn migration seasons consistent among migratory species (e.g., *Zugunruhe* timing and intensity, and effects of age and sex)? Are birds that display high *Zugunruhe* generally more active birds, or is migratory activity a completely independent activity trait?

In our efforts to answer these questions, our first objective is to develop robust analysis methods for *Zugunruhe* data. When looking at nocturnal activity data, researchers are often faced with noisy time series that contain putative migration-related signals whose clarity varies among species and individuals. In particular, it has been challenging for existing methods to simultaneously:

a) Determine the presence or absence of *Zugunruhe* in the nocturnal activity profile of a bird. In many studies, some proportion of birds may show very little or inconsistent bouts of nocturnal activity. Depending on study-specific criteria, records of these birds could get dropped completely from the study, remain included in population measures of "migratoriness" (e.g., for average *Zugunruhe* profiles; Berthold 1988a), or be assigned the status of "non-migrants" (e.g., in studies of partial migrant populations).
b) Define the onset, completion and duration of the *Zugunruhe* period. Nocturnal activity frequently occurs outside of the migration period of wild conspecifics. *Zugunruhe* has

often been distinguished from other nocturnal activity by thresholds and cut-off practices based on informed guesses or varying subsidiary criteria (e.g., occurrence of moult) that are not always comprehensively reported.

c) Assess the consistency of *Zugunruhe* estimates when measured over more than one migration period. Data from individuals are often presented for a single migration period, leaving open whether the behaviour is a stable, age-independent trait—expected, for example, for some genetic studies (Berthold 1988a).

d) Distinguish inter-individual differences in *Zugunruhe* from differences in activity that may be unrelated to migration. Many studies have quantified activity only for seasonal time windows designated as migration periods, and during the night but not the day. While it is likely that inter-individual variation in nocturnal activity during the migration season correlates with variation in migratory disposition, alternative explanations are possible and should be accounted for. For example, such variation may be explained by individual differences in overall activity levels, which have been reported across animal species (e.g. van Oers and Naguib 2013).

Although most studies address a subset of these issues in some manner, analytic processes have often lacked robust, objectively defined, and thus transferrable criteria (Pulido et al. 1996; Helm and Gwinner 2006). Considerations of how to process the data substantially influence the outcome of a study. For example, the results of studies using *Zugunruhe* to classify captive birds as putative migrants or residents will vary depending on the choice of time windows, threshold levels, and processing of noisy *Zugunruhe* profiles.

We address these analytical issues by presenting an automated procedure based on changepoint analysis. Using a single algorithm for birds of all migratory phenotypes, we determine presence, timing and intensity of *Zugunruhe* on an individual level. Specifically, we assign presence or absence of *Zugunruhe* to all individuals, thereby deriving proportions of putative migrants based on *Zugunruhe* for all populations, and we use activity data from the full annual cycle of individuals to obtain robust estimates of the timing of *Zugunruhe*.

Using these quantifications, we then analyse activity data of the stonechat populations and their hybrids with the following objectives:

1. Examine population-level variation in the occurrence, timing and intensity of Zugunruhe. Based on field-derived differences in migratoriness, we test the prediction that birds originating from resident and partially migratory populations (Kenyan and Irish) are more likely to refrain from engaging in *Zugunruhe*; if they do show *Zugunruhe*, we expect

intensities to be lower than in obligate short- and long-distance migrants (Austrian and Siberian). We therefore anticipate that intensity and duration will increase in the following sequence: Kenyan < Irish < Austrian < Siberian. We expect this pattern to hold for both spring and autumn seasons. Likewise, we test the prediction that timing of *Zugunruhe* relates to reported population differences in annual cycles, phenology, and migratory strategy (Helm 2009). In spring, we expect partially migratory populations to engage in *Zugunruhe* earlier than obligate short- and long-distance migrants. Conversely, for autumn we expect that long distance migrants will be the first, and partial migrants the last, to leave the breeding grounds.

2. Examine hybrid phenotypes. We investigate whether timing, prevalence, and intensity of *Zugunruhe* in hybrids are intermediate relative to parental phenotypes.

3. Identify differences between autumn and spring migration periods. Based on field evidence that migration is often more compressed and intense in spring than autumn (Alerstam 2011), we predict that *Zugunruhe* profiles are also more intense in spring than in autumn. We expect this pattern to be consistent among the three migratory populations, and possibly also in residents.

4. Examine the consistency of activity with age. Based on the premise that *Zugunruhe* reflects genetically programmed migratory traits, we test the prediction that *Zugunruhe* is consistently displayed over the lifetime of a bird. Because in weakly migratory species patterns may be flexible (Schwabl and Silverin 1990, Hegemann et al. 2015), we also test the prediction that *Zugunruhe* traits should have lower consistency in partial and short-distance migrants compared to the most migratory population. We first examine nocturnal restlessness during the postfledging phase, before the end of postjuvenile moult (referred to as "juvenile restlessness"). Then, we focus on *Zugunruhe* and test for changes in timing, intensity, and frequency of occurrence with age, whether such changes differ among populations, and whether they also apply to year-round diurnal and nocturnal activity.

5. Compare analyses based on assignment of individuals as either migrants or residents to population-wide analyses. Classification of birds as either showing *Zugunruhe* (i.e., putative migrants) or not showing *Zugunruhe* (i.e., putative residents) filters nocturnal restlessness data prior to further analysis. *Zugunruhe* studies differ in whether or not individuals are divided by behaviour in this manner. To assess the effects of this classification on conclusions about *Zugunruhe* in stonechats, we compare outcomes of our analyses of birds identified as showing *Zugunruhe* to overall population-wide analyses of diurnal and nocturnal activity.

6. Examine the relationship between Zugunruhe and daytime activity. The few studies that have investigated how daytime activity changes during *Zugunruhe* suggest that birds compensate for sleepless nights by slight increases in daytime rest (Rattenborg et al. 2004, Fuchs et al. 2006). We therefore quantify the extent to which increased nocturnal activity will be partially compensated by reduced daytime activity.

7. Examine whether more intense Zugunruhe *is a characteristic of generally more active birds.* We test whether activity levels of birds covaried between migratory and non-migratory contexts. We compare nocturnal activity levels of individuals during migration seasons (*Zugunruhe*) to (a) nocturnal activity levels during "neutral", non-migratory seasons, and to (b) diurnal activity levels during non-migration seasons. We posit that a positive correlation between activity levels of these periods will weaken the assumption that individual variation in *Zugunruhe* uniquely represents individual variation in migratory propensity.

METHODS

Birds and Experimental Setup

Origin and Maintenance of Birds: We present data from 976 migration periods (minimal duration 90 days) from 414 stonechats of the following four population groups: *S. rubicola rubicola* from Austria (hereafter referred to as "Austrian" or by code A); *S. rubicola hibernans* from Ireland (hereafter "Irish" or I); *S. torquatus axillaris* from Kenya (hereafter "Kenyan" or K); and *S. maurus* from Kazakhstan (hereafter "Siberian" or S) (Figure 1A). We also included hybrid Austrian × Kenyan stonechats (n = 16, all A×K F1) and Austrian × Siberian stonechats (A×S; n = 80). Austrian × Siberian included F1 crosses (n = 56) and backcrosses (AxS with Austrian parent: n = 16; AxS with Siberian parent: n = 8). Hereafter, we refer to the four populations and the hybrid groups simply as "populations" (for details see Supplementary material Appendix 1, and Helm 2003, 2009).

For the birds in this study, the sex ratio was consistently nearly balanced in all groups (overall: 193 females, 221 males). The vast majority (330) were offspring of captive stonechats from our breeding scheme (i.e. at least 2nd generation in captivity), and were born between 1998 and 2006. Between 1997 and 2004, 84 birds were taken from the field, usually as nestlings, with the exception of two Irish stonechats that were collected as wintering adults in January 2003. After fledging, birds were housed individually indoors in recording cages (60 x 40 x 40 cm) (Gwinner et al. 1995). Birds were kept under constant mild temperatures (c. 20°C) and under light exposure of ca. 300 lx during daytime and 0.01 lx at night, simulating

natural photoperiodic change as described below. We assessed postjuvenile moult by inspection of wing and 19 defined body areas (Helm and Gwinner 1999). To focus on the main phase of moult, we defined its onset and end when birds crossed a threshold of at least 5 simultaneously moulting body areas.

Photoperiodic Conditions: A main purpose of the breeding experiments were comparisons of the stonechat populations under identical conditions (i.e., "common garden", Noordwijk et al. 2006). Birds were kept indoors under one of three photoperiods that all simulated naturally fluctuating photoperiods. The vast majority (n = 356) were kept under conditions that simulated day length experienced by Austrian stonechats around the annual cycle; two Irish birds experienced summer day lengths mimicking those of their native location (52.5°N). A further 37 birds were exposed to simulated day length experienced by Siberian stonechats (Siberian and Austrian stonechats and their hybrids). Finally, 8 birds were exposed to both European and Irish day lengths during different years, and 11 were exposed to both European and Siberian day lengths during different years. In our analyses, if data were used from day length simulations other than those of Austrian stonechats, we included photoperiod as a covariate. The data reported are numbers of daily or nightly 10-minute intervals during which activity was detected by passive infrared detectors.

Data Preparation and Processing

Data Preparation (for details, see Supplementary material Appendix 1): For our primary *Zugunruhe* analysis, we only analysed nocturnal activity data after a bird had started postjuvenile moult (Figure 2). Overall, missing data represented 1.0% of our dataset and were handled as explained in Supplementary material Appendix 1. We split the annual cycle into two halves to quantify spring and autumn migration periods separately. To do so, we determined population-specific cut-off dates as the mid-points of summer and winter periods when nocturnal activity was minimal.

Changepoint Analysis for Identifying and Characterising Periods of Zugunruhe (see Supplementary material Appendix 1, Figure A1): We applied changepoint analysis, developed to identify changes in the statistical properties of time series data, in the R package "changepoint" (Killick and Eckley 2014). This algorithm assesses the mean and variance of time series data and identifies any changes in these properties (or absence thereof). Accordingly, we classified a migration period of a given bird as showing *Zugunruhe* if at least one changepoint was identified. A changepoint is identified if its addition to the model sufficiently improves the log-likelihood enough to overcome a penalty value used to prevent

too many changepoints from being identified. Changepoint analysis of spring and autumn data provided us with one or more time segments for each migration period (Supplementary material Appendix 1, Figure A1). When the analysis identified one or more changes, we classified a contiguous sequence of elevated segments as *Zugunruhe*.

Defining Timing and Intensity of Zugunruhe: For each migration period, we calculated several *Zugunruhe* timing metrics. Onset and end dates were given by the starting and ending dates of the contiguous elevated *Zugunruhe* period (see Supplementary material Appendix 1, Figure A1); duration was inferred as the number of days between start and end dates. We defined "Mean day" as the average day from the elevated period, weighted by nightly activity levels. To derive a consistently defined measure of intensity, we first calculated the mean activity level during the contiguous 15-day period with highest overall activity (hereafter "uncorrected peak intensity"). We then calculated "corrected" peak intensity as the difference between uncorrected peak intensity and the mean of winter and summer baseline activity levels flanking the migration period. This correction accounts for consistent, year-round inter-individual differences in activity levels. We also calculated a bird's overall mean intensity as the average activity level over an entire elevated *Zugunruhe* period. Lastly, for comparability with the practice of some studies on *Zugunruhe*, we conducted a supplemental analysis in which we normalized our data according to the concurrent length of night (Owen and Moore 2008); we illustrate some comparative findings in Supplementary material Appendix 1.

Statistical Analyses

Factors Affecting Occurrence of Zugunruhe: To identify which factors influence *Zugunruhe*, we modelled the proportion of spring and autumn migration periods with and without *Zugunruhe* by an analysis of deviance with binomial errors ("glm" function in the R base "stats" package). The initial model comprised the categorical predictors of population (including hybrid groups), sex, age (first year or older), migratory season (spring or autumn), and all possible interactions. See Supplementary material Appendix 1 for details.

Consistent Individual Propensity to Engage in Zugunruhe: We additionally examined intra-individual patterns of *Zugunruhe* for birds with activity data for more than two periods (spring or autumn). We analysed variation in the proportion of birds that always, sometimes, or never engaged in *Zugunruhe* during the periods for which they were monitored. We compared proportions using the *pairwise.prop.test* function in R and corrected for multiple comparisons with the Holm-Bonferroni method (Holm 1979).

Variation in Zugunruhe Timing and Intensity: We examined overall variation in timing and intensity of *Zugunruhe* using linear mixed models (packages lme4 and lmerTest in R; Bates et al. 2015; Kuznetsova et al. 2015) as detailed in Supplementary material Appendix 1.

Population-wide Nocturnal and Diurnal Activity During the Migration Periods: In addition to the procedure described above, we analysed overall nocturnal and diurnal activity levels during the migration periods of all individuals, regardless of the *Zugunruhe* status assigned to them by changepoint analysis, for compatibility with earlier analyses (e.g., Blackcaps *Sylvia atricapilla*, Berthold 1988a). This required definitions of migration periods that were independent of changepoint analysis, as explained in Supplementary material Appendix 1. Diurnal activity of this data set was used to test for age-related changes in activity levels during the migration seasons. We also analysed these data by an approach that has sometimes been used in the literature, correcting the amount of nocturnal activity for the length of night. This follows the rationale that activity levels may be limited by night length, but it has the disadvantage of confounding activity level with time of year, which determines night length.

Covariation of Diurnal and Nocturnal Activity Levels: To examine how daytime activity varied relative to *Zugunruhe* (defined using changepoint analysis), we tested diurnal activity levels before, during, and after birds engaged in *Zugunruhe* with linear mixed models. We also studied the association between nocturnal and diurnal activity levels within individuals during both migration seasons and the 30-day neutral summer and winter periods. See Supplementary material Appendix 1.

Covariation of activity between migratory and non-migratory contexts: We used two approaches to test whether high nocturnal activity levels during *Zugunruhe* periods were specific to a migration context, or, alternatively, reflected generally elevated activity levels of populations or individuals. First, we compared nocturnal activity during and outside of migration periods to test whether individuals with higher *Zugunruhe* activity were generally more active at night. Secondly, we compared *Zugunruhe* to diurnal activity during the non-migration seasons to test the hypothesis that individuals with high *Zugunruhe* were generally more active birds. We used linear mixed models (see Supplementary material Appendix 1).

RESULTS

Using changepoint analyses on a total of 976 migration periods (autumn: 552; spring: 424) from 414 individual birds, we detected *Zugunruhe* in 80.9% migration periods (autumn: 396; spring: 394, excluding 17 periods that were ambiguous).

Occurrence of *Zugunruhe* in Different Populations

Our final model predicting the frequency with which birds engaged in *Zugunruhe* comprised population, age, season, and the population × season interaction.

Effect of Population and Season): We found significant differences in *Zugunruhe* frequency among populations, and these varied between seasons. In autumn, Kenyan, Austrian and Irish populations showed a significantly lower proportion of periods with *Zugunruhe* than Austrian × Siberian and Siberian birds. In spring, the proportion of migration periods showing *Zugunruhe* was significantly elevated compared to autumn for Austrian ($z = 2.66$, $P = 0.0078$) and Irish ($z = 3.31$, $P = 0.0009$) stonechats. Figure 3 details these patterns.

Effects of Age and Sex (Figure 3CD): The proportion of periods during which birds engaged in *Zugunruhe* was significantly lower for older birdsduring both spring and autumn ($z = -7.43$, $P < 0.0001$). This observation was consistent across all populations. There was no significant effect of sex on frequency of *Zugunruhe*.

Consistency of *Zugunruhe* Within Individuals

We examined whether birds monitored for two or more migration periods (counting spring or autumn; $n = 296$ birds) always, sometimes, or never engaged in *Zugunruhe* (Figure 4). Pooling all population groups, 63.9% of individuals always engaged in *Zugunruhe* and only 3.0% of birds never exhibited *Zugunruhe*. The remaining 33.1% were mixed records, when birds changed between showing and not showing *Zugunruhe*. Among birds with mixed records and data from their first autumn, 62.0% (49/79) showed *Zugunruhe* during that first autumn (significantly different from 50% by Binomial test, $P = 0.042$). Patterns also differed starkly among populations, with the lowest proportions of consistent *Zugunruhe* in Kenyan (18%) and Irish (44%) stonechats and the highest in Siberian and Siberian × Austrian stonechats (86-88%). The proportion of birds showing mixed patterns was highest in Kenyan and Irish stonechats (53-55%) and lowest in Siberian and Siberian × Austrian stonechats (13%). The proportion of birds that never engaged in *Zugunruhe* was significantly greater than zero only in the Kenyan group (29%).

Timing of *Zugunruhe*

Effect of Population: Populations varied significantly in all aspects of *Zugunruhe* timing (Figure 5AB). Among migratory populations, Siberian stonechats began autumn *Zugunruhe* earlier than all other groups; Irish birds started afterwards, followed by Austrian birds. Kenyan stonechats, with a small sample size (n = 8), had start dates not significantly different from Irish or Austrian birds. Irish and Siberian stonechats all showed mean autumn *Zugunruhe* at approximately the same time, but Austrian birds had significantly later mean dates; Kenyan stonechats were similar to all three. Irish stonechats ended *Zugunruhe* significantly earlier than Austrian and Siberian birds (which themselves had similar end dates); Kenyan stonechats showed intermediate end dates that did not significantly differ from those of the other populations. Irish, Kenyan, and Austrian birds all showed relatively short autumn *Zugunruhe* durations compared to Siberian stonechats.

In spring, onset, mean, and end dates were earliest for Irish stonechats, followed by Austrian stonechats. Kenyan and Siberian populations showed *Zugunruhe* periods that were later than those of the other two populations but not significantly different from one another. The duration of the spring *Zugunruhe* period was longest in Irish birds, significantly shorter in Austrian stonechats, and significantly shorter still in Siberian stonechats. For Kenyan stonechats, duration did not significantly differ from Austrian or Siberian birds, possibly because the sample size for Kenyan stonechats was small ($n \geq 6$). Within populations, the dates of *Zugunruhe* onset were generally more synchronous than the date of *Zugunruhe* completion. Compared to autumn, duration of spring *Zugunruhe* was significantly longer for Irish (effect = 51.06 days, t = 12.03, $P < 0.0001$) and Austrian stonechats (effect = 27.51 days, t = 7.66, $P < 0.0001$), but shorter for Siberian birds (effect = -49.62 days, t = -10.63, $P < 0.0001$). Thus, in autumn, Siberian long-distance migrants started *Zugunruhe* earliest and showed the longest durations, while in spring, Siberian birds started among the latest and showed the shortest durations.

Hybrids: Austrian × Siberian hybrids generally showed intermediate timing relative to parental birds. In autumn, onset dates, mean dates, and durations of Austrian × Siberian stonechats were intermediate and significantly different from parental values, but end dates were all similar. In spring, timing of onset and mean *Zugunruhe* were intermediate and significantly different from parental values; the end date for hybrids was not significantly different from Siberian birds and duration was not significantly different from Austrian birds. Austrian × Kenyan stonechats showed autumn timing characteristics that were similar to those of both parental groups; spring timing was not significantly different from Austrian birds but

significantly earlier than Kenyan birds. *Zugunruhe* profiles of hybrids are shown in Figure 5C-F.

Effects of Age and Sex: Young stonechats showed high levels of juvenile nocturnal restlessness before they finished postjuvenile moult (Figure 2). Nocturnal activity beginning after moult start was interpreted as *Zugunruhe*. In autumn (Supplementary material Appendix 2, Figure A2), the onset, mean, and end dates of *Zugunruhe* of young birds occurred 2-3 weeks later than those of older birds (onset: effect = -14.08 days, 240.72 d.f., t = -5.05, P < 0.0001; mean: effect = -19.19 days, 195.11 d.f., t = -8.11, P < 0.0001; end: effect = -22.25 days, 355 d.f., t = -8.35, P < 0.0001). There was no significant main effect of age on autumn duration (189.78 d.f., t = -0.02, P = 0.9872), but there was a significant interaction for Siberian birds, which showed shorter *Zugunruhe* periods in older birds (effect = -25.44 days, 162.96 d.f., t = -2.6, P = 0.0101). In spring, older birds of all groups had slightly later *Zugunruhe* start dates (effect = 4.98 days, 283.8 d.f., t = 4.73, P < 0.0001), earlier end dates (non-significant; effect = -7.12 days, 105.9 d.f., t = -1.72, P = 0.0875), and shorter durations (effect = -15.29 days, 113.2 d.f., t = -3.01, P = 0.0032). Mean date did not change with age (effect = 0.24 days, 167.46 d.f., t = 0.19, P = 0.8477).

In autumn, sex had no effect on timing. In spring, we found an effect of sex on onset date, with females showing significantly later onset (effect = 4.57 days, 171.01 d.f., t = 4.46, P = 0.0001) and later mean *Zugunruhe* dates (effect = 2.95 days, 161.28 d.f., t = 2.24, P = 0.0265), but no difference in end dates; overall, females therefore showed shorter spring durations (effect = -6.64 days, 163.39 d.f., t = -2.27, P = 0.0247). We tested for an interaction between sex and population and found that the difference between male and female spring *Zugunruhe* onset dates in Kenyan stonechats was significantly greater than that of the other populations (effect = 30.3 days, 349.66 d.f., t = 4.03, P < 0.0001). There was no significant interaction between population and sex for mean date or duration.

Intensity of *Zugunruhe*

We quantified *Zugunruhe* intensity as both the mean level of nocturnal activity (Supplementary material Appendix 2, Figure A3) during a given *Zugunruhe* period and the peak nocturnal activity relative to a bird's winter and summer levels (Figure 6). The two metrics yielded similar results.

Effect of Population: Populations differed significantly in ways that generally aligned with their approximate degree of migratoriness (Figure 6). Surprisingly, Irish stonechats, which are partial migrants, showed peak *Zugunruhe* intensities that were greater than or similar to those

of obligate Austrian migrants in both autumn and spring and comparable to long-distance Siberian migrants in spring (Figure 6AB). For some, but not all, groups, intensity was significantly greater in spring compared to autumn: Irish (corrected peak: effect = 16.90, t = 10.54, $P < 0.0001$; overall mean: effect = 11.7, t = 9.37, $P < 0.0001$), Austrian (corrected peak: effect = 12.49, t = 9.70, $P < 0.0001$; overall mean: effect = 9.32, t = 8.76, $P < 0.0001$), and Austrian × Siberian (corrected peak: effect = 9.05, t = 5.27, $P < 0.0001$; overall mean: effect = 7.91, t = 6.03, $P < 0.0001$).

Hybrids: Austrian × Siberian stonechats showed corrected peak intensities that were significantly higher than the intensities of Austrian birds but not significantly different from those of Siberian stonechats (Figure 6CD). Like their Austrian parents (and unlike their Siberian parents), Austrian × Siberian hybrids showed a significant difference between levels of autumn and spring *Zugunruhe*. Austrian × Kenyan stonechats showed spring peak intensities lower than Austrian birds, and not significantly different from those of Kenyan birds; autumn intensities of all three groups were similar.

Effects of Age and Sex: (Supplementary material Appendix 2, Figure A3). Average autumn peak *Zugunruhe* intensity and mean intensity were generally significantly lower after the first year (corrected peak: effect = -4.55, 303.58 d.f., t = -3.36, P = 0.0009; overall mean: effect = -3.06, 262.97 d.f., t = -3.13, P = 0.002). There was no significant overall population × age interaction. We found no effect of age on spring intensity (corrected peak: effect = -0.80, 260.01 d.f., t = -0.67, P = 0.5025; overall mean: effect = -0.25, 267.55 d.f., t = -0.26, P = 0.7929), nor an effect of sex on intensity during either migration period in any population.

Age Effects on Nocturnal and Diurnal Activity

Migration Periods: Across all populations, the intensity of diurnal activity during the migration periods showed declines after the first year. Paralleling the patterns in nocturnal activity, these declines occurred both in autumn (effect = -11.00, 344.66 d.f., t = -9.41, $P <$ 0.0001) and spring (effect = -5.88, 158.44 d.f., t = -3.26, P = 0.0014). There were no significant interactions between population and age in these models.

Winter (see Supplementary material Appendix 2, Figure A3): Nocturnal activity during winter was subtly but significantly lower after the first year (effect = -0.97, 268.32 d.f., t = -3.5, P = 0.0005); diurnal winter activity showed a stronger reduction (effect = -12.02, 348.59 d.f., t = -8.43, $P < 0.0001$).

Summer (see Supplementary material Appendix 2, Figure A3): The first summer for which we have sufficient data was the summer approximately one year after hatching. Comparing activity levels during this summer to those during subsequent years revealed no effects of age on nocturnal activity (effect = 0.5, 215.06 d.f., $t = 1.58$, $P = 0.1154$), but a significant drop in diurnal activity (effect = -10.29, 106.89 d.f., $t = -4.68$, $P < 0.0001$).

Within-individual Effects: We examined the consistency of *Zugunruhe* measurements in the same individual in different years, although we were constrained by the small number of same-bird measurements in our dataset (sample size from 20-67, depending on the measurement). Overall, subsequent *Zugunruhe* patterns were correlated within individuals, but these patterns were largely driven by population differences (Supplementary material Appendix 2, Figure A4). After standardizing for population, individual consistency was much lower (Supplementary material Appendix 2, Figure A5). Our data suggest that spring *Zugunruhe* intensities show the highest within-individual consistency of all our timing or intensity metrics (Supplementary material Appendix 2, Figures A5 and A6).

Population-wide Nocturnal Activity During the Migration Periods

Comparison between Populations: Using the complete dataset of all populations, we visually compared activity profiles of hybrids with those of their parental populations (Figure 5C-F) and those of the three migratory populations (Figure 7). Although birds without assigned *Zugunruhe* were included in this data set, all main patterns persisted, including distinctly elevated nocturnal activity in Irish, relative to Austrian, birds. In some cases, reductions of activity over age were accentuated by inclusion of assigned non-migrants. In autumn, population-level nocturnal activity was most apparent in first-year birds (Figure 7A) but decreased drastically in later years in Austrian and Irish individuals (Figure 7C). In contrast, Siberian birds continued to show autumn nocturnal activity at levels on par with their first year. In spring, all migrant populations showed little appreciable change in nocturnal activity with age (Figure 7BD).

Quantitative comparisons of population-wide activity levels during the most active two-month periods of each population yielded results that were similar to those of peak intensity (Supplementary material Appendix 2, Figure A6, top row). In particular, Irish partial migrants showed high mean and peak activities in spring that were not significantly different from those of Siberian long-distance migrants, and also significantly higher than those of Austrian short-distance migrants. In autumn, however, Irish stonechats showed mean and

peak activities that were significantly lower than those of Siberian birds and similar to those of Austrian birds.

Nocturnal Activity as Proportion of Night Length: We detected generally minor differences in results when analysing *Zugunruhe* activity as a proportion of night length as opposed to in absolute units of time (Supplementary material Appendix 2, Figure A6, bottom row). The exception was that Siberian stonechats, and to a lesser extent Austrian × Siberian stonechats, showed a higher relative peak activity level compared to the original analysis. This is because Siberian stonechats began *Zugunruhe* relatively late in spring and relatively early in autumn, when the nights were shorter. Some birds were active for nearly 100% of certain nights in spring.

Covariation of Diurnal and Nocturnal Activity

Across all birds, we observed distinct differences in a bird's mean diurnal activity depending on whether it was also engaging in *Zugunruhe*. In the first 15 days of *Zugunruhe*, diurnal activity was significantly lower than in the 15 days preceding *Zugunruhe* onset in autumn (effect = -7.74, 844.04 d.f., t = -5.1, $P < 0.0001$); in spring, however, this effect was much reduced, suggesting that birds compensated less for their nocturnal restlessness (interaction effect = 6.87, 848.2 d.f., t = 4.28, $P < 0.0001$). A decrease in diurnal activity at the start of *Zugunruhe* was more pronounced for first year birds, regardless of the season (interaction effect = -3.51, 845.62 d.f., t = -2.19, P = 0.0289). We found no significant interactions with population or sex. In the 15 days following *Zugunruhe* completion, diurnal activity in autumn increased relative to the preceding 15 days (effect = 3.13, 863.94 d.f., t = 2.7, P = 0.007), and even more so in spring (interaction effect = 6.33, 862.58 d.f., t = 3.8, P = 0.0002). There were no significant interactions with age, population, or sex.

Individual birds showed significant negative relationships between diurnal and nocturnal activity during periods of *Zugunruhe*: at the level of the individual day, birds were less active during days following high-activity nights (Supplementary material appendix 2, Figure A7; autumn: effect = -0.09, 202.5 d.f., t = -5.3, P < 0.0001; spring: effect = -0.20, 242.43 d.f., t = -10.36, P < 0.0001). Overall, the fixed effect of diurnal activity explained approximately 4.0% of the variation in nocturnal activity during autumn *Zugunruhe* periods and 6.5% in spring. During summer and winter, diurnal and nocturnal activity did not covary significantly on a daily basis (effect for both periods = -0.02, 259.89 d.f., t = -0.11, P = 0.91). During migration periods, neither sex nor population had a significant effect on diurnal

activity, whereas during summer and winter, females were on average more active (effect = 5.81, 552.24 d.f., t = 3.99, P < 0.0001).

Zugunruhe and Activity Outside a Migration Context

On a population level, when all individuals were included, nocturnal activity during migration periods was greatly elevated over summer and winter baseline levels for all populations studied (Figure 8, top row). Within individuals, nocturnal activity levels during spring migratory periods were positively associated with those during summer and winter after accounting for age, sex, and population (winter: effect = 0.05, 358 d.f., $t = 3.41$, $P = 0.0007$; summer: effect = 0.07, 181.66 d.f., $t = 3.78$, $P = 0.0002$). This was also true for activity during autumn migratory periods (winter: effect = 0.06, 318.81 d.f., $t = 4.19$, $P < 0.0001$; summer: effect = 0.02, 2.03 d.f., t = 1.32, P = 0.3167); the non-significant effect of summer may have been due to a lack of data (none for first year birds) and thus very low power.

Nocturnal activity during migration periods also explained variation in diurnal activity during summer and winter, both for spring (winter: effect = 0.32, 290.41 d.f., $t = 4.33$, $P < 0.0001$; summer: effect = 0.50, 146.51 d.f., $t = 3.56$, $P = 0.0005$) and autumn (winter: effect = 0.29, 241.94 d.f., $t = 3.66$, $P = 0.0003$; summer: effect = 0.13, 65.66 d.f., $t = 0.63$, $P = 0.5341$). This indicates, for example, that a bird more active than another by an average of 100 minutes per night during the migration season also averaged 13-50 minutes more activity during the day at other times of the year.

Overall, therefore, more active individuals during the migration periods tended to also be more active during other times of the annual cycle, both during the day and at night.

DISCUSSION

Population-specific Patterns of *Zugunruhe*

Our analyses reveal clear population-specific differences in the migratory programmes of stonechats, akin to documented differences in many traits of life history, physiology, morphology, and biological time-keeping (e.g., Gwinner et al. 1983, 1995, Helm et al. 2005, 2009, Helm 2009, Tieleman et al. 2009, Baldwin et al. 2010, Fuchs et al. 2014, Versteegh et al. 2014). These population-level differences were largely consistent with our predictions: long-distance migratory Siberian stonechats showed by far the strongest migratory tendencies and most consistent behaviour. Siberian birds rarely failed to engage in *Zugunruhe* and consistently showed high *Zugunruhe* intensities in both spring and autumn. Among the

migrant populations, Siberian stonechats were the first to initiate *Zugunruhe* in autumn and the last to do so in spring. Siberian birds had the longest *Zugunruhe* period in autumn and the shortest in spring. This extended autumn *Zugunruhe* period is likely related to the long distances travelled by this population, paired with a possibly slower pace of autumn migration (Yamaura et al. 2016; but see Raess 2008). Alternatively, the long fall *Zugunruhe may* reflect a necessity to facultatively remain in migratory condition because of unpredictable resource availability (Helms 1963). Although short, the spring *Zugunruhe* periods of Siberian stonechats were exceptionally intense (peak activities were near 100% of the night length). This may reflect the need for a rapid long-distance return journey to the breeding grounds within a tightly constrained time window (i.e. late enough that spring has arrived—but not too late for breeding in a short season, Alerstam 2006, 2011). This view is supported by spring field records from stonechats (Raess 2008).

On the other end of the migration spectrum, resident Kenyan stonechats clearly showed the weakest migratory tendencies, but they still regularly engaged in *Zugunruhe*, especially first-year birds (Helm 2006, Helm and Gwinner 2006). Most Kenyan birds showed *Zugunruhe* at least once, but also refrained from engaging in *Zugunruhe* at least once, including the overwhelming majority of older birds. Under temperate photoperiods in the laboratory, Kenyan birds showed well-defined population timing, starting at about the same time as Austrian birds in autumn, but significantly later in spring. In both seasons, *Zugunruhe* profiles of Kenyan birds had relatively short durations and low intensities. Although the present analysis finds relatively low levels, it corroborates the persistence of *Zugunruhe* and several of its features in African Stonechats. The interpretation of this behaviour is still unclear and merits further investigation (Zink 2011).

The Irish and Austrian populations are currently classified as members of the same species (European Stonechat), and are both medium distance migrants that reach Mediterranean winter quarters. However, in contrast to obligate Austrian migrants, British Isles stonechats are partial migrants, with roughly half of the birds remaining at or near the breeding sites in winter (Helm et al. 2006). Our study population originated from a coastal site in Ireland where birds overwinter, such that our sample could have been biased towards resident phenotypes (Helm 2003). Nonetheless, we found that *Zugunruhe* behaviours in these two populations were generally similar, showing intermediate phenotypes of timing and incidence compared to Kenyan and Siberian birds. Counter to predictions for partial migrants (Berthold 1988a, Pulido et al. 1996, Pulido and Berthold 2010), we could not detect a proportion of Irish stonechats showing no *Zugunruhe*. Most unexpectedly, Irish birds

exhibited significantly higher spring *Zugunruhe* intensity than Austrian birds. These patterns are difficult to explain if *Zugunruhe* is expected to correspond closely to actual migration, and if partial migrant populations are expected to show greater dimorphism of migratory phenotypes (Berthold 1988a). They are more easily reconciled with regulation of migration where *Zugunruhe* may mark a window of opportunity during which environmental factors act to repress or activate actual migration (Helms 1963, Gwinner and Czeschlik 1978, Chan 2005, Helm 2006).

Austrian and Irish stonechats clearly differed from Siberian long-distance migrants. Both European populations showed significantly longer *Zugunruhe* periods during spring compared to autumn; this pattern was opposite to the one observed in Siberian stonechats. The long spring period of nocturnal restlessness is likely to represent *Zugunruhe*, rather than summer restlessness (Gwinner and Czeschlik 1978) because it ceased when the birds' reproductive organs matured (Helm and Gwinner 2005), just like migration of free-living stonechats (Raess and Gwinner 2005). Instead, the early, and long, spring restless periods of both European populations may reflect the readiness of these short-distance migrants to react to variation in local conditions that may allow for an early return to the breeding grounds, or, contrarily, for maximising arrival condition by pausing migration at favourable stop-over sites (Alerstam 2006, 2011). We have no convincing explanation for the short *Zugunruhe* window in autumn relative to Siberian stonechats, but speculate that it may reflect the shorter migration distance of the European populations.

The primary difference we detected between the two European populations related to the onset of *Zugunruhe* and matched our predictions. Irish stonechats showed by far the earliest spring migratory tendencies of all populations, regularly starting by late January. This early endogenous spring window fits well with field data, including those from ringing recoveries (Helm et al. 2006). Early spring arrival is expected both because of the more temperate environment of the British Isles, allowing for suitable environmental conditions earlier in the year, and because returning partial migrants will compete with resident conspecifics for territories upon arrival (Lack 1943, 1944). However, Irish birds also started *Zugunruhe* significantly earlier in autumn compared to their continental counterparts. This finding is more challenging to interpret in ecological terms. In terms of biological time-keeping mechanisms, it confirms a shift of the entire annual cycle of Irish compared to Austrian stonechats: annual cycle organisation and photoperiodic responses of both populations were identical, but migrations, reproduction and moult were all advanced by approximately one month in Irish stonechats (Helm 2003, 2009, and unpublished data).

*Hybrid Phenotypes***:** Our data show that hybrid stonechats express behavioural traits that are generally intermediate with respect to their parental groups. This is most obvious for the timing measures of Austrian × Siberian hybrids compared to Austrian or Siberian groups, and it indicates a strong genetic basis for both timing and intensity of migratory restlessness. These findings of intermediate phenotypes parallel data from other traits in stonechats, including timing of reproduction and moult, immune traits, metabolic measures, and life history traits (e.g., Gwinner et al. 1995, Helm et al. 2009, Versteegh et al. 2014, but see Tieleman et al. 2009). They also align with findings from other crossbreeding studies of migratory birds, including Silvereyes (*Zosterops lateralis*, Chan et al. 2005) and Blackcaps (Berthold 1988a, Helbig 1996), although inheritance patterns in Quail (*Coturnix coturnix*) appeared to be biased towards resident types (Deregnaucourt et al. 2005).

*Sexes***:** In contrast to the extensive differences between populations, the sexes of stonechats exhibited similar endogenous migratory programmes, in line with their unusual behaviour of wintering in heterosexual pairs (Gwinner et al. 1994). We found no effect of sex on the frequency with which birds abstained from *Zugunruhe* or on the intensity or autumnal timing of *Zugunruhe*. However, males started spring *Zugunruhe* on average 4.6 days earlier than females. Protandry in spring migratory timing has been documented in the wild in many species, and it is seen as advantageous that males arrive to defend territories as early as possible (e.g., Kokko et al. 2006, Copack and Pulido 2009, Alerstam 2011). Our findings add to the increasing evidence that such differences between the sexes can be hard-wired. Maggini and Bairlein (2012) have recently shown that in Wheatears, protandry of *Zugunruhe* persisted even in the absence of environmental cues such as photoperiod, and is therefore part of the birds' circannual programme (Gwinner 1986, 1996).

Age, Season and Individual Activity Levels

*Development and Age***:** In our study, juvenile stonechats showed strong nocturnal restlessness even before the beginning of postjuvenile moult. Likewise, hand-raised first year birds of other species also showed periods of elevated nocturnal activity after fledging, but prior to finishing postjuvenile moult (Gwinner 1990, Mukhin 1999). Wild birds are unlikely to commence migration during intense moult (Jenni and Winkler 1994), but postfledging movements have been associated with movements to moulting areas or explorative behaviours (Mukhin et al. 2005, Brown and Taylor 2015). Prospecting and training flights of young birds during the summer may be relevant for the development of celestial compass systems and establishment of navigational targets for return migration (Mukhin et al. 2005). Stonechats

have been reported to move locally in juvenile flocks, for example to suitable moulting sites (Urquhart 2002), and ringing recoveries confirm such early-life movements (Helm et al. 2006). Juvenile restlessness in captivity could therefore represent a true urge to move, but one that differs from actual migration.

Subsequently, the expression of the stonechat endogenous migratory programme changed profoundly with age in ways that depended on season and population. Generally, occurrence and intensity decreased with age, and these decreases were most conspicuous in the more weakly migratory populations (Kenyan, Austrian, Irish, compared to Siberian) and in the lower-intensity migratory season, autumn. Across all populations, older stonechats were approximately twice as likely to abstain from *Zugunruhe* compared to first year birds. Decreases in overall activity with age are well known from many animal species, including vertebrates and invertebrates (Ingram 2010) and could well be unrelated to migration. We therefore used our data on activity outside of a migration context to assess whether the reductions in *Zugunruhe* represented general age-related patterns. We identified a consistent, but slight, reduction of activity levels with age for nocturnal activity also during summer and winter, and for diurnal activity around the annual cycle. In contrast to *Zugunruhe*, this reduction was consistent for the populations and seasons. Our findings thus suggest that the decrease in *Zugunruhe* in older birds was predominantly associated with migratory programming, rather than a general ageing process.

The magnitude of the age-related reductions in *Zugunruhe* was astonishing. Age effects on *Zugunruhe* are rarely discussed in the literature, and where they have been reported, patterns were inconsistent, for example between the sexes of European blackbirds (Lundberg 1988, Schwabl and Silverin 1990). It is possible that the reductions derived from long periods spent in captivity (Schwabl and Silverin 1990), or from modifications of the migration programme by prior experience (Ketterson and Nolan 1983, 1988). For example, based on experiments with Dunnocks (*Prunella modularis*), Schwabl et al. (1991) speculated that birds may recognise previous wintering locations and accordingly reduce *Zugunruhe* when exposed to them. Whatever the interpretation, it is interesting to note that in stonechats, age effects were absent in the most migratory population (i.e., Siberian stonechats).

We also found effects of age on the timing of *Zugunruhe*. Autumn timing was consistently earlier in older birds by approximately 2-3 weeks across populations, but duration did not change. These findings are consistent with those from other annual cycle events in stonechats, including earlier reproductive cycles and earlier moult in second-year compared to first-year stonechats (Helm et al. 2009). In the field, adults of many bird species commence

autumn migration before juveniles (Newton 2008), presumably because juveniles benefit from a longer stay on the breeding grounds for maturation and the completion of postjuvenile moult. In spring, older stonechats started *Zugunruhe* slightly later but ended it earlier, possibly primed by previous photoperiodic experience (Sockman et al. 2004).

*Seasons***:** Overall, our analysis demonstrates stark differences between spring and autumn *Zugunruhe*. *Zugunruhe* was more difficult to measure in autumn than in spring because of its more drawn out time profile and lower, more variable intensity. Autumn *Zugunruhe* was also confounded by juvenile restlessness and is known to be affected by other early-life effects, in particular by variation in hatching date (Helm and Gwinner 2006). Intensity of *Zugunruhe* was much higher in spring than in autumn for Austrian and Irish stonechats. Siberian birds appeared to make greater use of the available night time in spring than in autumn, but their high activity levels in both seasons were statistically inseparable.

These seasonal differences correspond well with *Zugunruhe* data of other species and with observations of wild birds. Many species migrate more rapidly during spring than autumn (Alerstam 2006, Newton 2008, Nilsson et al. 2013, Bäckman et al. 2016, Horton et al. 2016), and several aspects of migratory physiology reflect this faster pace. For example, when being re-fed after a fasting period, Blackcaps pause *Zugunruhe* in autumn, but not in spring (Fusani and Gwinner 2005). Such differences may result from higher selection pressure on the timing of spring migration because of its proximity to the breeding season, relative to the apparently more "casual" pace of autumn migration (e.g., Lack 1943, 1944, Helms 1963, Both et al. 2004, Alerstam 2006, Newton 2008). In our data, this interpretation is further supported by findings from diurnal activity. During *Zugunruhe*, mean diurnal activity was lower than before or after *Zugunruhe*, in accordance with the idea that birds require more rest to compensate for the increase in nocturnal activity (Rattenborg et al. 2004, Fuchs et al. 2006, Bäckman et al. 2016). The drop in mean diurnal activity during *Zugunruhe* was less pronounced during spring, indicating that birds generally maintained high diurnal activity levels in spring. However, in both seasons, stonechats compensated for increased nocturnal activity on a day-to-day basis by reducing diurnal activity levels after highly active nights, and this effect was at least as clear in spring as in autumn.

Finally, unlike for intensity, differences in the duration of *Zugunruhe* between the seasons were not consistent between populations (e.g. spring migration was shorter than autumn migration for Siberian stonechats, but the opposite was true for Irish stonechats); this type of heterogeneity might be expected from bird migration theory (Alerstam 2006, 2011).

*Individual Activity Levels***:** Within individuals, *Zugunruhe* occupied a unique position in the annual cycle, with no significant elevations of nocturnal activity detected outside the migration seasons. However, intensity of *Zugunruhe* also covaried with individual differences in overall activity and was positively correlated with activity levels outside of the migration season, both during daytime and night-time. This finding implies that *Zugunruhe* intensity is not solely a measure of migratory tendency but also contains some information about a bird's overall behavioural phenotype, including, for example, possible differences in "personality" or physiology (e.g., Mettke-Hofmann et al. 2005, Reale et al. 2007, van Oers and Naguib 2013). Breeding programs for high levels of *Zugunruhe* (e.g., in Blackcaps, Berthold 1988a) may have thus selected in part for generally high locomotor activity, although in our study within-individual correlations between activities were low relative to the Blackcaps' large selection response. Locomotor activity levels are known to be highly heritable. For example, genetic studies of mice found a QTL (quantitative trait locus) for the amount of activity, indicating high potential for selection (Mayeda and Hofstetter 1999).

Because variation in *Zugunruhe* intensity can predict variation in activity in other behavioural contexts, *Zugunruhe* intensity may be less useful than previously believed as a measure of migratory tendency. However, this depends on whether heightened overall activity levels covary with migration in the wild. Consequently, there is a need for further research on the relationship between *Zugunruhe* and other behavioural traits (Marchetti and Baldaccini 2003, Nilsson et al. 2010). In future genetic experiments on *Zugunruhe*, data should be collected on traits that shed light on the migratory context of behaviour (Noordwijk et al. 2006).

Changepoint analysis as a quantitative tool for *Zugunruhe*

Our analytic approach allowed us both to classify presumed migrants and non-migrants and identify of periods of *Zugunruhe* on the individual level for the vast majority of birds from all taxa and age groups. Overall, the algorithm performed equally well for classifying *Zugunruhe* regardless of a population's migratory phenotype. The parameters of the method can easily be adjusted to fit other phenotypes or answer different questions. In contrast to fixed exclusion criteria (e.g., Maggini and Bairlein 2010), changepoint analysis has the advantage of assessing changes in nocturnal activity in the context of a given bird's behavioural profile, thus accommodating individual differences by using an individual-specific baseline. Consequently, changepoint analysis is presumably less prone to bias from behavioural differences or recording methods. Some studies have applied individual-specific criteria, e.g., nocturnal

activity relative to diurnal activity (Ramenofsky et al. 2008), or white-noise techniques (Helm and Gwinner 2006). However, unlike changepoint analysis, these measures were not specific to the seasonal features of *Zugunruhe*. In comparison to autocorrelation techniques (Helm and Gwinner 2006), changepoint analysis classified fewer birds as exhibiting *Zugunruhe,* presumably because of its focus on major changes in nocturnal activity profiles. Importantly, and in contrast to commonly used "eyeballing" methods, changepoint analysis is objective and repeatable as long as its settings are documented. Strong contrasts and sharp delineation between *Zugunruhe* and neutral periods, especially during spring migration, were easiest to measure unambiguously (Supplementary material Appendix 2, Figure A1AC). However, even in cases with noisy data, the decisions made by the analytic approach were objective compared to methods of manual classification of raw data. Delineation of *Zugunruhe* by changepoint analysis was also more adaptable to the features of *Zugunruhe* than methods based on fixed thresholds (e.g., Pulido et al. 1996: Owen and Moore 2008), and general algorithms such as "edge detectors" (Helm and Gwinner 2005).

Our findings on *Zugunruhe* intensity were robust to variation in analysis method: population-level comparisons of raw activity data largely mirrored results of *Zugunruhe* intensity. This suggests that more conventional analytic methods are sufficient for detecting coarse intensity patterns. Overall, we feel that the main advantage of our approach is its general applicability, and we hope to aid its implementation with our provided R script (Supplementary material Appendix 3).

Conclusions: Implications for Avian Migration Studies

Our study shows that *Zugunruhe* is a robust component of the annual cycle in the stonechat complex: the behaviour was present in four populations with drastically different migratory phenotypes. Characteristic differences in *Zugunruhe* between the populations were apparent in our common-garden set-up, supporting the view that genetic variation drives differences in the underlying migration programme (see also e.g. Berthold 2001, Maggini and Bairlein 2010, Ketterson et al. 2015). A heritable basis of the main features of *Zugunruhe* was further indicated by intermediate patterns in hybrids, in line with findings from other crossbreeding studies of migratory birds, and matching evidence from wild species. For example, tracking data from wild Swainson's Thrushes (*Catharus ustulatus*) revealed intermediate routes taken by hybrids from a migratory divide (Delmore and Irwin 2014). The largely consistent evidence for genetic migration programmes is encouraging for future studies of the evolutionary architecture of movement behaviour (e.g., identifying the specific genes that are

responsible for such variation, their regulation, and their interactions), which could combine *Zugunruhe* with genomic tools (Petersen et al. 2013, Liedvogel and Lundberg 2014).

In our stonechat study, the differences between populations imply high evolutionary lability of migratory traits as well as some phenotypic plasticity of individuals, for example with increasing age. In agreement with findings from other species, *Zugunruhe* levels were higher and more robustly programmed in spring than in autumn (e.g., Helms 1963, Maggini and Bairlein 2010). Several comparisons between populations indicated that the timing of spring and autumn migration can be modified independently. Remarkably, however, we observed an apparent coupled change across seasons within European Stonechats: Irish birds showed a consistent phase shift in timing compared to Austrian birds throughout the annual cycle, despite their overlapping wintering ranges (Helm et al. 2006, Helm 2009). Similarly consistent phase differences (often referred to as carry-over effects) within species have recently also been reported in field studies of migratory waders and songbird (Conklin et al. 2010, Briedis et al. 2016) and may thus have an inherited basis.

We also found patterns that are not commonly reported, despite possibly being widespread. For example, stonechats of all groups showed juvenile nocturnal restlessness during their postfledging phase, sometimes even before the start of moult (Mukhin 1999). A better understanding of this behaviour could provide important cues for studies of avian navigation, prospecting and dispersal. In addition, the striking reductions of *Zugunruhe* with age could indicate plasticity and learning processes that are yet to be understood, requiring testing in wild birds over several years of their life. Furthermore, our comparisons of diurnal and nocturnal activity across the annual cycle suggest that individuals that showed higher levels of *Zugunruhe* were also generally more active birds. This calls for closer study of relationships between *Zugunruhe* and other behaviours, including personality traits (Oers and Naguib 2013), and for cautious interpretation of findings from selective breeding experiments (Berthold et al. 1988a).

Clearly, many questions remain about the interpretation of *Zugunruhe*. Our findings of undiminished *Zugunruhe* in partially migrant Irish stonechats and confirmation of *Zugunruhe* in Kenyan residents underscore an urgent concern: the parts of this behaviour that align with actual migration need to be distinguished from those that indicate an environmentally sensitive preparedness to migrate (Merkel 1956, Helms 1963, Gwinner and Czeschlik 1978, Helm 2006). This view is supported by exciting new research from wild birds. Bäckman et al. (2016) have provided the first annual-cycle data of activity of a free-living migratory bird, a Red-backed shrike (*Lanius collurio*). Intriguingly, the same species had earlier been recorded

in captivity (Gwinner and Biebach 1977). The overall timing of migration is well-matched in both studies. However, in agreement with observations from stop-over sites, the wild bird showed relatively few nights with migratory flights, whereas its captive conspecifics showed several months of continued *Zugunruhe*. Another recent study, on European Blackbirds (*Turdus merula* Zúñiga et al. 2016), found that radio-tracked wild migrants showed no increase in nocturnal activity until the night of departure from the breeding grounds, whereas captive birds slowly built up *Zugunruhe* over several weeks. The physiological and ecological mechanisms that affect alternations between flight and stopover mode are now under intense investigation, using both *Zugunruhe* and tracks of free-flying birds (Fusani et al. 2009, 2013, Goymann et al. 2010, Eikenaar et al. 2014, Skrip et al. 2015). Dissection of the genetic and environmental regulators of migration will not only aid migration research, but also allow important advances for understanding how genes and environment interact to shape complex behaviour.

We believe that *Zugunruhe* will continue to be a powerful tool in the study of avian migration. If used with circumspection and in combination with new tools, from molecular methods to new tracking technologies (Alerstam 2011, Liedvogel and Lundberg 2014, Ketterson et al. 2015), *Zugunruhe* will reveal new answers to ancient questions about the migration of birds (Alerstam 1990).

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Supplementary material

- **Appendix 1:** Additional methods.
- **Appendix 2:** Seven figures with associated figure legends.

- **Appendix 3:** Tools for changepoint analysis in R, containing an annotated script, functions and an example subset of data from stonechats. To run the script, please store all R files in a single folder.

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FIGURE CAPTIONS

Figure 1. (**A**) Stonechat range map highlighting the locations and migratory phenotypes of the four populations: Partially migratory Irish stonechats *Saxicola rubicola hibernans*, medium distance migrants *S. rubicola rubicola* from Austria, resident *S. torquatus axillaris* from Kenya, and long-distance migrants *S. maurus* from Siberia (Kazakhstan). Arrows indicate migratory routes between breeding and wintering grounds. We further included the following two hybrid groups: Austrian × Siberian and Austrian × Kenya. (**B**) Adjustment of the timing of *Zugunruhe* in response to the day lengths experienced over a bird's posthatching period, for captive Austrian and Kenyan stonechats. The onset ages of *Zugunruhe* for Austrian stonechats are shown as blue triangles (dashed blue line); the onset ages for Kenyan stonechats are shown as orange dots (solid orange line). Stonechats hatching later in the breeding season compensated for this by commencing *Zugunruhe* at earlier ages. Inlay: pairs of Austrian (left) and Kenyan (right) stonechats. Modified after Helm and Gwinner (2006).

Figure 2. Nocturnal activity relative to moult completion in juvenile Irish, Austrian and Siberian stonechats. Although stonechats do not migrate until after the completion of postjuvenile moult, young birds showed "juvenile restlessness" before this time, especially 50 or more days before moult completion (negative values along the x-axis). This activity typically reached a minimum 20-30 days before moult completion, after which *Zugunruhe* began. Activity level is defined as the number of active ten-minute periods during the night

for an individual bird. For each day, a boxplot is shown that describes the activity levels of birds in each population. Wide coloured bars corresponding to a given day represent the interquartile range (middle 50%) of activity values for Siberian stonechats on that day. Thin coloured lines extend outwards to the most extreme data point that is not an outlier, where an outlier is defined as exceeding a distance of 1.5 times the interquartile range from the bar. For clarity, outlier points are not shown.

Figure 3. The proportion of seasons during which stonechats showed *Zugunruhe*, by population and age. Proportions were analysed with binomial errors; error bars show one standard error. Bar charts in the top row (**A**, **B**) directly contrast autumn and spring seasons side-by-side, while the bottom row (**C**, **D**) directly contrasts birds in their first year from older birds. Kenyan stonechats frequently abstained from *Zugunruhe* (spring and autumn), as did Irish and Austrian populations (autumn only).

Figure 4. Consistency of individual birds' *Zugunruhe* behaviour by population. Shown are the proportions of birds for each population that engaged in *Zugunruhe* always (i.e., "migrants"), sometimes (middle row: "mixed") and never (bottom row: "residents"). Included are birds for which data were available for at least two seasons (spring or autumn); numbers on the x-axis are total numbers of birds included from each group, and error bars are 95% confidence intervals. Letters shown above bars indicate significant pairwise differences: groups that do not share a letter are significantly different. For example, in the "Always" category, Kenyan stonechats share an 'a' with Irish birds and Kenyan-Austrian hybrids, but not with the remaining groups, from which they differ significantly. Among non-hybrid groups, Siberian stonechats had the highest proportion of migrants and Kenyan stonechats had the highest proportion of "residents." Irish, Kenyan, and Austrian groups all had substantial numbers of "mixed" individuals that sometimes engaged in *Zugunruhe*.

Figure 5. Timing of *Zugunruhe* by population. (**A**) and (**B**) show the dates of onset, mean, and end of autumn and spring *Zugunruhe*, respectively, determined with changepoint analysis. Numbers under boxplots show the number of migration periods in the given category; there are multiple migration periods for some birds with multiple years of data. Boxplots show interquartile range, and whiskers extend to the most extreme data point that is not an outlier (see legend of Figure 2). Outliers are not shown for clarity. Rows of letters at the top of plots indicate significant pairwise differences within each timing category: groups that do not share

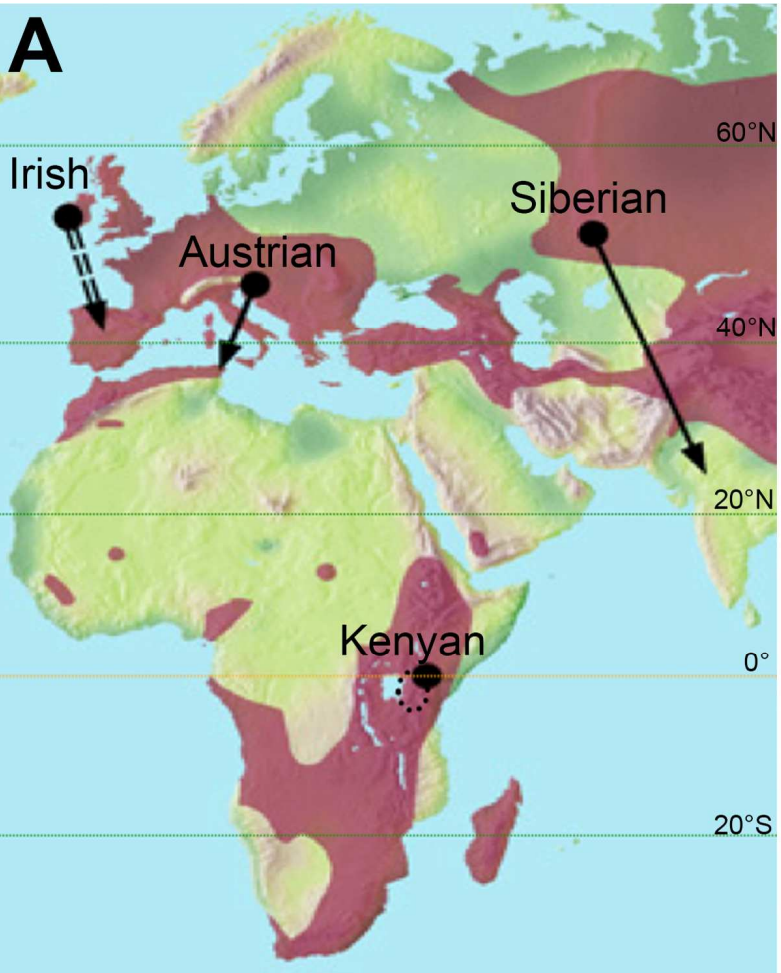
the same letter are significantly different. (**C**) to (**F**): Population level activity profiles of hybrids and their parental populations during autumn (**C, E**) and spring (**D, F**) migration periods. Activity level is quantified as the number of active ten-minute periods during the night for an individual bird. (**C**) and (**D**) compare nocturnal activity in Siberian stonechats, Austrian stonechats, and Austrian × Siberian hybrids. (**E**) and (**F**) compare nocturnal activity in Kenyan stonechats, Austrian stonechats, and Austrian × Kenyan hybrids. Lines show medians, and coloured bars show the interquartile ranges (middle 50%) of activity values corresponding to that day. Data shown are smoothed by fully overlapping 30-day windows, incremented by one day.

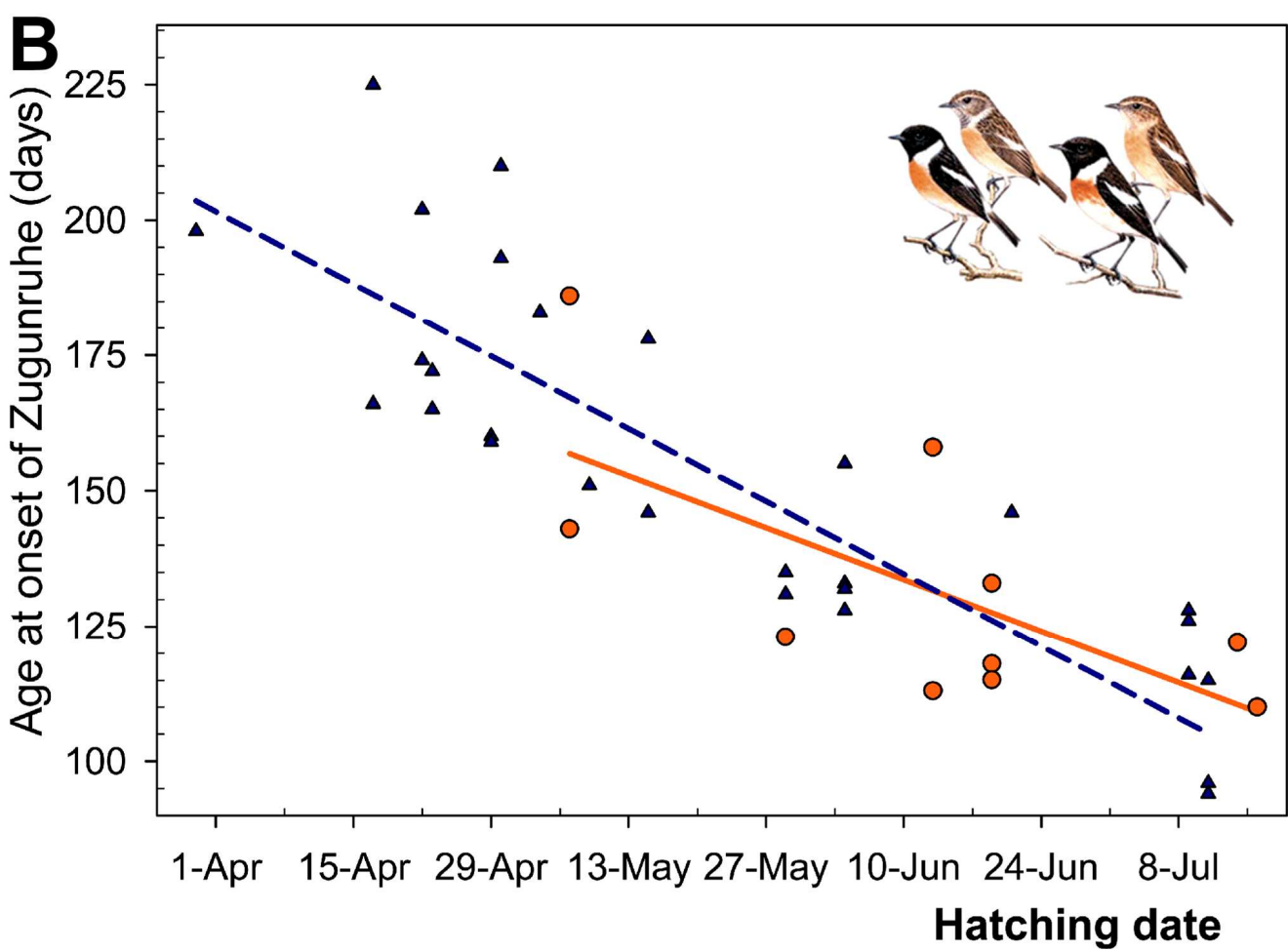
Figure 6. Intensity of *Zugunruhe* by population. (**A**) and (**B**) compare *Zugunruhe* with nocturnal activity during summer and winter (**A:** autumn, **B:** spring). (**C**) and (**D**) compare hybrids with their parental populations (**C:** autumn, **D:** spring). Activity level is defined as the number of active ten-minute periods during the night for an individual bird. Intensity of *Zugunruhe* (i.e., peak intensity) values are calculated as the mean activity level during the most-active 15-day period of *Zugunruhe*, as identified by changepoint analysis. Summer and winter values are the mean level of nocturnal activity across the least-active 30-day periods for each population. In (**C, D**), we show individual-specific corrected peak, calculated by subtracting the mean activity of flanking summer and winter periods from peak intensity. Numbers under boxplots show the number of migration periods in the given category; there are multiple periods for some birds with multiple years of data. Boxplots as in Figure 2. Letters shown at the top of plots indicate significant pairwise differences: groups that do not share a letter are significantly different.

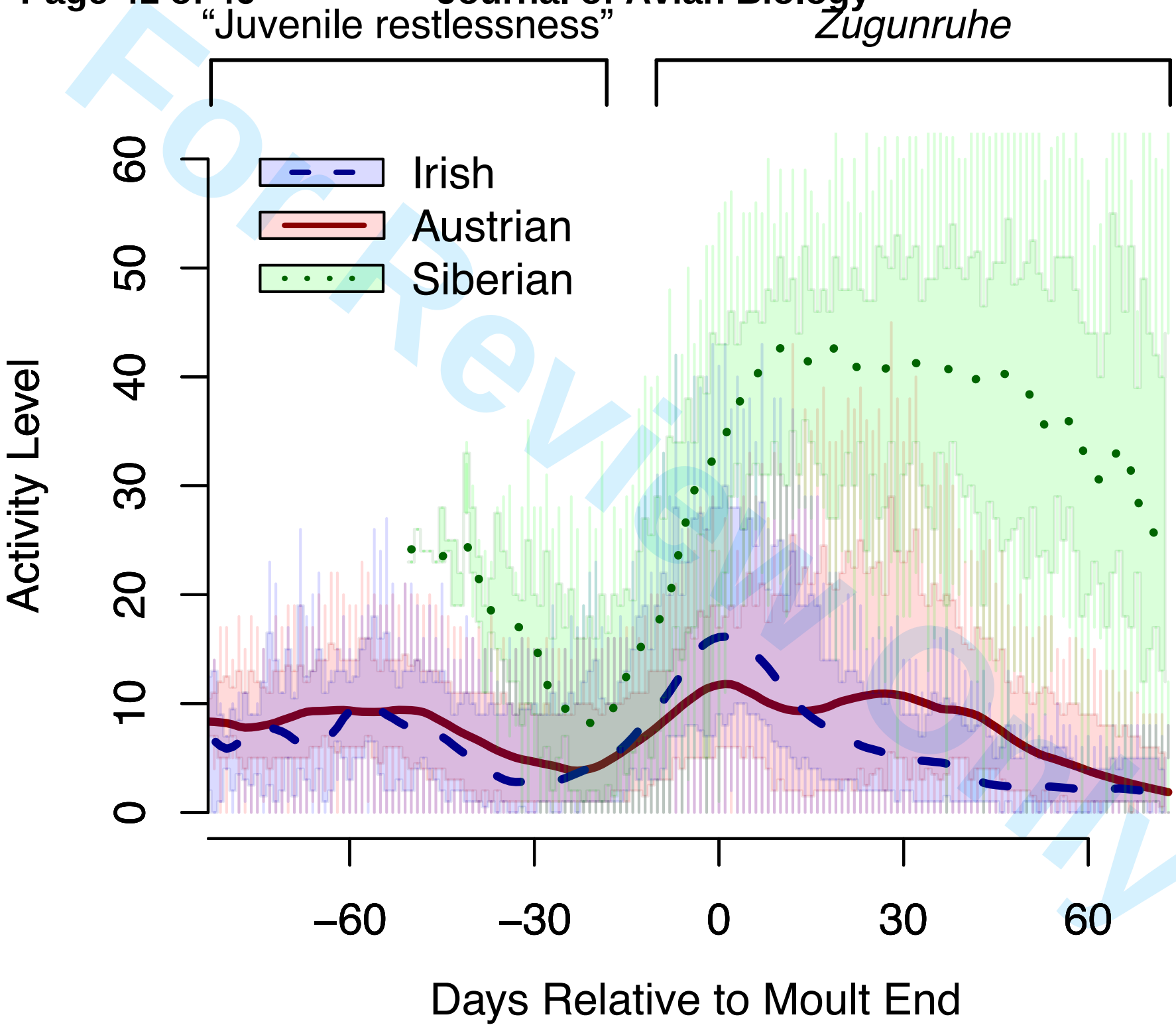
Figure 7. Population-level nocturnal activity in Siberian, Austrian and Irish stonechats, contrasting different age groups during the autumn and spring migration periods (first year (**A**) and (**B**) versus later years (**C**) and (**D**) for autumn and spring, respectively). Activity level is quantified as the number of active ten-minute periods during the night for an individual bird. Lines show medians and coloured bars show the interquartile range (middle 50%) of smoothed activity values corresponding to that day. Data shown are smoothed by fully overlapping 30-day windows, incremented by one day. For details, see Figure 5 (**C-F**).

Figure 8. Comparison between overall nocturnal and diurnal activity levels by season for each population. Boxplots show activity levels for each migration season flanked by activity levels

during summer and winter “neutral” seasons (for definitions, see Figure 6). The boxplots show daily activity levels as the number of 10-minute active periods for night (top row) and day (bottom row); boxplots as in Figure 2.


A
60°N
40°N
20°N
0°
20°S
Irish
Austrian
Siberian
Kenyan


B
Age at onset of Zugunruhe (days)
225
200
175
150
125
100
1-Apr
15-Apr
29-Apr
13-May
27-May
10-Jun
24-Jun
8-Jul
Hatching date



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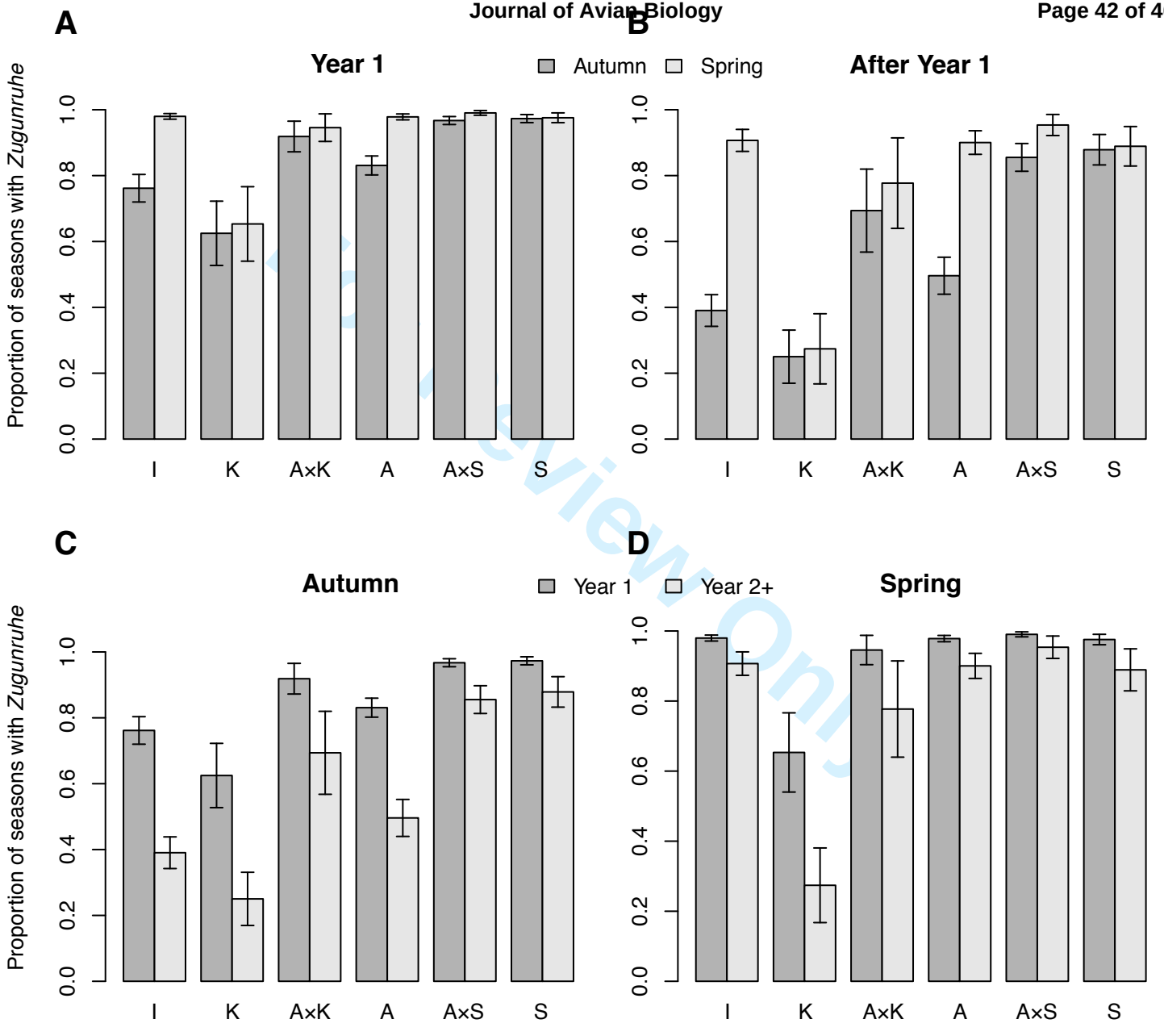

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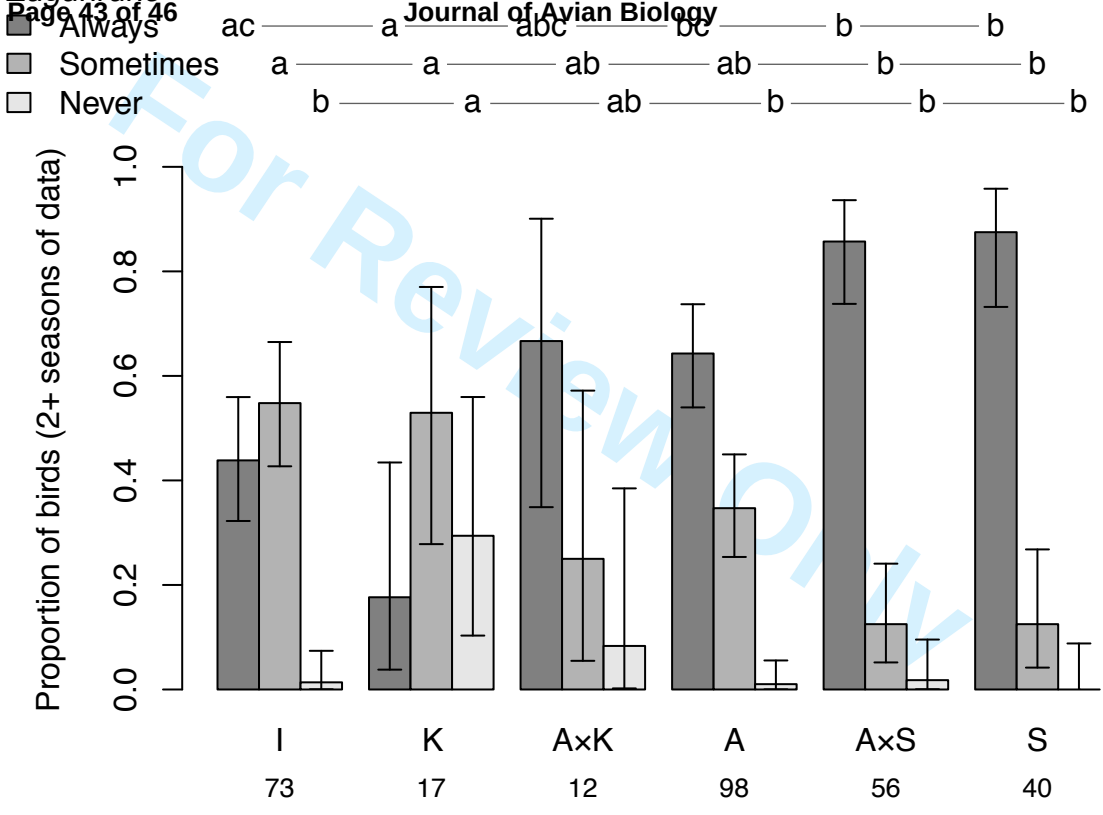

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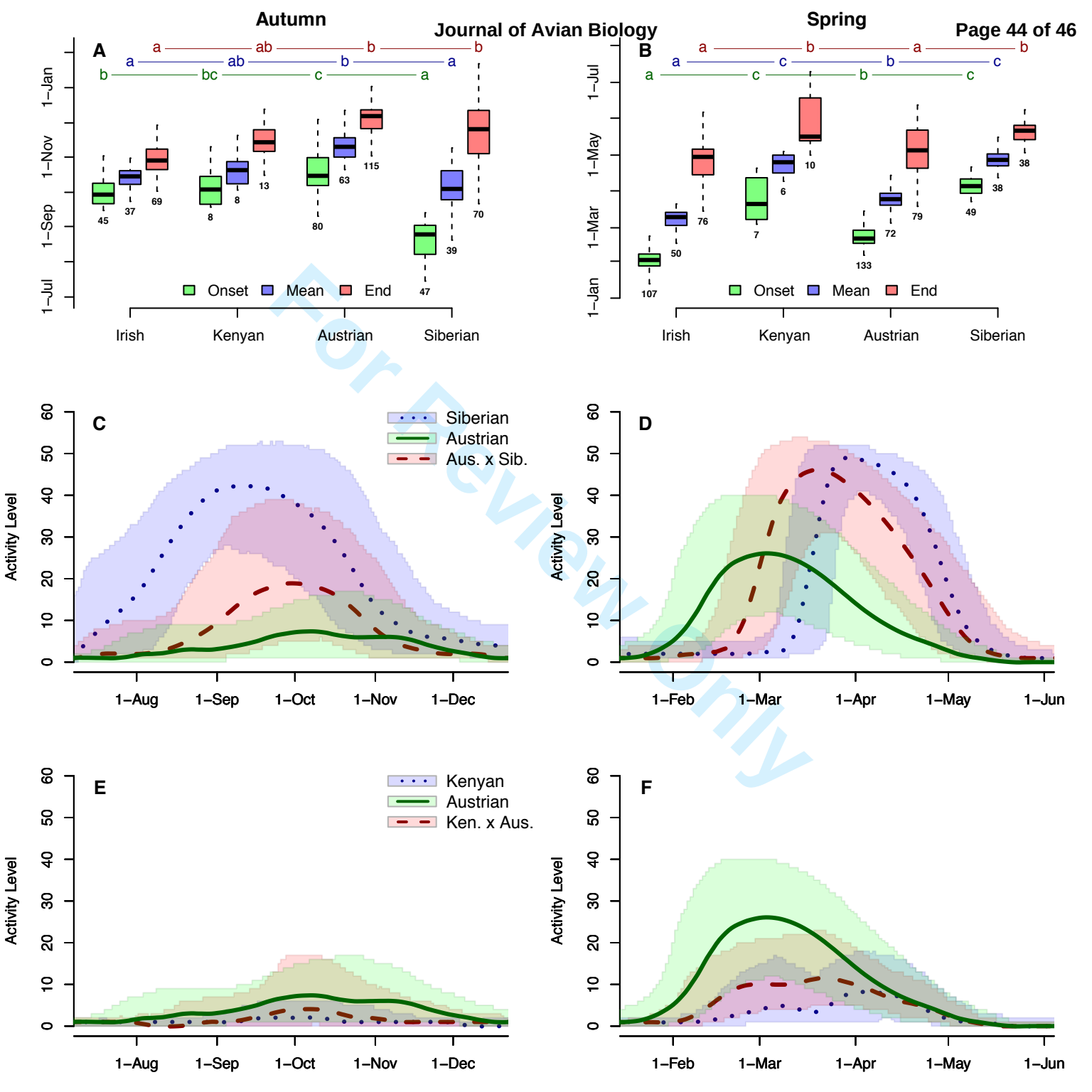

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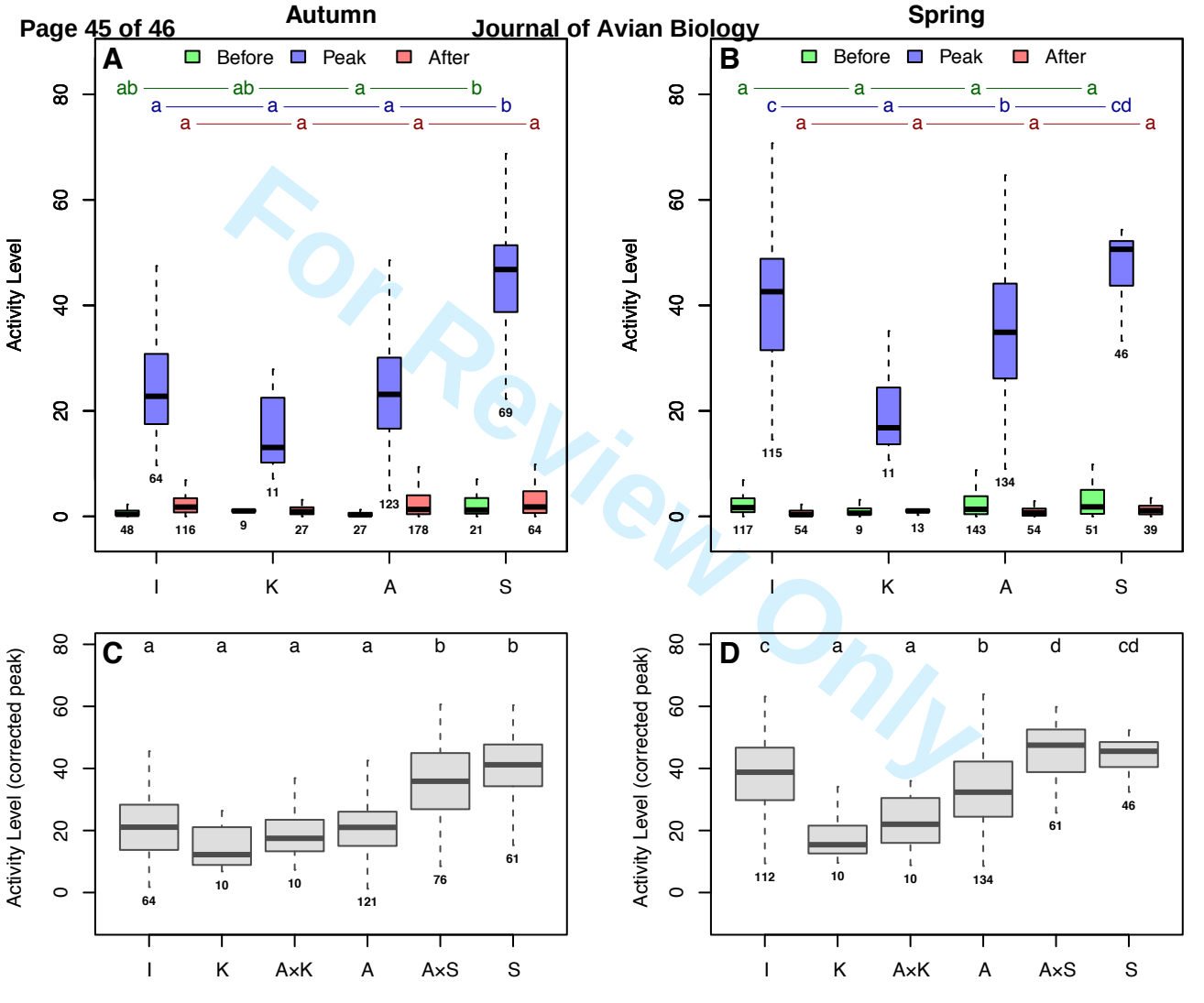

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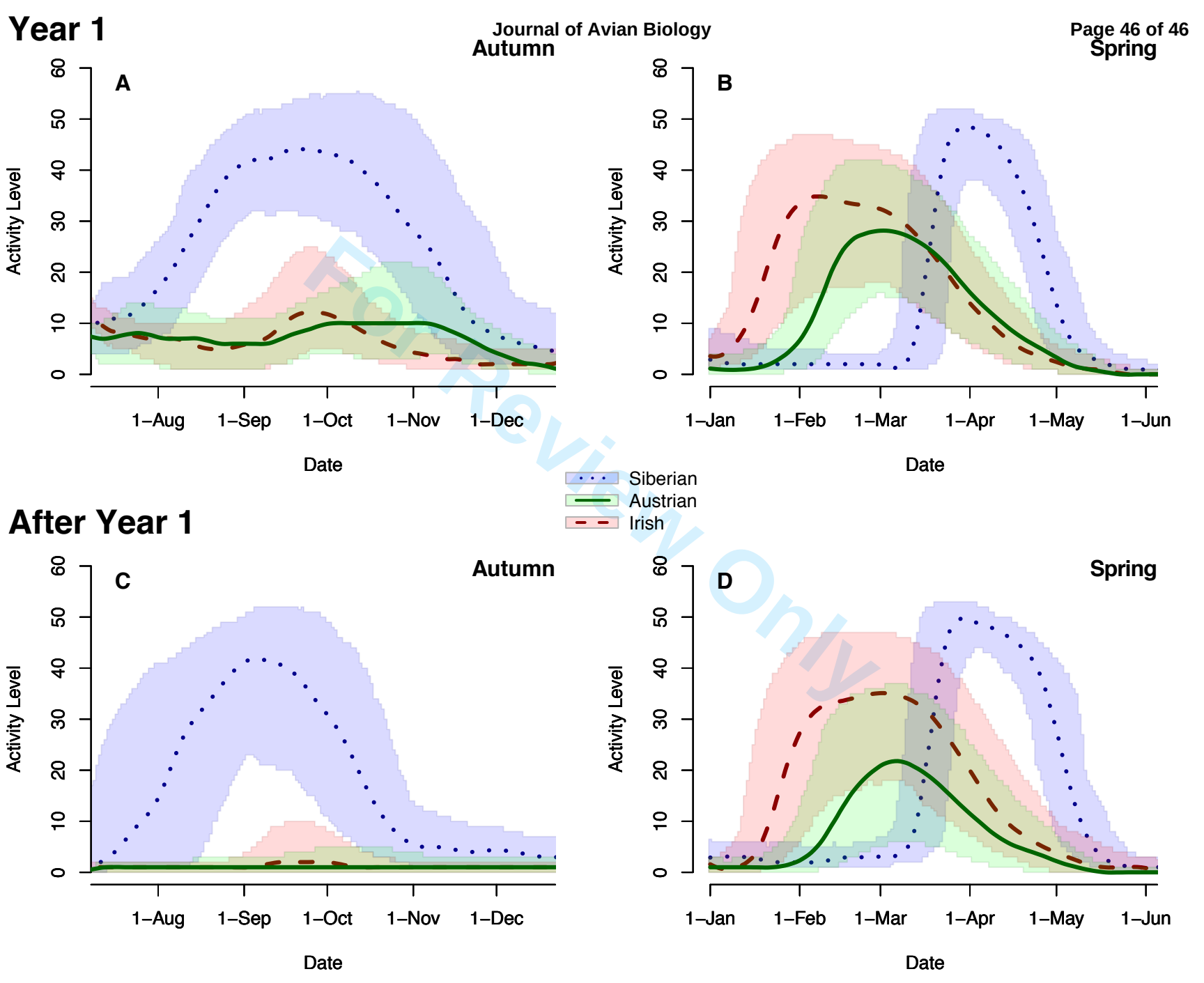

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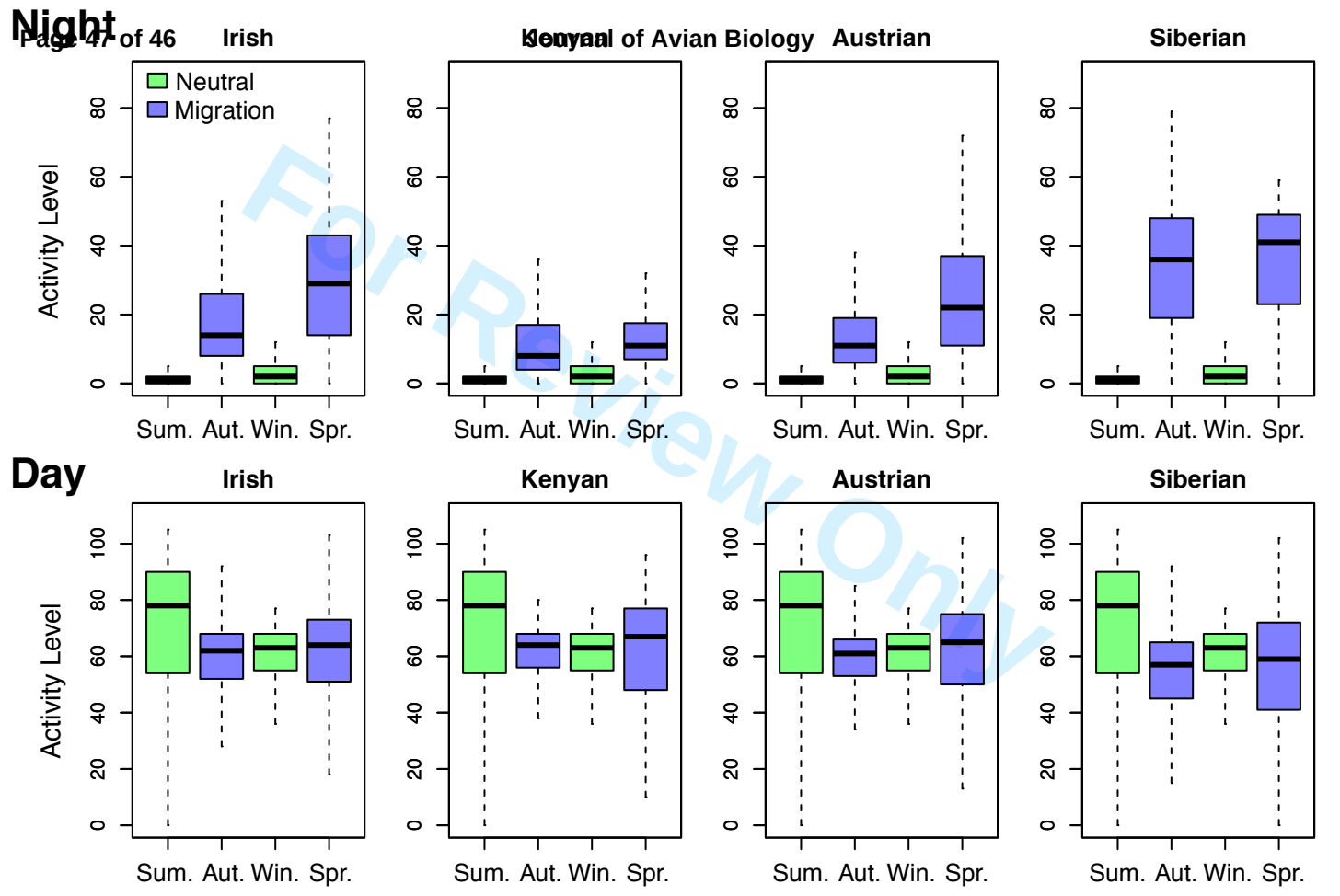

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