Supplementary material for:

Krist M, Munclinger P, Briedis M & Adamík P (2021) The genetic regulation of avian migration timing: combining candidate genes and quantitative genetic approaches in a long-distance migrant, submitted to *Oecologia*.

Supplementary methods

Estimation of male arrival date to the breeding site

We caught collared flycatcher males at the time of their spring arrival to Velký Kosíř from 2013–2016. In this period, 381 nest-boxes were located at five plots in an oak (*Quercus petraea*) forest. We simultaneously trapped males in all these nest-boxes. Each year, 11–15 catching sessions (days) were carried out. During each trapping session, a team of ca 10 field assistants activated string traps in all nest-boxes that did not contain any nest or that contained a nest without any progress in nest-building from the last nest-box check. We monitored the study area several times a week following the first record of the collared flycatcher in the database for birdwatchers in the Czech Republic (<https://birds.cz/avif>). We then started to catch flycatchers the day after we recorded the first singing male in our study area (range April 12th–16th in the four years). Catching lasted about a month, with the last session between May 15th–19th. We started to catch flycatchers at 7–8 a.m. and finished about at 14–15 p.m. We planned the subsequent session according to the number of newly arrived males. When many new males arrived, the next session was carried out the next day or within a few days, while the interval was prolonged when only a few new males arrived (see the Dryad Digital Repository for the dates of all catching sessions). We started to catch flycatchers in most nest-boxes as they were empty at the start of the season. The number of nest-boxes available for catching steadily declined as the season progressed and tits and flycatchers started to breed. Each newly arrived male was brought to the field station placed in the centre of the study area for measurements, ringing and blood sampling, which was done by tibial venipuncture. Males were also aged based on their plumage characteristics (second year of life or older) and then either brought back to the plot where they were caught (2013) or released directly from the field station (2014–2016).

The arrival date of each male was calculated as the mean date between the first capture and the previous catching session. We validated this method using 16 males for whom we had both catching data and the true arrival data from geolocators in 2016 (see below for methods of geolocation). Our estimate of arrival date correlated strongly with the true arrival date ($F_{1,14} = 133.6095$, $p < 0.001$, $R^2 = 0.905$, $n = 16$, Supplementary Figure 1a). A further option for checking our ability to catch males soon after their arrival is to compare our estimates of arrival date to laying dates in nests attended by them. In total, 281 out of 520 males caught on arrival were found breeding in our nest-boxes. For 18 of these, the estimated arrival date was later than the laying date of the first egg (i.e. negative mating speed, mean = -3.28, range: -0.5 to -15.5, Supplementary figure 1b). This happens sometimes due to mate replacement, i.e. the adoption of a widowed female and nest by a newly arrived male (Lifjeld et al. 1997, Sheldon et al. 1999), but we consider most of these cases to be caused by our inability to catch males before they attracted their primary female. After the primary female started to build a nest, we stopped catching in that nest-box and the males could only be caught if they tried to attract a secondary female. Therefore, we excluded these presumed inaccuracies from the dataset. If we assumed a similarly low frequency of ca 6% ($18/281 \times 100$) for these inaccuracies in the rest of the data for which we could not validate since the arriving males did not breed at our plots (i.e. $520 - 281 = 239$ arrivals), we would have about 15 such cases in our sample of 502 analyzed arrivals. Together with the good congruence between our estimate of arrival date and the true arrival date from geolocators, this low frequency of inaccurate values suggests that our estimates captured most of the phenotypic variation in the timing of male arrival.

Lifjeld JT, Slagsvold T, Ellegren H (1997) Experimental mate switching in pied flycatchers: Male copulatory access and fertilization success. *Animal Behaviour* 53:1225-1232. doi: 10.1006/anbe.1996.0430

Sheldon BC, Davidson P, Lindgren G (1999) Mate replacement in experimentally widowed collared flycatchers (*Ficedula albicollis*): determinants and outcomes. *Behavioral Ecology and Sociobiology* 46:141-148. doi: 10.1007/s002650050603

Supplementary Figure 1 Validation of the catching date as the arrival date. (a) Relationship between the spring arrival date inferred from geolocators and our estimate of the arrival date from the midpoint between the date of first catching of the male and the date of the previous catching session. Both dates are expressed as the day of the year (1 = January 1st). (b) Distribution of mating speeds. The speed is expressed as the difference between the laying date of the first egg in the nest this male attends and the male's estimated arrival date. Negative values mean that the first egg was laid before we caught the attending male.

Supplementary Table 1 Results of the general linear model relating the estimated arrival date (day of the year, 1 = January 1st) to male age and year (n=502). The reference category was the arrival of an old individual in the year 2013.

Parameter	Estimate	SE	t	p
Intercept	111.14	0.55		
Age (young)	7.10	0.72	9.82	<0.001
Year (2014)	2.44	0.82	2.97	<0.001
Year (2015)	4.83	0.85	5.67	0.003
Year (2016)	1.33	0.87	1.53	0.126