<https://helda.helsinki.fi>

Climate-driven build-up of temporal isolation within a recently formed avian hybrid zone

Sirkiä, Päivi M.

2018-02

Sirkiä , P M , McFarlane , S E , Jones , W , Wheatcroft , D , Ålund , M , Rybinski , J & Qvarnstrom , A 2018 , ' Climate-driven build-up of temporal isolation within a recently formed avian hybrid zone ' , Evolution , vol. 72 , no. 2 , pp. 363-374 . <https://doi.org/10.1111/evo.13404>

<http://hdl.handle.net/10138/328208>

<https://doi.org/10.1111/evo.13404>

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Climate-driven build-up of temporal isolation within a recently formed avian hybrid zone

Keywords: speciation, pre-zygotic isolation, temporal segregation, ecological speciation, competitive exclusion, reinforcement

Short running title: Climate-driven temporal isolation

Päivi M. Sirkä^{1,2‡*}, S. Eryn McFarlane^{1‡}, William Jones¹, David Wheatcroft¹, Murielle Ålund¹, Jakub Rybinski¹, and Anna Qvarnström¹

¹ Animal Ecology/Department of Ecology and Genetics, Norbyvägen 18d, SE-752 36 Uppsala, Sweden

² Finnish Museum of Natural History, Zoology Unit, University of Helsinki, Finland

‡ co-first authors

*** Correspondence to**

Päivi M. Sirkä, Finnish Museum of Natural History, Pohjoinen Rautatiekatu 13, FIN-00100 Helsinki, Finland. Tel. +358505951782, email: paivi.sirkia@helsinki.fi

Authors' contributions

AQ planned and coordinated the collection of the data, which was collected by all authors. PMS, SEM and AQ wrote the manuscript with all authors contributing. SEM performed the statistical analysis of data. All authors gave final approval for publication.

Acknowledgements

We thank numerous persons collecting the long-term data in the field.

Funding

This work was supported by the Swedish Research Council (awarded to A.Q.), the Academy of Finland (awarded to P.M.S.), and the Natural Sciences and Engineering Research Council (awarded to S.E.M.).

Data archival location: Dryad

Climate-driven build-up of temporal isolation within a recently formed avian hybrid zone

Abstract

Divergence in the onset of reproduction can act as an important source of reproductive isolation (i.e. *allochronic isolation*) between co-occurring young species, but evidence for the evolutionary processes leading to such divergence is often indirect. While advancing spring seasons strongly affect the onset of reproduction in many taxa, it remains largely unexplored whether contemporary spring advancement directly affects allochronic isolation between young species. We examined how increasing spring temperatures affected onset of reproduction and thereby hybridization between pied and collared flycatchers (*Ficedula* spp.) across habitat types in a young secondary contact zone. We found that both species have advanced their timing of breeding in 14 years. However, selection on pied flycatchers to breed earlier was weaker, resulting in a slower response to advancing springs compared to collared flycatchers and thereby build-up of allochronic isolation between the species. We argue that a pre-adaptation to a broader niche use (diet) of pied flycatchers explains the slower response to raising spring temperature, but that reduced risk to hybridize may contribute to further divergence in the onset of breeding in the future. Our results show that minor differences in the response to environmental change of co-occurring closely related species can quickly cause allochronic isolation.

Introduction

Global climate change is increasingly affecting populations and communities on a broad scale (e.g. Parmesan 2006; IPCC 2014). As a short-term response to climate change species may expand or

26 contract distribution ranges or in the worst-case scenario be driven towards extinction (e.g. Tayleur
27 et al. 2015). Alternatively, organisms may locally adjust, either plastically or genetically, to
28 changed conditions by altering the timing of key events, such as the timing of breeding and
29 migration patterns of adults, or their growth and development (Thackeray et al. 2016). One
30 important consequence of distribution range changes is that young species that have genetically
31 diverged, but are still phenotypically similar, come into secondary contact at an increasing speed
32 and may compete over resources and interfere sexually. The formation, location and movement of
33 hybrid zones, which are geographical places where young species meet and form hybrid offspring,
34 have been shown to be greatly affected by both past (Hewitt 1996) and present climatic conditions
35 (Taylor et al 2014). Thus, large-scaled changes in the environment such as global warming are
36 likely to have significant effects on speciation processes and/or on patterns of competitive
37 exclusion. Long-term monitoring of hybrid zones has therefore been identified as an important
38 source of information that can increase our general understanding of how species are affected by
39 climate change (Harr and Price 2014, Taylor et al 2015, Qvarnström et al 2016) but this potential
40 remains largely unexplored. For example, while there are studies on phenology responses across
41 years in multiple taxa (e.g. Davis et al. 2010, Møller et al. 2008), no previous studies have, to our
42 knowledge, investigated whether co-occurring young species respond phenologically differently to
43 ongoing climate change within hybrid zones and what the possible consequences of such
44 differences in responses might be. This is a big omission as effects on the timing of key
45 reproductive events may affect temporal segregation in breeding between young species and
46 thereby processes of both hybridization and competition in areas of co-occurrence.

47
48 In general, diverged timing of breeding can facilitate co-existence of young species both by acting
49 as a pre-zygotic barrier (i.e. allochronic isolation Hendry and Day 2005; Taylor and Friesen 2017)
50 that prevents gene flow or other forms of costly reproductive interferences (Gröning and Hochkirch

2008) and by reducing competition over resources (MacArthur and Levins 1967; Schoener 1974; Richards 2002). For example, 13- versus 17- year periodical cicadas of the genus *Magicicada* can apparently co-exist despite having otherwise identical ecology and similar mating signals. These periodical cicadas diverged into three different species groups each containing 13-year and 17-year species 4-2.5 million years ago (Sota et al. 2013). Because 17-year cicadas occur in the northern parts of eastern USA, the slower lifecycle is considered to be an adaptation to a colder climate (Koyama et al. 2015). However, establishing the relative importance of allochronic isolation arising as a consequence of (i) allopatric divergence due to different exposure to climate preceding secondary contact, (ii) different responses to climate change during sympatric conditions, or (iii) allochronic isolation as a direct response to selection against hybridization and competition in sympatry is difficult when past selection pressures need to be reconstructed. The same problem is true for most established cases of allochronic isolation including flowering time in plants (Ellis et al. 2006), migration and breeding in salmon (Quinn et al. 2000), breeding in stickleback fish (Lackey and Boughman 2017), diel activity in moth (Devries et al. 2008) and timing of spawning in algae and corals (Knowlton et al. 1997; Clifton and Clifton 1999).

While the distinction between reproductive isolation arising during divergence in (i) allopatry or (iii) sympatry has been an intensively debated scientific question (Coyne and Orr 2004), reproductive isolation affected by different responses to environmental change in sympatry (ii) have received comparatively little scientific attention probably because closely related species often are expected to respond in the same way to identical selection pressures. However, different histories of distribution ranges and therefore histories of independent evolution in geographic isolation should influence how species react to current conditions. Whether and how different local responses to ongoing climate change affect interactions between young species when they co-occur remains largely unknown.

76

77 Collared flycatchers are small insectivorous passerine birds that are one of several species that are
78 expanding their breeding range northward in response to global climate change (Huntley et al
79 2007). In this study, we focus on a population that is located at the northern edge of their breeding
80 range, in a young hybrid zone with the closely related pied flycatchers (Qvarnström et al. 2010;
81 Qvarnström et al. 2016). Our goal is to investigate whether different local responses to climate
82 change in timing of reproduction between the two species may lead to allochronic isolation. Well-
83 timed breeding is known to have a strong effect on the reproductive success of birds breeding in the
84 temporal zone (e.g. Both et al. 2006) and both flycatcher species rely on seasonal peaks in their
85 preferred food, caterpillar larvae (Cramp and Simmons 2006; Burger et al. 2012). Several
86 populations of collared and pied flycatchers have therefore advanced their breeding due to increased
87 spring temperatures, which causes earlier development of leaves on the deciduous trees and
88 therefore advanced peaks in caterpillar larvae (Both et al. 2004; Weidinger and Král 2007).
89 However, it remains unknown whether the responses to increased spring temperatures differ in
90 magnitude between populations of the two flycatcher species that live under sympatric conditions
91 and how allochronic isolation is affected. Since collared flycatchers experience a steeper decline in
92 reproductive success as the breeding season progresses as compared to pied flycatchers
93 (Qvarnström et al. 2005; Qvarnström et al. 2009), we expect collared flycatchers to be selected to
94 more closely track the advancing peak in food abundance as the spring temperature is increasing.
95

96 While both species have advanced their laying during the last fourteen years we do find a relatively
97 steeper advancement in the timing of breeding of collared flycatchers, which is consistent with a
98 relatively stronger phenotypic response to earliness of springs and selection favoring early breeding
99 in this species. Increased temporal segregation is however only obvious within the low quality
100 habitat type while in the high quality habitat both species appear to have advanced their onset of

101 breeding to a similar extent. Late breeding pied flycatcher males experienced a reduced likelihood
102 of hybridization, but we observe a lower degree of temporal segregation (and thereby allochronic
103 isolation) in the high quality habitats, where both selection against hybridization and interspecific
104 competition are stronger due to a higher relative proportion of heterospecific individuals. This
105 suggests that selection against hybridization or competition are unlikely to drive temporal isolation.
106 Together, these results imply that allochronic isolation between the two *Ficedula* species in this
107 young hybrid zone is caused by minor ecological differences between the two species that render
108 collared flycatchers more sensitive to mismatched onset of breeding relative to the advanced peak
109 of caterpillar larvae abundance.

110

111 **Materials and Methods**

112 *Study system and sampling of long-term data*

113

114 Pied and collared flycatchers diverged less than one million years ago (Nadachowska-Brzyska et al.
115 2013) most likely during allopatric conditions. The Swedish hybrid zone arose when collared
116 flycatchers expanded their breeding range northwards where pied flycatchers were already present.
117 This hybrid zone comprises of two islands of the Baltic Sea: Gotland (that probably was colonized
118 around 150 year ago, Lundberg and Alatalo 1992) and Öland (that collared flycatchers are known to
119 have colonized only around 50 years ago, Qvarnström et al. 2009). Our study was performed on the
120 island of Öland, where mixed species pairings occur regularly (Qvarnström et al. 2010), but
121 selection against heterospecific pairings is strong (Wiley et al. 2009). Based on our breeding
122 records, we have not detected any hybrid individuals (including both male and female hybrids) that
123 managed to produce offspring (Svedin et al. 2008; Ålund et al. 2013). However, Ellegren et al.
124 (2012) detected a low level of introgression from pied flycatchers to collared flycatchers indicating
125 gene flow. Thus, either not all hybrids may be completely sterile or complete hybrid sterility is a

126 relatively new phenomenon. After over-wintering in Africa, males arrive at the breeding grounds in
127 northern Europe in late April and early May and start defending natural breeding holes or nest-
128 boxes (Alatalo et al. 1994; Pärt and Qvarnström 1997). Flycatcher males compete fiercely over nest
129 boxes collared flycatcher being dominant over pied flycatcher. Females arrive on average a few
130 days after the males and choose their male based both on a male's characteristics and the territory
131 he defends (Alatalo et al. 1986; Dale and Slagsvold 1996; Sirkkiä and Laaksonen 2009). The onset of
132 egg laying varies yearly depending on the prevailing spring temperatures (e.g. Both et al. 2004).
133 Females lay five to seven eggs, which hatch in the beginning of June. Both parents feed their
134 offspring with insects for approximately two weeks in the nest and two weeks after fledging.
135

136 The relative proportion of breeding pied flycatchers varies from less than 10% to 100% across the
137 different woodlots on Öland (for detailed distribution of the breeding pairs of two species across
138 different habitat types over time see Rybinski et al. 2016). More than twenty separate nest-box
139 plots, each consisting of 25–350 boxes, have been established in order to study the dynamics of
140 interspecific interactions. Study areas were monitored in a standardized way (e.g. Qvarnström et al.
141 2009) and data on pairing patterns and the date of onset of egg laying, and number of fledged
142 offspring were collected yearly since 2002. In each year, all breeding birds are caught, identified by
143 species, ringed with unique aluminum rings and sampled for blood. We calculated the relative
144 fitness (after Lynch and Walsh 1998) of each female as the number of nestlings fledged from her
145 nest divided by the average number of nestlings fledged by other females of the same species over
146 the course of the entire study. Because none of the monitored hybrids managed to produce any
147 offspring (Svedin et al. 2008; Ålund et al. 2013) each female paired with either a heterospecific or
148 hybrid male had her fitness set to zero. This purposely over estimates the possible selection (as
149 females can and do mediate the effects of heterospecific social mates using extra pair paternity
150 (Cramer et al. 2016)), to be able to examine the worst-case scenario of heterospecific pairing.

151

152 ***Prevailing temperature, habitat composition and food availability***

153 Temperature data is from the Swedish Meteorological and Hydrological Institute's (SMHI)
154 published data for the weather station Norra Udde on Öland. Spring warmth sums are commonly
155 used to indicate the earliness of the spring (e.g. Visser and Holleman 2001; Charmantier et al. 2008)
156 and as a measure of earliness of the spring phenology capturing the general pattern of earliness and
157 lateness of the season preceding the arrival of flycatchers (late April-early May) we used April
158 warmth sums (with a base temperature +5 degrees, representing the bud-burst of tree leaves). To
159 test if species respond to general temperature pattern during arrival, nest building and beginning of
160 average egg laying period, we used the mean temperature for the 30-day period preceding the mean
161 laying date for each species.

162

163 The seasonal peak in abundance of preferred food differs dramatically across different habitat types
164 used by the birds within the hybrid zone, varying from highly seasonal food peaks in rich
165 broadleaved deciduous forests to lower but more stable food abundance in poor quality mixed
166 forests (Fig. S2) (Veen et al. 2010; Vallin et al. 2012; Rybinski et al. 2016). Early breeding in
167 synchronization with the food peak is therefore relatively more important in the broadleaved
168 caterpillar rich habitats (e.g. Both and Visser 2001; Burger et al. 2012). It is therefore important to
169 take into account habitat quality when examining selection acting on the onset of breeding. To
170 compare different habitat types with varying temporal availability of preferred food items we used
171 the most common 11 tree species (Supplementary figures S1, S2) to be able to cover the habitat
172 gradient from rich deciduous forest to relatively poor mixed forests. We calculated the amount of
173 caterpillars available on the occupied breeding territories. To do this, we first estimated caterpillar
174 biomass associated with different tree species and then combined this estimate with nest-box point
175 estimates of habitat tree species composition and finally extrapolated this point data to get habitat

176 type estimates for the foraging range of each breeding pair of flycatchers (see details in
177 Supplementary Information and Rybinski et al. 2016).

178

179 *Statistical analyses*

180

181 To determine whether the start of reproduction (here date of laying the first egg) was affected by
182 spring temperature differently in pied and collared flycatchers, we used a mixed effects model,
183 where laying date was the response variable, and year of study, mean temperature over the thirty
184 days preceding the mean laying date and species were included as fixed effects, with all possible
185 interactions. Additionally, we included both male and female identity as random effects.
186 To test whether the start of reproduction has advanced over the study years at different speeds in the
187 two species we used a mixed effects model, where we included the year of study, the species and
188 the habitat quality, with all possible interactions, as fixed effects and each male and female
189 individual identities as a random effects to account for repeated measures. We examined these
190 relationships using only conspecific pairs.

191

192 We tested whether there was evidence of temporal isolation, i.e. whether temporal segregation
193 affected pied flycatchers' risk of hybridizing with collared flycatchers using a generalized linear
194 mixed effects model with a logistic regression (link = logit) function where hybridization is a binary
195 response variable and laying date and habitat quality are fixed effects. We tested this separately in
196 male and female pied flycatchers as the likelihood of hybridization differs between sexes. We
197 binned habitat quality into above and below average to assist with model convergence.

198

199 Finally, we evaluated possible differences in the selection gradients acting on laying date in the two
200 study species. To do this, we used standardized laying date (the difference between individual

201 laying date and the species specific mean laying date over the course of the study, divided by the
202 standard deviation in laying date), and species, as well as the interactions between laying date and
203 species, with relative fitness (the number of nestlings fledged divided by the average number of
204 nestlings fledged by other individuals of the same sex of the same species over the course of our
205 study period) as the response variable (Lande and Arnold 1983). We also included habitat quality as
206 a covariate, and individual identity and year as random effects. This allowed for us to test for
207 directional selection in both pied and collared flycatchers, as well as to directly compare selection
208 between the two species. In a second model, we added standardized laying date squared, as well as
209 an interaction between laying date squared and species to test for stabilizing selection, and the
210 difference in stabilizing selection between the two species. These terms were added in addition to
211 all of the previously mentioned terms in the model. We estimated stabilizing selection in a separate
212 model from linear selection because linear and quadratic selection will be correlated unless all traits
213 are multivariate normal (Brodie 1992, Lande and Arnold 1983). Additionally, we report stabilizing
214 selection as twice the regression estimate (Lande and Arnold 1983, Stinchcombe et al. 2008). We
215 did not include mixed pairs in any of these analyses.

216

217 All statistical analyses were done in R Statistical Software version 3.2.3 (R Core Team 2014). The
218 linear mixed effects models (including the generalized linear mixed effects) were fitted with the
219 ‘lme4’ package (Bates et al. 2015), which was functionally expanded with the ‘lmerTest’ package
220 (Kuznetsova et al. 2014) that implements Satterthwaite’s approximations to degrees of freedom
221 (Flint et al. 1984) and provides tests of significance. All models fit within the assumptions of a
222 mixed effects model framework, as judged visually.

223

224 **Results**

Spring temperature sums preceding the arrival of flycatchers on breeding grounds have considerably increased on Öland during the last 50 years since collared flycatchers colonized the island (slope = 1.97 ± 0.38 , $t = 5.21$, $df = 49$, $p < 0.0001$) and this spring warming trend is similar during the subset of 14 years (2002-2015) when the present study was carried out (slope = 5.29 ± 2.69 , $t = 1.97$, $df = 13$, $p = 0.073$). During the study period we recorded breeding data for 2621 (80%) collared flycatcher pairs, 461 (14%) pied flycatcher pairs, and 180 (6%) mixed-species pairs between collared and pied flycatchers.

Responses to spring temperature and temporal segregation in onset of breeding

When testing whether spring warmth influenced the onset of egg laying of the two *Ficedula* species we found a significant effect of the interaction between species, spring temperature and year (*difference in slope between species* = -0.653 ± 0.12 , $t = -5.52$, $p = 3.7e-8$, Table S1) on the onset of egg laying. This interaction shows that both species advanced their onset of breeding when the spring was relatively warm but that collared flycatchers showed a stronger response than pied flycatchers (Fig. 1). As a consequence, there has been a build-up of temporal segregation (difference in the mean onset of breeding) between the two flycatcher species during the last fourteen years. Thus, while both species have advanced their laying during the last fourteen years (roughly from 3 to 5 days on average), a more pronounced advancement in the timing of breeding of collared flycatchers means that temporal segregation between the onset of breeding between pied and collared flycatcher females has increased over the study period. When we further examined how the onset of breeding have changed over time in the different habitat types we found a significant effect of a three-way interaction between year, habitat quality and species (*difference in slope between species* = -0.338 ± 0.15 , $t = -2.31$, $p = 0.021$, Supplementary table S2) on the onset of egg laying. This analysis reveals that increased temporal segregation is only obvious within the

low quality habitat type (Fig. 2A). Within the high quality habitat both species appear to have advanced their onset of breeding to a similar extent across the study period (Table S2; Fig. 2B).

Temporal isolation (i.e. effects of temporal segregation on reproductive isolation)

In order to investigate the consequences of increased segregation in the onset of breeding we tested whether the timing of breeding affected pied flycatchers' hybridization risk (whether they paired with conspecific or heterospecific individuals). We found that male pied flycatchers tend to be more likely to hybridize early than late in the season ($estimate = -0.191 \pm 0.10$, $Z = -1.88$, $p = 0.060$, Table S3, Fig. 3), when the effect of habitat quality is controlled for. However, female pied flycatchers were not significantly more likely to hybridize at earlier laying dates ($estimate = -0.019 \pm 0.02$, $Z = -0.91$, $p = 0.365$, Table S4). Thus, temporal segregation is beneficial because late breeding pied flycatcher males tend to reduce their risk of hybridization with collared flycatchers.

Selection acting on timing of breeding

We found that there were significantly different patterns of selection on laying date in pied and collared flycatchers ($F_{1, 2289.8} = 23.71$, $p = 0.0090$, Table S5), when using nestling fledging success as our relative fitness metric and controlling for habitat quality. There was significant directional selection acting on collared flycatchers ($\beta = -0.106 \pm 0.012$, Fig. 4A) to breed earlier, but not on pied flycatchers (β of -0.030 ± 0.029 , Fig. 4A). We furthermore found little evidence for stabilizing selection in either species ($F_{1, 2296.7} = 1.00$, $p = 0.316$, Table S5, Fig. 4B), when controlling for habitat quality and using nestling fledging success as relative fitness metric. However, there was a significant difference in this non-linear selection acting on the two species ($F_{1, 2290.4} = 3.81$, $p = 0.051$, Figure 4B with an estimated γ of -0.023 ± 0.025 in collared flycatchers and γ of 0.085 ± 0.050 in pied flycatchers). Taken together, the linear component of selection (Table S5) shows that there is stronger selection against late breeding in collared flycatchers and the stabilizing component of

274 selection contribute by showing that breeding extremely early also appear slightly more costly to
275 collared flycatchers.

276

277 **Discussion**

278 We investigated whether there has been a build-up of allochronic isolation *per se* (when controlling
279 for differences in habitat use) between pied and collared flycatchers during the last fourteen years
280 within a young *Ficedula* hybrid zone. We find that both flycatcher species have advanced their
281 timing of breeding during the last 14 years, probably in response to the ongoing climate change,
282 which are causing locally increased spring temperatures. However, pied flycatchers have responded
283 to increased spring temperatures more slowly (laying date advanced roughly 2.5 days for pied
284 flycatchers as compared with 4 days for collared flycatchers), leading to allochronic isolation,
285 which was most evident within the poor habitat type. The relationship between yearly varying
286 spring temperatures and the onset of breeding was less steep in pied flycatchers than in collared
287 flycatchers. Below we suggest that the observed fast build-up of allochronic isolation is indeed a
288 side-effect of the two species responding differently to large scale environmental change during
289 these sympatric conditions (i.e. secondary contact) and that these different responses were
290 facilitated by a broader niche use in pied flycatchers in terms of variety of insect types eaten
291 Lundberg and Alatalo (1992). This broader diet likely already evolved during allopatric conditions,
292 and allows pied flycatchers to use a broader temporal niche in sympatry. We also present evidence
293 against an alternative hypothesis, i.e. that allochronic isolation resulted from later breeding of pied
294 flycatchers in response to selection against hybridization and competition with collared flycatchers.
295 However, the present situation (later breeding pied flycatchers avoiding hybridization with collared
296 flycatchers) opens up an opportunity for selection against hybridization to further strengthen
297 temporal segregation between the two species in the future. Finally, we discuss whether the build-

up of temporal isolation should be considered an important part of the speciation process in this particular case or if this process mainly is relevant in the context of long-term co-existence.

Overall we found an advancing trend in the onset of breeding in both collared and pied flycatchers across habitat types during a 14-year study period. Avian phenology is known to be currently tracking the fast change in climate in the temporal zone (Møller and Fiedler 2004; Pearce-Higgins and Green 2014) and the observed advancing trend in timing of breeding is likely a response to warmer spring temperatures due to global climate change and a corresponding advancement of the peaks in food availability. The temperatures experienced during the breeding season of birds have increased in Sweden over the past few decades (e.g. Lindström et al. 2013; Tayleur et al. 2016) and both flycatcher species apparently track the advancing springs, especially in high quality habitats, where temporal segregation is not yet taking place.

There are at least three possible non-mutually exclusive explanations for the increasing difference in the onset of breeding of the two flycatcher species within the caterpillar larvae poor habitat. First, stronger selection for early breeding in collared flycatchers may drive temporal segregation. Collared flycatchers pay a relatively higher cost of late breeding in terms of reduced offspring condition and increased mortality than do pied flycatchers (Qvarnström et al. 2005; Qvarnström et al. 2007; Qvarnström et al. 2009) and appear specialized to the richest habitats with high food availability (e.g. Qvarnström et al. 2009). As a consequence, this species seems to be less flexible in their onset of breeding as compared to pied flycatchers, which also breed in a wide variety of habitats (Lundberg and Alatalo 1992; Mäntylä et al. 2015). In addition, it may be that collared flycatchers are unable to respond to the right cues to match the low and late peak of food abundance in the caterpillar poor environment. It has been recently suggested that pied flycatchers are better adapted to the seasonal changes in temperature and food availability by being able to adjust the

323 resting metabolic rate of their offspring to the prevailing environmental conditions (McFarlane, S.E.
324 personal communication). In the present study, we find evidence for stronger selection in favor of
325 early breeding in collared flycatchers than in pied flycatchers using the long term data collected in
326 the hybrid zone (14 years of data). Taken together, the observed increased segregation in onset of
327 breeding, especially within the poor habitat, could be a side effect of a narrower niche use (stronger
328 dependence on the peak in larvae abundance) of collared flycatchers that evolved already during
329 allopatric conditions, which constrains their onset of breeding to a larger extent.

330

331 Second, another possible explanation for the observed segregation in breeding time could be that
332 competition between the two species may force pied flycatchers to breed later than would be
333 optimal. For example, a comparison between closely related species of North American birds that
334 were involved in aggressive interactions revealed that subordinate species often started to breed
335 later as compared to their dominant rivaling species (Freshwater et al. 2014). During the period of
336 secondary contact between the two flycatcher species on Öland, pied flycatchers have been
337 progressively expelled into poorer quality habitats (Rybinski et al. 2016), which has resulted in
338 increased temporal segregation, due to differences in optimal timing of breeding between habitats
339 (Vallin et al. 2012). In the present study we control for the effects of habitat but one may still argue
340 that pied flycatchers could possibly avoid the intense interference competition over nest boxes
341 taking place early in the season by delaying the onset of breeding also *within* habitat types.

342 However, two facts contradict this alternative explanation. First, competition over nest boxes is
343 stronger in the caterpillar larvae rich and preferred habitat type where temporal segregation was not
344 observed. Second, if pied flycatchers were forced to shift their timing of breeding later than optimal
345 we would have expected to find a higher cost of late breeding and therefore stronger directional
346 selection for earlier breeding in this species rather than in collared flycatchers. We therefore
347 consider this alternative explanation unlikely.

348

349 Finally, selection to avoid hybridization (reinforcement Fisher 1930; Dobzhansky 1937) may also
350 favor increased difference in the onset of breeding between the two species. In other words, rather
351 than being a consequence or side-effect of a difference in how the two species respond to a
352 changing climate as argued above, temporal isolation could have been a direct response to selection
353 against hybridization acting in the hybrid zone. Hybridizing is costly as female and male hybrids
354 have serious fertility problems (Svedin et al. 2008; Ålund et al. 2013) and especially pied
355 flycatchers (the rarer species) experience a high risk of pairing with a heterospecific partner in this
356 young hybrid zone on Öland. However, the observed temporal segregation mainly takes place in
357 poor quality habitat where the numbers of collared flycatchers are low and thus where the risk of
358 hybridization for pied flycatcher is lowest. Moreover, since we currently only find evidence for a
359 reduced hybridization risk among relatively late breeding male pied flycatchers (Figure 3) and not
360 among relatively late breeding female pied flycatchers, this cannot explain the observed relaxed
361 selection for early breeding in female pied flycatchers. Thus, we can conclude that the observed
362 temporal segregation in the poor quality habitat is most likely a side-effect of pied flycatchers
363 having a broader niche use (range and proportion of various insect species in their diet) than
364 collared flycatchers, making them less obliged to match their onset of breeding with the climate-
365 driven seasonal advancement of the peak of larvae abundance.

366

367 The difference in the rate of laying date advancement that we observed between the two flycatcher
368 species is rather dramatic with 2.5 days advancement in pied flycatchers as compared with 4 days in
369 collared flycatcher during a period of only 14 years. Moreover, temporal (allochronic) isolation is
370 already manifested as lowered risk of hybridization among late breeding pied flycatcher males and
371 is likely to further build up in the near future and benefit both species. A crucial question then
372 becomes how important the build-up of this reproductive barrier is in the specific context of the

373 flycatcher speciation process. The lack of evidence of fertile hybrids in our breeding records (Ålund
374 et al. 2013) suggests that the studied build-up of temporal isolation should be considered a post-
375 speciation process (see e.g. Butlin 1987) that may facilitate co-existence between the two species
376 without strictly speaking being part of the speciation process *per se*. The most important
377 consequence of the observed build-up of temporal isolation in the young hybrid zone on Öland is
378 probably the reduced risk of maladaptive mixed species pairing (and production of malfunctioning
379 hybrids) in pied flycatchers. This reduces the risk that the combined effects of interspecific
380 competition and hybridization drive pied flycatchers to local extinction. Currently the cost of
381 producing hybrid offspring in terms of lost opportunity to produce fertile purebred offspring is a
382 larger threat to the pied flycatcher population than their genome being swamped by gene flow since
383 gene flow is low at this very late stage of speciation. However, we cannot rule out the possibility
384 that the mechanism by which temporal isolation is build up in this hybrid zone has also played a
385 role in previous episodes of geographical contact at earlier stages of the flycatcher speciation
386 process.

387

388 In general, the evolution of temporal isolation appears to be less straightforward than the evolution
389 of many other ecological reproductive barriers. While divergence in timing of breeding among
390 geographically isolated populations experiencing different local climates should evolve relatively
391 fast, this may not ensure temporal isolation at secondary contact since the populations are
392 experiencing the same local climate. Disruptive selection caused by competition over resources
393 needed for reproduction may then be an important prerequisite for maintained or increased level of
394 segregation in timing of breeding. However, as illustrated by our present study, minor ecological
395 differences that have evolved in allopatry may make populations (or young species) respond
396 differently to variation in the local climate or in this case to a directional change in the local
397 climate. Should we expect this to be a general phenomenon? Restricted temporal availability of

398 resources needed for reproduction, which often is the case in temperate regions, may constrain the
 399 evolution of temporal segregation in timing of breeding among sympatric species that rely on
 400 similar resources. For example, reviews on flowering plants (e.g. Rathcke and Lacey 1985; Lowry
 401 et al. 2008) have concluded that although pre-zygotic isolation is on average twice as strong as post-
 402 zygotic isolation, divergence in flowering time often has a relatively small influence on
 403 reproductive isolation compared to other pre-zygotic barriers such as habitat and pollinator isolation
 404 (but see also Silvertown et al. 2005). By contrast, temporal isolation seems to play an important role
 405 in some insects (e.g. Harrison 1985; Ordning et al. 2010; Boumans et al. 2017) amphibians (e.g.
 406 Hillis 1981) and in particular in free-spawning marine animals such as corals and tropical green
 407 algae (Knowlton et al. 1997; Clifton and Clifton 1999). Marine environments are often relatively
 408 stable suggesting that the evolution of temporal isolation through competition (e.g. Butlin 1987;
 409 Howard 1993; Pfennig and Pfennig 2009) or reinforcement (Fisher 1930; Dobzhansky 1937) or due
 410 to different responses to changes in the local climate (this study) may be less constrained by abiotic
 411 temporal effects on resource availability. In the flycatcher case, we see a larger degree of temporal
 412 segregation in the habitats where the temporal peak of food availability is relatively lower and
 413 broader, as compared to high but narrow peak of larvae found in the deciduous forest. Another
 414 potential evolutionary solution to this problem (a restricted time window with available resources
 415 for breeding) is that the temporal segregation in timing of breeding spans long enough to match
 416 alternative yearly optimal times for breeding as observed in periodical cicadas (Sota et al. 2013).
 417
 418 In the young hybrid zone on the Baltic island Öland, Sweden, temporal segregation between
 419 collared and pied flycatchers is partly built-up as a side effect of the subdominant species (pied
 420 flycatchers) being displaced into a poorer habitat type with a late peak in food abundance (Vallin et
 421 al. 2012, Rybinski et al 2016). In the current study, we focus on the build-up of temporal
 422 segregation *within* each habitat type, which is most relevant from the perspective of reproductive

423 isolation (*allochronic isolation*). We find that an increased divergence in the onset of breeding
424 between the two species is only evident among birds breeding within the poor habitat. Our analyses
425 show that pied flycatchers pay a lower cost of late breeding than collared flycatchers across habitat
426 types suggesting that the explanation to the observed patterns of habitat specific temporal
427 segregation depends on a difference in how the two species react to breeding in the poorer habitat
428 type; pied flycatchers adjust their onset of breeding to the later and more stable food peak in the
429 poor habitat while collared flycatchers do not. In addition, we found that relatively late breeding
430 pied flycatcher males are less likely to hybridize suggesting that there is a potential for
431 reinforcement of temporal isolation through selection against hybridization. Thus, the observed
432 habitat specific build-up of temporal isolation most likely is a side-effect of an initially broader
433 niche use in the sub-dominant species, which makes them relatively less obliged to match their
434 onset of breeding with the peak in larvae abundance and therefore also less responsive to the
435 climate-driven advancement of this peak.

436

437 Our study demonstrates the difficulty to make general predictions of the consequences of climate
438 change across populations, species and habitats. We show here that even closely related species
439 with the same preferred niche have different prerequisites to adapt to increasing temperatures. This
440 in turn influences hybridization patterns in the hybrid zone as the build-up of temporal segregation
441 results in temporal isolation. Furthermore, while there is ample evidence for the drastic negative
442 consequences of environmental change, our results suggest that changing environmental pressures
443 might sometimes facilitate cohabitation (and thus potentially prevent extinction) of very closely
444 related species through different speed of adaptation to new conditions and thus opening of new
445 temporal niches used only by one of the species. However, the prevalence of this remains to be
446 explored. We call for caution when making predictions on the consequences of environmental

447 changes across locations, populations or species and suggest more fine-tuned studies of the
448 reactions of interacting species to similar selective pressures over time.

449

450

451

452 **References**

- 453 Alatalo, R. V., L. Gustafsson, and A. Lundberg. 1994. Male coloration and species recognition in
454 sympatric flycatchers. *Proceedings of the Royal Society of London Series B-Biological*
455 *Sciences* 256:113-118.
- 456 Alatalo, R. V., A. Lundberg, and C. Glynn. 1986. Female pied flycatchers choose territory quality
457 and not male characteristics. *Nature* 323:152-153.
- 458 Ålund, M., S. Immler, A. M. Rice, and A. Qvarnström. 2013. Low fertility of wild hybrid male
459 flycatchers despite recent divergence. *Biology Letters* 9:4.
- 460 Brodie, E. D. 1992. Correlational selection for color patterns and antipredator bahavior in the garter
461 snake *Thamnopsis ordinoides*. *Evolution* 46:1284-1298.
- 462 Bates, D., M. Machler, B. M. Bolker, and S. C. Walker. 2015. Fitting Linear Mixed-Effects Models
463 Using lme4. *J. Stat. Softw.* 67:1-48.
- 464 Both, C., A. V. Artemyev, B. Blaauw, R. J. Cowie, A. J. Dekhuijzen, T. Eeva, A. Enemar, L.
465 Gustafsson, E. V. Ivankina, A. Järvinen, N. B. Metcalfe, N. E. I. Nyholm, J. Potti, P. A.
466 Ravussin, J. J. Sanz, B. Silverin, F. M. Slater, L. V. Sokolov, J. Török, W. Winkel, J.
467 Wright, H. Zang, and M. E. Visser. 2004. Large-scale geographical variation confirms that
468 climate change causes birds to lay earlier. *Proceedings of the Royal Society B-Biological*
469 *Sciences* 271:1657-1662.
- 470 Both, C., S. Bouwhuis, C. M. Lessells, and M. E. Visser. 2006. Climate change and population
471 declines in a long-distance migratory bird. *Nature* 441:81-83.
- 472 Both, C. and M. E. Visser. 2001. Adjustment to climate change is constrained by arrival date in a
473 long-distance migrant bird. *Nature* 411:296-298.
- 474 Boumans, L., S. Hogner, J. Brittain, and A. Johnsen. 2017. Ecological speciation by temporal
475 isolation in a population of the stonefly *Leuctra hippopus* (Plecoptera, Leuctridae). *Ecol.*
476 *Evol.* 7:1635-1649.

477 Burger, C., E. Belskii, T. Eeva, T. Laaksonen, M. Mägi, R. Mänd, A. Qvarnström, T. Slagsvold, T.
478 Veen, and M. E. Visser. 2012. Climate change, breeding date and nestling diet: how
479 temperature differentially affects seasonal changes in pied flycatcher diet depending on
480 habitat variation. *Journal of Animal Ecology* 81:926-936.

481 Butlin, R. 1987. Speciation by reinforcement. *Trends Ecol. Evol.* 2:8-13.

482 Charmantier, A., R. H. McCleery, L. R. Cole, C. Perrins, L. E. B. Kruuk, and B. C. Sheldon. 2008.
483 Adaptive phenotypic plasticity in response to climate change in a wild bird population.
484 *Science* 320:800-803.

485 Clifton, K. E. and L. M. Clifton. 1999. The phenology of sexual reproduction by green algae
486 (Bryopsidales) on Caribbean coral reefs. *J. Phycol.* 35:24-34.

487 Coyne, J. A. and H. A. Orr. 2004. *Speciation*. Sinauer Associates.

488 Cramer, E. R. A., E. Stensrud, G. Marthinsen, S. Hogner, L. E. Johannessen, T. Laskemoen, M. C.
489 Eybert, T. Slagsvold, J. T. Lifjeld, and A. Johnsen. 2016. Sperm performance in conspecific
490 and heterospecific female fluid. *Ecol. Evol.* 6:1363-1377.

491 Cramp, S. and K. E. L. Simmons. 2006. *Birds of the Western Palearctic* interactive version 2.0.1.
492 BirdGuides Ltd, Oxford University Press, Sheffield, Oxford.

493 Dale, S. and T. Slagsvold. 1996. Mate choice on multiple cues, decision rules and sampling
494 strategies in female pied flycatchers. *Behaviour* 133:903-944.

495 Davis, C. C., C. G. Willis, R. B. Primack, and A. J. Miller-Rushing. 2010. The importance of
496 phylogeny to the study of phenological response to global climate change. *Philos. Trans. R.*
497 *Soc. B-Biol. Sci.* 365:3201-3213.

498 Devries, P. J., G. T. Austin, and N. H. Martin. 2008. Diel activity and reproductive isolation in a
499 diverse assemblage of Neotropical skippers (Lepidoptera : Hesperiiidae). *Biological Journal*
500 *of the Linnean Society* 94:723-736.

501 Dobzhansky, T. 1937. *Genetics and the Origin of Species*. Columbia University Press, New York.

502 Ellegren, H., L. Smeds, R. Burri, P. I. Olason, N. Backström, T. Kawakami, A. Künstner, H.
 503 Mäkinen, K. Nadachowska-Brzyska, A. Qvarnström, S. Uebbing, and J. B. W. Wolf. 2012.
 504 The genomic landscape of species divergence in *Ficedula* flycatchers. *Nature* 491:756-760.
 505 Ellis, A. G., A. E. Weis, and B. S. Gaut. 2006. Evolutionary radiation of "stone plants" in the genus
 506 *Argyroderma* (Aizoaceae): Unraveling the effects of landscape, habitat, and flowering.
 507 *Evolution* 60:39-55.
 508 Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.
 509 Flint, V. E., R. L. Boehme, Y. V. Kostin, and A. A. Kuznetsov. 1984. A Field Guide to Birds of the
 510 USSR. Princeton University Press, Princeton.
 511 Freshwater, C., C. K. Ghalambor, and P. R. Martini. 2014. Repeated patterns of trait divergence
 512 between closely related dominant and subordinate bird species. *Ecology* 95:2334-2345.
 513 Gröning, J. and A. Hochkirch. 2008. Reproductive interference between animal species. *Quarterly*
 514 *Review of Biology* 83:257-282.
 515 Harr, B. and T. Price. 2014. Climate Change: A Hybrid Zone Moves North. *Curr. Biol.* 24:R230-
 516 R232.
 517 Harrison, R. G. 1985. Barriers to gene exchange between closely related cricket species. 2. life-
 518 cycle variation and temporal isolation. *Evolution* 39:244-259.
 519 Hendry, A. P. and T. Day. 2005. Population structure attributable to reproductive time: isolation by
 520 time and adaptation by time. *Molecular Ecology* 14:901-916.
 521 Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and
 522 speciation. *Biological Journal of the Linnean Society* 58:247-276.
 523 Hillis, D. M. 1981. Premating isolating mechanisms among three species of the *Rana pipiens*
 524 complex in Texas and Southern Oklahoma. *Copeia* 2:312-319.

525 Howard, D. J. 1993. Reinforcement - Origin, dynamics, and fate of an evolutionary hypothesis. Pp.
526 46-69 in R. G. Harrison, ed. *Hybrid Zones and the Evolutionary Process*. Oxford University
527 Press, New York.

528 Huntley, B., R. E. Green, Y. C. Collingham, and S. G. Willis. 2007. A climatic atlas of European
529 breeding birds. The RSPB and Lynx Editions, Barcelona.

530 IPCC. 2014. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and*
531 *Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the*
532 *Intergovernmental Panel on Climate Change*. Pp. 1132 in C. B. Field, V. R. Barros, D. J.
533 Dokken, K. J. Mach, M. D. Mastrandrea, Bilir T.E., M. Chatterjee, K. L. Ebi, Y. O. Estrada,
534 R. C. Genova, B. Girma, E. S. Kissel, A. N. Levy, S. MacCracken, P. R. Mastrandrea, and
535 L. L. White, eds, Cambridge, United Kingdom and New York, NY, USA.

536 Knowlton, N., J. L. Mate, H. M. Guzman, R. Rowan, and J. Jara. 1997. Direct evidence for
537 reproductive isolation among the three species of the *Montastraea annularis* complex in
538 Central America (Panama and Honduras). *Mar. Biol.* 127:705-711.

539 Koyama, T., H. Ito, S. Kakishima, J. Yoshimura, J. R. Cooley, C. Simon, and T. Sota. 2015.
540 Geographic body size variation in the periodical cicadas *Magicicada*: implications for life
541 cycle divergence and local adaptation. *Journal of Evolutionary Biology* 28:1270-1277.

542 Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2014. lmerTest: Tests for random and
543 fixed effects for linear mixed effect models (lmer objects of lme4 package) R package
544 version 2.0-11. <http://cran.r-project.org/package=lmerTest>.

545 Lackey, A. C. R. and J. W. Boughman. 2017. Evolution of reproductive isolation in stickleback
546 fish. *Evolution* 71:357-372.

547 Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution*
548 37:1210-1226.

549 Lindström, Å., M. Green, G. Paulson, H. G. Smith, and V. Devictor. 2013. Rapid changes in bird
550 community composition at multiple temporal and spatial scales in response to recent climate
551 change. *Ecography* 36:313-322.

552 Lowry, D. B., J. L. Modliszewski, K. M. Wright, C. A. Wu, and J. H. Willis. 2008. The strength
553 and genetic basis of reproductive isolating barriers in flowering plants. *Philos. Trans. R.*
554 *Soc. B-Biol. Sci.* 363:3009-3021.

555 Lundberg, A. and R. V. Alatalo. 1992. *The pied flycatcher*. T & AD Poyser, London.

556 Lynch and Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer, Sunderland, MA,
557 USA.

558 Macarthur, R. and R. Levins. 1967. Limiting similarity convergence and divergence of coexisting
559 species. *American Naturalist* 101:377-+.

560 Mäntylä, E., P. M. Sirkiä, T. Klemola, and T. Laaksonen. 2015. An experimental test of whether
561 pied flycatchers choose the best territory for rearing the young. *Current Zoology* 61:604-
562 613.

563 Møller, A. P. and W. Fiedler. 2004. *Birds and climate change*. Academic Press, Oxford.

564 Møller, A. P., D. Rubolini, and E. Lehikoinen. 2008. Populations of migratory bird species that did
565 not show a phenological response to climate change are declining. *Proceedings of the*
566 *National Academy of Sciences of the United States of America* 105:16195-16200.

567 Nadachowska-Brzyska, K., R. Burri, P. I. Olason, T. Kawakami, L. Smeds, and H. Ellegren. 2013.
568 Demographic divergence history of Pied Flycatcher and Collared Flycatcher inferred from
569 whole-genome re-sequencing data. *PLoS Genet.* 9:e1003942.

570 Ordning, G. J., R. J. Mercader, M. L. Aardema, and J. M. Scriber. 2010. Allochronic isolation and
571 incipient hybrid speciation in tiger swallowtail butterflies. *Oecologia* 162:523-531.

572 Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annu. Rev.*
573 *Ecol. Evol. Syst.* 37:637-669.

574 Pearce-Higgins, J. W. and R. E. Green. 2014. Birds and climate change: impacts and conservation
 575 responses. Cambridge Univ. Press.

576 Pfennig, K. S. and D. W. Pfennig. 2009. Character displacement: Ecological and reproductive
 577 responses to a common evolutionary problem. *Quarterly Review of Biology* 84:253-276.

578 Pärt, T. and A. Qvarnström. 1997. Badge size in collared flycatchers predicts outcome of male
 579 competition over territories. *Animal Behaviour* 54:893-899.

580 Quinn, T. P., M. J. Unwin, and M. T. Kinnison. 2000. Evolution of temporal isolation in the wild:
 581 Genetic divergence in timing of migration and breeding by introduced chinook salmon
 582 populations. *Evolution* 54:1372-1385.

583 Qvarnström, A., J. V. Kehlenbeck, C. Wiley, N. Svedin, and S. A. Sæther. 2007. Species
 584 divergence in offspring begging intensity: difference in need or manipulation of parents?
 585 *Proceedings of the Royal Society B-Biological Sciences* 274:1003-1008.

586 Qvarnström, A., A. M. Rice, and H. Ellegren. 2010. Speciation in *Ficedula* flycatchers. *Philos.*
 587 *Trans. R. Soc. B-Biol. Sci.* 365:1841-1852.

588 Qvarnström, A., N. Svedin, C. Wiley, T. Veen, and L. Gustafsson. 2005. Cross-fostering reveals
 589 seasonal changes in the relative fitness of two competing species of flycatchers. *Biology*
 590 *Letters* 1:68-71.

591 Qvarnström, A., C. Wiley, N. Svedin, and N. Vallin. 2009. Life-history divergence facilitates
 592 regional coexistence of competing *Ficedula* flycatchers. *Ecology* 90:1948-1957.

593 Qvarnström, A., M. Ålund, S. E. McFarlane, and P. M. Sirkiä. 2016. Climate adaptation and
 594 speciation: particular focus on reproductive barriers in *Ficedula* flycatchers. *Evol. Appl.*
 595 9:119-134.

596 Rathcke, B. and E. P. Lacey. 1985. Phenological patterns of terrestrial plants. *Annual Review of*
 597 *Ecology and Systematics* 16:179-214.

598 Richards, S. A. 2002. Temporal partitioning and aggression among foragers: modeling the effects of
599 stochasticity and individual state. *Behavioral Ecology* 13:427-438.

600 Rybinski, J., P. M. Sirkkä, S. E. McFarlane, N. Vallin, D. Wheatcroft, M. Ålund, and A.
601 Qvarnström. 2016. Competition-driven build-up of habitat isolation and selection favoring
602 modified dispersal patterns in a young avian hybrid zone. *Evolution* 70:2226-2238.

603 Schoener, T. W. 1974. Resource partitioning in ecological communities. *Science* 185:27-39.

604 Silvertown, J., C. Servaes, P. Biss, and D. Macleod. 2005. Reinforcement of reproductive isolation
605 between adjacent populations in the Park Grass Experiment. *Heredity* 95:198-205.

606 Sirkkä, P. M. and T. Laaksonen. 2009. Distinguishing between male and territory quality: females
607 choose multiple traits in the pied flycatcher. *Animal Behaviour* 78:1051-1060.

608 Sota, T., S. Yamamoto, J. R. Cooley, K. B. R. Hill, C. Simon, and J. Yoshimura. 2013. Independent
609 divergence of 13-and 17-y life cycles among three periodical cicada lineages. *Proceedings*
610 *of the National Academy of Sciences of the United States of America* 110:6919-6924.

611 Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008.
612 Estimating nonlinear selection gradients using quadratic regression coefficients: Double or
613 nothing ? *Evolution* 62:2435-2440.

614 Svedin, N., C. Wiley, T. Veen, L. Gustafsson, and A. Qvarnström. 2008. Natural and sexual
615 selection against hybrid flycatchers. *Proceedings of the Royal Society B-Biological Sciences*
616 275:735-744.

617 Tayleur, C., P. Caplat, D. Massimino, A. Johnston, N. Jonzen, H. G. Smith, and A. Lindstrom.
618 2015. Swedish birds are tracking temperature but not rainfall: evidence from a decade of
619 abundance changes. *Glob. Ecol. Biogeogr.* 24:859-872.

620 Tayleur, C. M., V. Devictor, P. Gauzere, N. Jonzen, H. G. Smith, and A. Lindström. 2016. Regional
621 variation in climate change winners and losers highlights the rapid loss of cold-dwelling
622 species. *Divers. Distrib.* 22:468-480.

623 Taylor, S. A., T. A. White, W. M. Hochachka, V. Ferretti, R. L. Curry, and I. Lovette. 2014.
 624 Climate-Mediated Movement of an Avian Hybrid Zone. *Curr. Biol.* 24:671-676.
 625 Taylor, S.A., Larson, E.L. and Harrison R.G. 2015. Hybrid zones: windows on climate change.
 626 *Trends in Ecology and Evolution* 30:398-406.
 627 Taylor, R. S. and V. L. Friesen. 2017. The role of allochrony in speciation. *Molecular ecology*.
 628 Thackeray, S. J., P. A. Henrys, D. Hemming, J. R. Bell, M. S. Botham, S. Burthe, P. Helaouet, D.
 629 G. Johns, I. D. Jones, D. I. Leech, E. B. Mackay, D. Massimino, S. Atkinson, P. J. Bacon, T.
 630 M. Brereton, L. Carvalho, T. H. Clutton-Brock, C. Duck, M. Edwards, J. M. Elliott, S. J. G.
 631 Hall, R. Harrington, J. W. Pearce-Higgins, T. T. Hoyer, L. E. B. Kruuk, J. M. Pemberton, T.
 632 H. Sparks, P. M. Thompson, I. White, I. J. Winfield, and S. Wanless. 2016. Phenological
 633 sensitivity to climate across taxa and trophic levels. *Nature* 535:241-U294.
 634 Vallin, N., A. M. Rice, R. I. Bailey, A. Husby, and A. Qvarnström. 2012. Positive feedback
 635 between ecological and reproductive character displacement in a young avian hybrid zone.
 636 *Evolution* 66:1167-1179.
 637 Veen, T., B. C. Sheldon, F. J. Weissing, M. E. Visser, A. Qvarnström, and G.-P. Sætre. 2010.
 638 Temporal differences in food abundance promote coexistence between two congeneric
 639 passerines. *Oecologia* 162:873-884.
 640 Weidinger, K. and M. Král. 2007. Climatic effects on arrival and laying dates in a long-distance
 641 migrant, the Collared Flycatcher *Ficedula albicollis*. *Ibis* 149:836-847.
 642 Wiley, C., A. Qvarnström, G. Andersson, T. Borge, and G. P. Sætre. 2009. Postzygotic isolation
 643 over multiple generations of hybrid descendents in a natural hybrid zone: how well do
 644 single-generation estimates reflect reproductive isolation? *Evolution* 63:1731-1739.
 645 Visser, M. E. and L. J. M. Holleman. 2001. Warmer springs disrupt the synchrony of oak and
 646 winter moth phenology. *Proceedings of the Royal Society B-Biological Sciences* 268:289-
 647 294.

648

649

650 **Figure legends**

651

652 **Figure 1.** The relationships between first egg laying dates of collared (black) and pied (grey)
653 flycatcher and mean temperature of a 30-day period preceding mean first egg laying dates for each
654 species. Both species advance their laying in warm springs but in collared flycatcher the
655 relationship is steeper than in pied flycatchers.

656

657 **Figure 2.** Temporal segregation in the onset of breeding between collared and pied flycatchers is
658 taking place in a) habitat with low quality but not in b) the habitat with high quality in the young
659 hybrid zone on Öland, Sweden, during a 14 year study period.

660

661 **Figure 3.** The onset of breeding of male pied flycatchers paired with heterospecific females (mixed
662 pairs) tends to take place earlier than in males paired with conspecific females (pure pairs).

663

664 **Figure 4.** Comparison of the relationships between breeding performance measured as number of
665 produced fledglings and onset of breeding between collared flycatchers and pied flycatchers while
666 controlling for habitat quality. (a) A significant linear selection gradient shows that late breeding is
667 costly in collared flycatcher (grey) while no evidence for similarly strong directional selection
668 favoring early breeding was found in pied flycatchers (black). (b) There is no strong evidence of
669 stabilizing selection in either species, although extremely early breeding appears slightly more
670 costly in collared flycatchers as compared to pied flycatchers. Raw data plotted. Points with similar
671 values are overlapping. Data shown include 2391 breeding events and 1873 females. In addition we
672 present the total numbers of breeding pairs of c) collared and d) pied flycatchers recorded during
673 2002–2015 as bars for each standardized laying date. Data were automatically split into 30 bins for
674 both species. We also present a smoothed function of relative fledging success (estimated separately

675 for each species) across these standardized laying dates. Hence, the majority of breeding pairs of
676 collared flycatchers bred later than would have been optimal for breeding performance while a
677 similar mismatch between the onset of breeding and peak performance was not observed in pied
678 flycatchers. Plots c) and d) were made using ggplot2 (Wickham, 2009), with the “loess” method.

Pairing Type

Mixed Pair

Pure Pair

10

20

30

40

50

Laying Date

Supplementary material

Breeding habitat quality in terms of food availability is expected to be tightly associated with reproductive performance in birds (Siikamäki 1998; Wright et al. 1998; Slagsvold and Wiebe 2007). Habitats however differ strongly in how food availability varies throughout the season (Both et al. 2010) and the importance of timing of breeding can vary strongly between different habitat types. It is thus highly important to take into account habitat quality when examining selection acting on timing of breeding.

To investigate build-up of allochronic isolation in different habitat types in the young contact zone on Öland we estimated habitat quality with the methods used by Rybinski et al. (2016) in the same system. To get an estimate of habitat quality we 1) estimated caterpillar biomass associated with different tree species and 2) combined this estimate with nest-box point estimates of habitat tree species composition and 3) extrapolated this point data to get habitat quality estimates for the foraging range of each breeding pair of flycatchers (see details below).

Measure of caterpillar biomass associated with tree species

Caterpillars are nutrient-rich, high-quality food items (Arnold et al. 2010; Eeva et al. 2010) and both collared and pied flycatchers primarily provide their offspring with caterpillars (Cramp and Simmons 2006; Burger et al. 2012). Caterpillars can contribute up to 80% of nestlings' diet and the higher their proportion in the diet the higher the fledgling mass (Burger et al. 2012). The availability and seasonal pattern of caterpillar larvae have been found to differ between tree species, however this knowledge is limited to a few studied tree species (van Asch and Visser 2007; Veen et al. 2010). Furthermore, the studies estimating caterpillar availability often use caterpillars associated

only with one tree species (most often oak). Temperate forests are however diverse in their tree species composition (see Table S1 below) and the role of habitat quality in terms of caterpillar availability for breeding birds remains largely unknown. We estimated larvae biomass by quantifying larvae fecal pellets, i.e. frass (a commonly used method e.g. Van Balen 1973; Visser et al. 2006) for 43 (in 2013) and for 48 (in 2014) individual trees from the 11 most common tree species in our study areas in 2013-2014. Frass biomass correlates highly with the biomass of larvae collected directly from branches (Visser et al. 2006). In our study the term “caterpillars” represents both *Lepidoptera* and *Hymenoptera* herbivorous larvae.

Larvae pellets were collected by custom-built traps that consisted of 50cm x 50cm wooden frames that were positioned approximately 50cm above the ground and about 1m from the tree trunk (following method used e.g. by Van Balen 1973; Visser et al. 2006; Veen et al. 2010). Each frame was fitted with a dense net that collected caterpillar droppings. The 41 (2013) and 48 (2014) trees from which frass were collected were selected so that they reflected the relative abundances of tree species in the studied areas (based on habitat composition data collected in 2007–2012). For the most common tree species (hazel, *Corylus avellana*; oak, *Quercus robur*; ash, *Fraxinus excelsior*; birch, *Betula* sp.; pine, *Pinus sylvestris*) two traps per tree were placed in three different study areas in 2013 and in four different study areas in 2014. For the rather common tree species (alder *Alnus* sp.; elm, *Ulmus* sp.; European hornbeam, *Carpinus betulus*; spruce, *Picea abies*; lind, *Tilia cordata*; maple, *Acer platanoides*) two traps per tree were placed in one study area plus additional two traps for spruce in 2014. The selected tree species for frass sampling represented 97 % of all the trees present in the study areas. One of the ash trees died early in the season 2013 and was excluded from the analysis. The frass sampling started soon after the bud burst of the earliest tree species and lasted until the end of the flycatcher breeding season in 2013 (19th of May until 26th of June) and in 2014 (5th of May until 25th of June). The nets from the traps were replaced every 4th day during

the season. Collected nets with frass inside were dried indoors and afterwards frass was separated from the coarse fraction of the litter with dense standard sized sieves. The samples were stored frozen to avoid any damage of sampled material for the rest of the field season. After the field season the remaining litter was separated manually in the laboratory. The amount of dry frass was weighed to an accuracy of 0.01 mg.

An estimate of caterpillar abundance during the nestling phase of the flycatcher breeding cycle was calculated as a sum of the mean daily frass values of each tree species in 2013-2014 (data from two to eight tree individuals depending on the species, see above). The period (from June 1st to June 25th) was chosen so that it covers 80% of the nestling phase of the flycatcher breeding cycle, as averaged from the whole 14-year dataset in our study areas. Our aim was to compare the overall differences between tree species, therefore no attempt was made to correct for the effect of the prevailing temperature that is known to affect larval fecal production (Tinbergen and Dietz 1994).

Point estimates of habitat tree species composition and food availability around specific nest-boxes

Tree species composition was measured around specific nest-boxes with a relascope (Bitterlich 1984), which is commonly used in forestry and also in ecological studies (e.g. Huhta et al. 1998). We calculated the basal area of different tree species around nest-boxes based on the relative size of the tree trunks assessed from a single point next to a nest-box. The method takes into account both the tree trunk size and the distance from the observer. The size of each individual tree trunk was estimated with the relascope (a scale kept at a fixed distance from the eye). Based on the size of the trunk compared to the scale in the relascope, each tree trunk was classified into one of three categories and assigned a value; (A) small (value 0); (B) medium sized (value 0.5) and (C) large (value 1). For birch (*Betula* spp.), alder (*Alnus* spp.), elm (*Ulmus* spp.) and willow (*Salix* spp), the

counts were done on the level of the genus and 19 tree taxa were recorded in total. A mature hazel bush, typically consisting of several thin trunks close together, was considered as one tree trunk. For each nest-box point estimate of habitat composition all basal area values per tree species were summed. These point estimates of habitat composition were obtained for 421 different nest-boxes evenly spread across our monitored breeding sites and, on average, four of the considered tree species were present within these points (mean 4.44 ± 0.15) with a range from 1 to 8 tree species (Fig. S1). There was also considerable variation in the relative abundances of the different tree species, ranging from an average of 0.4 individual trees (alder, spruce) to 16.1 (hazel) (Fig S1).

We then combined the caterpillar biomass data from each tree species with a nest-box point estimate of habitat tree species composition to have an estimate of food availability during the flycatchers' nestling phase for each specific nest-box sampling points. For each nest-box, we calculated the mean caterpillar biomass estimate weighted with abundances of different tree species. This point estimate of food availability thus combines the caterpillar biomass production associated with particular tree species and the relative densities around a particular flycatcher nest-box sampled.

Habitat quality (HQ) of foraging ranges used by breeding flycatcher pairs

We used the acquired point estimates of food availability (see above) to calculate the habitat quality (HQ) of foraging ranges used by breeding flycatcher pairs. Our estimate of HQ combines the information about caterpillar biomass production associated with particular tree species with the relative densities and canopy coverage of the trees within the foraging range of the birds around their nests sites. Flycatchers utilize relatively big areas in the surroundings of their nest when foraging as compared to the defended territory around their nest (Lundberg and Alatalo 1992). Pied flycatchers have been observed to fly up to 100 m or more from their nests in search of food (Huhta

et al. 1999). In order to get representative estimates of HQ, we calculated the average values of food availability, based on all available point estimates of food availability within a radius of 150 m from the focal nest. The HQ of the foraging range of each breeding flycatcher pair was hence extrapolated from the 421 point estimates of food availability in a way that allowed us to associate biologically meaningful HQ estimates with the majority of our breeding data. This extrapolation was based on GPS coordinates of all the nest boxes in our dataset that were collected in 2012 and 2013. Calculations were performed with the open source QGIS software (QGIS Development Team 2015).

First, 150m radius zones were created around each nestbox that was occupied by a breeding flycatcher pair. Then “Point statistics for polygons” SAGA algorithm (Olaya 2005; Cimmery 2007-2010) was used to obtain averaged HQ values. The algorithm used two layers: point layer – which was the layer containing point estimates of food availability; and polygon layer – which contained the feeding range zones around each breeding pair from our dataset. The point estimates of food availability overlapping with each polygon were used to calculate the extrapolated HQ values for the foraging range of flycatcher pair the polygon represented. This method resulted in a dataset with HQ estimates that incorporate habitat heterogeneity within the foraging range used by the breeding flycatcher pairs. For the sake of the algorithm used, the point estimate of food availability was represented as point data, however it is important to remember about its true, area like, nature (described above). Even though in the algorithm the data is associated with a fixed point in space, it still represents the average food availability based on the composition of the habitat around the focal nest-box. Moreover, the extrapolated HQ estimates for foraging range need to be also perceived as area data, namely the amount of caterpillars available within a 150 range from the occupied nest-box, rather than a point data corresponding to food available at the very spot where the nest was located.

Supplementary tables

Table S1. Collared and pied flycatchers respond differently to the earliness of spring, measured as the 30-day mean temperatures preceding the mean onset of laying. Presented are the results of a model testing the effect of study year, species (where collared flycatcher is the reference level), mean temperature and all possible interactions on laying date, with random effects of male and female identity.

Fixed Effects	Estimate	Std. Error	DF	t value	p value
Intercept	78.839	2.69	3167	29.28	<2e-16
Year	-5.416	0.33	3173	-16.57	<2e-16
Mean Temperature	-7.267	0.33	3174	-21.88	<2e-16
Species	-48.311	8.07	3110	-5.99	2.3e-9
Year:Temperature	0.645	0.04	3178	16.30	<2e-16
Year:Species	5.289	1.02	3122	5.17	2.5e-7
Temperature:Species	6.468	0.95	3113	6.81	1.2e-11
Year:Temperature:Species	-0.653	0.12	3125	-5.52	3.7e-8
Random Effects	Variance				
Male Identity	0.61				
Female Identity	2.65				
Residual	20.06				

Table S2: There is habitat dependent temporal segregation in the onset of breeding between collared and pied flycatchers during our 14-year study period. Results of a model testing the effect of study year, species (where collared flycatcher is the reference level), habitat quality and all possible interactions on laying date, where male and female identities were included as random effects.

Fixed Effects	Estimate	Std. Error	DF	t value	p value
Intercept	23.928	1.02	2837	23.40	<2e-16
Year	-0.563	0.11	2835	-5.08	4e-7
Habitat Quality	1.123	1.59	2914	0.71	0.479
Species	-2.394	0.77	2868	-3.13	0.002
Year:Species	0.371	0.17	2903	2.20	0.028
Year:Habitat Quality	0.241	0.08	2847	2.94	0.003
Habitat Quality:Species	1.256	1.40	2941	0.90	0.368
Year:Habitat Quality :Species	-0.338	0.15	2881	-2.31	0.021
Random Effects	Variance				
Male Identity	0.00				
Female Identity	1.74				
Residual	26.55				

Table S3: The proportion of male pied flycatchers breeding in mixed pairs with female collared flycatchers, compared to male pied flycatcher breeding with conspecific females, tends to decrease with the date of onset of breeding within a breeding season. Habitat quality is controlled for in the model. Late breeding male pied flycatchers tend to experience a lower risk of hybridization. The residual degrees of freedom for this model was 523, where there were 526 observations on 369 individual males.

Fixed effects	Estimate	Std. Error	Z value	p value
Intercept	-5.494	2.00	-2.74	0.006
Laying date	-0.191	0.10	-1.88	0.060
Habitat	-1.662	1.20	-1.39	0.165
Random Effects	Variance			
Individual	289.3			

Table S4: The proportion of female pied flycatchers breeding in mixed pairs with male collared flycatchers, compared to female pied flycatcher breeding with conspecific males, does not seem to be affected by laying date. Habitat quality is controlled for in the model. The residual degrees of freedom for this model was 598, where there were 602 observations on 501 individual females.

Fixed Effects	Estimate	Std. Error	z value	p value
Intercept	-2.464	0.62	-3.99	0.0001
Laying date	-0.019	0.02	-0.91	0.365
Habitat Quality	1.354	0.27	4.93	8.3e-7
Random Effects	Variance			
Female Identity	1.07			

Table S5: Here we have estimated standardized linear selection gradients for laying date in females in pied and collared flycatchers. Habitat quality is included in the model as a covariate. We used nestling fledging success as our fitness metric. Female identity and year of study were included as random effects. Only pure-species pairs were included in the model.

Fixed effects	Estimate	Std.Error	df	t value	p value
Intercept	1.004	0.040	79.4	25.40	< 2.00e-16
Standardized laying date	-0.097	0.011	1516	-8.78	< 2.00e-16
Species (Pied flycatcher)	-0.009	0.033	2021	-0.28	0.779
Habitat	0.003	0.023	2116	0.14	0.886
Standardized laying date * species (Pied flycatcher)	0.070	0.027	2290	2.62	0.009
Random effects	Variance				
Female identity	0.020				
year	0.007				
Residual	0.149				

Table S6: Here we have estimated standardized non-linear selection gradients for laying date in females pied and collared flycatchers. Habitat quality is included in the model. We used nestling fledging success as our fitness metric. Female identity and year of study were included as random effects. Only pure-species pairs were included in the model. Note that stabilizing selection should be interpreted as twice the regression estimates and standard errors reported here (Stinchcombe et al. 2008).

Fixed effects	Estimate	Std.Error	df	t value	p value
Intercept	1.014	0.040	85.6	25.33	< 2.00e-16
Standardized laying date	-0.091	0.012	1298	-7.60	5.77e-11
Standardized laying date ^2	-0.010	0.006	2232	-1.59	0.111
Species (Pied flycatcher)	-0.040	0.036	2081	-1.12	0.262
Habitat	0.003	0.023	2115	0.139	0.889
Standardized laying date * species (Pied flycatcher)	0.022	0.028	2296	0.564	0.573
Standardized laying date ^2 * species	0.041	0.021	2290	1.951	0.051
Random effects	Variance				
Female identity	0.020				
year	0.007				
Residual	0.148				

Supplementary Figures

Figure S1. Tree species composition around specific nest-boxes. The y-axis represents the mean ($\pm$ SE) numbers of recorded tree individuals for the 11 most common tree species and per nest-box point estimate of habitat composition. Tree species composition was measured with a relascope based on the relative size of the tree trunks assessed from a point next to a nest box (see above). The number of measured nest-box point estimates is 421.

Figure S2. Illustration of caterpillar larvae biomass (i.e. estimated as caterpillar frass) (means $\pm$ SE) produced across the nestling phase of the flycatcher breeding season (date 1 refers to 1st of May) in 11 common tree species in 2013–2014. Note different scaling of y-axis for the most caterpillar rich tree species on the first row compared to caterpillar-poorer tree species on the 2nd and 3rd rows.

References

- Arnold, K. E., S. L. Ramsay, L. Henderson, and S. D. Larcombe. 2010. Seasonal variation in diet quality: antioxidants, invertebrates and blue tits *Cyanistes caeruleus*. *Biological Journal of the Linnean Society* 99:708-717.
- Bitterlich, W. 1984. *The Relascope Idea: Relative Measurements in Forestry*. Commonwealth Agricultural Bureau, Slough, England.
- Both, C., C. A. M. Van Turnhout, R. G. Bijlsma, H. Siepel, A. J. Van Strien, and R. P. B. Foppen. 2010. Avian population consequences of climate change are most severe for long-distance migrants in seasonal habitats. *Proceedings of the Royal Society B-Biological Sciences* 277:1259-1266.
- Burger, C., E. Belskii, T. Eeva, T. Laaksonen, M. Mägi, R. Mänd, A. Qvarnström, T. Slagsvold, T. Veen, M. E. Visser, K. L. Wiebe, C. Wiley, J. Wright, and C. Both. 2012. Climate change,

- breeding date and nestling diet: how temperature differentially affects seasonal changes in pied flycatcher diet depending on habitat variation. *Journal of Animal Ecology* 81:926-936.
- Cimmery, V. 2007-2010. SAGA User Guide, Updated for SAGA Version 2.0.5.
- Cramp, S. and K. E. L. Simmons. 2006. Birds of the Western Palearctic interactive version 2.0.1. BirdGuides Ltd, Oxford University Press, Sheffield, Oxford.
- Eeva, T., S. Helle, J. P. Salminen, and H. Hakkarainen. 2010. Carotenoid composition of invertebrates consumed by two insectivorous bird species. *Journal of Chemical Ecology* 36:608-613.
- Huhta, E., J. Jokimäki, and P. Rahko. 1999. Breeding success of pied flycatchers in artificial forest edges: The effect of a suboptimally shaped foraging area. *Auk* 116:528-535.
- Huhta, E., J. Jokimäki, and P. Rahko. 1998. Distribution and reproductive success of the Pied Flycatcher *Ficedula hypoleuca* in relation to forest patch size and vegetation characteristics; the effect of scale. *Ibis* 140:214-222.
- Lundberg, A. and R. V. Alatalo. 1992. The pied flycatcher. T & AD Poyser, London.
- Olaya, V. 2005. A gentle introduction to SAGA GIS., <http://freecomputerbooks.com/A-Gentle-Introduction-to-SAGA-GIS.html>.
- QGIS Development Team, Q. D. 2015. QGIS Geographic Information System. Open Source Geospatial Foundation Project., <http://qgis.osgeo.org>.
- Rybinski, J., P. M. Sirkiä, S. E. McFarlane, N. Vallin, D. Wheatcroft, M. Ålund, and A. Qvarnström. 2016. Competition-driven build-up of habitat isolation and selection favoring modified dispersal patterns in a young avian hybrid zone. *Evolution* 70:2226-2238.
- Siikamäki, P. 1998. Limitation of reproductive success by food availability and breeding time in pied flycatchers. *Ecology* 79:1789-1796.
- Slagsvold, T. and K. L. Wiebe. 2007. Hatching asynchrony and early nestling mortality: the feeding constraint hypothesis. *Animal Behaviour* 73:691-700.
- Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: Double or nothing ? *Evolution* 62:2435-2440.
- Tinbergen, J. M. and M. W. Dietz. 1994. Parental energy-expenditure during brood rearing in the great tit (*Parus major*) in relation to body-mass, temperature, food availability and clutch size. *Functional Ecology* 8:563-572.
- van Asch, M. and M. E. Visser. 2007. Phenology of forest caterpillars and their host trees: The importance of synchrony. Pp. 37-55. *Annual Review of Entomology*. Annual Reviews, Palo Alto.
- Van Balen, J. H. 1973. Comparative study of breeding ecology of great tit *Parus major* in different habitats. *Ardea* 61:1-93.
- Veen, T., B. C. Sheldon, F. J. Weissing, M. E. Visser, A. Qvarnström, and G. P. Sætre. 2010. Temporal differences in food abundance promote coexistence between two congeneric passerines. *Oecologia* 162:873-884.
- Visser, M. E., L. J. M. Holleman, and P. Gienapp. 2006. Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia* 147:164-172.
- Wickham, H. (2009) *ggplot2: elegant graphics for data analysis*. 216 pp. Springer, New York.
- Wright, J., C. Both, P. A. Cotton, and D. Bryant. 1998. Quality vs. quantity: energetic and nutritional trade-offs in parental provisioning strategies. *Journal of Animal Ecology* 67:620-634.