

Variation in chronotype is associated with migratory timing in a songbird

(Supplemental Material)

Jeffrey L. Rittenhouse, Ashley R. Robart, Heather E. Watts

Biology Letters

2. Methods

(a) Animals

Data for this study came from previous experiments investigating the use of environmental cues to time spring migration in pine siskins ([1]; Robart and Watts unpublished). Birds for these experiments were captured in Eagle Point, OR (42°30'N, 122°49'W); Los Angeles, CA (34°07'N, 118°12'W); Leavenworth, WA (47°36'N, 120°50'W); Randle, WA (46°18'N, 121°32'W); and Jackson, WY (43°28'N, 110°49'W). The current study used data from three experimental groups (Table 1 of original manuscript) where subjects were held in individual cages on photoperiods mimicking naturally increasing day lengths. From the Robart et al. [1] study we used birds from the 'natural-day' treatment of the 'photoperiod experiment', which experienced a photoperiod mimicking 34°N. From the same study, we also included birds from the 'timing experiment,' which were held on a photoperiod mimicking 42°N. Finally, we included birds from a third experiment focusing on male pine siskins (hereafter 'males only' experiment; Robart and Watts unpublished); these birds were housed on a photoperiod that mimicked a latitude of 42°N. Although a few birds were subjects in more than one experiment, we used only data from the first experiment a bird participated in; thus, each bird is represented only once in the dataset.

In all experiments, birds were maintained with *ad libitum* access to Roudybush Small Bird Maintenance Diet (Woodland, CA) and a mixture of Nyjer thistle and sunflower heart seeds, as well as water and grit. Birds were housed such that they could hear, but not see, other birds. Birds included in the Robart et al. [1] study could hear both male and female conspecifics throughout the experiment. In the ‘males only’ experiment, only male conspecifics could be heard. In all three experiments, birds were handled every ~ 2 weeks to collect body measurements and small volumes of blood as previously described [1; data not presented].

(b) Activity data

All experiments commenced in the winter and continued into the spring, though the timing of each experiment varied (Table 1 of original manuscript). Despite this variation, data collection in all experiments captured the period when spring nomadic migratory transitions typically occur [1, 2]. For all experiments, activity of individual birds was recorded by a system of passive infrared motion sensors mounted on top of each cage and connected to a VitalView Data Acquisition System (Starr Life Sciences Corp., Oakmont, PA). This system continuously recorded movements for each bird and summed them in 10-minute intervals.

To determine when each bird began to express spring migratory restlessness, we examined activity data recorded between the hours of 23:00 and 3:00. We focused on this timeframe because pine siskins expressing migratory restlessness are typically quiescent between lights-off and 23:00 [2], and because activity preceding lights-on could indicate predawn behavior rather than migratory restlessness [2-4]. We considered a bird to have expressed migratory restlessness on a given night if at least seven 10-minute intervals contained ≥ 10 movements. This threshold was based on activity levels previously observed in migratory pine

siskins [1, 2]. To account for isolated nights of irregularly elevated activity, we only categorized a bird as expressing migratory restlessness when it had at least five consecutive nights of activity meeting our criterion for migratory restlessness. The date of onset of migratory restlessness was then defined as the first night of the ≥ 5 -night period. Nights following the day on which a blood sample was collected from a bird were excluded from our analysis—i.e. they would neither add to nor interrupt such a five-night period. Birds that never expressed nocturnal activity that met our criteria were excluded from the dataset. Additionally, we excluded two birds that had expressed migratory restlessness from the start of data collection, because we could not determine their onset date of migratory restlessness. This resulted in a sample of 16 males and 7 females that had expressed migratory restlessness.

To characterize a bird's chronotype, we used the onset time of nocturnal activity (i.e., the time when a bird ended its quiescence and initiated activity associated with migratory restlessness). We used this measure of chronotype, rather than the time of morning awakening, because it is independent of any immediate changes in light levels as it occurs in the middle of the dark phase. In our captive experiments, the transition between light and dark phases occurred abruptly, and almost all birds showed changes in activity levels that were closely synchronized with these transitions. Thus, the onset of morning activity in our captive setting may reflect masking effects of light [5] rather than chronotype.

We determined the onset time of nocturnal activity for each night after a bird began expressing migratory restlessness. To determine the onset of nighttime activity, we examined data across the entire night, first determining that a bird had entered a period of quiescence following lights off – defined as occurring when a bird had at least three consecutive 10-minute intervals with less than 10 movements each [2]. Once a bird had become quiescent, we defined

the onset of nocturnal activity to be the time when activity levels increased to an average of at least 10 movements per 10-minute interval for at least three intervals. By assigning the onset time of nocturnal activity to occur only after the quiescent period, we ensured that activity immediately following lights-off—likely a continuation of diurnal activity—was not included in our dataset. If the onset of nocturnal activity occurred fewer than 30 minutes before lights on in the morning, then we conservatively considered the bird in question to be inactive for that night as such activity likely indicates an early onset of diurnal activity. The number of nights for which we could determine the onset of nocturnal activity varied across birds (mean = 49.3 nights, range: 9 - 150 nights); this variation was driven by differences among birds in when they bird became migratory and differences in the duration of experiments. For analysis, we recorded the onset time of nocturnal activity as minutes relative to lights-off.

(c) Statistical analyses

Statistical analyses were performed using R 3.5.0 [6]. Our general approach was to use linear mixed models implemented with the lme4 package [7]. We visually inspected residual plots to check for deviations from normality and homoscedasticity. We also inspected plots of the estimated random effects (i.e., the conditional modes of the random effects) for deviations from normality. We tested for model effects using Satterthwaite's method in the lmerTest package [8].

We calculated repeatability for the onset time of nocturnal activity to assess whether individuals exhibited consistent chronotypes. We used a linear mixed model with onset of nocturnal activity as the dependent variable. Experiment and days since winter solstice (hereafter

‘date’; which was centered and scaled) were fixed effects and bird was a random effect (with random slopes with respect to date) in the model. Experiment was included in models to account for differences in experimental conditions across experiments. We tested for the effect of sex and the interaction of date and experiment; however, neither had a significant effect on the onset of nocturnal activity (sex: $t_{17.40} = 1.03, p = 0.32$; date x experiment_{timing}: $t_{75.26} = 1.31, p = 0.19$; date x experiment_{males-only}: $t_{12.84} = -1.20, p = 0.25$), and we removed them from the final model. Repeatability was estimated with the rptR package [9], using 1000 bootstrap samples to obtain a 95% confidence interval and p -value for our repeatability estimate.

To determine whether there was an association between chronotype and the timing of onset of migratory restlessness we used a linear mixed model. Following the approach of Graham et al. [10] and Helm and Visser [11], we used the onset of nocturnal activity—our measure of chronotype—as the dependent variable and included the onset date of migratory restlessness as a fixed effect. We also included experiment and date as fixed effects and bird identity with respect to date was fit using random slopes. Again, we tested for the effect of sex and the interaction of date (centered and scaled) and experiment, but we excluded them from the final model as they had no significant effect on the onset of nocturnal activity (sex: $t_{20.39} = 0.59, p = 0.56$; date x experiment_{timing}: $t_{75.09} = 1.24, p = 0.22$; date x experiment_{males-only}: $t_{12.80} = -1.35, p = 0.20$).

References

1. Robart A.R., McGuire M.M.K., Watts H.E. 2018 Increasing photoperiod stimulates the initiation of spring migratory behaviour and physiology in a facultative migrant, the pine siskin. *Royal Society Open Science* **5**(8), 180876.

2. Watts H.E., Robart A.R., Chopra J.K., Asinas C.E., Hahn T.P., Ramenofsky M. 2017 Seasonal expression of migratory behavior in a facultative migrant, the pine siskin. *Behav Ecol Sociobiol* **71**(1), 9.
3. Agatsuma R., Ramenofsky M. 2006 Migratory behaviour of captive white-crowned sparrows, *Zonotrichia leucophrys gambelii*, differs during autumn and spring migration. *Behaviour* **143**(10), 1219-1240.
4. Pérez J.H., Furlow J.D., Wingfield J.C., Ramenofsky M. 2016 Regulation of vernal migration in Gambel's white-crowned sparrows: Role of thyroxine and triiodothyronine. *Horm Behav* **84**, 50-56.
5. Mrosovsky N. 1999 Masking: history, definitions, and measurement. *Chronobiol Int* **16**(4), 415-429.
6. R Core Team. 2018 R: A Language and Environment for Statistical Computing. (3.5.0 ed. Vienna, Austria, R Foundation for Statistical Computing.
7. Bates D., Mächler M., Bolker B., Walker S. 2015 Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**(1), 1-48.
8. Kuznetsova A., Brockhoff P.B., Christensen R.H.B. 2017 lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software* **82**(13), 1-26.
9. Stoffel M.A., Nakagawa S., Schielzeth H. 2017 rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution* **8**(11), 1639-1644.
10. Graham J.L., Cook N.J., Needham K.B., Hau M., Greives T.J. 2017 Early to rise, early to breed: a role for daily rhythms in seasonal reproduction. *Behav Ecol* **28**(5), 1266-1271.
11. Helm B., Visser M.E. 2010 Heritable circadian period length in a wild bird population. *Proceedings of the Royal Society B: Biological Sciences* **277**(1698), 3335-3342.