

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Pied flycatchers ( <i>Ficedula hypoleuca</i> )	Wild-caught	N/A
Deposited Data		
Custom analysis scripts and associated data	This paper	<a href="https://doi.org/10.17632/6n38vwnwc7.1">https://doi.org/10.17632/6n38vwnwc7.1</a>
Software and Algorithms		
R statistical computing environment	<a href="https://cran.r-project.org">https://cran.r-project.org</a>	N/A
Other		
Local hourly ambient temperature data from two weather stations in southwest Germany	German weather service	<a href="https://www.dwd.de/EN/climate_environment/cdc/cdc_node.html">https://www.dwd.de/EN/climate_environment/cdc/cdc_node.html</a>

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Barbara Helm ([b.helm@rug.nl](mailto:b.helm@rug.nl)). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Pied flycatchers

The experimental subjects were pied flycatchers (*Ficedula hypoleuca*). All experimental procedures conformed to the relevant regulatory standards under permit by the state of Upper Bavaria, Germany.

METHOD DETAILS

Description of replication study in captivity

Pied flycatchers from southwest Germany were studied in the Max Planck Institute for Ornithology by the late Eberhard Gwinner in research on circannual rhythms and photoperiodism [9, 13, 27]. The original studies in the 1980s established the annual cycle of hand-raised birds under different photoperiodic cycles [13, 27]. One experiment in 1981 [27] mimicked the natural daylengths experienced by the birds during breeding, on migration, and in their West African wintering area (10° N). Under these conditions, captive young flycatchers underwent post-juvenile moult soon after independence and started autumn migratory restlessness at very young ages (Main text, Figure 1). Autumn migratory restlessness was often biphasic and extended into early winter. The substantial fat reserves deposited in autumn were also maintained until late winter. Thereafter, the flycatchers lost these fat reserves and undertook prenuptial winter moult of body plumage, as well as tertials and some inner secondaries (collectively “flight feathers”). Moult was followed by the start of spring migratory restlessness and gonadal growth.

In 2002, Gwinner, with help from author BH, replicated this study under identically mimicked conditions [13, 27], although sadly he did not live to see the full results. The goal of the replication was to test for evolutionary change in flycatcher timing since 1981. Gwinner collected flycatchers in 2002 from the same area as in 1981 (Figure S1), hand-raised them in the same way, and tested them under identical conditions, using original lighting devices and both, original and new recording methods.

In 2002, birds were collected in Lahr in southern Germany (48.3° N / 7.3° E; elevation 160 m asl; Figure S1) as nestlings and hand-raised as described earlier [48]. For precise replication, nestlings were collected at similar dates and ages (Figure S2). Because the timing of a bird’s hatching may influence the timing of subsequent events in its annual cycle [44], we tested whether hatch date was significantly different between cohorts. There was no detectable difference between the 1981 and 2002 cohorts tested by linear model (effect = 1.61 days,  $t_{30} = 0.97$ ,  $p = 0.34$ ), nor between males and females (effect = -2.49 days,  $t_{30} = -1.59$ ,  $p = 0.12$ ), and the interaction term was likewise not significant (effect = 2.08 days,  $t_{29} = 0.62$ ,  $p = 0.54$ ). In 1981, mean hatch date was ordinal (julian) day 148.5; in 2002, it was day 150. Hatch date was also included in all our models and was not a significant predictor of any timing trait, with the exception of the end of postjuvenile moult. Age at collection also did not differ (8.4 days in 2002; 9.0 days in 1981; t test,  $p = 0.52$ ).

Once independent, young birds were kept in individual cages (42x23x23cm) in climate-controlled chambers (ca. 20°C) with light provided by 40-W fluorescent bulbs in the daytime (400 lx at perch level) and by 10-W incandescent bulbs at night (ca. 0.01 lx). Birds were exposed to simulated local daylength until the approximate start of autumn migration. Thereafter, they were progressively shifted to the photoperiodic conditions they would naturally experience *en route* and at their West African wintering areas slightly