



**Figure 3. Differences in Timing Traits between 1981 and 2002**

Shown are means and one SE of timing of captive flycatchers, estimated from linear mixed-effects models. Negative values indicate that the event occurred earlier in 2002.

See also [Figures S1](#) and [S2](#).

### Field Data from Wild Flycatchers

To link the replicated laboratory experiment to responses in wild conspecifics, we analyzed data from a 46-year field study of nearby breeding flycatchers [34] ([Figure S1](#)), testing for changes in reproductive timing and sensitivity to local ambient temperature [35] ([Figure 4](#)). We expected the degree of advance in the spring phenology of captive birds to be comparable to that of wild conspecifics, although wild birds might show additional phenological plasticity of  $-1$  to  $-2$  days  $^{\circ}\text{C}^{-1}$  [16].

Field data indicated that wild flycatchers also advanced spring phenology ([Figure 4A](#)). During the interval between captive studies (1981–2002), wild flycatchers commenced egg-laying progressively earlier (slope:  $-0.53$  days  $\text{year}^{-1}$ , 95% CI  $-0.73$  to  $-0.34$ ), achieving an 11.2-day advance over those 21 years. Over the entire field time series (1973–2018), lay dates changed by  $-0.31$  days  $\text{year}^{-1}$  (95% CI  $-0.39$  to  $-0.24$ ).

Advances of breeding phenology in wild flycatchers were partly explained by ambient temperature on the breeding grounds ([Figure 4B](#)). We identified the time window in which mean temperature was most closely associated with lay date using R package *climwin* [35, 36]. This temperature-sensitive window occurred from March 29 to May 13 (1973–2018; [Figure S4](#); *climwin* randomization  $p < 0.001$ ). Mean temperature during this window increased rapidly, by  $0.080^{\circ}\text{C}$   $\text{year}^{-1}$  (95% CI  $0.012$  to  $0.15$ ) between the captive experiments, and by  $0.063^{\circ}\text{C}$   $\text{year}^{-1}$  (95% CI  $0.039$  to  $0.088$ ) from 1973–2018 ([Figure 4B](#)).

In addition to temperature sensitivity (i.e., phenological plasticity), flycatcher lay dates also showed directional change over time. Between captive experiments (1981–2002), flycatcher lay dates covaried with temperature by  $-1.5$  days  $^{\circ}\text{C}^{-1}$  (95%

CI  $-2.6$  to  $-0.32$ ) while advancing at a rate of  $0.41$  days  $\text{year}^{-1}$  (95% CI  $-0.6$  to  $-0.21$ ). From 1973 to 2018, plasticity was identical ( $-1.5$  days  $^{\circ}\text{C}^{-1}$ , 95% CI  $-2.3$  to  $-0.72$ ), but the rate of annual change was lower ( $-0.22$  days  $\text{year}^{-1}$ , 95% CI  $-0.3$  to  $-0.14$ ). Hence, our study interval captured a particularly strong directional change during a period of rapid warming. These figures fit well with studies of flycatchers in regions with strongly increasing spring temperature [10, 12, 16] ([Figure S4](#)). Accounting for the effect of year was important; a model including temperature as the sole predictor overestimated plasticity ( $-2.7$  days  $^{\circ}\text{C}^{-1}$ , 95% CI  $-3.5$  to  $-1.9$ ).

Beyond advancing egg-laying, wild flycatchers advanced the timing of the temperature-sensitive window itself ([Figure 4C](#)). The mean date of the best window advanced by  $0.83$  days  $\text{year}^{-1}$  (bootstrapped 95% CI  $-1.1$  to  $-0.45$ ) over 24 years. Lay dates early in our time series (e.g., 1973–1995) were best explained by breeding-ground temperatures from mid-April to mid-May, while lay dates in the later years (e.g., 1996–2018) were best explained by temperatures from late March to early May.

In summary, our captive experiment revealed advances in the timing of spring events that were not likely attributable to plasticity, since flycatcher cohorts monitored in 1981 and 2002 were raised and studied under replicated laboratory conditions. Spring advances of 9 days in captive birds mirrored advances in the lay dates of wild birds of 11 days during the same period. We also detected a potential delay in autumn timing and a tendency of earlier timing in late winter in the captive birds.

### Changes to the Timing Program

Our findings suggest that the circannual timing program of flycatchers has undergone phenotypic evolution. Circannual clocks are inherited [9] and track the time of year, even under constant experimental conditions. Importantly, they regulate organisms' timing responses to environmental factors, in particular, photoperiod and ambient temperature [9, 37]. Rapid micro-evolutionary change in the circannual program is feasible in songbirds and has been reported in Eurasian blackcaps (*Sylvia atricapilla*) [38, 39]. Timing changes may advance or delay the entire annual cycle [29], but in our captive flycatchers shifts were season specific.

In spring, the clear phenology advance of our flycatchers mirrored widely reported shifts in migratory birds [2, 5, 6, 10, 15, 25, 29], which may partly reflect high selection pressures linked to reproduction [4, 17, 18, 30]. Because early departure from African wintering quarters facilitates early arrival on the breeding grounds [40, 41], it is clear how selection for reproductive timing may translate into earlier preparation to depart Africa. Among the contributing traits, the strong advance of flight feather moult is expected because this moult is largely completed before birds migrate, whereas body moult may overlap with migration ([Figure 1](#)). A weaker signal for migratory restlessness timing may be due to the large sampling variance of that trait and our small sample size; migratory restlessness is a proxy for wild migratory behavior, and its timing is difficult to quantify with high precision [8, 42]. Our findings were robust when this trait was excluded to enable larger sample sizes (see [STAR Methods](#)). Last, as predicted for our protandric