THE EVOLUTIONARY DYNAMICS OF LAYING DATE IN THE PIED FLYCATCHER

Ficedula hypoleuca

PhD thesis
2021

Justine Le Vaillant

RECOMMENDED CITATION:
Le Vaillant J. (2021). The evolutionary dynamics of laying date in the pied flycatcher *Ficedula hypoleuca*. PhD Thesis. University of Seville, Spain.

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Doctoral Thesis

The evolutionary dynamics of laying date in the pied flycatcher *Ficedula hypoleuca*

Author:
Justine Le Vaillant

Supervisor:
Dr. Jesús Martínez-Padilla

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

University of Sevilla, 2021

This thesis have been funded by FPI grant from the Spanish Ministry of Economy and Competitiveness BES-2016-077694.

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Declaration of Authorship

I, Justine LE VAILLANT, declare that this thesis titled, "The evolutionary dynamics of laying date in the pied flycatcher *Ficedula hypoleuca*" and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed:

Date:

“Man, he took his time in the sun
Had a dream to understand
A single grain of sand
He gave birth to poetry
But one day’ll cease to be
Greet the last light of the library”

The Greatest Show On Earth, "Endless Forms Most Beautiful",
Nightwish 2015

This research presented in this thesis was carried out at the Department of Evolutionary Ecology, Estación Biológica de Doñana-CSIC, Spain

With visits and collaborative work at
UMIB/Research Unit of Biodiversity (Univ. Oviedo-CSIC-Princ. Asturias), Spain
and
Department of Animal Ecology at the Netherlands Institute of Ecology (NIOO-KNAW), Netherlands

Justine LE VAILLANT

Estación Biológica de Doñana-CSIC
Department of Evolutionary Ecology

Abstract

The evolutionary dynamics of laying date in the pied flycatcher *Ficedula hypoleuca*

Current global warming is unequivocally impacting biodiversity at an unprecedent rates. Such negative impact can be quantified on species distribution and diversity, but understanding biodiversity loss can only be fully achieved by considering the capacity of populations to track prevailing environmental conditions. Thus, an evolutionary approach is inevitable to comprehend how populations of species adapt under a scenario of climate change. This PhD follows a research agenda to quantify the variation of the timing of breeding laying date and the relative role of environmental variation on phenotypic selection along the evolutionary mechanisms that may explain local adaptation. Taking advantage of a long-term monitored population of pied flycatchers *Ficedula hypoleuca*, I detected a lack of temporal trend of laying date over 29 years despite highly variable environmental conditions during the same time period. A lack of repeatability of the date of breeding, suggesting a high variability among individuals in laying date and explaining variance of laying date. The intensity of phenotypic selection on laying date was strongest when environmental conditions were poor, specifically when minimum temperatures in April were lowest and May were highest. Despite negative selection on laying date, there was no evolutionary response at genetic level, suggesting that non-genetic mechanism may explain local adaptation. Accordingly, we found individual-by-environment interactions explaining phenological variation that can be partially explained by genotype-by-environmental interaction. This PhD stresses first, the crucial role that multiple environmental factors may play on the evolutionary dynamics of life-history traits and, second, that evolutionary mechanisms that do not involve genetic changes, namely genotype-by- environmental interactions, may play a pivotal role on local adaptation.

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General Introduction

Nothing in Biology Makes Sense Except in the Light of Evolution, Theodosius Dobzhansky 1973

Evolution

Evolution is defined as the change in the inherited traits of a population of organisms through successive generations. Since the theory established by Darwin (Darwin, 1859), evolution is one of the best-substantiated and supported scientific evidence and became a key concept in modern biology and ecology. According to the Modern Synthetic Theory of Evolution, evolutionary change is a continuous and gradual process of adaptation and speciation as result of natural, sexual and artificial selection (leaving aside mutation, genetic flow and genetic drift, see **Box 1**). Therefore, understanding, predicting and protecting biodiversity can only fully occur under the light of natural selection since it is a key mechanism that result in adaptive evolution. Evolution by means of natural selection is sustained on three main pillars: (1) variation of the phenotype in a population, (2) relative fitness benefits of an individual in the population, either by reproduction or survival values, by expressing a phenotype, and (3) that variation in traits show high additive genetic variation. Evolutionary change is usually a gradual and can be observed at a slow pace (Hendry & Kinnison, 1999; Kinnison & Hendry, 2001; Reznick & Ghalambor, 2001). Despite natural selection is a process that may end up in evolutionary change, or adaptive evolution, is not the cause of evolution. The cause of evolution and the engine that makes natural selection acting is environmental variation. If adaptive evolution has been attested during episodes of strong environmental change at long time periods of time (Darwin, 1859), evolution also operates at shorter ecological timescale and can be observed in experimental conditions (Reznick et al., 1990) or in wild populations (Grant & Grant, 1993). This so-called microevolution refers to the adaptive change in a phenotypic trait due to natural selection across few or several generations, observable at relatively short, usually ecological, timescale (Hendry & Kinnison, 2001).

A challenging task nowadays however is predicting and detecting an evolutionary response caused by environmental variation (MacColl, 2011; Wade & Kalisz, 1990). Indeed, the strength and direction of phenotypic selection may vary over time and depending on the environmental conditions that individuals in wild populations face (Chevin & Haller, 2014; Grant & Grant, 1995; Siepielski et al., 2009; Siepielski et al., 2013; Wood & Brodie III, 2016). In addition, the environmental conditions shape additive genetic variance of phenotypes, influencing the raw material that evolution can act upon, influencing the evolvability of phenotypes (Charmantier & Garant, 2005; Hoffmann & Merilä, 1999; Martínez-Padilla et al., 2017). Identifying those environmental factors that operate differently in populations is a complex task, making predictions of evolutionary change difficult to infer in wild populations. Moreover, the relative role of multiple environmental factors on natural selection and the ecological influence on local adaptation is relatively poorly understood (MacColl, 2011). Evolutionary ecology mainly focuses on abiotic environmental factors, as climate-driven factors but also considered multiple biotic factors such as pressure of predation, competition and parasites or many other density-dependant population factors. Evolutionary studies are done under a range of different ecological conditions and should always be described within an ecological context (Carroll et al., 2007; Wade & Kalisz, 1990). It is therefore of particular interest to understand how environmental variation can shape evolutionary process (i.e. eco-evolutionary dynamics ; Hendry, 2017; Pelletier et al., 2009; Schoener, 2011).

Box 1 : Others source of evolutionary adaptation

Evolutionary change, a shift in the frequency distribution of a phenotype, can occur under different genetic mechanisms (Hendry et al., 2018; Møller & Merilä, 2004) than natural selection:

Genetic flow increases the genetic variance and result in a shift in the mean trait in the population. It is mostly driven by the immigration rate and the exchange of genes and alleles between populations. In spatial heterogeneous environment, local selection pressures can affect the survival and the reproductive success of immigrant compared to locally adapted residents (Garant et al., 2007b). The scale and the pattern of selection depend of the dispersal status of the individual (Hanski et al., 2011; Postma & van Noordwijk, 2005). Non-random dispersal and gene flow can counteract or reinforce evolutionary change (Garant et al., 2007a).

Genetic drift is a stochastic fluctuation in allele frequencies causing a decrease in the variability of a trait and a deviation of the mean in a random direction. The population size and the proportion of inbreeding both affect the probability of genetic drift to occur, making the rate of evolution very difficult to estimate (Lanfear et al., 2014). However, genetic drift can mask the correlation between selection and evolution, leading to an apparent evolutionary stasis (Bonnet & Postma, 2018).

Genetic mutation is expected to increase genetic variance, however, because most mutations are random, deleterious, and unaffected by natural selection, the rate of mutation in a population do not affect considerably the rate of evolutionary change compared to genetic drift (Lehtonen et al., 2009).

Ecological changes affect evolution, however the fact that natural selection resulted in genetic change in a population is not always demonstrated. Indeed, variation in selection, often thought to be driven by environmental fluctuations (Bell, 2010; Chevin & Haller, 2014), does not necessarily translate in evolutionary change (i.e. evolutionary stasis, Bonnet and Postma, 2018; Merilä et al., 2001b. Such lack of genetic change of a phenotype in a population can be misunderstood as a lack of adaptation, but adaptation can occur in the absence of genetic change. The lack of evolutionary change can occur when individuals (or genotypes) express a different phenotype in response to different environmental conditions (Pigliucci, 2001), so- called phenotypic plasticity. Plasticity allows an organism to 'fit' its phenotype to the current environment and is an essential mechanism by which an individual can best response to short-term environmental changes (Scheiner, 1993). Environment can therefore shape the variation phenotype of a particular individual in a way to be plastic, and through process of adaptation and evolution acting on genotypes, leading genotypes in populations to be more plastic. However, if the environment changes more slowly than the generation time (e.g. a coarse-grained environment), a population will not experience selection for plasticity even if it is adaptive in the long-term (Rago et al., 2019). On the opposite, when environmental variance is high, selection on the appropriate phenotypic trait will be stronger (Brommer et al., 2005; De Jong, 1995), and plastic genotypes experience weaker selection because they are able to cope with a wider range of environments (Ghalambor et al., 2007). Although it allows for a rapid response to changing environmental conditions, adaptive plasticity have costs and limits are often hard to detect (DeWitt, Thomas et al., 1998; Murren et al., 2015; Pigliucci, 2005). Phenotypic plasticity can therefore not account for all adaptation, because plasticity can be maladaptive or neutral (Ghalambor et al., 2007; Hendry, 2016), but it can be considered as a good candidate in open populations when genetic changes are unrevealed.

Phenotypic plasticity is crucial to forecast species' response to environmental variation and to understand the evolutionary potential of populations to adapt. However, because a genetic change is required to prevent population extinction in the face of long-term environmental change, plasticity may at the same time promote or constrain genetic evolution (Ghalambor et al., 2007; Paenke et al., 2007). The Baldwin theory effect suggests that, plastic individuals survive better and drive the evolution by increasing plasticity (Baldwin, 1896; Crispo, 2007). When plasticity allows a population to persist in a changing environment, thereby enabling later genetic adaptation (i.e. genetic assimilation theory), plasticity may speed up microevolution (Kopp & Matuszewski, 2014; Waddington, 1953). This 'plasticity-first' hypothesis proposes that there is genetic variation in plastic responses that arise when exposed in a novel environment, which are then refined by evolutionary adaptation and further speed up genetic evolution (Lande, 2009; Levis & Pfennig, 2016; Schwander & Leimar, 2011). It is therefore, widely accepted that plasticity exhibits genetic variation and has the capacity to evolve (Pigliucci, 2005; Scheiner, 1993; Van Tienderen & Koelewijn, 1994; Via et al., 1995). However, the hypothesis that phenotypic plasticity can facilitate local adaptation remains controversial (De Jong, 2005; Levis & Pfennig, 2016; Pigliucci et al., 2006; Via et al., 1995) and need furthers investigations in field studies.

Glossary 1

Agent of selection/driver of selection: environmental factor (biotic or abiotic) that affects the fitness landscape of a population.

Adaptation: any heritable characteristic of an organism that improves its ability to survive and reproduce in its environment. Also used to describe the process of genetic change within a population, as influenced by natural selection.

Breeding value: sum of the additive effects of an individual's genes for a given trait. It quantifies the expected effect of the genes on polymorphic traits that it passes on to its offspring.

Eco-evolution dynamics: interplay between ecological and evolutionary dynamics in ecological times.

Evolutionary stasis: lack of evolutionary change over a period of time during the history of a species or a population.

Fitness: success of an individual in surviving and reproducing, measured by that individual's genetic contribution to the next and subsequent generations.

Genotype: genetic profile or set of genes shared by levels of relatedness of an individual, and defined the polymorphic traits or the phenotype of an organism.

G×E or Genotype-by-Environment interaction: influence of the environment on the phenotypic expression of different genotypes. The influence of the environment can result in changes of additive genetic variance along the environmental variable considered.

Heritability: proportion of total phenotypic variance explained by additive genetic variance of a trait.

I×E or Individual-Environment interaction: term to indicate that individual-specific trait values vary as a function of the environment. Presence of I×E suggests between-individual variance in phenotypic plasticity. When I×E are detected, it can be split in G×E and PE×E.

Microevolution: evolutionary changes in a short temporal scale of genotypes, or breeding values for polymorphic traits, such as changes in gene frequencies within a population.

PE×E or Permanent-by-environment effects: environmental effects on individual's phenotype that are constant across (or common to) repeated measures on that individual.

Phenotype: physical or functional characteristics observable of an organism, produced by the interaction of genotype environment during growth and development.

Phenotypic trait: characteristic that can be observed in an organism (morphological, physiological and behavioural) and that result from the interaction between the organism's genotype and its environment. Phenotypic trait is on what selection acts.

Phenotypic plasticity: differential phenotypic expression of genotypes along a gradient of environmental variation.

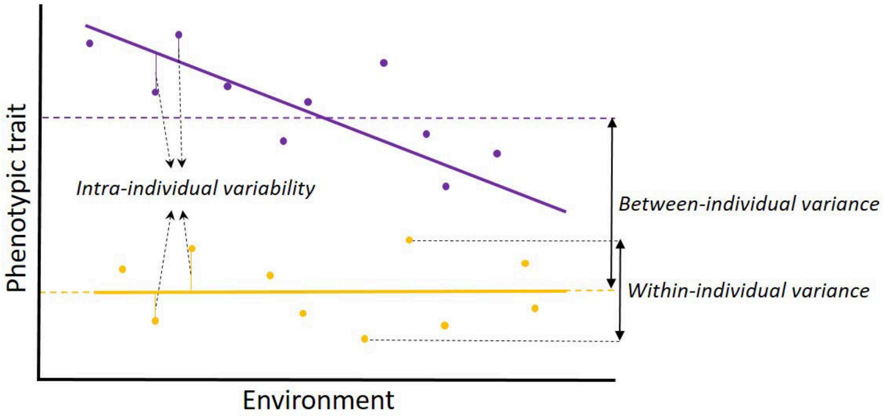
Population: group of organisms of the same species that are in close enough proximity to allow them to interbreed

Exploring evolution in natural populations

To understand ecological and evolutionary process on natural population we need to explore variation in phenotypic, its selection and how it is mediated by genetic effects. Several methods to study evolutionary mechanism have been developed in the recent years.

Phenotypic variation

Observed phenotypic variances in a trait can arise from the proportion of differences between-individuals and within-individual variance (i.e. repeatability) and can indicating a strong evidence for within-individual variations. This variance can be induced by environmental plasticity or a residual variance that cannot be explained by the environment (predictability or intra-individual variability; Dingemanse and Dochtermann, 2013; Stamps et al., 2012). Methods from quantitative genetics allow to separate genetic and non-genetic (or environmental) variance components and treated plasticity and intra-individual variability as individual-specific traits. There are multiple ways to measure variation at individual level, however, the reaction norm approach is the most commonly used to explain phenotypic variation in wild populations. Most approach in evolutionary ecology has been to work with individual-specific mean trait values, while within-individual variation in reaction norms was largely disregarded till recent years (Dingemanse et al., 2010). Variation in individual phenotypes among environments (individual variation in reaction norm or individual-environment interaction, i.e. I×E), denotes for within- individual differences in their plastic response to environment, while differences in reaction norm of individuals denotes for between- individual variations.



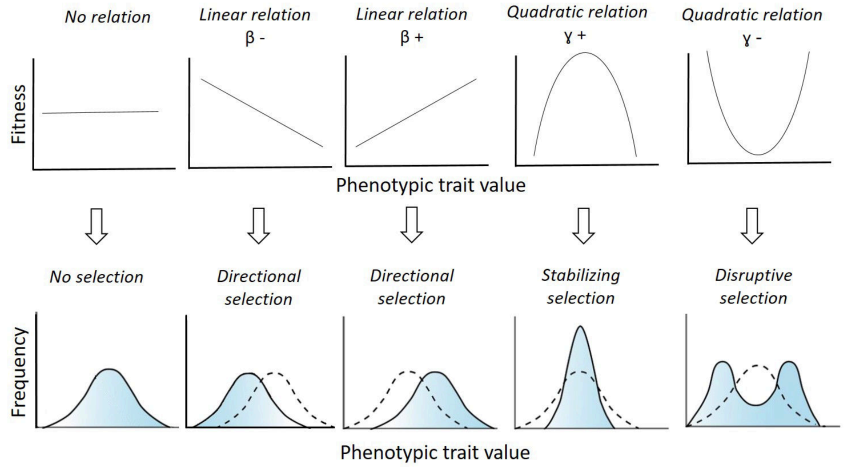
Reaction norms of two individuals (coloured lines) with repeated trait measures (dots) along an environmental gradient. The residual variance around the (expected) reaction norm shows the intra-individual variability (or predictability). Within-individual variance is composed of plasticity and intra-individual variability, while between-individual variance is the variance of mean trait values (dotted lines).

Phenotypic selection

Measuring selection at either phenotypic or genetic levels requires a proper quantification of fitness, since relative fitness benefits of expressing a phenotype is what will determine selection on a such trait in a population. However, either the definition or choosing the right measure of fitness is not a simple task. Selection at phenotypic level can be quantified through the selection differential (S), as the covariance between the phenotypic trait and the relative fitness (Falconer & Mackay, 1996; Walsh & Lynch, 2018). Using the relative fitness benefits is more accurate for measuring the direction and strength of selection on phenotype. The selection differential depend of the phenotypic variance in the population (V_P) and is related to the measure of selection of a trait, it can be achieved by direct measures of fitness in relation to the phenotype (Lande & Arnold, 1983). However, it is not an ideal measure for comparing selection strengths among different traits and populations because S does not consider the total phenotypic variance of the trait in the population. On the contrary, the selection gradient (linear β or non-linear γ) is the covariance between relative fitness (ω') and standardized trait divided by the variance of the total phenotypic trait (σ_p^2) (Matsumura et al., 2012):

$$\beta = cov[(\omega', trait)/\sigma_p^2]$$

Selection gradient measures the sensitivity of fitness to changes in a trait's value, making a more direct measure of selection strength in natural populations. The strength, direction and form of selection at phenotypic level can be further quantified using selection gradients. As such, the slope of the association between relative fitness and a standardised trait determines the intensity of selection (strength of selection) and so the 'rate' of the phenotypic change. The value of the gradient of selection (positive or negative) will define the direction of selection. Finally, the type of the relation (linear, quadratic or higher-level polynomial) shapes the form of the selection acting on a given trait.



The coefficient of the regression (gradient of selection) between relative fitness and the phenotypic trait gives information about the direction and the rate of selection (selection differential) acting on the trait.

Additive genetic variance

Despite selection acts on individual phenotypic traits, evolution and phenotypic selection, are quantified at population level when such selection impact on the genetic architecture of such trait. Polymorphic traits, like in most life-history or morphological traits, are assumed to be encoded by a single locus or large number of gene loci, inherited according Mendelian rules. Quantitative genetics provides a powerful statistical tool to split additive genetic variance from other residual variances at explaining the total phenotypic variance of a polymorphic trait in a population. The quantitative genetics approach was developed for inferring the evolutionary dynamics of phenotypic traits (Walsh & Lynch, 2018). It has been proved as a robust framework for the analysis of the evolutionary architecture of phenotypic traits and the shaping role of environmental variation of the evolutionary dynamics of polymorphic traits in wild populations (Charmantier et al., 2014b). Analysis in a quantitative genetic framework includes information about the relatedness between individuals or a breeding design data (e.g. cross-fostering experiments). By doing so, pedigrees can be assembled both in wild and populations reared under more controlled environmental conditions (e.g. laboratory or breeding design). The so- called 'animal model' include individual-specific measurements and relatedness matrices (pedigree) to estimate quantitative genetic parameters. 'Animal models' are mI×Ed-model where individual's phenotypic variance is split in genetic and variances controlling for fI×Ed variables (Kruuk, 2004). It was originally developed by animal breeders, but is increasingly used in the recent years in many fields in evolutionary ecology using mammals, birds, plants, fish or insects in wild or captive conditions.

Specifically, a quantitative genetics approach allows partitioning the phenotypic variation (V_P), into its genetic (V_G) and a residual non-genetic component, usually defined as the environmental variation (V_E):

$$V_P = V_G + V_E$$

V_G is composed of additive genetic variance (V_A; the additive effect of a gene), dominance variance (V_D ; the dominance effect of a genes attributable to allele interactions) and epistatic variance (V_I ; epistatic interaction between loci). VA is considered as the most important evolutionary parameter for population as it will determine the short-term evolutionary potential of a trait (the 'evolvability' of the trait), particularly when it is corrected by the mean of the targeted trait (CV_A or I_A – see Garcia-Gonzalez et al., 2012; Houle, 1992). V_I and V_D are usually ignored for simplicity and their small effect in wild populations. V_E refers to a general environmental variance whereas (V_{PE}) denoted as permanent 'environment variance'. V_{PE} represents the variation between individuals that cannot be attribute to (additive) genetic effects but rather environmental effect that are constant across individuals (among individual variance). Residual variance (V_R) denotes for the specific environmental variance attributable to the variance in measures of individuals (within-individual variance). Therefore, the phenotypic variance is usually defined as:

$$V_P = V_A + V_{PE} + V_R$$

The amount of genetic variation (V_G) is often measured for the broad-sense of heritability H of a trait, representing the common measure of a population's evolutionary potential. However, much emphasis in quantitative genetics studies laid on estimating the additive variance V_A as the narrow sense heritability h^2, therefore the use of heritability as a measure of evolvability is often misleading and cannot be used as evolutionary potential in the context of natural selection particularly because of the covariation between Va and the environment (Hansen et al., 2011). The (narrow-sense) heritability can been also measured using different approaches Postma, 2014), including parent-offspring regressions, full-sib analyses, selection lines and 'animal models' (see Kruuk, 2004). The existing bias about using parent-offspring regressions and others methods using similarity between relatives, is that there may be non-genetic causes of resemblance (shared environment) that upwards the estimated heritability (van der Jeugd & McCleery, 2002). Indeed, heritability estimate is based on individual relatedness since they are more similar than unrelated individuals and share some common genes.

Infer evolution with heritability estimate

Traditionally, heritability has been used to predict the expected evolutionary response of a trait (see Postma, 2014). The response to selection per generation R is given by the product of h^2 and selection differential S, often referred as the breeders' equation:

$$R = h^2 \times S$$

R is the change of the mean trait value from one generation to the next and S the covariance between relative fitness and the mean phenotypic value between generations (Lande & Arnold, 1983). However, because of the covariation between fitness and other phenotypic traits, the use and reliability of the 'breeder's equation' in natural population is limited and should be considered carefully (Morrissey et al., 2010). In addition, because heritability provides little information on the absolute amount of additive genetic variation, it can be a poor measure of the 'evolvability' of a trait (see above and Hansen et al., 2011). By using the alternative selection gradient as a measure of selection, we can predict response to selection with the Lande equation (Lande, 1979):

$$R = V_A \times \beta$$

Many studies failed to demonstrated an apparent evolutionary response for various heritable traits (Merilä et al., 2001a; Merilä et al., 2001b). The possible cause of this 'evolutionary stasis' is related to the interrelation of natural selection with environmental change. Inaccurate estimates of phenotypic selection gradient due to environmental variation will bias the estimation of response to selection (Kruuk et al., 2003). To counteract this issue, it has been proposed to estimate selection at the genetic level rather phenotypic level (Rausher, 1992; Stinchcombe et al., 2002), using for example the 'Robertson-Price equation' (Price, 1970). By taking into account the genetic covariance between a trait and its (relative) fitness, the Price's equation may be a better predictor of the cross-generation response to selection

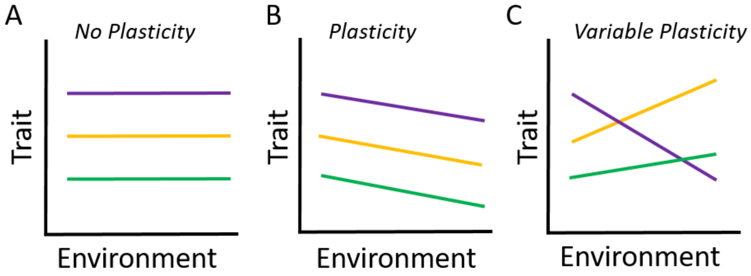
(Hadfield, 2008; Morrissey et al., 2010). However, the use of the Robertson-Price equation applying the second theorem of evolution, does not explain whether the expected response to selection is due to natural selection or any other evolutionary mechanism (Morrissey et al., 2010 see also **Box 1**). Thus, few predictions from methods derivate heritability estimates match the actual responses to selection (Pujol et al., 2018) and provides an unreliable proxy of evolutionary change in natural populations.

Infer evolution using Best Linear Unbiased Predictors ($BLUPs$)

Animal model is powerful method for the estimation of quantitative genetic parameters in natural populations. By separating environmental and genetic effects on both population and individual level (see further details above), it provides accurate and precise estimates of genetic variation (Kruuk, 2004). More specifically, they allow for the quantification of breeding values which cannot be directly measured on an individual for a given trait. The breeding value is defined as the expected effect of the genes that it passes on to its offspring (Falconer & Mackay, 1996; Kruuk, 2004; Walsh & Lynch, 2018)). Adjusting to the mean population trends, the predicted breeding values (PBV) can be used to test whether the observed response to selection deviated from the expected response (Kruuk, 2004; Postma, 2006). By taking advantage of animals models and accounting for potentially confounding random environmental variables and others fI×Ed effects, the Best Linear Unbiased Predictors, or $BLUPs$, allows for a better prediction of the breeding values (Kruuk, 2004; Postma, 2006). However, although it is powerful method to predict individual breeding value and test a change over time, $BLUPs$ may be biased and anti-conservative if models do not take into accounts error in prediction of breeding values (with for example power problem due to pedigree information) and genetic drift to infer an evolutionary change (Hadfield et al., 2010). Therefore, a proper estimation of microevolutionary change using $BLUP$s is to compared the inferred variances or temporal changes in $BLUP$s to those expected by chance, i.e. by genetic drift (Hadfield et al., 2010).

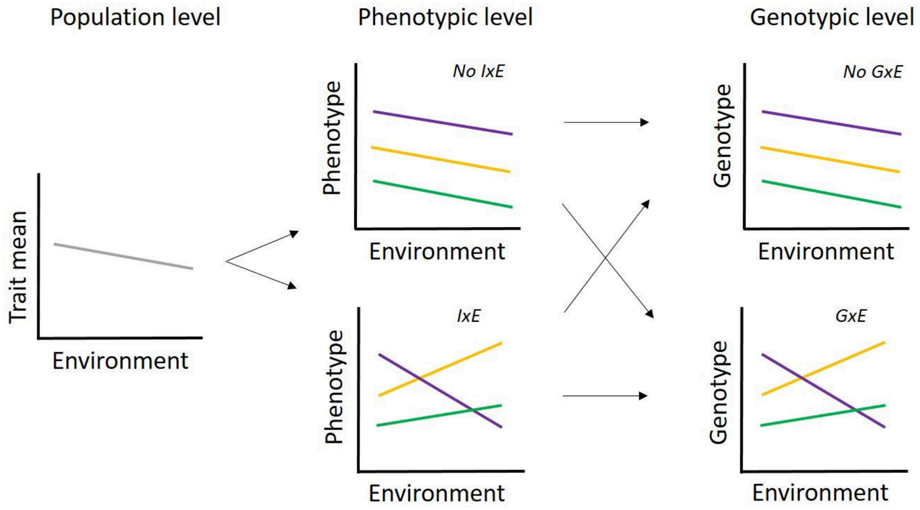
No genetic change: reaction norm approach to explore G×E interactions

Despite selection acting on a trait may lead to an evolutionary change, local adaptation can also occur without claiming a change in the genetic architecture of a trait or change in breeding values relative to those expected from a random change within a quantitative genetic approach. Detecting such lack of genetic change can occur because genotypes are plastic enough to express different phenotypes over a gradient of environmental conditions. This plastic response can have a genetic basis on which selection can act upon. The reaction norm approach is commonly used to quantify traits variation in a natural setting but it also allows the integration of phenotypic plasticity into a quantitative genetic framework. The reaction norm is defined when an individual (or genotype) expresses different phenotypes in function of an environmental variable (Via et al., 1995). It is usually quantified as linear association, where every phenotypic individual (or genotype) trait values are regressed against an environmental gradient or across different conditions (Nussey et al., 2007). It is described by the intercept or elevation, which reflect the trait value in the average environment and a slope, which describes the sensitivity of the trait to the environment (i.e. the plasticity of the trait).



Linear reaction norm from different individuals or genotypes (colour lines). On the figure A, there is no plastic response to the environmental gradient (no slope), whereas the mean response differs across environment (difference in the intercepts, V_G). In the two others scenarios B and C, there is phenotypic plasticity however the response is similar on the figure B (parallel slope, V_G+V_E), while responses differ in sensitivity on figure C (variability in slope, $V_G \times$E).

Plasticity indicates the property of individual reaction norms, however, it can also refer to a population-level attribute to how the mean phenotypic trait between-individual (genotypes) changes in response to the environment (Pigliucci, 2005). If reaction norms represent different genotypes in a population, thus the phenotypic plasticity within a population can be described by the means of individual reaction norms. Representation of different reaction norms of individuals inform about the variation in plasticity across the entire population. The intercept of the reaction norm reveals the relative performance across environment between individuals, whereas slopes of reaction norm indicate the degree of respective plasticity in response to the environment.



Levels of phenotypic plasticity at individual level. The mean trait population can be decomposed by variation in plasticity at the individual level in their response to the environment (I×E). Such I×E interaction can underly variation in plasticity at the genetic level (G×E) or by experienced different environment (PE×E).

Between-individual phenotypic variation can be decomposed into genetic and non-genetic components (so-called ‘permanent environmental’, PE×E) using quantitative genetic models (Kruuk & Hadfield, 2007). At the population level, if genotypes express a different phenotype across environment (genetic variation of reaction norm or genotype-by-environment- interaction, i.e. G×E), it suggests that plasticity has a genetic basis on which selection can act upon. When I×E occurs but not G×E, it may suggest that individuals differ in their plasticity. Such differences in plasticity may emerge when individuals differ consistently in their phenotypic expression along a same environment (i.e. PE×E). There are few empirical studies for G×E in plasticity because of a lack of power in methods and statistic issues, but evidence for I×E is more commonly found (Brommer, 2013). Genotype-by-environment interactions may lead to local adaptation because the differences in reaction norms of genotypes may favour the evolution of phenotypic plasticity in heterogeneous environments (Via & Lande, 1985).

Environmental change and local adaptation

Climate change and habitat loss are a major cause of threat of biodiversity and challenge species persistence during the last few decades. The impact of habitat loss on populations is so fast that it does not allow evolution to occur. However, the climate change in the last decades may allow populations of some species to track such changes and adapt to new conditions (Brown & Brown, 2000; Garant et al., 2004). Organisms can potentially respond to spatial and temporal environmental variation, including climate change, via dispersion, microevolution or phenotypic plasticity. However, dispersal mainly depends on the species' dispersal ability and the availability of unaffected habitat by climate change, neither of which may be sufficient to prevent extinction risk (Thomas et al., 2004). Phenotypic plasticity may enable populations to cope with changing environments (Scheiner, 1993; Scheiner et al., 2019; Yeh & Price, 2004), but in the long term, such responses are unlikely to be sufficient (Gienapp & Brommer, 2014; Gienapp et al., 2014) and evolutionary adaptation will be necessary for population persistence (i.e. evolutionary rescue ; see Bell, 2013; Bell and Gonzalez, 2009; Carlson et al., 2014). In addition, to assess the ecological consequences of climate change, it is essential to predict the rate at which population can adapt (Shaw & Etterson, 2012; Visser, 2008). Thus, our comprehension of the influence of climate change to predict population viability can only be fully accomplished by disentangling the influence of the evolutionary mechanisms on local adaptation (Both & Visser, 2005; Gienapp et al., 2014; Kingsolver & Buckley, 2017; Visser et al., 2015).

How species will respond to climate change is a major challenge in evolution and conservation, by therefore investigating the impact of environment on natural selection. To understand the influence phenotypic selection and the impact on local adaptation on populations, we need to consider multiple environmental factors (biotic and abiotic) acting at different scales (local and global) (MacColl, 2011; van de Pol et al., 2016). However, identifying the single or multiple factors that change phenotypes and drives natural selection (e.g. agent of selection) is challenging in wild populations (Merilä & Hendry, 2014). Climate change is assumed to increase mean temperatures, alter precipitation rates and modify the frequency and intensity of extremes weather events (IPCC, 2014). Modifications are also predicted to unequally affect areas spatially and temporally, which often lead to phenotypic variation among populations and caused dramatic changes for ecosystem dynamic, community and populations (Parmesan, 2006). In addition, climate change can have a knock-on effect on multiple taxa from different trophic levels and affect simultaneously the full ecological network (Schleuning et al., 2016; van der Putten et al., 2010). Climate change is therefore characterised by a rapid ecological change with short-term evolutionary consequences. However, evolutionary mechanisms can be complex and reflect the interplay of several factors, whose intensity may in turn fluctuates over time. Establishing the relation between climate and the impact on populations has been largely explored and widely accepted in recent years (Parmesan, 2006; Parmesan & Yohe, 2003); but predictions on evolution on a trait across populations, time and habitats remain an unresolved task (Thomas et al., 2004).

Avian phenology of breeding

Most observations of climate change responses have focused on alterations of species' phenology (from the Greek *phainomai* "to appear"). Phenology is the timing of periodic life-history events, such as leaf unfolding in trees, flowering in plants, appearance of fruits or insects and start in reproduction or migration departure in birds (Menzel et al., 2006; Parmesan & Yohe, 2003; Root et al., 2003; Walker et al., 2019; Walther et al., 2002). Changes in reproductive phenology are one of the most pervasive responses observed to global climate change in a wide variety of taxa (Cleland et al., 2012; Dunn & Winkler, 2010; Réale et al., 2003; Visser & Both, 2005). Thus, the breeding date in avian species is intensively studied to understand whether populations are able to respond to climate change (Charmantier et al., 2008; Nussey et al., 2005c). In addition, the timing of breeding is crucial for birds in wild populations because of the strong relationship with reproductive investment and performance (Dunn, 2004), in particular for the optimal clutch size (Verhulst et al., 1995) and the benefits of multiple brooding (Husby et al., 2009). Hence, the phenology on multiple avian population have been collected in several long-term studies allowing comparisons between populations and to provide early warning signs of the impact of climate change over time and space. Under climatic change, temperate zone birds have thus been detected to advance their breeding season (Brown et al., 1999; Crick et al., 1997; Crick & Sparks, 1999; Dunn & Winkler, 1999, 2010; Visser et al., 2004), as well as the duration of their reproduction (Hällfors et al., 2020) and incubation behaviour (Simmonds et al., 2017).

The effects of climate change on phenology is generally related to temporal changes in optimal condition (Visser & Both, 2005). The deterioration of ecological condition as breeding season advances (date hypothesis) is associated with an increase of the cost of reproduction (Dunn, 2004; Low et al., 2015). This scenario has been reported in multiples studies where early breeding individuals have, on average, a higher reproductive success than late breeding ones (Goodenough et al., 2009; Öberg et al., 2013; Verhulst et al., 1995). More specifically, food abundance is expected to display a peak which increases the need to match the maximum food demand of offspring, through an optimal breeding time, with peak food availability to optimise reproductive success (Both et al., 2009a; Naef-Daenzer & Grüebler, 2016; Visser et al., 2006). For example, because warmer temperatures predict insect emergence to occur earlier in the breeding season (Both & Visser, 2005; Visser, 2008; Visser et al., 1998), insectivorous species are expected to breed earlier in warmer spring (Both & Visser, 2001; Brommer et al., 2005; Brommer et al., 2003; Schaper et al., 2012). This hypothesis suggests that the impact of climate change on wild population may be mediated through these so-called 'mismatches' (see **Box 2**) (Burgess et al., 2018; Dunn et al., 2011; Jones & Cresswell, 2010; Mayor et al., 2017; Renner & Zohner, 2018; Samplonius et al., 2021; Thomas et al., 2001), which result as a major consequences for fitness and population persistence (Both et al., 2006a; Bowler et al., 2019; Møller et al., 2008; Reed et al., 2013a; Saino et al., 2011; Simmonds et al., 2020; Visser & Gienapp, 2019). However, the influence of this mismatch may blurry as the food diversity increases in the population, where multiple prey species may emerge at different times, and depending of the nestling diet between habitats (Burger et al., 2012; Sanz, 1998).

Bird populations are responding to changing environmental conditions. From an evolutionary perspective, breeding time can be a trait under evolutionary selection. Some populations are unable to track climate change and increasing temperatures can speed up microevolution (Husby et al., 2011a) while other populations can respond expressing phenotypic plasticity (Husby et al., 2010). Plasticity enables individuals to match their habitat conditions to adjust to environmental changes (Nussey et al., 2007) and reduce their extinction risk (Vedder et al., 2013). Nevertheless, a plastic response no necessarily means an optimisation of reproductive success, and a similar response between populations can differ in magnitude and consequences. For instance, avian populations capable to correctly respond to the new environmental conditions and advancing their laying date have a stable demography (Charmantier et al., 2008), while populations with a similar but lower responses were declining (Both & Visser, 2001; Visser et al., 2006). Moreover, a plastic response is efficient if the environmental factors used as predictors the phenology of birds (i.e. the cues, see **Box 3**) remain the same during an environmental change (Charmantier & Gienapp, 2014). However, plasticity of breeding date might be sufficient to explain population adaptive response to climate change through micro-evolution. In that purpose, the potential of evolution of plasticity could be confirmed when the variation in the trait is heritable and under directional selection (Charmantier & Gienapp, 2014; Rago et al., 2019).

Glossary 2

Phenology: the study of cyclically recurring biological events, such as the seasonal timing of tree leafing, insect hatching, or animal migration and reproduction.

Trophic level: the position that an organism occupies in the food chain. Primary consumers are herbivores, and secondary consumers are omnivores or carnivores.

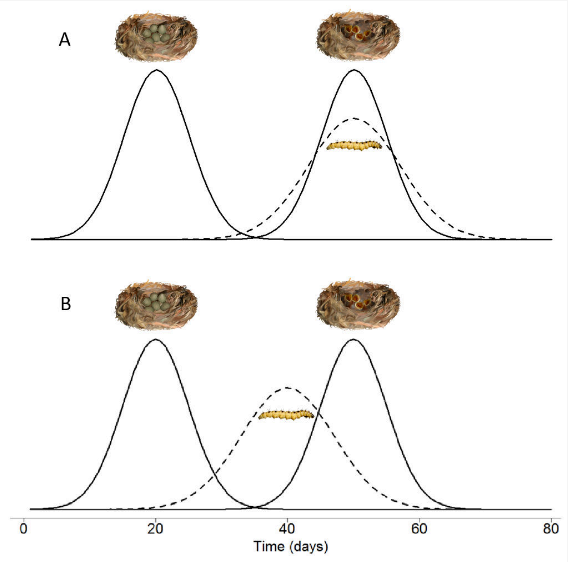
Phenological response: the extent to which phenology responds to biotic or abiotic variables.

Phenological asynchrony: when the peak consumer demand for a resource does not coincide with the peak availability of that resource.

The match-mismatch hypothesis: trophic asynchrony or phenological mistiming with negative consequences for consumer fitness or population size.

Box 2 : Optimal time window and phenological mismatch

The phenology of animals is expected to be synchronized with the lowers trophic level (Both et al., 2009a), such as insectivorous birds reproduction rely on the abundance of insects, as the main food source for the nestlings. Reproductive success is thus dependant on the availability of food preys, resulting in an optimal time window for reproduction (Naef-Daenzer & Grüebler, 2016). However, this is narrow time window is changes from year to year and shifts in peak food availability may cause a 'phenological mismatch' between trophic levels. However, the mismatch hypothesis is highly dependent of the model species and the diet of specific the population, and by consequence cannot be imply in all phenological studies.



The optimal time window is dependant on the maximum of availability of preys for raising the nestling (schema A). The reproductive success is compromised when the preys advance their phenology, without a shift in the timing of brood highest demand, creating a phenological mismatch (schema B).

In a model study of great tits, *Parus major*, from a Dutch population, breeding phenology relies mainly on the caterpillars abundance of the winter moth, *Operophtera brumata*, in relation to its main host plant the common oak, *Quercus robur*. These caterpillars show a clear peak in mid-May, depending on the blossoming of oaks and spring temperature. Breeding success of great tits is highest when the energy demand of the 9-12 days old nestlings matches with the peak of caterpillars biomass (Reed et al., 2013a; Visser et al., 2006). With warmers springs, the phenology of trees and consequently the biomass peak of caterpillars have advanced by about 2 weeks, whereas the timing of nestling of greats tits have only advanced of 5 days, leading to a mismatch (Visser et al., 2006; Visser et al., 1998), which may impact the population viability (Visser & Gienapp, 2019).

Box 3 : Phenological cues

Predicting phenological changes is essential for biodiversity conservation. However, environmental variation is common in temperate latitudes which have large annual variations in day length, temperature and precipitation. Organisms have to use cues to time their activities over the seasonal change in such a way to maximize their fitness. Because the timing available for breeding season is crucial (i.e. 'optimal time window'), individuals have to develop strategies to accurately synchronise their start of breeding with prevailing environmental conditions. However, a distinction has to be made between cues that birds use to breed and the environmental factor affecting the selection on breeding period (i.e. 'drivers of selection'). Because the period of laying eggs takes place weeks before the time of peak of food availability, the cues use by birds to start breeding can be different from the environmental factors when selection takes place (Visser et al., 2004). In fact, the reliability of cues and phenological sensitivity may explain the difference in phenological plasticity between trophic levels, leading to a stronger selective pressure on consumers phenology (Cleland et al., 2012; Gienapp et al., 2014).

Mechanism of synchronization between biological rhythms and environmental cycle are widely recognized to be generated by circadian clock analogous to a circannual clock. There is a consensus that the photoperiod (variation in day light) is the main cue used by organisms, including migrate ones, to time their annual cycle stages (Gwinner, 1996). However, if photoperiod has been proved to determine the gonadal development or neuroendocrine system for the onset of bird breeding (Verhagen et al., 2019b), it does not have influence on laying date (Salis et al., 2019). Migrate birds have to used supplementary cues to track year-to-year changes in optimal condition for reproduction. If the cues that birds use to time their reproduction are no longer a reliable information for predicting the breeding conditions, there is more chance to produce a phenological mismatch ('cue' hypothesis). However, in a context of global change photoperiod is not a reliable cue used by birds to fine-tune the timing of egg laying. In that sense, photoperiod cannot be an adaptive cue for reproductive phenology of birds.

Local temperature is an important factor used to study the impact of global change in wild population. Temperature-derived factors are probably frequent cues used by many birds, which may explain why most birds that breed in north temperate zones begin to lay their eggs earlier in warmer springs (Both & Visser, 2001; Brommer et al., 2005; Brommer et al., 2003; Crick & Sparks, 1999; Schaper et al., 2012; Visser et al., 2004). High temperature causally advance the timing of breeding (Brown et al., 1999; Mccleery & Perrins, 1988; Meijer et al., 1999; Verhagen et al., 2020;

Visser et al., 2009). However, warmers springs can be correlated with an advancement in insect availability and thus the optimal conditions for feeding chicks (van Noordwijk et al., 1995; Visser & Both, 2005; Visser et al., 2006; Visser et al., 1998). The temperature-related budburst of trees can also be used by insectivorous birds to track the optimal period of insect emergence (Both et al., 2009a; Buse et al., 1999; Van Asch et al., 2013; Visser & Holleman, 2001).

However, the optimal timing of breeding is not only determined by food availability associated with temperature. Additional cues, which may include rainfall (Imlay et al., 2018; Sockman & Courter, 2018), extreme weather events such as drought and hard freezes (Marrot et al., 2017), predation density (Both et al., 2009a) and breeding density (Ahola et al., 2012; Wilson et al., 2007b) might change in a yearly basis affecting reproductive success and impacting on fitness.

Differences between long-term studies on avian populations

Phenology and distribution of laying date from long-term studies are spread out around Europe and permit to highlight differences in birds' behaviour and multiple responses to climate change. A major distinction reside between resident and migrant's species (Both et al., 2009b; Kluen et al., 2017; Pearce-Higgins et al., 2015; Wilcove & Terborgh, 1984). In migrant species, early arrival to the breeding grounds is favoured, because more resources (territories, mates or food) are available for the first than last individuals within a season (Jonzén et al., 2007). However, migratory species are in disadvantage for optimize their breeding time to the local environmental change in the breeding habitat, and often, populations are in declines (Both et al., 2006a; Jones & Cresswell, 2010; Møller et al., 2008; Saino et al., 2011). Because the clues in the wintering site no reflect the phenology in breeding habitat (see **Box 3**), the departure from migration is independent of the timing phenology in local habitat (Both & Visser, 2001). This difference makes migrant species interesting models to understand and compare the ecological impact of environmental change on reproduction with resident birds, as the great tit (*Parus major*), another well-studied species in Europe. In addition to habitat degradation in stop-over sites, non-breeding and breeding grounds (Howard et al., 2020), the climate change also alter the interaction between migrants and residents birds and increase the breeding competition among them (Potti et al., 2021; Samplonius et al., 2018; Samplonius & Both, 2019; Wittwer et al., 2015).

Environmental conditions for migrant birds are different in wintering grounds than from local ones. For instance, in pied flycatchers (*Ficedula hypoleuca*), some studies aimed to demonstrate that northern flycatchers can be affected by environmental conditions in the wintering grounds or along the migration route (Ahola et al., 2004; Both et al., 2006b; Both & Visser, 2005). These studies add supporting evidences that migration distance, speed and the arrival date are constrained and limit the optimal window for breeding time in migratory birds, suggesting the 'arrival constraint hypothesis' (Both & Visser, 2001). The interval between arrival time and the beginning of breeding is limited and push individuals to be more reactive for finding the best mate (Potti, 1999) or storing fat (Sandberg & Moore, 1996). The migration distance play a role in the ability to respond to changing conditions earlier in the season and advancing the arrival date (Butler, 2003; Clark et al., 2014; Koleček et al., 2020). Long-distance migrants are supposed in that sense to be more maladaptive to adjust their life cycle (Both et al., 2006b; Both et al., 2009b; Coppack et al., 2008; Jonzén et al., 2006; Lehikoinen et al., 2019; Møller et al., 2008; Rubolini et al., 2007) and more sensitive to climate change (Howard et al., 2020; Lomas Vega et al., 2021; Saino et al., 2011; Sanz et al., 2003).

Because the breeding site is dependent on local condition, the reaction to climate change is also specific to each population. A difference found in Europe is that the increase of temperature is less important in southern part of Europe (Both et al., 2004) and that temperature rise just before the laying date and alter the climatic pattern (Sanz et al., 2003). Hence, breeding populations across Europe are no affected in the same way by the same climatic factors, like for example the North Atlantic Oscillation index (NAO; Hurrell, 1995) which has more effect on northern birds populations (Both et al., 2006b; Jonzén et al., 2006; Sanz et al., 2003). The climatic variation and stochastic events encounter during the migration are restrained by distance, that is why the evolutionary strategy of population must be different among studies (Both et al., 2004; Both et al., 2006b). For instance, populations of pied flycatchers in Central Europe have advanced their laying date but not arrival date (Both et al., 2004; Both & Visser, 2001; Sanz et al., 2003), whereas Finnish pied flycatchers have advanced arrival (Ahola et al., 2004; Jonzén et al., 2006) but not laying date (Laaksonen et al., 2006). As different pied flycatcher populations may winter in different areas or experience different temperatures during their migration, this could account for inter-population differences in ability to advance breeding phenology (Both & te Marvelde, 2007; Moore et al., 2005).

Given the lack of consistent results that quantifies the evolutionary dynamics of laying date in relation to changing environmental conditions in wild populations, I will tackle in this PhD the basic material needed for natural selection to act upon. To do so, I took advantage of one of the most valuable long-term and individual-based monitored population of birds: the pied flycatcher.

Study model

The european flycatchers of the genus *Ficedula* (family Muscicapidae) are insectivorous birds very prone to use nest boxes, that made them easy to track during the reproductive period, becoming a study model in ecology and evolution (Lundberg & Alatalo, 1992). The pied flycatcher (*Ficedula hypoleuca*) is a small passerine (11-13g) and a solitary migratory bird who breeds in natural or artificial holes in temperate forests across Europe and eastern Russia, wintering in the sub-Saharan subtropical region of western Africa (Clements et al., 2021; Lundberg & Alatalo, 1992; Ouwehand et al., 2016). With long-term population studies available across Europe (Figure 1a), a prerequisite necessary to understand the evolution of birds' phenology, pied flycatcher populations are well-known study model for its response to climate change (Both et al., 2006a; Both & Visser, 2005; Sanz et al., 2003).

Male and female pied flycatcher of the subspecies *Ficedula hypoleuca iberiae* (**Witherby1928**). During the breeding season, the sexual dimorphism is contrasting with males exhibit a black or greyish plumage with a prominent white forehead patch (with fluctuating size and form), whereas females are greyish-brown.

This PhD is based on a pied flycatchers breeding population, which has been continuously monitored since 1987 (although with reduced fieldwork in 2002 and 2003) in la Hiruela, in Central Spain. From all populations breeding in Europe, this population in the Iberian Peninsula is the one of the southernmost long-term study populations in this species (Figure 1a). Weekly monitoring of nest-boxes on the study site (more than 200 boxes) during the breeding season allowed a long-time record breeding date (date of first laid egg), clutch size, number of hatchlings and number of fledglings of the population over the years (see trends in **Box 4**). Breeding individuals were captured using a nest-box trap, measuring body size (tarsus length ±0.01 mm and wing length ±1mm), weighed (±0.1 g) and aged as either 1 year old or older following the criteria of (Karlsson et al., 1986). Adult breeding males were individually marked with colour and metal rings and only with a metal ring for females. All fledglings were ringed and measured (body mass and tarsus length). Offspring rings enabled following their fate in the following years as proxy of recruitment and to know the (social) individual relatedness in the population (pedigree).

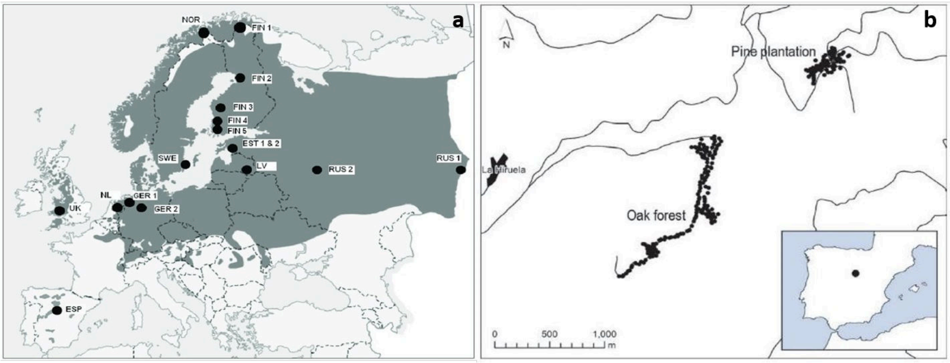
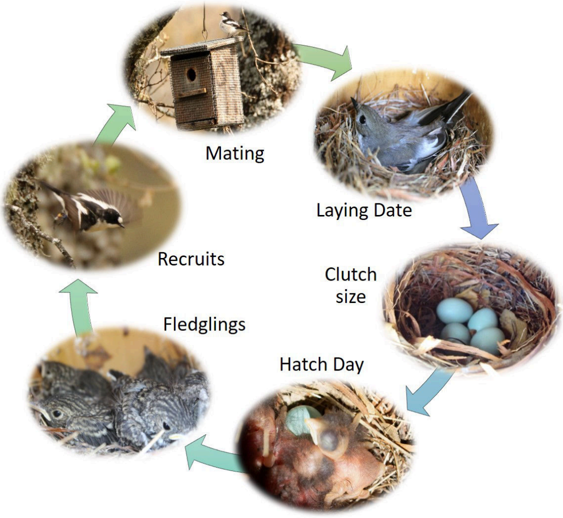


Figure 1: a: Distribution of breeding range of pied flycatchers in Europe, where each dot show s long-term monitored populations (adapted in Ruuskanen et al., 2011).
b: Map of the two study areas; each black dot denotes a nest box monitored for at least 22 years. Black lines indicate roads (map from Potti et al., 2018).

The study site has two main areas, one a mature oak forest of 9.3 ha (Madrid, 41°04′N 3°27′W) and a close pine plantation of 4.8 ha (Castilla La Mancha 40°40′N 4°8′W), away 1.1 km from each other (Figure 1b). The two study sites are suitable breeding habitats for flycatchers and the breeding density of population increased (see **Box 4**) since the installation of wooden nest boxes in 1984 in the oak forest and from 1988 in the pine forest (see Camacho et al., 2013; Potti et al., 2018 for more details). The two areas are comparable in term of climatic conditions but differ in vegetation structure and composition. Caterpillars is the most important prey for nestlings, and because it is considering to be of high-quality nutritional quality and will impact the timing of breeding (Arnold et al., 2010), oak forest are assumed to be of higher quality for breeding pied flycatcher. However, the assumption that coniferous forests are a low-quality habitat for flycatchers can be questioned in our population (Camacho et al., 2015; Potti et al., 2018). Speculating that the difference among these two sites in the vegetation and diversity of insects, we cannot assume a similar phenology of the emergence if insects between both forests, which could explain the principal difference in reproductive traits between the two habitats (see **Box 5**). However, neither the diet composition and prey size difference between forest types, neither the latitude explain the difference in breeding date observed between populations across Europe (Sanz, 1997, 1998). Consequently, breeding data from both areas will be treated as the same population but controlling for potential consistent differences among these two habitats (see Camacho, 2018 for further comparison and implications between both habitats).



Breeding cycle of long-term individuals monitoring of pied flycatchers provides information on life-history traits and individual relatedness, essential for investigating evolutionary dynamics in the wild.

Usually, individuals in this population come back from wintering grounds from late April to early May according the age of individuals and their experience (Potti, 1998a). Like in other protandric species, males are the first to arrive to the breeding areas to monopolize a breeding territory before the arrival of females few weeks later (Bell et al., 2021b; Canal et al., 2012). Pied flycatcher first reproduction is delayed until the second, or more rarely the third year of life (see **Box 7**, with a strong fidelity for the breeding site (Montalvo & Potti, 1992; Potti & Montalvo, 1991b). On average, 30% of the breeding adults every year are immigrants of unknown origin whereas 25% of the native birds disperse between habitats (Camacho et al., 2013). Pied flycatchers are socially monogamous, but polygamy is common in this species where a small proportion of males can have a secondary female in addition to their social pair, (see Canal et al., 2021 and extra-pair paternity, despite annual variation, is of around 15% in our population (Canal et al., 2012). Females lay between four and six eggs in a single brood every year, second clutches are occasional and mostly result from a reproductive failure of the previous one. Incubation lasts 12-13 days and fledglings leave the nest between 15 and 19 days after hatching.


Laying date


Incubation : 12 days


Age 1 day


Age 5 days


Age 10 days


Age 15 days

Box 4 : General population pattern

The clutch size of the population decreased over years (r=-0.008±0.004, df=2826, P<0.001) as the hatching success (r=-0.005±0.002, df=2836, P=0.02) and the numbers of recruits (r=-0.005±0.002, df=3009, P=0.007). Although there is no temporal trend for numbers of fledglings (r=-0.002±0.003, df=3008, P=0.53) and female survival over the study period (r=0.0007±0.001, df=2937, P=0.55).

Considering both habitats, the nest-boxes occupancy (breeding flycatcher nests on the total of nest-boxes available) and the population density (number of total breeding pairs) have increased over the years (Figure 7, respectively r=0.01±0.28, df=26, P<0.01; r=2.89±0.28, df=26, P<0.01) for an average of population growth rate of 1.1% per year since 1984. There is an increase of population density in both forest patches (oak forest r^2=0.578, df=29, P<0.001 ; pine forest r^2=0.889, df=25, P<0.001), however when comparing the saturation of nest box occupation as a proxy of nest-box quality (Potti et al., 2018), the population growth was greater in the oak forest (oak forest r^2=0.738, df=29, P<0.001 ; pine forest r^2=0.408, df=25, P<0.001).

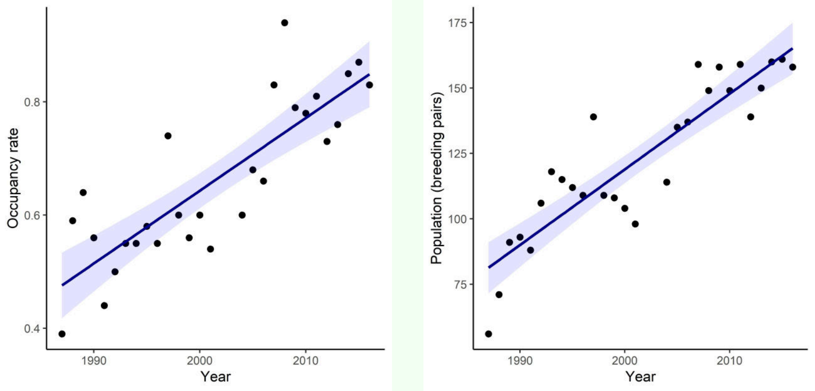


Figure 2: Temporal trend in nest-boxes occupancy rate (left) and population density (right). Solid line represents the trend of population over time with 95% CI.

Box 5 : Comparison of the two habitats

Between both habitats, the laying date is in average early for the oak forest than the pine forest (Figure 3, mean oak forest (1987-2016) = 20.93, mean pine forest (1988-2016) = 22.64, linear mixed model estimate ± SE=2.249 ± 0.309, t=7.281, df=1306, p <0.001). Note that the first next-boxes have been put in 1988 in the pine forest (see Camacho et al., 2013 for further details).

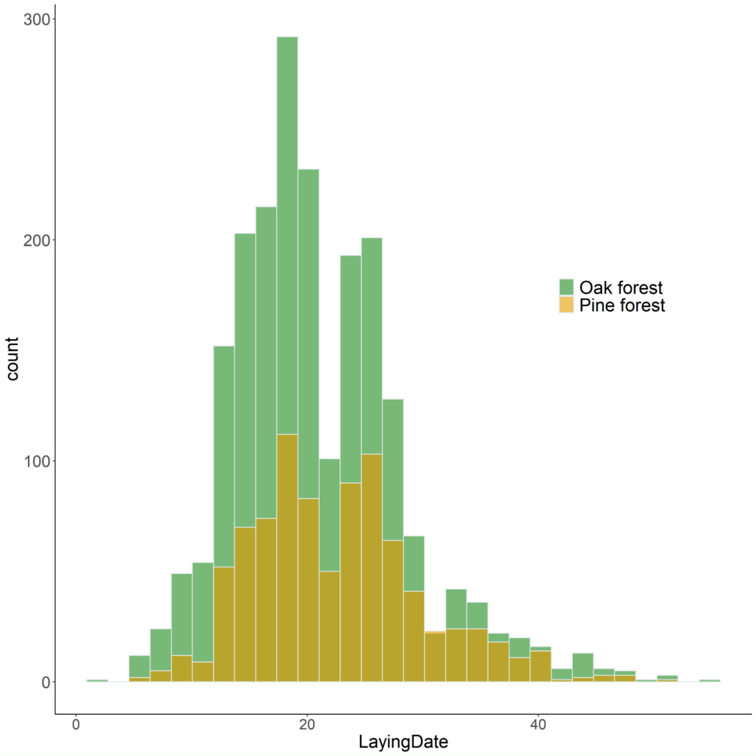


Figure 3: Histogram of the frequency distribution of laying date (1 = 1st May) from 1987 to 2016 for the oak forest (green) and from 1988 to 2016 for the pine forest (yellow).

The clutch size is higher in the oak forest (mean oak =5.53, mean pine =5.34; =-0.18±0.03, t =-4.918, df = 1434, p-value < 0.001), whereas the fledgling success during the breeding period is similar between both sites (mean oak forest = 4.31, mean pine forest = 4.34; =0.049 ± 0.062, t=0.792, df = 1314, p = 0.429). However, the reproductive success (number of recruits the following years), is higher in the pine forest (mean oak forest =0.52, mean pine forest =0.72; =0.230 ±0.033, t =7.034, df = 2896, p-value< 0.001).

Individual-dependant effect on breeding date

Life-history trait expression changes with age in many species with a large variation in reproductive fitness among individuals (e.g. fecundity). Reproductive traits have been many times observed to be age-dependant, with senescence strongly impacting reproductive performances (Nussey et al., 2013). In the Spanish flycatcher's population, after an increase in reproductive success at two years of age, there is a small but decreasing reproductive performance in relation to age, which might be attributed to senescence (see **Box 6**). However, according to the "selection hypothesis", age is also related to an increase in reproductive success because of selection on individual quality, with individuals improving in condition and experience as they age (Curio, 1983; Pärt, 1995). Variation in reproductive performance should therefore be the result of two independent processes: age-specific reproductive effort and effects of individual quality as breeders (McCleery et al., 2008). Selection against individuals of inferior quality (e.g. selection hypothesis) is partially supported since individuals improve their performance with age (**Box 6**, Table 1) and experience (**Box 7**, Table 2), before the supposed age of senescence (after five years old in southern population of the pied flycatcher (Sanz & Moreno, 2000). The age at recruitment and number of breeding events confirm that age pays a major role in the increase in breeding success whereas experience influence might be an indirect related effect of age (Pärt, 1995) as it have been shown experimentally in flycatchers populations (Cichoñ, 2003; Fay et al., 2021; Harvey et al., 1985). Therefore, because of the indirect effect of breeding date on reproductive performance, age-related experience effect on breeding date should be consider further.

A seasonal decline in reproductive success is observed in many passerines in the temperate zones, and could be interpreted as a consequence of optimal reproductive investment. Physiological and ecological differences could result in 'good' quality females laying large clutches early in the season and 'poor' quality females laying small clutches later in the season (McNamara & Houston, 2008). Nutritional and body condition of females might also limit the fecundity of birds and so impose cost and selection on breeding date (Price et al., 1988). Therefore, the timing of breeding reflects the difference in quality between early and later breeders (Møller, 1994; Verhulst & Nilsson, 2008; Verhulst et al., 1995), implying that individuals arriving to the breeding grounds in lower body condition are more limited to optimise their reproductive success (see **Box 7**). In migrant species, early arrival to the breeding grounds is favoured, because more resources (territories or mates) are available for the first than last individuals within a season (Kokko, 1999), whereas early individuals suffer more from non-favourable condition during early spring than late individuals. Pied flycatchers with greater previous breeding experience (or older individuals) tended to arrive earlier in spring, lay eggs earlier and have larger clutches (**Box 7**) (Both et al., 2016; Potti, 1998a). However, individuals of high phenotypic quality may start to breed at an early age and, at the same time, be able to achieve high reproductive performance, irrespective of breeding experience (Pärt, 1995). Experienced and females in better body condition are supposed to arrive first at breeding grounds outcompeting other females for best mates and territories (Canal et al., 2012). Males can therefore also have an indirect effect on the breeding date of females (Brommer & Rattiste, 2008; Evans et al., 2020). The phenotypic quality of individual pied flycatchers also mediate the breeding status and the probability of being involved in a polygynous event, and by consequence have an influence on the reproduction success (Canal et al., 2021; **Box 7**, Table 3). Despite, we cannot access to the quality of females as breeders per se, body mass, as a metric of female condition (Labocha & Hayes, 2012) and partner effect, through the polygamy status and male identity, should therefore be taken into account in analyses that aim to explore variance on breeding date.

Box 6 : Age-dependant effect on breeding date

Breeding date shows a typical age-senescence pattern with an early laying date after the first age and a progressive later breeding at older age (Figure 4). On average, laying date of one year old females and inexperienced birds is delayed in relation any other ages (Table 1). Second years old females and older are more similar in their breeding date and seem to reach a plateau after the first year of breeding.

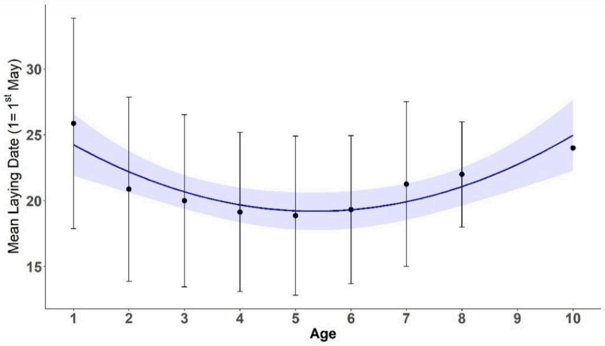


Figure 4: Average annual laying dates (±SD; 1 = 1st May) by age of individuals. Laying date display a quadratic relation (blue line with 95% CI) with age, a common pattern of an age-dependant trait.

The effect of senescence in reproduction is described by the reproductive success of females according their age (Table 1). The reproductive success measured in the current year (fledglings' number) or following years (recruit number) show a similar age-dependent pattern as laying date (Table 1, Figure 4). One year old females have on average less fledglings and recruits than 2- and 3-years old females. Six-year-old and older females have less fledglings than the last age category, but higher number of recruits of all ages. The age at the first reproduction does not strongly influence the reproductive fitness and the timing of breeding (Table 1). Early breeding date is associated with higher fledglings (r=0.068, df=1587, p<0.001) and higher number of recruits (r=0.020, df=1587, p<0.001) and for all age category.

Table 1: Mean and SD (± Standard Deviation) of laying date, number of fledging and recruits according to known age of individuals.

Age	**1 y/o**	**2-3 y/o**	**4-5 y/o**	**6 y/o**
Laying Date ± SD	25.873±7.988	20.569±6.849	19.052±6.043	20.063±5.761
Fledglings ± SD	3.995±1.407	4.465±1.474	4.525±1.496	4.074±1.523
Recruits ± SD	0.512±0.782	0.586±0.851	0.529±0.771	0.653±0.872
n	646	1706	465	95
Age at recruitment	**1 y/o**	**2 y/o**	**3 y/o**	**4-5 y/o**
Laying Date ± SD		21.164±7.381	20.566±7.039	20.167±7.240
Fledglings ± SD		4.422±1.508	4.434±1.571	4.367±1.51
Recruits ± SD		0.537±0.832	0.530±0.687	0.500±0.629
n		820	83	30

Box 7 : Individual-dependant condition impact on laying date

Females in our breeding population (n=1475) recruit mostly between one and two years old (see **Box 6**, Table 1), with a large number of females reproducing twice (n=403) or more (n=311). The number of reproductions of females through years reflect her experience and, in theory, their quality as a breeder the given year. More experienced females have an early breeding date than less-experiences females (Table 2). Moreover, first time breeders have a smaller number of fledglings and recruits than more experienced females.

Table 2: Laying date, reproductive fitness and SD (standard deviation) as function of the breeding experience of the individual (number of reproductive events). The difference in laying date (days), is the mean difference of individual laying day compared to the mean laying date of the population for the given year.

Number of reproduction	**1st**	**2nd**	**3rd**	**4**
Laying Date ±SD	23.041±7.967	19.840±6.099	19.579±6.142	19.051±6.011
Fledglings ± SD	4.247±1.485	4.516±1.420	4.506±1.510	4.363±1.543
Recruits ± SD	0.526±0.801	0.619±0.871	0.553±0.811	0.647±0.840
Difference in laying date ±SD	0.935± 7.465	-1.872±5.182	-2.399±4.927	-2.927± 4.84
n	1579	776	342	215

Female with higher body mass breed early (r^2=-0.725, df=2742, $p<0.001$) and have a better reproductive performance with bigger clutch size (r^2=0.090, df=2627, $p<0.01$), more fledgling (r^2=0.129, df=2738, $p=0.01$) and recruits (r^2=0.131, df=2739, $p<0.001$) than lighter females.

The polygamous status of the breeding female influenced breeding date and fitness (see further implications in Canal, 2012), with individuals involved in a polygamous pair as primary females have early and better reproductive success than secondary females, with or without male assistance (Table 3, see also Canal et al., 2021 for a more detailed description in relation to laying date).

Table 3: Laying date, reproductive fitness with SD (± Standard Deviation) according the breeding status of the individual. Monogamy: female in a monogamous pair; Primary: female in a polygamous pair, as primary female; Secondary A: female in a polygamous pair, as secondary female with assistance of the male; Secondary nA: female in a polygamous pair, as secondary female without assistance of the male.

Mating Status	**Monogamy**	**Primary**	**Secondary A**	**Secondary nA**
Breeding Date ± SD	20.994±6.983	17.648±5.472	25.649±5.728	24.970±9.200
Fledglings ± SD	4.512±1.373	4.594±1.405	3.956±1.460	2.882±1.430
Recruits ± SD	0.577±0.797	0.766±1.016	0.342±0.689	0.221±0.514
n	2441	128	114	68

Thesis objectives and outlines

The main aim of this thesis is to investigate the role of environmental variation on the evolutionary dynamics of laying date in a Spanish population of pied flycatcher. To consider the evolutionary dynamics of laying date, I will need to test the main evolutionary prerequisites needed for natural selection to occur. First, I studied the consistency (i.e. repeatability) of the laying date behaviour in females of our population among years to understand the pattern of variability of reproductive timing (**Chapter I**). Then, I explored the relative influence of multiple environmental variables on selection at phenotypic level on laying date (**Chapter II**). After exploring and detecting the most important drivers of selection on laying date, I quantified the potential influence of those environmental factors on the genetic change in laying date using quantitative genetic models (**Chapter III**). Given the lack of microevolutionary changes in laying date, I explored the potential evolutionary role of phenotypic plasticity against different environmental variables (**Chapter IV**). Finally, I further explored the influence of within-individual variation at explaining total phenotypic variance of laying date (**Chapter V**). Therefore, in this PhD I developed a research schedule to comprehend the mechanisms that may explain local adaptation in a wild bird population.

Chapter I - Repeatability of reproductive events

Variation in climatic factors imposed changes on the timing of breeding in many populations of birds. However, environmental conditions are not uniform among populations and individuals may respond differently (Rubolini et al., 2007). In migratory species, more affected by climate change with observed decline in population persistence, it is essential to understand how populations can cope with rapid environmental change. Therefore, exploring the variation of phenotypic traits among individuals is crucial for a conservative perspective. Repeatability is a measure for the consistency of a labile trait and can be inferred to appraise the variation of a trait (Falconer & Mackay, 1996). This chapter explores the repeatability of timing in migratory behaviour and breeding timing among females and years. This work might support the understanding of migratory birds breeding strategy and the long-term flexibility of avian population to cope with changing environment.

Chapter II – Detecting the relative effect of environmental variables in selection on laying date.

How organisms interact with its environment and how environment shape evolutionary process is a major concern in evolutionary studies. Identifying the environmental factors affecting natural selection is crucial to understand population adaptation to prevailing environmental conditions (Hendry, 2017). Climate change is acting as a major selective pressure on wild populations, in addition to habitat loss and others human-induced factors. To anticipate the response of a population to a changing environment, the identification of the environmental variable which mostly influences the covariation between laying date and fitness is required. Natural selection must favour the breeding time of females that optimise fitness. How and which factors influence phenology through selection are key to predict the impact of environmental change on bird populations.

Chapter III – Response to selection and evolutionary change

Several non-exclusive processes can explain phenotypic changes at the population level in response to environmental change. However, because of the lack of a micro-evolutionary response in breeding time in birds, the interplay between selection and genetic variation is not well understood. Quantitative genetics allows phenotypic variation to be partitioned into heritable and non-heritable component. With these models and the pedigree of the population available it is possible to quantify the additive genetic variance of from non-genetic sources of variances. By knowing the genetic contribution of each individual to the total phenotypic variance, I can track whether there is a genetic change of laying date over time (Gienapp et al., 2008; Merilä, 2012). Quantitative genetics models offer a new perspective to explore evolutionary response; but have been used here to explore the potential influence of environmental factors on the evolutionary change of laying date.

Chapter IV – Genetic merit of plasticity

To forecast the evolutionary response of selection of a phenotypic trait in a population is necessary to reckon the transmissible part of phenotypic variation from a generation to the next. However, estimates of 'evolutionary potential' or 'evolvability' of a trait in a population is shaped by the genetic part or heritance of the trait (Houle, 1992). Plasticity can be selected for only when genetic variation underlies genotype-by-environment interactions (G×E). Genetic variation for plasticity implies that plasticity can evolve, which suggests that adaptive phenotypic plasticity can occur in natural populations by responding to natural selection (Pigliucci, 2005). However, the presence and magnitude of G×E vary dramatically across populations and also depending on the environmental variables considered. In this chapter, I explore the potential role of G×E interactions under different local and global environmental variables making use of quantitative genetic models.

Chapter V – Phenotypic variation and individual effects

The key concern in evolution is the variation of phenotypes and how this variation is mediated by individuals. However, variation within- individuals (genotypes) drives differences in fitness among individuals and is therefore on interest in evolutionary ecology (Dingemanse et al., 2010; Piersma & Drent, 2003; Westneat et al., 2015). If phenotype responds to fluctuating environmental conditions across an individual's life, it may suggest that the same individual expresses plasticity in its phenotype. Behavioural, as much as life-history traits are known to be phenotypically plastic (Scheiner, 1993). Life-history traits are usually labile traits, reversible and express repeatedly with variation during an individual's life- time, and phenotypically plastic responses are expected to be shaped by the current environmental conditions that individuals experience (Brommer, 2013). Difference in plasticity among individuals (between-individuals) and within individuals inform about the latent ability of adaptive plasticity inside the population. Therefore, in the last chapter of this PhD, I further explored the within individual variation of laying date taking into account the additive genetic variance of the trait.

The major questions of this thesis are therefore:

- Is the expression of a labile trait predictable among individuals and time? (**Chapter I**, p33)
- What environmental factors influence selection on laying date? (**Chapter II**, p51)
- Can environmental variation shape genetic changes of laying date in short-time periods? (**Chapter III**, p 79)
- Can phenotypic plasticity evolve under different environmental factors? (**Chapter IV**, p99)
- To what extent within-individual variation may explain total variance of laying date under changing environmental conditions? (**Chapter V**, p115)

Addressing these questions are crucial for understanding and predicting how populations will respond to ongoing climate change, which becomes key not just for the ecological knowledge in which a population is settled, but also for conserving species diversity by understanding their evolutionary adaptations.

Chapter 1

Low repeatability of breeding events reflects flexibility in reproductive timing in the Pied Flycatcher *Ficedula hypoleuca*

Justine Le Vaillant, Jaime Potti, Carlos Camacho, David Canal
& Jesús Martínez-Padilla

Individual flexibility in breeding time is essential to respond to unpredictable changes in environmental conditions. Repeatability quantifies the consistency of the expression of phenotypes over time due to differences between individuals. Here, we estimate the repeatability of breeding date (laying date of first egg), hatching date and timing of pre-breeding events in a population of pied flycatchers *Ficedula hypoleuca* monitored over three decades in central Spain. We found low repeatabilities of breeding and hatching dates (respectively, R=0.135 and R=0) and among-year fluctuations (R=0.276 and R=0.218) in the expression of these traits. Repeatabilities of mating dates and of the interval between mating and egg laying were also very low (respectively R=0.053 and R=0) and among years (respectively R=0.218 and R=0.172), suggesting that pied flycatcher females are flexible to adjust their schedule of breeding to current breeding conditions. We interpret the low consistency of traits related to breeding phenology as a strong support for female phenotypic plasticity in breeding timing and the potential of such trait to respond to changing environmental conditions.

Ardeola 2022. 69(1):21-39

Introduction

Unpredictable environmental changes, exacerbated by ongoing global change, have strong effects on the biological timing of seasonal events (Parmesan & Yohe, 2003; Root et al., 2003; Walther et al., 2002). As a consequence, population responses to changing environmental conditions are commonly described from individual changes in the expression of their phenology-based traits (Ahola et al., 2004; Both et al., 2004; Lehikoinen et al., 2004; Menzel et al., 2006). Variability in such individual responses to environmental fluctuations has fitness consequences and may enhance the probability of population persistence (Both et al., 2006a; Jones & Cresswell, 2010; Møller et al., 2008; Saino et al., 2011), with major implications for the evolutionary dynamics of those traits (Cleasby et al., 2015) However, population and individual responses to environmental variation are rather heterogeneous (Radchuk et al., 2019). Thus, population-specific studies addressing the variability of such phenological responses are crucial to improve our understanding on how the populations may adapt to environmental change.

Repeatability is a measure of the consistency of a trait over time and can therefore be inferred to appraise the response of a labile trait due to environmental variation (Falconer & Mackay, 1996; van Noordwijk et al., 2006). Formally, repeatability is the expected within-individual correlation among measurements, also called intra-class correlation coefficient (Bell et al., 2009; Sánchez-tójar et al., 2021; Sokal & Rohlf, 1995). Repeatability estimates have crucial behavioural and ecological implications and have also been used as a proxy of evolvability for multiple phenotypic traits (Falconer & Mackay, 1996). The evolutionary relevance of repeatability lays on the idea that it was initially thought that it can set up the upper limit of heritability since it takes into account both environmental and genetic variances, but the latter assumption is not always true (Dohm, 2002). The most common approach for estimating repeatability considers the proportion of the between-individual variance (σ^2_α) relative to the total phenotypic variance (σ^2_P or V_P), represented by the sum of the between- and within-individual variance ($\sigma^2_\alpha + \sigma^2_\epsilon$, respectively; Nakagawa and Schielzeth 2010). Specifically, a high repeatability in a trait over a given period of time suggests a high individual consistency, either because high σ^2_α or low σ^2_ϵ, whereas an absence or low level of repeatability would imply that there is no or little individual consistency overtime in the expression of that trait. Thus, repeatability of life history traits could underline individual differences in response to environmental change.

Most climate change studies have focused on time of breeding, a key reproductive decision for temperate-zone birds because of the importance of optimizing the onset of breeding to match the prevailing environmental conditions (e.g. food availability; Both et al., 2009a; Naef-Daenzer and Grüebler, 2016; Visser et al., 2006). Migratory birds are at a disadvantage compared to resident birds for optimizing the timing of breeding according to the local environment, because adjustment to environmental conditions is constrained by their settlement dates after spring migration (Both et al., 2005; Both et al., 2006a; Both & Visser, 2001). Therefore, the study of variation in breeding phenology is crucial for migratory species (Both et al., 2006a; Both et al., 2006b; Jones & Cresswell, 2010; Møller et al., 2008; Saino et al., 2011). However, generalisation of repeatability estimates across populations is not straightforward (Cleasby et al., 2015) and estimates of repeatability of breeding (egg-laying) date differ between populations (see review in Table 1). Indeed, repeatabilities of laying dates are relative to the sex- and age-demographic structure of populations (Hochachka, 1993; Winkler et al., 2020), the quality of the breeding sites (Murphy, 2004) and may also be dependent on their residency status, on the distance to wintering grounds or on the trajectory of the migratory journey (Lourenço et al., 2011). In addition, many bird studies assessing effects of climate change that have quantified repeatability were focused on spring migration and other labile traits, such as pre-nuptial moult, migration routes and speed, and departure and arrival dates in spring (Both et al., 2016; Pulido, 2007). Quantifying and comparing repeatability estimates for arrival dates, the time elapsed between arrival and breeding (pre-laying period) and breeding dates is therefore required for a better understanding of the phenological responses of migratory birds to changing environmental conditions at the time of breeding (van Noordwijk et al., 2006).

We capitalised on three decades of individual-based phenological information of a European migratory passerine, the pied flycatcher *Ficedula hypoleuca*, to assess the degree of repeatability of mating date (onset of nest construction), breeding time (date of first egg laid), time elapsed between mating and egg laying (hereafter, pre-laying period), and hatching date (day the majority of the clutch hatched). Although there is annual variation in arrival date at breeding territories in the pied flycatcher, arrival date is constrained by intrinsic rhythms and therefore more consistent in females and among years (Both et al., 2016; Tomotani et al., 2018) than other phenological traits such as breeding time, although is more sensitive to local environmental variation (Bell et al., 2009; Both & Visser, 2001). Given the presence of individual phenotypic plasticity in breeding dates in response to environmental variation (Thorley & Lord, 2015; Visser et al., 2006), we therefore would expect low repeatabilities of breeding dates in female pied flycatchers. We predict that pre-breeding phenological traits (mating date and duration of the pre-laying period) will display higher repeatabilities because they should be primarily influenced by the more consistent time schedule of migration. Further, given that our study site consists of two different habitats, a mature oak forest and a managed pine forest (e.g. Camacho et al., 2015), we can make specific predictions for these habitats. The oak forest is assumed to be of higher quality for breeding pied flycatchers (Lundberg & Alatalo, 1992). Indeed, the oak forest provides a large source of caterpillars, whereas the pine forest is more constant in prey diversity and abundance (but of lesser quality). Breeding conditions are constrained by food availability (Both et al., 2009a; Visser et al., 2006) and the environmental conditions are supposed to be more restricted in the deciduous than in the pine forest in early spring (see Camacho et al., 2015). Consequently, we expect differences in phenology, and hence in the repeatability of related breeding traits, between the two habitats, with a higher repeatability in the more stable pine forest.

Table 1: Review of publications that have studied repeatability of laying date in avian populations.
Sex (F: females, M: males) and segment of the population on which the analyses of repeatability have been done (^{a} Absolute laying date; ^{r} Relative laying date: standardized with respect to the annual mean or from the day of the first occurrence, specific of each study). N is the number of individuals and Years the numbers of study years. Error estimates for repeatabilities are presented as (±SE) or [CI 95%]; and when specified, significance levels: ns > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001. Underlined vales of repeatability denotes corrected values from Lessells and Boag, 1987.

References	**Species**	**Status**	**Population**	**N**	**Years**	**Repeatability**
Verhoeven et al., 2020	*Limosa l. limosa*	Migrant	F^{geolocator}	65	7	0.54 [0.28;0.75]**
			F^{observation}	650	7	0.24 [0.17;0.31]**
Winkler et al., 2020	*Tachycineta bicolor*	Migrant	F^{a}, all females	867	15	0.15 [0.08;0.22]***
			F^{a}, older	612	15	0.34 [0.23;0.44]***
Sauve et al., 2019	*Cepphus grylle mandtii*	Resident	F	954	42	0.14 [0.11;0.17]
Morrison, 2016	*Eudyptes c. filholi*	Migrant	F	34	2	0.86 (0.04)n.s
Both et al., 2016	*Ficedula hypoleuca*	Migrant	F^{a}	217	7	0.22 (0.03)***
			M^{a}	257	7	0.06 (0.04)n.s
Bourret and Garant, 2015	*Tachycineta bicolor*	Migrant	F	130	4	0.32*
			M	90	4	0.81*
Thorley and Lord, 2015	*Cyanistes caeruleus*	Resident	F	726	12	0.43 [0.34–0.51]
Lourenço et al., 2011	*Limosa l. limosa*	Migrant	F	70	4	0.18 (0.004)**
			M	48	4	0.16 (0.05)*
Murphy, 2004	*Tyrannus tyrannus*	Migrant	F	64	13	0.284 ***
Christians et al., 2001	*Sturnus vulgaris*	Resident	F^{r}	35	3	-0.23 *
Banbura and Zielinski, 2000	*Hirundo rustica*	Migrant	F	6	4	0.51 (0.26)*
Svensson, 1997	*Cyanistes caeruleus*	Resident	F	59	4	0.48***
Catry et al., 1999	*Catharacta skua*	Resident	F	278	7	0.47***
Potti, 1999	*Ficedula hypoleuca*	Migrant	F	312	5	0.15*
Phillips and Furness, 1998	*Skuas Stercorarius*	Resident	F^{a}	13	3	-0.16n.s
			M^{a}	12	3	0.52**
Sydeman and Eddy, 1995	*Uria aalge*	Resident	F	37	8	0.20
Hochachka, 1993	*Melospiza melodia*	Migrant	F, Age 1-2	97	15	0.19*
			F, Age 1-3	65	15	0.25*
			F, Age 2-3	50	15	0.38*
Perdeck and Cavé, 1992	*Fulica atra*	Resident	F	124	20	0.32**
			M	126	20	-0.06n.s
Montalvo and Potti, 1992	*Ficedula hypoleuca*	Migrant	F	126	5	0.20**
			M	89	5	0.13n.s
Wiggins, 1991	*Tachycineta bicolor*	Migrant	F	28	3	0.61 ***
Goodburn, 1991	*Pica pica*	Resident	F, same territory	16	29	0.54***
			M, same territory	11	28	0.54***
			F, different territory	4	19	-0.09n.s
			M, different territory	4	13	-0.04n.s
Leafloor and Batt, 1990	*Anas platyrhynchos*	Resident	F	40	2	0.66 (0.008)***
Korpimaki, 1990	*Aegolius funereus*	Resident	F	35	13	0.03
			M	52	13	-0.21
Gauthier, 1989	*Bucephala albeola*	Migrant	F	23	5	0.57***
Hamann and Cooke, 1989	*Chen caerulescens*	Migrant	F	99	9	0.22**
Pietiainen, 1989	*Strix uralensis*	Resident	F	51	11	0.21
Wanless and Harris, 1988	*Uria algae*	Resident	F	38	5	0.45***
Meijer et al., 1988	*Falco tinnunculus*	Resident	F	58	8	0.00 n.s
Newton and Marquiss, 1984	*Accipiter nisus*	Resident	F	135	11	0.23***
			M	20	11	0.63***

Material methods

Literature review

Before estimating the repeatability of breeding parameters, we conducted a literature review restricted on the breeding period to highlight the variation in estimates across studies and population for this crucial life-history trait. We reviewed all published studies that quantified repeatability of laying dates using the Web of Science bibliographic database in October 2020. We first conducted a literature search for the terms "laying date" AND "repeatability", which resulted in 61 references. Alternative search terms as 'breeding date' or 'breeding time' did not yield improved results over those with the term 'laying date'. From all references obtained, we only considered those studies that were performed in wild bird populations and analyzed repeatability of laying date (23 references). In addition, we complemented our search with publications (n = 7) cited in other articles, but not sorted out by Web of Science, that also gave repeatability estimates based on the variance components method described by Lessells and Boag 1987.

Study system

This study in based on 29 years of nest-box monitoring dataset. Phenological traits were collected between 1987 and 2016, except 2002-2003, in a population of pied flycatchers in central Spain (La Hiruela, 41°04′ N, 3°27′ E). Birds breed in nest boxes (n=237) distributed between two habitats (see Supplementary material Appendix A, Figure 1), an old deciduous forest dominated by oaks (*Quercus pyrenaica*) and a nearby (1 km away) mixed coniferous plantation dominated by *Pinus sylvestris* (see Camacho et al., 2015 for a description of the study area). Males are the first to arrive at the breeding site, by the third week of April to search and hold a nesting site, before the arrival of females about one week later (Canal et al., 2012). Individuals in prime condition –typically around 2-3 years old– return earlier than young and senescent individuals (Potti, 1998a). Every year, nest-boxes are inspected every 2-3 days from the time of arrival of the first males until the beginning of incubation and daily around hatching date. The day of the first lay egg occurs around the third week of May (with 1=1st of May, mean±SD: 20.819 ± 6.866), on average 10 days (10.015 ± 3.946) after the onset of nest construction (i.e pairs mating; 10.243 ± 7.361). Hatching date typically occurs during the first week of June (38.699 ± 6.5993), after an incubation period of, on average, 12 days.

Birds breeding in nest-boxes have been systematically captured and ringed for the last three decades. All breeding individuals are captured using a nest-box trap, marked with metal rings and then aged as either one year or older following the criteria of Karlsson et al., 1986. For each individual, we record "mating date", as the day in which the construction of the nest was first recorded (as indicated by nest material deposition, for details see (Potti, 1999; Potti et al., 2021), "pre-laying period" as the time elapsed between mating and laying date, "laying date", as the day of the first laid egg and "hatching date", as the day the majority of the clutch hatched. We considered mating date as a proxy of female arrival date, because the exact arrival dates are unknown for most females (Lundberg & Alatalo, 1992; Potti, 1999). Based on countless field observations of ringed females, we assumed that the female which started building a nest uninterruptedly became the breeder in that nest (Potti et al., 2021). In the analyses, we excluded eventual replacement clutches in the same year.

Statistical analysis

Repeatability of phenological traits (mating date, pre-laying period, breeding date and hatching date) of the same females in different years is expressed here as the ratio of between-individual phenotypic variance to the total phenotypic variance (Nakagawa & Schielzeth, 2010). Repeatability estimates vary between 0 (no consistency) and 1 (perfect consistency), with 'consistency' as a narrow sense for reproducibility in the same environment (Biro2015; Stamps, Briffa, Biro, 2012), and the inverse of repeatability (1-Repeatability) can be interpreted as the proportion of variance of measurements related to measurement error or phenotypic plasticity (Nakagawa & Schielzeth, 2010). Neither laying date nor mating date, hatching date or pre-laying period followed a normal distribution, thus analyses were conducted using Poisson distributions. Significance of repeatabilities was checked using 84% confidence intervals, since the absence of overlap between 84% confidence intervals is equivalent to a z test at the 0.05 level (Julious, 2004). Repeatability values with 84% CI were estimated with a bootstrap value of n=1000, using the '`rtpR`' package (Nakagawa & Schielzeth, 2010; Stoffel et al., 2017) in `R` statistical software (R core team 2019).

We ran separate models for laying date, mating date, hatching date and pre-laying period. Since ontogenetic changes in laying date can influence repeatability (Hochachka, 1993; Winkler et al., 2020), we focused on the older segment of the population (2-year-old individuals) to minimise the confounding effects of such age-related shifts (see Supplementary material appendix 1, Figure 2). For completeness and comparison, the results of the analysis taking into account all age groups are also shown. Because time-related changes can seriously affect our estimates of repeatability (Biro & Stamps, 2015), the effect of year was considered as a random intercept. Female identity was also included as a random intercept to account for the individual effect on repeatability. Thus, we calculated both a repeatability for the groups of year and female. Further, as males may influence female breeding decisions (Evans et al., 2020; Moiron et al., 2020a), we repeated the models above including male identity as random intercept. We ran all models for each habitat separately (oak vs. pine forest).

Results

The repeatabilities of laying date and hatching date were for years (Table 2, Figure 1a) and females (Table 2, Figure 1b). The repetabilities of mating date and duration of the pre-laying period among years were also low (Table 2, Figure 1a), while female repeatability was very low for mating dates (Table 2, Figure 1b) and was effectively 0 for the duration of the pre-laying period (Table 2, Figure 1b).

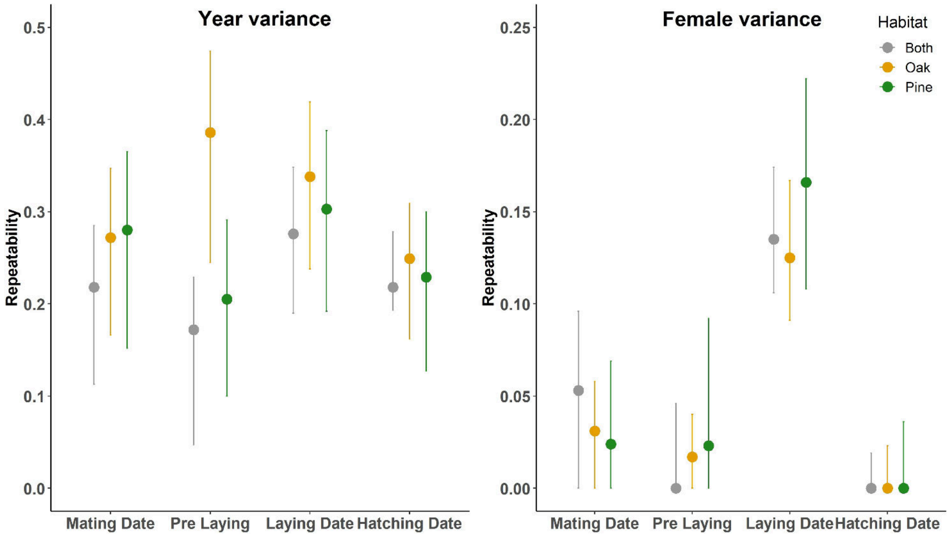


Figure 1: Repeatabilities of Mating Dates, Pre-Laying periods Laying Dates and Hatching Dates by habitat among years (1a, left) and within females (1b, right) in a pied flycatchers population. Mean estimates are shown for the oak forest, the pine forest and both habitats combined. 84% confidence intervals are displayed: non-overlapping 84% confidence intervals are equivalent to z tests at the 0.05 confidence level.

The repeatabilities of mating date, laying date, pre-laying period and hatching date among years were similar between habitats (Table 2, Figure 1). Likewise, there were no differences between habitats in the female repeatability for the phenological traits tested (Table 2, Figure 1). There were no differences neither in repeatabilities estimates for the four breeding traits when comparing all the age-group in the population (Table 3) and repeatabilites estimated using only 2-year-old individuals of the population (Table 2).

Discussion

Estimating the variability of phenological traits in birds is crucial for understanding the potential of individuals to respond to environmental change. It is known that migratory birds may often adjust their breeding phenology in response to environmental conditions. Consistent with this idea, we found a considerable flexibility in breeding schedules of pied flycatchers. In particular, female repeatability of laying date was low, and even smaller for mating date, duration of the pre-laying period and hatching date. Contrary to our expectations, there was no clear influence of breeding habitat on the repeatability of any of the

Table 2: Repeatability estimates considering for the phenological events (mating date, duration of pre-laying period, laying date and hatching date among years and within females in the oak forest, the pine forest and both habitats combined) when only considering 2-year-old females. R= repeatability estimate with CI= Confidence Interval 84%

Phenological trait	Mating date			Pre-laying period		
Habitat	Both habitats	Oak forest	Pine forest	Both habitats	Oak forest	Pine Forest
Among years	R=0.218 CI[0.113; 0.285] p <0.001	R=0.272 CI[0.166; 0.347] p <0.001	R=0.280 CI[0.152; 0.365] p <0.001	R=0.172 CI[0.047; 0.229] p <0.001	R=0.386 CI[0.245; 0.474] p <0.001	R=0.205 CI[0.100; 0.291] p <0.001
Within females	R=0.053 CI[0;0.096] p=0.005	R=0.031 CI[0;0.058] p=0.08	R=0.024 CI[0; 0.069] p=0.253	R=0 CI[0; 0.046] p=0.5	R=0.017 CI[0; 0.040] p=0.24	R=0.023 CI[0; 0.092] p=0.337
Observations	1219	858	361	1217	856	361
Number years	23	22	19	23	22	19
Number females	681	499	197	681	499	197

Phenological trait	Laying Date			Hatching date		
Habitat	Both habitats	Oak forest	Pine forest	Both habitats	Oak forest	Pine forest
Among years	R=0.276 CI[0.190; 0.348] p <0.001	R=0.338 CI[0.238; 0.419] p <0.001	R=0.303 CI[0.192; 0.388] p <0.001	R=0.218 CI[0.193; 0.278] p <0.001	R=0.249 CI[0.162; 0.309] p <0.001	R=0.229 CI[0.127; 0.300] p <0.001
Within females	R=0.135 CI=[0.106; 0.174] p <0.001	R=0.125 CI[0.091; 0.167] p <0.001	R=0.166 CI[0.108; 0.222] p <0.001	R=0 CI[0;0.019] p=0.5	R=0 CI[0;0.023] p=1	R=0 CI[0; 0.036] p=1
Observations	1764	1237	527	1757	1230	527
Number years	29	29	27	29	29	27
Number females	773	556	240	773	556	240

Table 3: Repeatability estimates considering all females (regardless the age) for the phenological events (mating date, duration of pre-laying period, laying date and hatching date) among years and within females in the oak forest, the pine forest and both habitats combined. R= repeatability estimate with CI= Confidence Interval 84%

Phenological trait	Mating date			Pre-laying period		
Habitat	Both habitats	Oak forest	Pine forest	Both habitats	Oak forest	Pine Forest
Among years	R=0.188 CI[0.109; 0.251] p<0.001	R=0.235 CI[0.143; 0.301] p<0.001	R=0.280 CI[0.153; 0.355] p<0.001	R=0.153 CI[0.081; 0.205] p<0.001	R=0.363 CI[0.235; 0.450] p<0.001	R=0.200 CI[0.093; 0.271] p<0.001
Within females	R=0.097 CI[0.055; 0.125] p<0.001	R=0.089 CI[0.041; 0.122] p<0.001	R=0.024 CI[0; 0.065] p=0.221	R=0 CI[0; 0.039] p=0.5	R=0.012 CI[0; 0.041] p=0.323	R=0.002 CI[0; 0.067] p=0.487
Observations	1405	981	424	1405	977	424
Number years	23	22	19	23	22	19
Number females	714	519	217	714	519	217

Phenological trait	Laying date			Hatching Date		
Habitat	Both habitats	Oak forest	Pine forest	Both habitats	Oak forest	Pine Forest
Among years	R=0.221 CI[0.146; 0.281] p<0.001	R=0.267 CI[0.181; 0.337] p<0.001	R=0.249 CI[0.155; 0.326] p<0.001	R=0.189 CI[0.124; 0.244] p<0.001	R=0.228 CI[0.149; 0.285] p<0.001	R=0.197 CI[0.110; 0.261] p<0.001
Within females	R=0.155 CI=[0.130; 0.193] p<0.001	R=0.133 CI[0.099; 0.174] p<0.001	R=0.193 CI[0.143; 0.258] p<0.001	R=0.037 CI[0.009; 0.057] p=0.004	R=0.008 CI[0; 0.029] p=0.32	R=0.036 CI[0;0.072] p=0.0797
Observations	2103	1460	640	2094	1451	640
Number years	29	29	28	29	29	28
Number females	776	560	249	776	560	249

phenological traits here considered (Table 2 and Figure 1). Also, repeatabilities estimates did not differ between females older than 1 year (Table 2) and the whole set of reproductive females regardless of age (Table 3), nor were significantly influenced by mate identity (Table 4, Table 5).

Table 4: Repeatability estimates considering all females (regardless the age) with male identity as random factor considered for the phenological events (mating date, duration of pre-laying period, laying date and hatching date) among years and within females in the oak forest, the pine forest and both habitats combined.
R= repeatability estimate with CI= Confidence Interval 84%

Phenological trait	Mating date			Pre-laying period		
Habitat	Both habitats	Oak forest	Pine forest	Both habitats	Oak forest	Pine Forest
Among years	R=0.202 CI[0.120; 0.261] p<0.001	R=0.256 CI[0.148; 0.329] p<0.001	R=0.215 CI[0.101; 0.289] p<0.001	R=0.162 CI[0.094; 0.217] p<0.001	R=0.371 CI[0.239; 0.468] p<0.001	R=0.211 CI[0.096; 0.295] p<0.001
Within females	R=0.061 CI[0.017; 0.087] p=0.002	R=0.052 CI[0; 0.081] p=0.01	R=0.008 CI[0; 0.048] p=0.416	R=0 CI[0; 0.041] p=1	R=0.024 CI[0; 0.049] p=0.149	R=0 CI[0; 0.061] p=1
Observations	1316	920	396	1312	916	396
Number years	23	22	19	23	22	19
Number females	696	507	207	696	507	207

Phenological trait	Laying date			Hatching Date		
Habitat	Both habitats	Oak forest	Pine forest	Both habitats	Oak forest	Pine Forest
Among years	R=0.239 CI[0.157; 0.306] p<0.001	R=0.294 CI[0.195; 0.368] p<0.001	R=0.257 CI[0.155; 0.345] p<0.001	R=0.198 CI[0.127; 0.255] p<0.001	R=0.236 CI[0.155; 0.297] p<0.001	R=0.196 CI[0.109; 0.261] p<0.001
Within females	R=0.137 CI=[0.105; 0.173] p<0.001	R=0.112 CI[0.079; 0.148] p<0.001	R=0.177 CI[0.127; 0.243] p<0.001	R=0.003 CI[0; 0.021] p=0.41	R=0 CI[0; 0.021] p=1	R=0.011 CI[0;0.044] p=0.337
Observations	1949	1356	590	1945	1352	590
Number years	29	28	27	29	28	27
Number females	774	557	244	774	557	244

Table 5: Repeatability estimates considering 2-year-old females of the population with male identity as random factor considered for the phenological events (mating date, duration of pre-laying period, laying date and hatching date) among years and within females in the oak forest, the pine forest and both habitats combined.
R= repeatability estimate with CI= Confidence Interval 84%

Phenological trait	Mating date			Pre-laying period		
Habitat	Both habitats	Oak forest	Pine forest	Both habitats	Oak forest	Pine Forest
Among years	R=0.228 CI[0.138; 0.292] p<0.001	R=0.185 CI[0.101; 0.252] p<0.001	R=0.170 CI[0.070; 0.242] p<0.001	R=0.178 CI[0.099; 0.240] p<0.001	R=0.152 CI[0.077; 0.214] p<0.001	R=0.113 CI[0.035; 0.172] p<0.001
Within females	R=0.022 CI[0; 0.048] p=0.155	R=0.077 CI[0.013; 0.113] p=0.010	R=0.068 CI[0; 0.016] p=0.063	R=0 CI[0; 0.047] p=1	R=0 CI[0; 0.057] p=0.1	R=0 CI[0; 0.072] p=1
Observations	1146	729	340	1144	727	338
Number years	23	23	21	23	23	21
Number females	660	414	189	660	413	188

Phenological trait	Laying Date			Hatching date		
Habitat	Both habitats	Oak forest	Pine forest	Both habitats	Oak forest	Pine forest
Among years	R=0.289 CI[0.198; 0.365] p<0.001	R=0.231 CI[0.142; 0.301] p<0.001	R=0.237 CI[0.140; 0.321] p<0.001	R=0.213 CI[0.140; 0.269] p<0.001	R=0.187 CI[0.114; 0.245] p<0.001	R=0.208 CI[0.109; 0.272] p<0.001
Within females	R=0.120 CI=[0.087; 0.161] p<0.001	R=0.166 CI[0.125; 0.225] p<0.001	R=0.121 CI[0.603; 0.182] p=0.001	R=0 CI[0; 0.018] p=1	R=0 CI[0; 0.025] p=1	R=0.036 CI[0;0.072] p=0.115
Observations	1635	1138	493	1631	1136	491
Number years	29	28	27	29	28	27
Number females	757	488	217	757	488	217

The repeatability of breeding time provides an indication of the consistency of reproductive timing over time. Repeatability of laying date can be low for two reasons: high within-individual variation or low between-individual variation (Nakagawa & Schielzeth, 2010). For example, the onset of spring migration is characterized by high levels of between-individual variation, but very low levels of within-individual variation and, consequently, individuals are typically highly repeatable in their timing of migration (Conklin et al., 2013). In this regard, it has been shown that the timing of spring migration has not advanced in Dutch populations of the pied flycatcher, despite the benefits of arriving and breeding early in response to climate warming at those latitudes (Both & Visser, 2001). In contrast to the onset of migration, the timing of breeding is more dependent on the local environmental conditions, thus showing high plasticity and is therefore less repeatable (Bell et al., 2009). Indeed, the low repeatability of laying date among years (R=0.276) in the study population is similar to the estimates reported in other pied flycatcher studies (R=0.22, Both et al., 2016, R=0.20 Montalvo and Potti, 1992) and smaller than in other migratory birds (mean R=0.35, Table 1). Moreover, in our population, the repeatabilities among years of mating date, pre-laying interval and hatching date (respectively, R=0.218 , R=0.172 and R=0.218) were rather small. Low estimates of among-year repeatability in these variables may be explained by a low inter-annual consistency in females and thus are suggestive of high individual variation in these reproductive-related traits over the years. Moreover, our results imply that the timing of mating and, in turn, the duration of the pre-laying interval, are more unpredictable than the breeding date itself, suggesting a lack of knock-on effect (Lourenço et al., 2011). The lower repeatability estimates of mating date relative to egg laying could be the consequence of an unaccurate estimation of mating dates, since we used the onset of nest contruction as proxy of it, which can be linked to a higher uncertainty compared to initiation of laying. However, this potential source of bias is unlikely to account for the inconsistencies in the timing of breeding of pied flycatchers, because mating date seems to be also dependent on the yearly environmental fluctuactions.

The female repeatability for laying date was small (R=0.14), suggesting the ability of individuals to modulate this trait as a function of the current or expected environmental conditions. This finding is fairly consistent with the results found in the same population two decades ago using a shorter (5 years) time series (R=0.15, Potti, 1999). Moreover, the repeatabilities of mating date (R=0.053), duration of the pre-laying period across several annual breeding events (R=0) and hatching date (R=0) was negligible. These estimates differ from those reported for the same population during the first years of the study when females were found to have moderate repeatable mating dates (R = 0.29) and duration of pre-laying periods (R = 0.16, Potti, 1999). We cannot rule out that the apparent decrease in repeatability of breeding phenology over time is an effect of the observed increase of density, and therefore mate availability, in this population (Camacho et al., 2019). On the one hand, the density-driven concomitant increase in intra-sexual competition for mates could enhance protandry and reduce the ability of females to breed at the preferred time (Canal et al., 2012). On the other hand, the increased availability of males could translate into greater uncertainty and likely increase the time required to choose a male (Dale et al., 1990; Dale et al., 1992). Alternatively, a possible explanation for the discrepancies in the repeatability of reproductive-related traits between these two study periods can be that, in the last decades and due to selection pressures (Le Vaillant et al., 2021), females might be more flexible after migration to adjust the time schedule to an optimal breeding period by fine-tuning the time of mating and the duration of the pre-laying period. In addition, the lack of repeatability in hatching period between individuals, provides additional insights on the flexibility of flycatchers to modulate the timing of hatching and adjust reproduction to environmental conditions (Both & Visser, 2005; Tomás, 2015). Indeed, laying date has been shown to be highly plastic, but also repeatable within females facing warmer springs (Thorley & Lord, 2015).

Low individual repeatability of breeding time means that environmental variation is the cause of a large proportion of phenotypic variation in reproductive phenology, which may be interpreted as an adaptation to fluctuating environmental conditions. While annual variation in breeding timing might reflect individual phenotypic plasticity, among-year variation in repeatability suggests that, in some years, environmental effects alter the expected trait expression of individuals. Indeed, although laying dates have been found to be repeatable in a Dutch pied flycatcher population (Both et al., 2016), the repeatability was lower in some dyads of consecutive years because environmental conditions altered the trait value of individuals from their mean expression. Here, we observe low repeatability at the individual and year levels, but also a similarity of estimates in both habitats. This suggests that, regardless of the structural differences between both habitats, such as for instance food abundance or occupancy density, the phenology between areas is pretty similar (see Table 2 and Figure 1) and individuals may respond similarly to the selective forces acting on laying date related to climatic variation. Moreover, despite that male can have an indirect genetic effect on the evolution of reproductive phenological traits (Evans et al., 2020; Moiron et al., 2020b), mate identity did not change significantly the repeatability of such traits (Table 4, Table 5). The expression of phenological traits are therefore mainly influenced by environmental factor and under the control of females (Amininasab et al., 2017).

A low individual repeatability in laying dates has adaptive significance, as bird populations are able to respond to natural variation in food abundance due to climatic conditions (Korpimaki, 1990; Sydeman & Eddy, 1995). However, repeatability cannot be used as an index of the (un)predictability of reproductive timing, as environmental effects over time cannot be accounted for (Biro & Stamps, 2015; Cleasby et al., 2015; Niemelä & Dingemanse, 2017; Wilson, 2018), (but see Sánchez-tójar et al., 2021). In our population, the low estimates of among-year repeatability indicate that yearly environmental variation may influence the strength of selection on laying date (see also Le Vaillant et al., 2021). Speculatively, the lower estimates of repeatability in our population compared to other studies in passerines species (mean R = 0.37, Table 1), may suggest that environmental fluctuations strongly affect the study population and that pied flycatchers may display more plastic responses than other migratory birds. However, we have to emphasize that the number of females tracked (n= 776) across a long period of time (29 years) in our study may support accurate repeatability estimates through fluctuating environments compared to other studies in repeatability (Table 1). Differences in environmental conditions due to latitude or altitude may also explain the variability of response, since the study population of Iberian pied flycatchers is located at the southern end of the species breeding range , and also at higher altitude, than most study populations in central and northern Europe (Both et al., 2005; Both et al., 2006b; Nicolau et al., 2021).

Repeatability is also a useful index of individual quality or condition (Sydeman & Eddy, 1995; Winkler et al., 2020) and can increase in older individuals (Bell et al., 2009; Brommer & Class, 2015; Hochachka, 1993; Winkler et al., 2020). Although we did not detect a lower repeatability when including yearling individuals (Table 3), future research should focus on the effects of individual quality, and experience on the repeatability of breeding timing, thus helping to disentangle the contribution of environmental and individual conditions to plasticity in breeding dates.

Acknowledgments

We thank the many volunteers, technicians and friends, too numerous to mention individually, that helped with data collection. We thank Barbara Tomotani for constructive suggestions improving the draft. We also thank two anonymous reviewers and the associate editor for constructive suggestions that greatly improved a previous draft of the manuscript. JLV and CC received financial support from the Spanish Ministry of Economy and Competitiveness (respectively, BES-2016-077694 and SVP2013067686). D.C. was supported by grants from the Spanish Ministry of Education and Science (I3PBDP2005) and the Hungarian Academy of Sciences (MTA Premium Postdoctoral Research Program; ID: 2019-353). J.M.-P. is funded by ARAID foundation. This paper was funded by project CGL2015-70639 of the Spanish Ministry of Economy and Competitiveness, project CGL2015-70639-P/BOS of the Spanish Ministry of Science, Innovation and Universities, and the project PID2019-104835GB-I00 granted by the Spanish Ministry of Science and Innovation.

Supplementary Material

Appendix A

The phenological traits in both areas are similar (t-test) despite a reduce number of years and nestboxes available in the pine forest : for mating date (mean oak=40.218; pine=40.299, t= -0.190, df = 1403, p-value = 0.849), the prelaying period (mean oak= 9.761; mean pine= 10.599, t = -3.666, df = 1399, p-value < 0.001), laying date (mean oak= 20.379; pine= 21.861, t = -4.576, df = 2098, p-value < 0.001) and the hatching date (mean oak: 38.218; pine= 39.827, t = -5.169, df = 2089, p-value < 0.001).

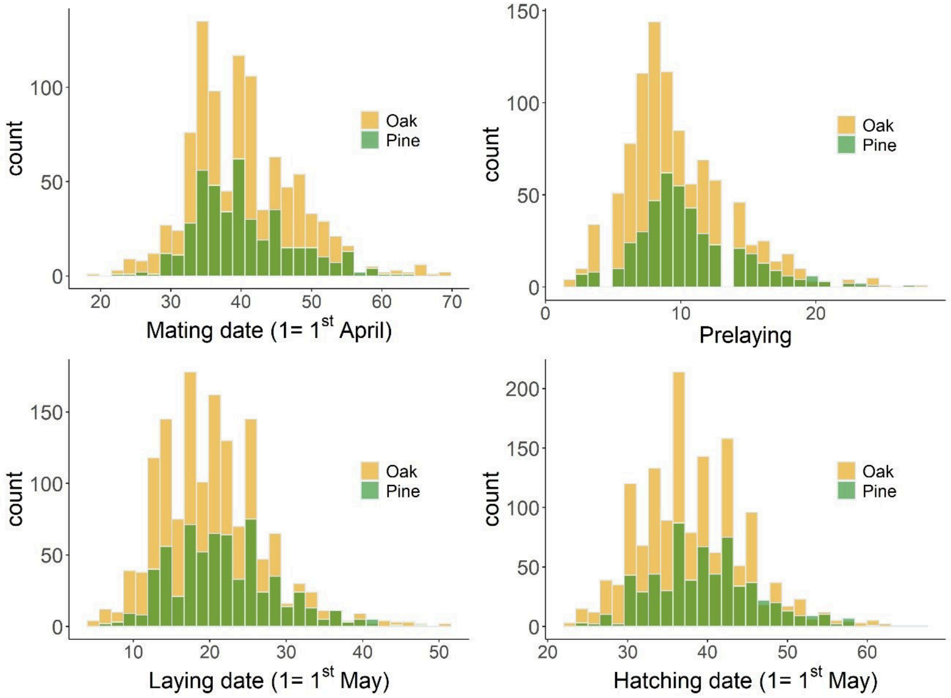


Figure 1: Distribution of the phenology events in the two habitats.

Note that many females reproduced several times in both habitats (with therefore the possibility to have a repeatability estimate for such females in both habitats) whereas some females may have not reproduce at all or only once in each habitat and cannot be included in the repeatability analyses.

Appendix B

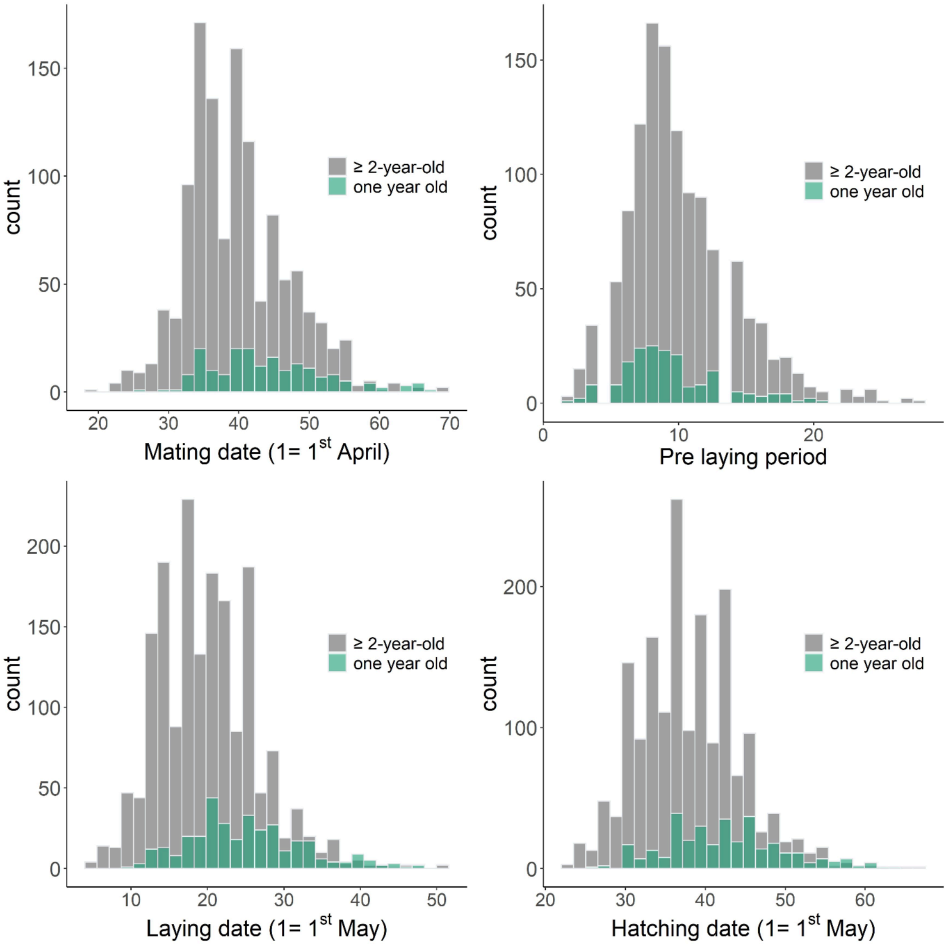


Figure 2: Number of females with phenology events by age.

Females recruit at the age of one or two years old. However, to discard the effect of experience and condition due to the age, repeatability models have also been run without data on females before the age of two years old, when we observed a major shift in phenological traits.

```
# Analysis with repeatbility
LAY<-read.csv("Individual Laying Analysis.csv",header=T,sep=";",dec=".")
nrow(LAY) #n=2103  for 776 females

library(nlme)  #Provide SD + function lme()
library(rptR)  #Repeatability package

# Repeatability estimation using the glmm method and log link
##Both habitat
rep1<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"),CI= 0.84l data=LAY,link="log")
print(rep1) #or summary(rep1)

###In different habitat
oak<-LAY[which(LAY$Habitat==1),]  #1460 for 560 females
pine<-LAY[which(LAY$Habitat==2),]  #640  for 249 females
repoak<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=oak,link="log")
reppine<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=pine,link="log")

####With others traits
rep2<-rptPoisson(MatingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"),CI= 0.84, data=LAY,link="log")
rep3<-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), CI= 0.84, data=LAY,link="log")
rep5<-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"),CI= 0.84, data=LAY,link="log")

#Within habitat
repoakM<-rptPoisson(MatingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=oak,link="log",CI= 0.84)
reppineM<-rptPoisson(MatingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=pine,link="log",CI= 0.84)
repoakP<-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=oak,link="log",CI= 0.84)
reppineP<-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=pine,link="log",CI= 0.84)
repoakH<-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=oak,link="log",CI= 0.84)
reppineH<-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=pine,link="log",CI= 0.84)

#########Same analysis with only 2 year-old or older female
LAYa<-LAY[which(LAY$Age!=1),]  #-332 age 1 (<2 years old)
##With Both Habitats
rep1a<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=LAYa,link="log", CI= 0.84)
rep2a<-rptPoisson(MatingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=LAYa,link="log", CI= 0.84)
rep3a<-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=LAYa,link="log", CI= 0.84)
rep4a<-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=LAYa,link="log", CI= 0.84)

##Within Habitats
oakA<-LAYa[which(LAYa$Habitat==1),]  #1237 for 556 females
pineA<-LAYa[which(LAYa$Habitat==2),]  #527  for 240  females
```

```
#
repoakA<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=oakA, link="log", CI= 0.84)
reppineA<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=pineA, link="log", CI= 0.84)
repoakA2<-rptPoisson(MatingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=oakA, link="log", CI= 0.84)
reppineA2<-rptPoisson(MatingDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=pineA, link="log", CI= 0.84)
repoakA3<-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=oakA, link="log", CI= 0.84)
reppineA3<-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=pineA, link="log", CI= 0.84)
repoakA4<-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=oakA, link="log", CI= 0.84)
reppineA4<-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year), grname=c("Female","Year"), data=pineA, link="log", CI= 0.84)

#########Same with Mate as random factor
#For all age
rep1m<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=LAY, link="log", CI= 0.84)
rep2m<-rptPoisson(MatingDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=LAY, link="log", CI= 0.84)
rep3m<-rptPoisson(Prelaying  ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=LAY, link="log", CI= 0.84)
rep4m<-rptPoisson(HatchDate  ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=LAY, link="log", CI= 0.84)

##Within habitat
oak<-LAY[which(LAY$Habitat==1),]   #1460 for 560 females
pine<-LAY[which(LAY$Habitat==2),]   #640 for 249  females
#
repoakM <-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=oak, link="log", CI= 0.84)
reppineM<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=pine, link="log", CI= 0.84)
repoakM2 <-rptPoisson(MatingDate~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=oak, link="log", CI= 0.84)
reppineM2<-rptPoisson(MatingDate~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=pine, link="log", CI= 0.84)
repoakM3 <-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=oak, link="log", CI= 0.84)
reppineM3<-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=pine, link="log", CI= 0.84)
repoakM4 <-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=oak, link="log", CI= 0.84)
reppineM4<-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=pine, link="log", CI= 0.84)

##For female of 2 year-old or older
rep1am<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=LAYa, link="log", CI= 0.84)
rep2am<-rptPoisson(MatingDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=LAYa, link="log", CI= 0.84)
rep3am<-rptPoisson(Prelaying  ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=LAYa, link="log", CI= 0.84)
rep4am<-rptPoisson(HatchDate  ~ 1+(1|Female)+(1|Year)+(1|Male), grname=c("Female","Year"), data=LAYa, link="log", CI= 0.84)
```

```r

##with both habitat
oaka<-LAY[which(LAYa$Habitat==1),]  # 1237 for 492 females
pinea<-LAY[which(LAYa$Habitat==2),]  # 527 for 222  females
#
repoakaaM <-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname
    =c("Female","Year"), data=oaka,link="log", CI= 0.84)
reppineaaM<-rptPoisson(LayingDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname
    =c("Female","Year"), data=pinea,link="log", CI= 0.84)
repoakaaM2 <-rptPoisson(MatingDate~ 1+(1|Female)+(1|Year)+(1|Male), grname
    =c("Female","Year"), data=oaka,link="log", CI= 0.84)
reppineaaM2<-rptPoisson(MatingDate~ 1+(1|Female)+(1|Year)+(1|Male), grname
    =c("Female","Year"), data=pinea,link="log", CI= 0.84)
repoakaaM3 <-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year)+(1|Male), grname
    =c("Female","Year"), data=oaka,link="log", CI= 0.84)
reppineaaM3<-rptPoisson(Prelaying ~ 1+(1|Female)+(1|Year)+(1|Male), grname
    =c("Female","Year"), data=pinea,link="log", CI= 0.84)
repoakaaM4 <-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname
    =c("Female","Year"), data=oaka,link="log", CI= 0.84)
reppineaaM4<-rptPoisson(HatchDate ~ 1+(1|Female)+(1|Year)+(1|Male), grname
    =c("Female","Year"), data=pinea,link="log", CI= 0.84)

############### Graphs with the 84% confidence interval
library(ggplot2)
library(gridExtra)

#Put results in the same table
RR<-read.table("Repeatability.txt",header=T,sep="\t",dec=".")
RR[, "Variable"] <- factor(RR[, "Variable"] , levels=c("Mating Date", "Pre
     Laying", "Laying Date", "Hatching Date"))

# for years
plotY<- ggplot(RR[which(RR$Group=="Year"),], aes(x=Variable, y=
    Repeatability, group=Habitat, colour=Habitat)) + theme_classic() +
   geom_point(shape=19, size=4,  position=position_dodge(width=0.5)) +
    ylim(0,0.5)  +
   geom_errorbar(width=.05, aes(ymin=ci8, ymax=ci92),  position=position_
    dodge(width=0.5)) +
    scale_color_manual(values=c("#999999", "#E69F00", "forestgreen"))+
   theme(legend.position='none', axis.text=element_text(size=14, face="
    bold"),axis.title=element_text(size=14,face="bold"))

# for females
plotF<-ggplot(RR[which(RR$Group=="Females"),], aes(x=Variable, y=
    Repeatability, group=Habitat, colour=Habitat)) + theme_classic() +
   geom_point(shape=19, size=4,position=position_dodge(width=0.5) ) +
     ylim(0,0.25) +
   geom_errorbar(width=.05, aes(ymin=ci8, ymax=ci92),position=position_
    dodge(width=0.5)) +
    scale_color_manual(values=c("#999999", "#E69F00", "forestgreen"))+
   theme(legend.position = c(0.9, 0.9),  axis.text=element_text(size=14,
    face="bold"),axis.title=element_text(size=14,face="bold"), legend.
    title = element_text(size=14),legend.text = element_text(size=12))
```

Chapter 2

Fluctuating selection driven by global and local climatic conditions leads to stasis in breeding time in a migratory bird

Justine Le Vaillant, Jaime Potti, Carlos Camacho, David Canal
& Jesús Martínez-Padilla

The origin of natural selection is linked to environmental heterogeneity, which influences variation in relative fitness among phenotypes. However, individuals in wild populations are exposed to a plethora of biotic and abiotic environmental factors. Surprisingly, the relative influence of multiple environmental conditions on the relative fitness of phenotypes has rarely been tested in wild populations. Identifying the main selection agent(s) is crucial when the target phenotype is tightly linked to reproduction and when temporal variation in selection is expected to affect evolutionary responses. By using individual- based data from a 29-year study of a short-lived migratory songbird, the pied flycatcher *Ficedula hypoleuca*, we studied the relative influence of 28 temperature- and precipitation-based factors at local and global scales on selection on breeding time (egg laying) at the phenotypic level. Selection, estimated using the number of recruits as a proxy for fitness, penalized late breeders. Minimum temperatures in April and May were the environmental drivers that best explained selection on laying date. In particular, there was negative directional selection on laying date mediated by minimum temperature in April, being strongest in cold years. In addition, nonlinear selection on laying date was influenced by minimum temperatures in May, with selection on laying date changing from null to negative as the breeding season progressed. The intensity of selection on late breeders increased when minimum temperatures in May were highest. Our results illustrate the complex influence of environmental factors on selection on laying date in wild bird populations. Despite minimum temperature in April being the only variable that changed over time, its increase did not induce a shift in laying date in the population. In this songbird population, stabilizing selection has led to a three-decade stasis in breeding time. We suggest that variation in the effects of multiple climatic variables on selection may constrain phenotypic change.

Journal of Evolutionary Biology 2021.00:1–13

Introduction

Understanding the evolutionary mechanisms underlying local adaptation is compulsory for forecasting population persistence under current scenarios of environmental change (Both & Visser, 2005; Gienapp et al., 2014; Visser et al., 2015). Multiple biotic and abiotic factors acting concurrently can influence phenotypic selection and thus shape the evolutionary trajectories of phenotypes over small spatial and temporal scales, driving their trajectories in contemporary times (Evans & Gustafsson, 2017; Husby et al., 2011b; Parmesan, 2006; Pigeon et al., 2016). Most studies of phenotypic selection in nature often focus on a single potential environmental factor, yet most phenotypes in populations are presumably under pressure from multiple environmental variables. As a consequence, our understanding of the nature, importance, and interdependence of the plethora of factors that influence phenotypic selection in natural populations is limited (MacColl, 2011).

Examining the role of multiple environmental factors in selection is particularly relevant in the current context of climate change (Bonnet et al., 2017; Evans & Gustafsson, 2017; Husby et al., 2011b; Marrot et al., 2018; Marrot et al., 2017; Visser et al., 2015). Alarmingly, trends in mean temperature and temperature ranges, but also in the frequency and intensity of rainfall and extreme weather events are changing at unprecedented rates (IPCC, 2014),influencing the form and intensity of selection (Marrot et al., 2018; Marrot et al., 2017; Siepielski et al., 2017). Migratory birds are particularly sensitive to such profound environmental change for several reasons. First, migratory birds travel on a biannual basis over long distances and rely on endogenous energy stores to support the energetic demands of long-distance flight (Lindström, 2003). Second, migratory birds are exposed to a range of challenges across their distribution range, and face the double challenge of scheduling annual events in separate regions (Marra et al., 2015). Third, at the time of departure, migrating birds lack information about current conditions at the destination, limiting their ability to make adjustments sufficiently in advance (Kokko, 1999; Møller, 1994). Finally, migratory birds are usually on a tight schedule to mate, raise offspring, moult, prepare for migration, and settle at their wintering destination (Alerstam & Lindström, 1990). Because of the sequential and cyclical nature of annual events (Marra et al., 2015; McNamara & Houston, 2008), conditions during any of these stages may have carry-over influences on individual performance in subsequent stages (Bogdanova et al., 2011; Kokko, 1999). Therefore, migratory birds are common models in studies exploring climate-mediated effects on the evolutionary dynamics of phenotypes.

Many studies have suggested that changes in mean spring temperatures influence selection on breeding time of migratory birds, yet the evidence is mixed and inconclusive (Bowers et al., 2016; Dobson et al., 2017; Goodenough et al., 2010; Husby et al., 2011b; Marrot et al., 2018; Marrot et al., 2017; Matthysen et al., 2011; Porlier et al., 2012; Reed et al., 2009; Townsend et al., 2013; van Noordwijk et al., 1995; Visser et al., 2015; Visser et al., 1998 – see also Supplementary Material - Appendix A - Literature review for further details). This lack of general consensus suggests that additional climate components may influence phenotypic selection on breeding time.

In addition to local climate factors, global climatic phenomena can act as strong selective agents in migratory species. However, phenotypic responses to selection may differ across distributional range, e.g. the effects of environmental conditions at the southern edge of the distribution can influence reproduction but should not necessarily do so in the same way in northern breeding areas. The North Atlantic Oscillation (NAO hereafter) is a major source of interannual variability in the atmospheric circulation, whose changes are derived from the difference of atmospheric pressure at sea level between the Icelandic Low and the Azores High (Hurrell et al., 2003). As a result, the influence of NAO on surface temperature and precipitation, as well as on ecosystems, is also greatest in winter. In birds, and particularly in migratory species, NAO in winter (NAOw hereafter) is considered a reliable proxy of the environmental conditions that populations experience in their wintering grounds (Johnston et al., 2016). NAOw might influence the fuel deposition rate of birds before spring departure, thereby affecting the total duration of migration and the timing of arrival at the breeding grounds (Both et al., 2016; Both & Visser, 2005; Ouwehand et al., 2017; Potti, 1998a). In fact, negative selection on recruitment rate has been reported for the warmest and driest winters (positive NAOw values - Muñoz-Díaz and Rodrigo, 2004, and therefore harshest years, so that selection favours individuals laying early in the season because earlier breeders produce more recruits than later ones(Visser et al., 2015).

Narrowing down to breeding time, perhaps the best-known life-history trait in evolutionary ecology (Crick, 2004; Jenouvrier, 2013), it is surprising that our knowledge about selection on this trait at the phenotypic level in the best-studied taxa -wild birds- is mainly based on temporal changes in mean spring temperatures (Bowers et al., 2016; Dobson et al., 2017; Goodenough et al., 2010; Husby et al., 2011b; Marrot et al., 2018; Marrot et al., 2017; Matthysen et al., 2011; Porlier et al., 2012; Reed et al., 2009; Townsend et al., 2013; van Noordwijk et al., 1995; Visser et al., 2015; Visser et al., 1998) – see also Appendix A - Literature review for further details). Thus, further understanding is needed of how and to what extent multiple climatic variables shape selection on life-history traits like breeding time.

Here, we used a long-term (29 years) data set on a Mediterranean pied flycatcher (*Ficedula hypoleuca*) population to explore the relative influence of a global and 28 local climatic variables on the strength, form, and direction of selection on laying date. We used the yearly number of recruits produced by each breeding female as a proxy of fitness. The number of recruits is perhaps the best approximation of the individual contribution to the population of breeding animals in wild populations, both from a genetic and phenotypic perspective (Bonnet et al., 2017; Visser et al., 2015). As argued above, temperature and precipitation may play a key role in selection on laying date in wild bird populations. Nevertheless, the pattern of climate change, particularly in relation to current scenario of global warming, might differ among mean, minimum and maximum temperatures, and therefore, it is necessary to incorporate these proxies into selection analyses as potential environmental drivers (Rebetez & Reinhard, 2008). Such consideration is particularly relevant in the Mediterranean area where this pied flycatcher population breeds, since changes in temperature and precipitation patterns are expected for this region (Giorgi & Lionello, 2008). Hence to explore the relative influence of multiple environmental variables in selection on laying date in the study population, we used indexes derived from minimum, mean and maximum temperature and precipitation during different periods of the breeding cycle.

The pied flycatcher is a hole-nesting migratory passerine that has been used as a model for investigating the effect of climate change on birds' breeding phenology (Both et al., 2006b; Cadahía et al., 2017; Visser et al., 2015). This small insectivorous bird winters in West Africa (Ouwehand et al., 2016) and breeds in temperate forests across North Africa, Europe and West Asia (Lundberg & Alatalo, 1992). Our aim feeds from, first, the inconclusive effect of local temperature- based metrics on selection on laying date among populations of different or the same species (Bowers et al., 2016; Dobson et al., 2017; Goodenough et al., 2010; Husby et al., 2011b; Marrot et al., 2018; Marrot et al., 2017; Matthysen et al., 2011; Porlier et al., 2012; Reed et al., 2009; Townsend et al., 2013; van Noordwijk et al., 1995; Visser et al., 2015; Visser et al., 1998); second, from the lack of knowledge on the influence of global climatic factors on selection on breeding time in migratory species; and, finally, on the relative role of several environmental variables acting on selection on laying date in wild populations of birds. Since wintering conditions can have a deep impact on reproductive performance of migrant species due to carry over effects (Finch et al., 2014), we hypothesize that wintering conditions will have the strongest impact on selection on laying date relative to local climatic conditions.

Material and Methods

Study system and general procedures

Data were collected between 1988 and 2016 during a long-term study of pied flycatchers breeding in nest-boxes in central Spain (La Hiruela, 41°04′ N, 3°27′ W). Because of limited field effort in 2002 and 2003 compared to other years, these years were excluded from analyses (Potti et al., 2013).

The population occupies an old deciduous forest dominated by oaks (*Quercus pyrenaica*) and a mixed coniferous plantation dominated by *Pinus sylvestris* located 1.1 km apart (see Camacho et al., 2015 for a description of the study area). Breeding seasons last from the third week of April, with the arrival of the first individuals to the breeding areas, to the first fortnight of July, when all nestlings have fledged. Individuals in prime condition, typically around 2-3 years old, arrive earlier to the breeding areas than young and senescent individuals (Potti, 1998b). First males arrive, on average, about one week before females, establish a territory around a nesting site and try to attract a female (Potti & Montalvo, 1991a).

Nest-boxes (n=237) were inspected every 2-3 days to record laying date (date of the first laid egg), clutch size, number of hatchlings, and number of fledglings. Replacement clutches due to e.g. predation during incubation represent less than 1% of all clutches (45 documented cases out of 1696 clutches monitored during 1987-2004) and were therefore excluded from the analyses. Breeding individuals were captured using a nest-box trap, individually marked with coloured (only males) and metal (males and females) rings, measured for tarsus length (±0.05 mm), weighed (±0.1 g), and aged as either one-year old or older following the criteria of Karlsson et al., 1986. All nestlings were ringed at the age of 13 days with metal rings, enabling us to establish their fate (returned/not returned) in the following years as a proxy of recruitment, and to evaluate the number of recruits (reproductive success). This population of pied flycatchers shows high breeding site fidelity (96% return to the patch of first reproduction) and local recruitment rates (up to 22%) are among the highest reported for the species (Canal et al., 2014; Potti & Montalvo, 1991b), suggesting that most fledglings that survive to breeding age return to our nest-box area. Immigrants represent about 30% of the population, and the proportion of immigrants in relation to population density has been constant over time (Camacho et al., 2019). We are confident that our recruitment estimates are reliable and unbiased for several reasons: i) we capture virtually all breeding adults in the population (Camacho et al., 2017); ii) there is no familial resem- blance in dispersal patterns, meaning that the offspring of dispersers are not more prone to disperse than those of residents (Camacho et al., 2015); iii) breeding outside the study plots, either in the surroundings (as indicated by surveys conducted during the breeding seasons; pers. obs.) or in more distant areas, including other study populations of Iberian flycatchers (as indicated by ring recoveries), is an extremely rare event (pers. obs.; Potti and Montalvo, 1991a; iv) mark-recapture analyses have shown that variation in the probabilities of survival (transition) and recapture do not vary over time (S. Santoro et al., *submitted*).

The size of the population increased from 56 pairs in 1987 to 158 pairs in 2016. The average population growth rate was around 4% per year, with a strong initial demographic increase that slowed from 1994 onwards (Camacho et al., 2019).

Environmental variables

Global and local temperature- and rainfall- derived indexes were used as environmental variables. Meteorological data were taken from the only available official weather station that covers the three decades considered in this study (`https://opendata.aemet.es/centrodedescargas/inicio`; Colmenar Viejo - 40°39′N 3°45′W). Since this station is 50 km away from the study area, we compared the annual data on mean temperature and rainfall with those from a station closer (15km and data from 1997 to 2019) to our study area. Temperature (mean, maximum and minimum) and rainfall did not differ between stations in their temporal trends (see Appendix B), supporting the validity of the long-term climatic data used in the analyses.

We calculated mean, maximum and minimum values for daily temperature and rainfall indexes before the start of egg formation (from 10 to 3 days before laying date or pre-laying period Potti, 1999, during the laying period (from 3 days before laying the first egg to the date of the last egg laid (Birkhead et al., 1997), and monthly in April and May, in addition to spring (April to June, 'Spring') (Table 1). Finally, as a proxy of oak leafing and subsequent caterpillar emergence, we used the cumulative number of warmer-than-average days during the onset of the egg-laying period, considering an average temperature of 10.5° across years for the relevant time window of laying date in our population (26th of April to 26th of May). This variable is commonly used for predicting plant and insect phenology, but can be understood as well as a potential cue to the peak of food abundance (Saino et al., 2011; Simmonds et al., 2019). As global indexes of climatic variation, we used values of the North Atlantic Oscillation from the monthly Hurrell's PC-Based North Atlantic Oscillation Index (`https://climatedataguide.ucar.edu/climate-data/hurrell-north-atlantic-oscillation-nao-index-pc-based`). We averaged the monthly values of NAO during winter (from January to March).

Overall, we considered 14 temperature- and 13 precipitation-based variables, and 1 NAO-derived variable (Table 1). To avoid redundant information, we checked for collinearity between all environmental variables and predictors by computing variance inflation factors (VIF) using the `vif` function of the package `car` (Fox & Weisberg, 2019). We only considered those variables that had a VIF lower than 3 Zuur et al., 2010 - see Table 1), ensuring independence of variables corresponding to potentially different ecological processes.

Statistical analyses

Temporal trends in climatic variables and phenotypic traits

All statistical analyses were performed using `R` version 4.0.4 (R Core Team, 2017). We ran Linear Models (LMs) to test for temporal trends in all environmental variables considered in the analyses (one value by year for each variable). We ran the models considering yearly values of climatic variables as the dependent term, and year as the continuous explanatory term.

We used Linear Mixed Models (LMMs) with laying date as dependent variable to explore the temporal trend in laying dates by using the `lme4` package 1.1.26 (Bates et al., 2015) considering a Gaussian distribution. We considered year (as a continuous variable), habitat (class: oak vs. pine) and bird age (class: one-year vs. older) as explanatory terms. We also included year as a random term to account for pseudo-replication and stochastic variation among years (see e.g. Evans and Gustafsson, 2017 for a similar approach) and also individual female identities to account for repeated records of the same birds across years.

Overall and environment-mediated selection on laying date

Generalized Linear Mixed Models (GLMMs) were performed with the `glmmTMB` package v1.0.2.9000 (Brooks et al., 2017) to formally explore overall and environment-mediated selection on laying date. We estimated selection by using relative fitness (number of recruits divided by the mean number of recruits per year) as dependent variable in our models. The yearly number of recruits was scored for each female and assumed to follow a Poisson distribution. We z-transformed laying dates (zLD hereafter) within each year to a mean of 0 and a standard deviation of 1 to control for the environmental covariance across years between fitness and the trait (Garant et al., 2007b; Gienapp et al., 2006; Marrot et al., 2018).

When exploring the overall pattern of selection on laying date, we pooled all data and regressed relative fitness on the linear and quadratic term of zLD. We included the random terms of female identity to control for repeated measures of the same female, and year to control for non-independent measures within years. As fixed effects, we used zLD as explanatory term, and year as a continuous variable, female mating status (primary or secondary, see below and (Canal et al., 2021), age (1-year old or older) and habitat type to control for consistent differences in fitness between the deciduous and the coniferous forest (Camacho et al., 2015).Since primary females may benefit from higher number of offspring and recruits compared with secondary ones (Alatalo et al., 1981), female mating status was categorized into monogamous, primary and secondary females with or without male help. Those fixed variables were kept in all models.

Overall selection patterns were analysed without considering neither temporal trends nor any environmental variable as explanatory terms in the models, but maintaining the random structure described above. We considered nonlinear selection patterns to explore changes in the slope along the predictor and explicitly tested for stabilizing (or disruptive) selection. Such pattern of selection requires being explicitly tested in a phenotype like laying date since this trait has changed differently over time in multiple populations, going from profound shifts leading to an earlier onset of breeding to no temporal changes (Both et al., 2004). In the latter case, a lack of temporal change can be indicative of weak or stabilizing selection, suggesting the need for exploration of non-linear time trends in selection on laying date.

To explore the effect of a particular mean-centred environmental factor on selection on laying date (LD), we included the interaction between zLD and the given environmental variable (E). Temporal trends in selection on laying date were tested by including the interaction between zLD and year (Y). Non-linear selection and temporal patterns were also tested for each environmental factor by including the zLD, year or both in their quadratic forms. For each environmental factor that had a VIF >3 (see above and Table 1), we modelled selection on LD of a given environmental factor (E) by *i*) testing its mediating effect on fitness ($zLD \times E$), *ii*) its temporal trend ($zLD \times E + zLD \times Y$), *iii*) exploring non-linear selection patterns ($zLD^2 \times E + zLD \times Y$), *iv*) testing the quadratic effect of temporal trends ($zLD \times E + zLD \times Y^2$) and, finally, *v*) testing the influence of the non-linear and environment-mediated selection on LD and non-linear temporal trends ($zLD^2 \times E + zLD \times Y^2$). We modelled the among-year variation in intercepts and slopes in all models. By doing so, we were able to account for the uncertainty in the estimates of selection gradients in populations where selection may fluctuate in strength and direction (Morrissey & Hadfield, 2012). Additionally, female identity and year were included as random terms in all models.

As we considered multiple climatic factors that may shape selection on laying date (Table 1; those that had a $VIF > 3$), we run a set of 53 a priori chosen models to get the most plausible ones at explaining selection on laying date. These models included full (all 5 combinations described above for all environmental variables considered; see Appendix D) and null models (only random terms; see Appendix D). We adopted this method since running all possible combinations to detect the most plausible models is undesirable (Burnham & Anderson, 2002). In all models, we maintained all fixed terms described above (zLD, year, habitat, female mating status and age), along with the random structure of the models. Top-ranked models that had an $AIC < 7$ from the best one were averaged (Burnham et al., 2011) using the package `MuMIn` v. 1.43.17 (Barton & Barton, 2020). Models that had an $AIC > 7$ were not considered further.

Results

Temporal trends of climatic variables and laying date

Over the study period, the annual number of nests ranged from 33 to 136 nests (mean ± SD: 73 ± 36), with a mean ± SD of 0.57 ± 0.81 recruits by female and year (0.90 ± 1.20 per female throughout its life). There was negligible overall variation in breeding time over the 29 years of the study (Figure 1, estimate = -0.004; p = 0.605; mean breeding time=22.2 ± SD 6.8, where day 1 = 1 May). In addition, only one of the 28 annual climatic conditions examined in the models showed a significant temporal trend (Figure 2, Table 1). Specifically, minimum temperatures in April have increased over the study period (Table 1).

Selection on laying date

Overall, there was non-linear selection on the number of recruits (Figure 3), relative recruit number against standardized laying date; estimate = -0.288 ; SE 0.0314, z = -2.570, p = 0.011). In general, despite the yearly variation in selection (see Appendix C), early breeding birds produced more recruits and had higher recruitment rate than late-breeding birds. However, the negative non-linear association between fitness and laying date shows that the slope of the regression between fitness and laying date changed over time, turning from null to negative as the season progressed (Figure 3). In other words, the intensity of negative selection on laying date was not constant but increased as the breeding season advanced.

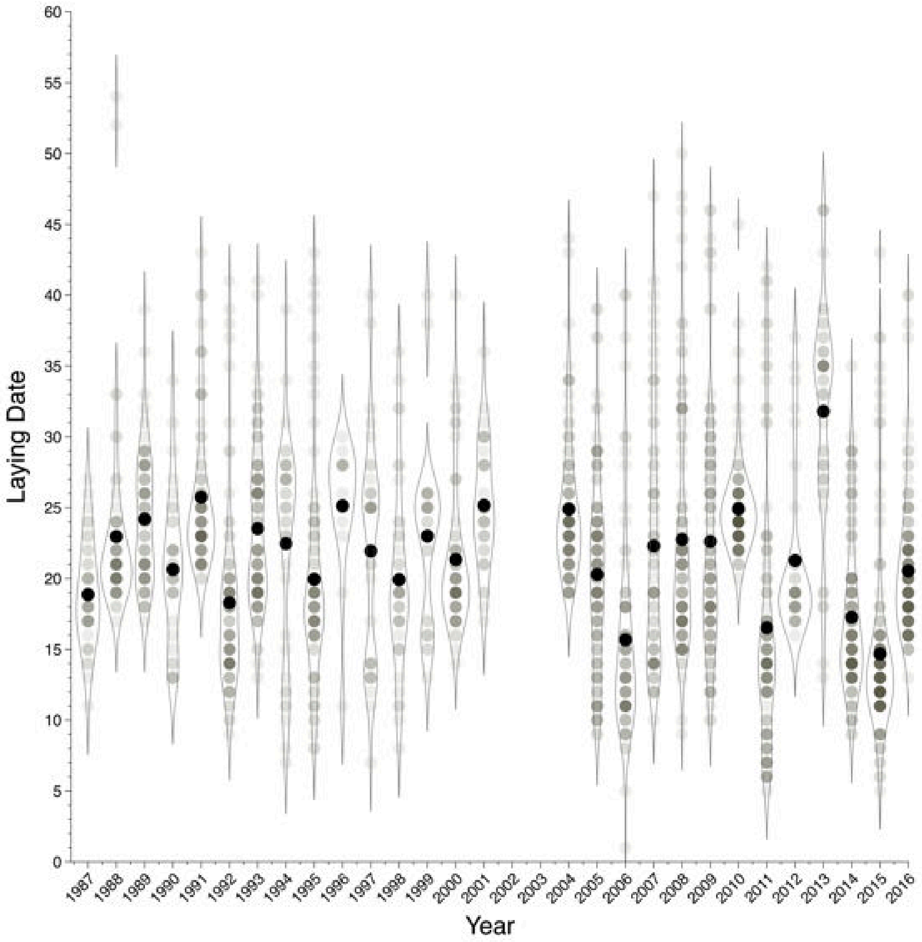


Figure 1: Temporal trends in annual mean laying dates (1=1st May). For a full description of the statistical analyses, see "Material and methods" section. Grey dots represent the laying dates of individual females (nests) each year, but increase in grey intensity suggest that more females start breeding in the same day. Black dots show the mean laying date for every year and "violin" areas represent the density of the sampled laying dates of all females each year.

As to the role of environmental variation in selection on laying date, two environmental factors, minimum temperatures in April and May, shaped selection in both a linear and a non-linear way (Table 2 and Figure 4 – for a full list of 53 models run, see Appendix D). The strength of directional selection varied according to the minimum temperatures in April, so that selection penalised late breeders when minimum temperatures in April were low, whereas early breeders enjoyed fitness benefits under these circumstances (Figure 4). Our results also showed that the intensity of selection progressed from null to negative as the breeding season progressed, conditional on the minimum temperatures in May. Specifically, the intensity of selection on late breeders increased when minimum

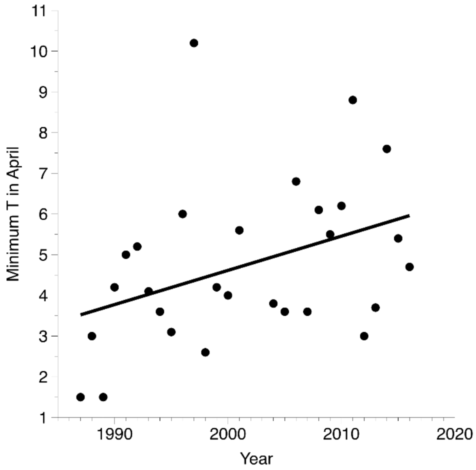


Figure 2: Temporal trends in absolute minimum April temperatures experienced by a long-term studied Mediterranean pied flycatcher population.

Table 1: Description and selection of environmental variables and their temporal variation in a pied flycatcher population in central Spain. Period denotes the time interval at which the environmental variables were considered (see Material and Methods for further details). Also shown are the estimates ± SE of the temporal trends (year) and p-value for each variable, in bold those with p-values < 0.001. In addition, as stated in the main text, only variables with a VIF value < 3 were considered as environmental factors in the models, which are highlighted in italics and lack values in the order column. Order denotes the step at which the variable was excluded according to its VIF.

	Period	**Environmental variable**	**Estimate ± SE**	**P**	**Order**	**R2**	**Tolerance**	**VIF**
Local Temperature	Spring	MeanTSpring	0.029 ± 0.021	0.192	18	0.715	0.285	3.507
	PreLD	TempPreLD	0.006 ± 0.038	0.868	7	0.983	0.017	60.254
		TempMinPreLD	-0.024 ± 0.035	0.491	17	0.787	0.213	4.692
		TempMaxPreLD	0.035 ± 0.041	0.402		0.365	0.635	1.575
	LD	TempLD	-0.002 ± 0.038	0.969	3	1.000	0.000	12247.193
		TempMinLD	-0.024 ± 0.036	0.513		0.283	0.717	1.394
		TempMaxLD	0.041 ± 0.045	0.370	15	0.809	0.191	5.240
	April	MeanTApril	0.042 ± 0.038	0.278	14	0.869	0.131	7.623
		MinTApril	**0.084 ± 0.04**	**0.044**		0.210	0.790	1.266
		MaxTApril	0.017 ± 0.05	0.732		0.389	0.611	1.638
	May	MeanTMay	0 ± 0.034	0.996	9	0.964	0.036	27.747
		MinTMay	0.004 ± 0.046	0.928		0.278	0.722	1.386
		MaxTMay	0.039 ± 0.051	0.445	13	0.906	0.094	10.659
	Spring	MeanPSpring	0.029 ± 0.156	0.855	5	0.994	0.006	168.369
Local Precipitation	PreLD	PrecipPreLD	0.043 ± 0.307	0.889	6	0.985	0.015	65.071
		PrecipMinPreLD	0.013 ± 0.018	0.462		0.336	0.664	1.506
		PrecipMaxPreLD	-0.641 ± 1.212	0.601	12	0.915	0.085	11.772
	LD	PrecipLD	-0.398 ± 0.329	0.237	4	0.995	0.005	194.973
		PrecipMinLD	-0.004 ± 0.002	0.090		0.422	0.578	1.729
		PrecipMaxLD	-1.868 ± 1.278	0.156	11	0.958	0.042	23.775
	April	MeanPApril	0.309 ± 0.271	0.265	8	0.977	0.023	42.826
		MinPApril	NA	NA	1	1.000	0.000	Inf
		MaxPApril	1.459 ± 2.21	0.515		0.210	0.789	1.266906
	May	MeanPMay	-0.016 ± 0.279	0.955	16	0.805	0.195	5.115
		MinPMay	NA	NA	2	1.000	0.000	Inf
		MaxPMay	-0.9 ± 1.806	0.623		0.367	0.633	1.580
Local T accumulated		DegreeDay	1.076 ± 2.056	0.605	10	0.961	0.039	25.455
Global climatic		NAOw	-0.034 ± 0.026	0.193		0.523	0.477	2.098

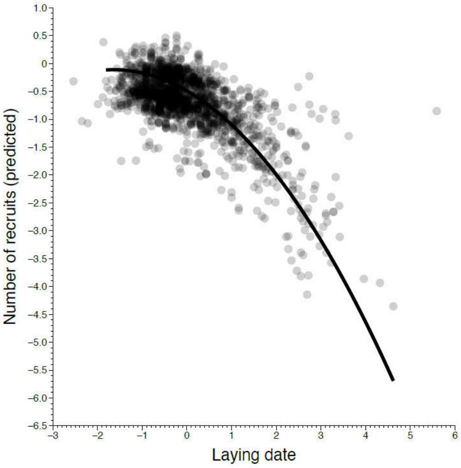


Figure 3: Pattern of overall selection on laying date in a pied flycatcher population in central Spain individually monitored for 29 years (nonlinear selection of relative number of recruits against annually standardized laying date).

temperatures in May rose, while no clear effect of minimum temperatures on selection on laying date was found in cold Mays (Figure 4). The above effects were conditional on the correcting effects of consistent differences due to habitat type, female age, mating status and the non-linear positive quadratic effect of year on fitness (Table 2). None of the precipitation-based variables considered, or the global climatic variable (NAOw), seems to be influencing selection on laying date in this population (Table 2).

Discussion

Our results align with the idea that local temperature is the main driver of selection on laying date in temperate-zone birds. Specifically, temperature- derived climatic factors during breeding time, namely minimum temperature in April and minimum temperature at the time of laying (in May), are the environmental variables that best shaped selection on laying date in our population. In addition, our results suggest three new insights regarding the role of environmental variation in selection at phenotypic level in wild populations. First, temperatures experienced during different time frames in the breeding season may impose a fitness cost on late breeders. Second, extreme values of two temperature-derived factors (minimum temperatures in April and May), but not their averages, are the major drivers of selection on laying date, supporting the idea that extreme climatic events are potential drivers of selection on breeding time in wild bird populations (Marrot et al., 2017; Schreiber & Schreiber, 1984; van de Pol et al., 2010). Finally, we found that the shape of selection on laying date may change from directional (minimum temperatures in April) to non-directional (minimum temperatures in May) depending on the environmental factor considered.

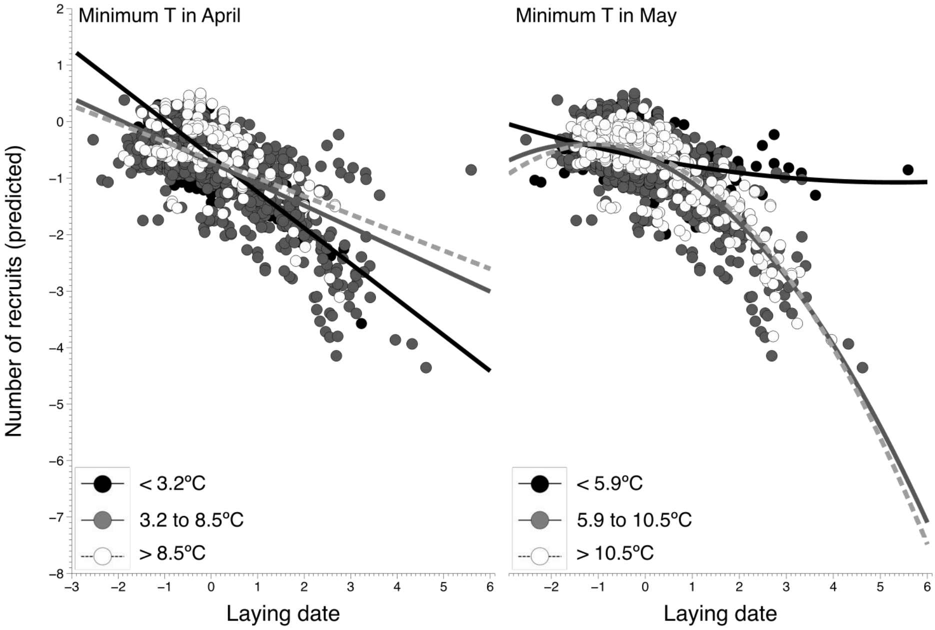


Figure 4: Selection pattern on standardized laying date in a spanish pied flycatcher population mediated by minimum temperature in April and May. Number of recruits are predicted values from the averaged model (see methods, results and Table 2) related to minimum temperatures in April (left) and May (right). Note that we categorized the minimum temperature in May at three levels (low (0-20 of the range; black dots and black line), intermediate (20-80 range, grey dots and grey line) and high (80-100, white dots and dashed line). These categories are split unequally to better show the change in the association between fitness and phenotype for each environmental variable.

Breeding cycle of long- distance migrants is particularly sensi- tive to current climate change scenarios (Both et al., 2006b; Both et al., 2009b; Coppack et al., 2008; Robinson et al., 2009). Accordingly, we found that climatic variation influences the individual covariance between fitness and laying date in our population. This result is consistent with previous findings in different bird species where selection on laying date is driven by temperature at the local scale (Husby et al., 2011b; Marrot et al., 2018; Visser et al., 2015; Visser et al., 1998). However, in contrast to previous work, we found that minimum temperatures in April and May are the paramount environmental factors shaping selection on laying date in the study population. By mid-April, pied flycatchers are just beginning to settle down from spring migration (Potti, 1998a), and the usually cold temperatures recorded at the study site during that time period may affect the reproductive performance of birds. Although we do not have a mechanistic explanation for this pattern, we speculate that a mismatch between the peak of food abundance and the peak

Table 2: Full model-averaged coefficients for all models with AICc < 7 that explored environmental variation on selection on laying date using number of recruits as proxy of fitness. CI and +CI, confidence limits for coefficient estimates at the 95% confidence intervals (CI); RVI, relative variable importance; SE, standard errors. Adjusted R^2 are included for each model and were calculated including all variables and interactions in a single model. "NoA" denotes secondary females without male assistance (see material and methods for further details)(Siepielski et al., 2017)

Variable	**Coefficient estimate**	**SE**	**RVI**	**-CI**	**+CI**
Intercept	-1.719	0.633	-	-2.956	-0.502
LD2	0.501	0.329	1.000	-0.151	1.133
LD	0.488	0.729	1.000	-0.933	1.804
Habitat	0.450	0.067	1.000	0.318	0.583
Mate - Primary	0.142	0.127	1.000	-0.106	0.391
Mate - Secondary	-0.229	0.187	1.000	-0.595	0.138
Mate - Secondary, NoA	-0.974	0.342	1.000	-1.643	-0.302
Age	-0.026	0.025	1.000	-0.074	0.022
LD*TempMinLD	-0.010	0.018	0.299	-0.043	0.024
TempMinLD	-0.008	0.015	0.362	-0.035	0.021
LD2*PrecipMinPreLD	-0.010	0.043	0.059	-0.082	0.067
LD*PrecipMinPreLD	0.000	0.026	0.105	-0.044	0.044
PrecipMinPreLD	0.012	0.033	0.189	-0.047	0.067
LD2*MaxPApril	0.001	0.009	0.023	-0.017	0.019
LD*MaxPApril	-0.001	0.010	0.034	-0.020	0.018
MaxPApril	-0.001	0.014	0.064	-0.028	0.027
LD2*TempMaxPreLD	0.000	0.001	0.004	-0.002	0.002
LD*TempMaxPreLD	-0.007	0.017	0.165	-0.036	0.024
TempMaxPreLD	0.004	0.011	0.238	-0.017	0.025
LD2*MaxPMay	0.006	0.021	0.086	-0.031	0.041
LD*MaxPMay	-0.012	0.033	0.133	-0.067	0.048
MaxPMay	0.039	0.058	0.574	-0.073	0.150
LD2*NAOw	0.002	0.012	0.073	-0.021	0.024
LD*NAOw	-0.079	0.054	0.856	-0.184	0.029
NAOw	-0.143	0.070	0.962	-0.277	-0.003
LD2*MaxTApril	-0.031	0.017	0.940	-0.064	0.004
LD*MaxTApril	-0.038	0.026	1.000	-0.088	0.015
MaxTApril	0.003	0.032	1.000	-0.058	0.066
LD2*MinTApril	0.044	0.023	0.966	-0.001	0.088
LD*MinTApril	0.051	0.026	1.000	0.001	0.101
MinTApril	-0.003	0.035	1.000	-0.073	0.065
LD2*MinTMay	-0.032	0.014	0.984	-0.059	-0.005
LD*MinTMay	-0.009	0.023	1.000	-0.055	0.036
MinTMay	0.050	0.029	1.000	-0.008	0.106
LD2*Year	0.001	0.008	0.012	-0.012	0.013
LD*Year	-0.001	0.019	0.094	-0.034	0.032
Year2	0.215	0.067	1.000	0.083	0.346
Year	-0.068	0.065	1.000	-0.194	0.061

of feed demands of young can be a plausible explanation. Since a cold April and warm May had the highest fitness cost for late breeders, it is likely that leaf burst and subsequent insect emergence and viability can be limited by extreme temperatures in those two months.

The idea that extreme climatic values shaped selection on laying date agrees with previous findings (Marrot et al., 2018). Specifically, Marrot *et al.* described that maximum temperatures in April were the main climatic factor shaping selection (using number of offspring produced) on laying date in a non-migrant passerine, blue tits (*Cyanistes caeruleus*), suggesting that warm temperatures may increase breeding mistiming, and reduce the number of offspring for late breeders. Our results are aligned with this idea, although the effect of warming in the study population is found in May and pointed to minimum temperatures instead. Perhaps, the difference in the warming period between the two studies is related to the later breeding phenology of migrants pied flycatchers compared to residents blue tits, at least in our study area (Potti, 2009; Potti et al., 2021). Overall, these results highlight the role that extreme values, rather than averages, may have at shaping fitness landscapes, reinforcing the idea that extreme climatic events should be considered when investigating selection on phenotypic traits (Arnold et al., 2001). Controversially, the patterns found here are in partial disagreement with previous findings in other pied flycatcher populations, where climatic conditions during breeding do not influence the temporal pattern of selection on breeding time (Visser et al., 2015). Instead, Visser et al., 2015 found that climatic conditions at the time of arrival of recruits play a role in shaping selection on the laying date with a stronger selection in the year of birth of those recruits. Although our study covering a wider range of climatic variables and finding that climatic conditions at the time of breeding had an influence on selection on laying date, further research is needed to investigate whether climatic conditions at arrival time of those recruits can also play a role on selection in our population.

This study does not support the idea that precipitation shapes selection on laying date (Siepielski et al., 2017). We considered 13 precipitation- based variables to test their potential selective role, and found that none of them had statistical support for explaining selection on laying date in our population. Mean and extreme values of precipitation throughout the study study period were insufficient to induce a change in the covariation between fitness and laying date, thus supporting that temperature, rather than precipitation, is the major environmental variable driving selection on this life-history trait. However, there is still a gap of knowledge of the relative role of precipitation in relation to that of temperature-based variables on selection in wild birds, since comparisons of the relative role of precipitation-derived variables in relation to temperature-derived variables are rather uncommon in the literature.

Our results Our results also disagree with previous studies showing that selection on laying date also operates during the non-breeding season (Ahola et al., 2004; Both et al., 2006b; Both & Visser, 2005; Finch et al., 2014) as we found that the chosen NAO- based index (NAO in winter) is not an environmental driver acting on selection on laying date in this pied flycatcher population. NAOw (January to March) may be seen as a proxy of the climatic conditions experienced by pied flycatchers wintering in sub-Saharan Africa during a crucial period for the survival of migratory birds (Johnston et al., 2016). Therefore, it seems that climatic conditions during breeding are the key factors at explaining selection on laying date, superseding the potential role that wintering conditions may play in such selection. However, care must be taken to reject the role of climatic conditions after departure to the wintering grounds or during winter in selection on laying date, since late fledgling in poor body condition in the season may impact their recruitment probability. We used NAOw, which is a broad scale index of climatic conditions, but the use of a similar approach used for minimum, mean and maximum temperature- and precipitation-based indexes at the precise wintering grounds would be advisable. Unfortunately, the location of the wintering grounds in our population is still inaccurate and climatic-derived indexed are only possible through global estimations.

Minimum temperature in May mediated selection on laying date in a nonlinear way, suggesting that the strength of negative selection gets stronger in warmer springs (Figure 4). Temperatures at settlement from spring migration and start of breeding have been suggested as major environmental drivers of selection on laying date in pied flycatchers (Both & Visser, 2001; Goodenough et al., 2011a; Goodenough et al., 2010; Visser et al., 2015). Our results support the notion that local climatic conditions shape selection on breeding time but, since the association is not linear, individuals experienced a more intense reduction in fitness benefits with the progress of the season in warmer springs (Figure 4). In fact, such a fitness cost disappears in cold months of May, which suggests i) a stronger selection pressure for late breeders in warm periods at the time of breeding and ii) potentially, a threat for population viability if warm Mays persist overtime and birds are unable to track such change. Over the nearly three decades of study, there has been considerable between-year heterogeneity in the climatic variables measured and, thereby, in the form and strength of selection on laying date. The strength of selection on laying date varied between years (Appendix C), suggesting that temporal heterogeneity in environmental conditions imposes changing selection pressures on the timing of breeding (Marrot et al., 2018). In light of our results, temporal inconsistencies in patterns of environmental variation emerge as a plausible explanation for the lack of temporal shifts in the timing of breeding of migratory birds (Ahola et al., 2012; Visser et al., 1998), a trend that has nevertheless been noted in northern populations of pied flycatchers (Both & Visser, 2001). However, despite the temporal increase in minimum temperatures in April, we also detected stabilizing selection on laying date depending on minimum temperatures at the time of laying, in May. Specifically, early breeders are not positively or negatively selected , but the intensity of selection against late breeders increases as the start of breeding delays, particularly when minimum temperatures in April and May decrease or increase respectively (Figure 3). Thus, the pattern of stabilizing selection may underlie the observed stasis of laying date over time in our population.

In summary, we have shown that selection on laying date is stabilizing in our population, and that it is also driven by multiple local climatic factors acting at the time of arrival and breeding. Overall, our results indicate that selection penalizes late breeders. However, temporal heterogeneity in climate variables, combined with the influence of two climatic factors differing in the form of selection and the breeding stage where they are acting on, has favoured a three-decade stasis of laying date in this population. Future studies exploring the factors that drive selection on any phenotypic trait should therefore assess the relative importance of the multiple environmental factors that individuals may face under changing natural conditions.

Acknowledgements

We thank the many volunteers, technicians and friends, too numerous to name individually, that helped with data collection over the three decades of study. All authors contributed to the drafts and gave final approval for publication. JLV was supported by a grant from the Spanish Ministry of Economy and Competitiveness (BES-2016-077694). CC received financial support from the Spanish Ministry of Science, Innovation and Universities (FJC2018-038412-I). DC was supported by a grant from the Hungarian Academy of- Sciences (MTA Premium Postdoctoral Research Program; ID: 2019-353). JM-P was supported by ARAID foundation and research projects granted by Spanish Ministry of Science and Innovation (PID2019-104835GB-I00; CGL2015-70639-P).

Supplementary Material

Appendix A - Literature review

We reviewed all published studies that quantified selection on laying date using the Web of Science bibliographic database. We aimed to synthesize the current knowledge available about the role of multiple environmental factors on selection on laying date in wild birds. We used the terms “selection” AND “laying date” OR “breeding time” OR “time of breeding” OR “egg-laying date”, which resulted in 535 references (accessed 17/9/2019). From all references obtained, we only considered those studies that 1) were performed in wild populations, and 2) quantified either selection gradients or selection differentials sensu Lande and Arnold, 1983. We complemented our search with the most comprehensive published compilations of estimates of selection on phenotypic traits (Siepielski et al., 2013; Siepielski et al., 2017 only for phenological traits).

Table 1: "Migration" column refers to the migratory (M) or sedentary (S) status of the species. There is specific mention whether a "selection test was performed" (yes or no), or the relative influence of environmental factors on selection (yes or no). "Selection", is the metric used to explore selection, being fecundity- (F) or viability- based (Survival, S) indexes. (*) selection was not calculated sensu Lande and Arnold, 1983. References [26-40], unless stated otherwise, used fecundity-based proxies of fitness to obtain selection estimates. Specifically, reference numbers refer to *Cyanistes caerueus* (26), *Cyanistes caerueus* using selection estimates based on viability-based fitness proxies and hatching date as phenotype included here as proxy of laying date (27), *Cygnus olor* (33), *Ficedula hypoleuca* (34), *Hirundo rustica* using arrival date as phenotype (35), *Lacerta agilis* using viability-based proxies of fitness (36) *Larus canus* (37), *Larus novaehollandiae scopulinus* (38), *Parus major* (28,39,40), *Parus major* using selection estimates based on viability-based fitness proxies (30) and *Tachycineta bicolor* (31,32).

Environmental variables	**Specific environmental variables**	**Species**	**Migration**	**Selection**	**Selection test**	**Environemental factor relative influence**	**Reference**
biotic and abiotic	biotic: population density abiotic: winter temperature and altitude	*Cinclus cinclus*	S	S	no	yes*	(1)
biotic and abiotic	biotic: population density, predation rate abiotic: spring temperatures	*Cyanistes caruleus*	S	F	yes	yes	(2)
biotic and abiotic	biotic: food peak, mismatch abiotic: spring temperature and rainfall	*Ficedula hypoleuca*	M	F	yes	no	(3)
biotic and abiotic	biotic: food abundance abiotic: average spring sea surface temperature, strength of upwelling in the Gulf of the Farallones, Northern Oscillation Index (NAO)	*Uria aalge*	M	F	yes	no	(4)
biotic and abiotic	biotic: food mismatch abiotic: spring temperatures during laying, incubation, hatchling, nestling and 30 days after laying	*Parus major*	S	S	yes	no*	(5)
abiotic	ice-out: early years or late years	*Bucephala clangula*	M	F	yes	no	(6)
abiotic	spring temperatures and rainfall	*Cyanistes caruleus*	S	F	yes	yes	(7)
abiotic	spring temperature - 4 populations	*Cyanistes caruleus*	S	F	yes	no	(8)
abiotic	spring temperature	*Cyanistes caruleus*	S	F	no	no	(9,10)
abiotic	NAO previous year	*Ficedula albicollis*	M	F	no	no	(11)
abiotic	spring temperature	*Ficedula hypoleuca*	M	F	no	no	(9,12,13)
abiotic	2 habitats	*Hydrobates pelagicus*	M	F	no	no	(14)
abiotic	interval laying date and food abundance	*Parus major*	S	F	no	no	(15)
abiotic	spring temperatures	*Parus major*	S	F	yes	no	(16)
Abiotic	spring temperatures	*Parus major*	S	F	no	no	(9,10,17)
abiotic	spring temperatures	*Periparus ater*	S	F	no	no	(9)
abiotic	spring temperatures	*Phoenicurus phoenicurus*	M	F	no	no	(9)
abiotic	spring temperature	*Setophaga caerulescens*	M	F	yes	no	(18)
abiotic	spring temperatures	*Sitta Europaea*	S	F	no	no	(9)
abiotic	Atlantic Multidecadal Oscillation; NAO; Southern Oscillation Index; sea surface temperature (STT) - reduced to SST	*Sterna hirundo*	M	F	no	no	(19)
abiotic	spring temperatures and rainfall	*Troglodites aedon*	S	F	yes	no	(20)
biotic	Population-level mismatch; Breeding density; Beech crop index; age composition	*Parus major*	S	F	yes	no	(21)
biotic	food mismatch	*Parus major*	S	F+S	yes	no	(22)
biotic	food abundance: peak height and width	*Parus major*	S	F	yes	no	(23)
biotic	terrritory size	*Parus major*	S	F	na	no	(24)
urbanization	Urban vs rural habitats	*Parus major*	S	F	yes	yes	(25)
none	none	Multiple species	S(8), M(5)	F	no	no	(26-40)

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Appendix B

We used the weather station located in Colmenar Viejo (40°39′ N, 3°45′ W) in or analyses. This weather station is the only one that has recorded the whole study period considered in this study. Since this station is 50km away for the study site, we compared the climatological data with another closer weather station that did not have the whole time series located in Buitrago de Lozoya (41°00′ N, 3°36′ W), 17.8kms away from our study site (in straight line).

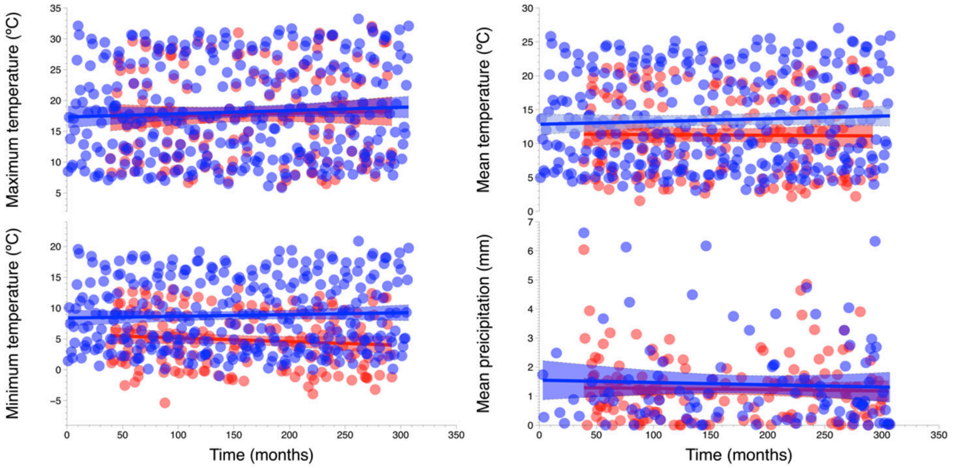
From January of 1997 to July 2019, we collected daily data from both stations and computed monthly values of precipitation, mean temperature, maximum temperature and minimum temperature. We compared whether the temporal trend on climatic values differed between the two weather stations. More formally, we tested whether the slope of the association between time and climatic values of one weather station 1 (Buitrago de Lozoya) differed from weather station 2 (Colmenar Viejo).

We run linear models where climatic variables of weather station 1 were the dependent variables and tested the interaction between time and climatic variables of weather station 2. The variable time was a consecutive number of months starting from 1 January of 1997 and 258 corresponding to July of 2019.

Temporal trends of any of the climatic variables did not differ between weather stations (Table below).

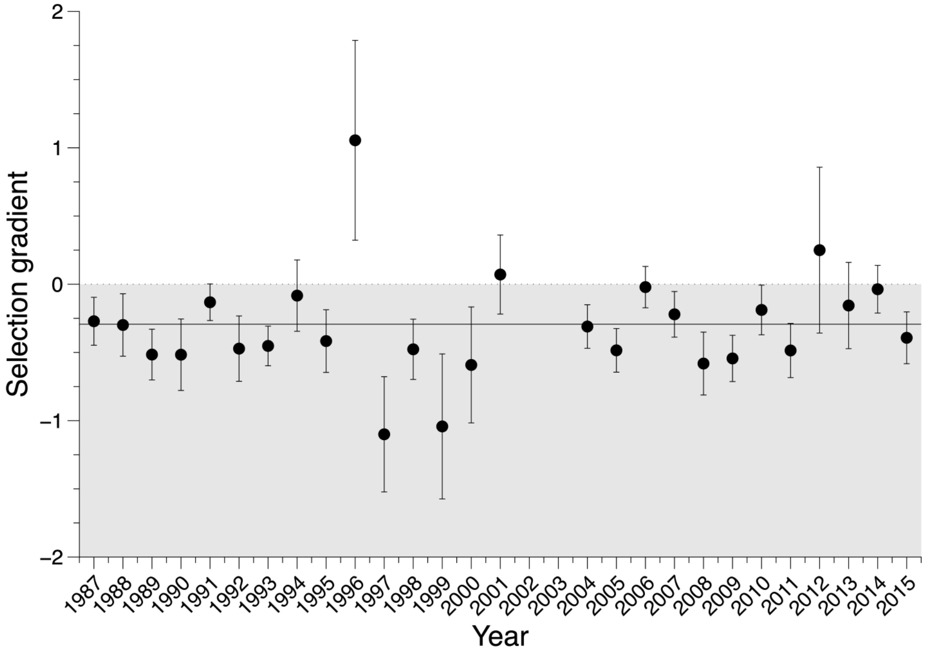
		Estimate ± SE	**t**	**P**
Precipitation	Time	*-0.0004 ± 0.0012*	-0.325	0.745
	Weather station2	*0.2435 ± 0.3760*	0.648	0.518
	Time*Weather station2	*-0.0003 ± 0.0019*	-0.198	0.843
Mean temperature	Time	*-0.0009 ± 0.0067*	-0.146	0.884
	Weather station2	*1.4289 ± 1.4034*	1.018	0.309
	Time*Weather station2	*0.0050 ± 0.0079*	0.634	0.526
Minimum temperature	Time	*-0.0059 ± 0.0055*	-1.071	0.285
	Weather station2	*2.6304 ± 1.1675*	2.253	0.025
	Time*Weather station2	*0.0087 ± 0.0065*	1.335	0.182
Maximum temperature	Time	*0.0040 ± 0.0079*	0.507	0.613
	Weather station2	*0.2277 ± 1.6591*	0.137	0.891
	Time*Weather station2	*0.0012 ± 0.0093*	0.132	0.895

In both graphs below, we show the temporal trends (months) and mean precipitation, mean temperature, maximum temperature and minimum temperature, as described above. It is represented for the two meteorological stations considered in this study, Buitrago de Lozoya (in red) and Colmenar Viejo (in blue). Temporal trends and confident intervals (95%) are also included as shaded colored areas accordingly.



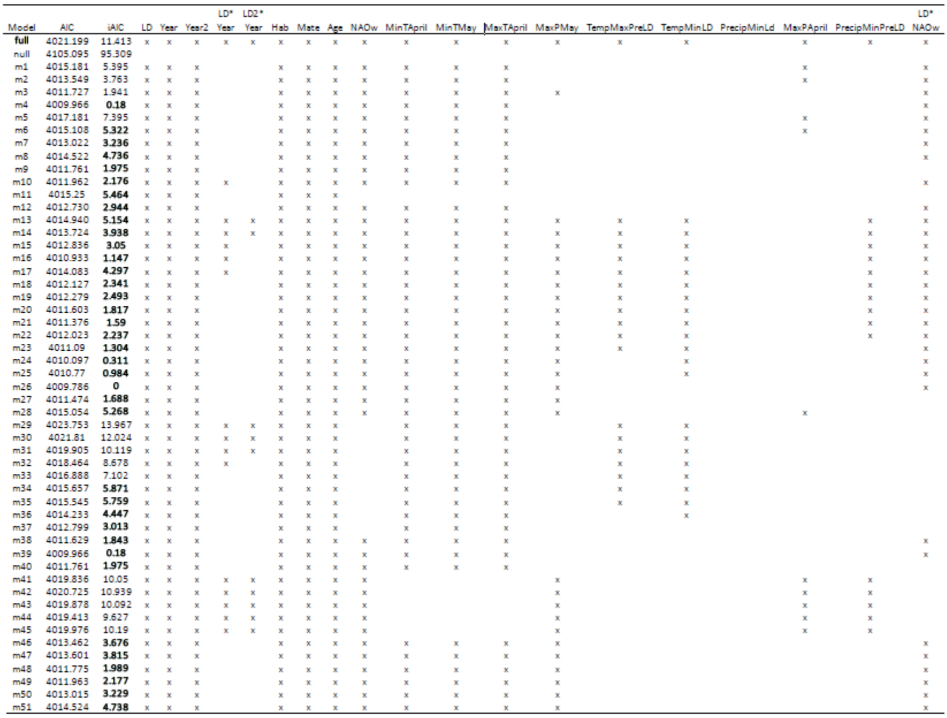
Appendix C

Temporal variation of selection on laying date. Selection gradients are represented for illustrative purposes, despite they do not represent any statistical analyses performed in this manuscript (see material and methods for further details). The grey area highlights negative selection gradients. The dotted line shows the lack of association between fitness and laying date and black line represents the mean value of the selection gradient on laying date in the population.



Appendix D

Full list of models run manually to explore the shaping role of multiple environmental variables on seleciton on laying date in a long-term monitored population of pied flycatchers (*Ficedula hypoleuca*). Only environmental variables that had a VIF<3 are included, as described in the text of the manuscript (Table 1). For each environmental variable, weincluded the linear and non-linear (quadratic) association with laying date, to explore linear or quadratic selection patterns. AIC values and differences in AIC values for each model (iAIC) are given, but highlithted in blue only those that had an iAIC<7 as estated in themain text of themanuscript. These models were averaged and the result of the averaged model is given in the Table 2. All other variables and interactions are explained in the main text.



Model	AIC	iAIC	LD	Year	Year2	LD* Year	LD2* Year	Hab	Mate	Age	NAOw	MinTApril	MinTMay	MaxTApril	MaxPMay	TempMaxPreLD	TempMinLD	PrecipMinLd	MaxPApril	PrecipMinPreLD	LD* NAOw
full	4021.199	11.413	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x
null	4105.095	95.309																			
m1	4015.181	5.395	x	x	x			x	x	x	x	x	x	x					x		x
m2	4013.549	3.763	x	x	x			x	x	x	x	x	x	x					x		x
m3	4011.727	1.941	x	x	x			x	x	x	x	x	x	x	x						x
m4	4009.966	**0.18**	x	x	x			x	x	x	x	x	x	x							x
m5	4017.181	7.395	x	x	x			x	x	x	x	x	x	x					x		x
m6	4015.108	**5.322**	x	x	x			x	x	x	x	x	x	x					x		x
m7	4013.022	**3.236**	x	x	x			x	x	x	x	x	x	x							x
m8	4014.522	**4.736**	x	x	x			x	x	x	x	x	x	x							x
m9	4011.761	**1.975**	x	x	x			x	x	x	x	x	x	x							
m10	4011.962	**2.176**	x	x	x	x		x	x	x	x	x	x	x							x
m11	4015.25	**5.464**	x	x	x			x	x	x											
m12	4012.730	**2.944**	x	x	x			x	x	x	x	x	x	x							x
m13	4014.940	**5.154**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x	x
m14	4013.724	**3.938**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x	x
m15	4012.836	**3.05**	x	x	x	x		x	x	x	x	x	x	x	x	x	x			x	x
m16	4010.933	**1.147**	x	x	x	x		x	x	x	x	x	x	x	x	x	x			x	x
m17	4014.083	**4.297**	x	x	x	x		x	x	x	x	x	x	x	x	x	x			x	x
m18	4012.127	**2.341**	x	x	x			x	x	x	x	x	x	x	x	x	x			x	x
m19	4012.279	**2.493**	x	x	x			x	x	x	x	x	x	x	x	x	x			x	x
m20	4011.603	**1.817**	x	x	x			x	x	x	x	x	x	x	x	x	x			x	x
m21	4011.376	**1.59**	x	x	x			x	x	x	x	x	x	x	x	x	x			x	x
m22	4012.023	**2.237**	x	x	x			x	x	x	x	x	x	x	x	x	x			x	x
m23	4011.09	**1.304**	x	x	x			x	x	x	x	x	x	x	x	x	x				x
m24	4010.097	**0.311**	x	x	x			x	x	x	x	x	x	x	x		x				x
m25	4010.77	**0.984**	x	x	x			x	x	x	x	x	x	x	x		x				x
m26	4009.786	**0**	x	x	x			x	x	x	x	x	x	x	x						x
m27	4011.474	**1.688**	x	x	x			x	x	x	x	x	x	x	x						
m28	4015.054	**5.268**	x	x	x			x	x	x	x	x	x	x	x				x		
m29	4023.753	13.967	x	x	x	x	x	x	x	x		x	x	x		x	x				
m30	4021.81	12.024	x	x	x	x	x	x	x	x		x	x	x		x	x				
m31	4019.905	10.119	x	x	x	x	x	x	x	x		x	x	x		x	x				
m32	4018.464	8.678	x	x	x	x		x	x	x		x	x	x		x	x				
m33	4016.888	7.102	x	x	x			x	x	x		x	x	x		x	x				
m34	4015.657	**5.871**	x	x	x			x	x	x		x	x	x		x	x				
m35	4015.545	**5.759**	x	x	x			x	x	x		x	x	x		x	x				
m36	4014.233	**4.447**	x	x	x			x	x	x		x	x	x			x				
m37	4012.799	**3.013**	x	x	x			x	x	x		x	x	x							
m38	4011.629	**1.843**	x	x	x			x	x	x	x	x	x	x							x
m39	4009.966	**0.18**	x	x	x			x	x	x	x	x	x	x							x
m40	4011.761	**1.975**	x	x	x			x	x	x	x	x	x	x							
m41	4019.836	10.05	x	x	x	x	x	x	x	x	x				x				x	x	
m42	4020.725	10.939	x	x	x	x	x	x	x	x	x				x				x	x	
m43	4019.878	10.092	x	x	x	x	x	x	x	x	x				x				x	x	
m44	4019.413	9.627	x	x	x	x	x	x	x	x	x				x				x	x	
m45	4019.976	10.19	x	x	x	x	x	x	x	x	x				x				x	x	
m46	4013.462	**3.676**	x	x	x			x	x	x	x	x	x	x	x						x
m47	4013.601	**3.815**	x	x	x			x	x	x	x	x	x	x	x						x
m48	4011.775	**1.989**	x	x	x			x	x	x	x	x	x	x	x						x
m49	4011.963	**2.177**	x	x	x			x	x	x	x	x	x	x	x						x
m50	4013.015	**3.229**	x	x	x			x	x	x	x	x	x	x	x						x
m51	4014.524	**4.738**	x	x	x			x	x	x	x	x	x	x	x						x

Model	AIC	iAIC	LD* MinTApril	LD* MinTMay	LD* MaxTApril	LD* MaxPMay	LD* TempMaxPreLD	LD* TempMinLD	LD* PrecipMinLd	LD* MaxPApril	LD* PrecipMinPreLD	LD2 * NAOw	LD2 * MinTApril	LD2 * MinTMay	LD2 * MaxTApril	LD2 * MaxPMay	LD2 * TempMaxPreLD	LD2 * TempMinLD	LD2 * PrecipMinLd	LD2 * MaxPApril	LD2 * PrecipMinPreLD
full	4021.199	11.413	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x		x	x
null	4105.095	95.309																			
m1	4015.181	5.395	x	x	x					x		x	x	x	x						
m2	4013.549	3.763	x	x	x					x			x	x	x						
m3	4011.727	1.941	x	x									x	x	x						
m4	4009.966	**0.18**	x	x	x								x	x	x						
m5	4017.181	7.395	x	x	x					x		x	x	x	x						
m6	4015.108	**5.322**	x	x	x					x		x	x	x							
m7	4013.022	**3.236**	x	x	x									x	x						
m8	4014.522	**4.736**	x	x	x								x		x						
m9	4011.761	**1.975**	x	x	x								x	x	x						
m10	4011.962	**2.176**	x	x	x								x	x	x						
m11	4015.25	**5.464**																			
m12	4012.730	**2.944**	x	x	x								x	x							
m13	4014.940	**5.154**	x	x	x	x	x	x			x	x	x	x	x	x	x				x
m14	4013.724	**3.938**	x	x	x	x	x	x			x	x	x	x	x	x					x
m15	4012.836	**3.05**	x	x	x	x	x	x			x	x	x	x	x	x					x
m16	4010.933	**1.147**	x	x	x	x	x	x			x		x	x	x	x					x
m17	4014.083	**4.297**	x	x	x	x	x	x			x		x	x	x	x					
m18	4012.127	**2.341**	x	x	x	x	x	x			x		x	x	x	x					
m19	4012.279	**2.493**	x	x	x	x	x	x			x		x	x	x						
m20	4011.603	**1.817**	x	x	x	x	x	x					x	x	x						
m21	4011.376	**1.59**	x	x	x		x	x					x	x	x						
m22	4012.023	**2.237**	x	x	x			x					x	x	x						
m23	4011.09	**1.304**	x	x	x			x					x	x	x						
m24	4010.097	**0.311**	x	x	x			x					x	x	x						
m25	4010.77	**0.984**	x	x	x			x					x	x	x						
m26	4009.786	**0**	x	x	x			x					x	x	x						
m27	4011.474	**1.688**	x	x	x			x					x	x	x						
m28	4015.054	**5.268**	x	x	x			x		x			x	x	x					x	
m29	4023.753	13.967	x	x	x		x	x					x	x	x		x	x			
m30	4021.81	12.024	x	x	x		x	x					x	x	x		x				
m31	4019.905	10.119	x	x	x		x	x					x	x	x						
m32	4018.464	8.678	x	x	x		x	x					x	x	x						
m33	4016.888	7.102	x	x	x		x	x					x	x	x						
m34	4015.657	**5.871**	x	x	x			x					x	x	x						
m35	4015.545	**5.759**	x	x	x								x	x	x						
m36	4014.233	**4.447**	x	x	x								x	x	x						
m37	4012.799	**3.013**	x	x	x								x	x	x						
m38	4011.629	**1.843**	x	x	x							x	x	x	x						
m39	4009.966	**0.18**	x	x	x								x	x	x						
m40	4011.761	**1.975**	x	x	x								x	x	x						
m41	4019.836	10.05				x				x	x					x				x	x
m42	4020.725	10.939				x				x	x					x				x	
m43	4019.878	10.092				x				x						x				x	
m44	4019.413	9.627				x				x										x	
m45	4019.976	10.19								x										x	
m46	4013.462	**3.676**	x	x	x			x		x			x	x	x					x	
m47	4013.601	**3.815**	x	x	x			x		x			x	x	x						
m48	4011.775	**1.989**	x	x	x			x					x	x	x						
m49	4011.963	**2.177**	x	x	x			x					x	x							
m50	4013.015	**3.229**	x	x	x			x						x	x						
m51	4014.524	**4.738**	x	x	x			x					x		x						

```
####### Selection on LD
### VIF - Environmental variables ####
library(fuzzySim)
library(dplyr)
y<-LD.final %>%
  group_by(Year) %>%
  summarise_at(vars("TempPreLD","TempLD","MeanTApril","MeanTMay","MinTApril","MinTMay","MaxTApril","PrecipPreLD",
                    "PrecipLD","MeanPApril","MeanPMay","MaxPApril","MaxPMay","MeanTSpring","MeanPSpring","DegreeDay","NAOw",
                    "TempMinPreLD","TempMaxPreLD","TempMinLD","TempMaxLD","MaxTMay","PrecipMaxPreLD","PrecipMinPreLD",
                    "PrecipMaxLD","PrecipMinLD","MinPApril","MinPMay"   ),
               funs(mean))
names(y)
VIF<-as.data.frame(y[(2:ncol(y))])
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "MinPMay")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "MinPApril")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "TempLD")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "PrecipLD")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "MeanPSpring")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "PrecipPreLD")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "TempPreLD")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "MeanPApril")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "MeanTMay")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "DegreeDay")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "PrecipMaxLD")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "PrecipMaxPreLD")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "MaxTMay")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "MeanTApril")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "TempMaxLD")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "MeanPMay")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "TempMinPreLD")]
multicol(VIF)
VIF<-VIF[,!(names(VIF) %in% "MeanTSpring")]
multicol(VIF)


# Overall selection ####
library(glmmTMB)
```

```r
sel.ally<-glmmTMB(Recruit~SDLayingDate+I(SDLayingDate^2)+Habitat+Mate+ClassAge+zYear+
                     (1|Female)+(1|Year.f)+(0+SDLayingDate|Year.f),
                   data=dataNA,
                   family="poisson"(link = "log"))
summary(sel.ally)

sel.ally.nq<-glmmTMB(Recruit~SDLayingDate+Habitat+Mate+ClassAge+zYear+
                        (1|Female)+(1|Year.f)+(0+SDLayingDate|Year.f),
                      data=dataNA,
                      family="poisson"(link = "log"))
summary(sel.ally.nq)

AIC(sel.ally,sel.ally.nq)

### Environment-mediated selection - models ####
# Only 3 models as examples.
# To run the rest of the models described in the chapter, environmental variables and interactions need to be included as required.

library(glmmTMB)
library(MuMIn)
names(dataNA)
data.dredge<-dataNA[,c(2,13,8,9,108,5,28,26,44,45,49,104,39,97,102,98,38,42,37,44,6)]
data.dredge.nona<-na.omit(data.dredge)
data.dredge.nona$Year.f<-as.factor(data.dredge.nona$Year)
names(data.dredge.nona)

AIC.models<-c("full", "null", "m1")
AIC.values<-NULL

{full<-glmmTMB(Recruit~SDLayingDate+zYear+Habitat+Mate+Age+
                 I(zYear^2)+
                 NAOw+SDLayingDate*NAOw+I(SDLayingDate^2)*NAOw+
                 #scale(PrecipMinLD)+scale(SDLayingDate)*PrecipMinLD+I(SDLayingDate^2)*scale(PrecipMinLD)+
                 MaxTApril+SDLayingDate*MaxTApril+I(SDLayingDate^2)*MaxTApril+
                 scale(MaxPMay)+SDLayingDate*scale(MaxPMay)+I(SDLayingDate^2)*scale(MaxPMay)+
                 TempMaxPreLD+SDLayingDate*TempMaxPreLD+I(SDLayingDate^2)*TempMaxPreLD+
                 PrecipMinPreLD+SDLayingDate*PrecipMinPreLD+I(SDLayingDate^2)*PrecipMinPreLD+
                 TempMinLD+SDLayingDate*TempMinLD+I(SDLayingDate^2)*TempMinLD+
                 MinTMay+SDLayingDate*MinTMay+I(SDLayingDate^2)*MinTMay+
                 scale(MaxPApril)+SDLayingDate*scale(MaxPApril)+I(SDLayingDate^2)*scale(MaxPApril)+
                 MinTApril+SDLayingDate*MinTApril+I(SDLayingDate^2)*MinTApril+
                 I(SDLayingDate^2)*zYear+SDLayingDate*zYear+
                 (1|Female)+(1|Year.f)+(0+SDLayingDate|Year.f),
               data=data.dredge.nona,
               family="poisson"(link = "log"))
  summary(full)
  AIC.values[1]<-AIC(full)
  AIC(full)} # full: 4021.199
```

```
{null<-glmmTMB(Recruit~
                 (1|Female)+(1|Year.f)+(0+SDLayingDate|Year.f),
               data=data.dredge.nona,
               family="poisson"(link = "log"))
  summary(null)
  AIC.values[2]<-AIC(full)
  AIC(null)} # null: 4105.095
{m1<-glmmTMB(Recruit~SDLayingDate+zYear+Habitat+Mate+Age+
               MaxPApril+NAOw+MinTApril+MinTMay+MaxTApril+
               #I(zYear^2)+I(SDLayingDate^2)+
               #SDLayingDate*MaxPApril+
               #SDLayingDate*MaxPApril+SDLayingDate*NAOw+SDLayingDate*MinTApril+SDLayingDate*MinTMay+SDLayingDate*MaxTApril+
               #I(SDLayingDate^2)*NAOw+I(SDLayingDate^2)*MinTApril+I(SDLayingDate^2)*MinTMay+I(SDLayingDate^2)*MaxTApril+
               #I(SDLayingDate^2)*zYear+SDLayingDate*zYear+
               (1|Female)+(1|Year.f)+(0+SDLayingDate|Year.f),
             data=data.dredge.nona,
             family="poisson"(link = "log"))
  summary(m1)
  AIC.values[3]<-AIC(m1)
  AIC(m1)} # m1: 4030.849

AICm.results<-as.data.frame(cbind(AIC.models,AIC.values))
AICm.results$AIC.values.n<-as.numeric(AICm.results$AIC.values)
AICm.results$DAIC<-AICm.results$AIC.values.n-min(AICm.results$AIC.values.n)
min(AICm.results$AIC.values)
```

Chapter 3

Response to selection of breeding date under environmental variation

Justine Le Vaillant, Jaime Potti, Carlos Camacho, David Canal, Jip Ramakers, Marcel Visser & Jesús Martínez-Padilla

Evolutionary responses to selection are determined by the strength of selection acting on a phenotypic trait in covariation with the heritable part of the trait. The lack of a micro-evolutionary response in breeding time in birds, despite consistent directional selection towards early breeding, is a textbook example where this expectation is not being fulfilled and the contributions of selection and genetic variation are not well understood. In a long-term study of an individually-based monitored population of pied flycatchers (*Ficedula hypoleuca*), we explore whether the genetic variance of laying date covaries with the environment. Using 'animal models', we also test whether there are phenotypic shifts over time in our population, which would be explained either by microevolution or genetic drift. We did not find evidence for selection on breeding time or support for genetic change or genetic drift. Furthermore, the additive genetic value of breeding time is small (around 11%). However, additive genetic variance does not display any trend related to the multiple environmental factors influencing selection for early breeding date. Despite interactions of environmental factors with selection and genetic variation (plasticity) being paramount in the study population, the lack of covariance between gradients of selection and additive genetic factors would no produce an evolutionary response and hence a change in breeding time.

Unpublished Manuscript

Introduction

Forecasting the evolutionary response to selection in phenotypic traits requires understanding the strength of selection acting on the traits and their genetic architecture. Response to selection is a fundamental tool to quantify and therefore understand local adaptation of populations under environmental change. However, the strength and direction of selection in a population vary with environmental conditions over time (Grant & Grant, 1995; Siepielski et al., 2013; Siepielski et al., 2017; Wood & Brodie III, 2016). Moreover, the magnitude of the genetic and environmental components in the expression of phenotypic variation may also change across environmental conditions (Kruuk et al., 2008). Thus, predictions of response to selection in natural populations are challenging and many studies have failed in demonstrating any apparent evolutionary response (Merilä, 2012; Merilä & Hendry, 2014; Merilä et al., 2001a; Merilä et al., 2001b). Despite the presence of directional selection at phenotypic level has been often detected, very little is known about the response to selection in a variable environment. However, such phenotypic selection does not imply evolution unless it is measured at a genetic level.

To understand and predict how populations can evolve under scenarios of environmental change, several methods have been developed. The breeder's equation (or Lande's equation, Lande, 1979), traditionally developed in animal breeding, is not the most suitable for wild populations (Morrissey et al., 2010). The limitation particularly occurs in situations where environmental covariance between the trait and fitness exists, or when other traits that are not included in the analysis influence the trait and fitness (Gienapp et al., 2014; Kruuk et al., 2008; Merilä et al., 2001b). The Roberson-Price equation has been proposed to estimate selection at the genetic rather than the phenotypic level (Rausher, 1992; Stinchcombe et al., 2002). However, this equation often fails to explain the lack of response in natural populations (see supplementary materials in Husby et al., 2011b, and Gienapp et al., 2006). Indeed, few studies in natural population match the predictions in responses to selection (Pujol et al., 2018) and evolutionary inferences to explain a phenotypic pattern need to include deeper description at the genotypic level (Merilä et al., 2001b). Shortcoming might also be due to the metric of the response to selection used, inaccurate estimates of selection (but see Dingemanse et al., 2021 and/or environmental factors considered for covariance between traits and fitness Rausher, 1992; Stinchcombe et al., 2002). As a better alternative, the 'random regression animal model' approach (i.e $RRAM$, see Kruuk, 2004, use covariance functions to fit variance components as a function of the environment. Each phenotype of an individual is modelled as its additive genetic merit (breeding value) as a function of the environment, hence allowing to test how environmental heterogeneity influences genetic variation. The predicted breeding values ($PBVs$) extracted from the selected animal model across cohorts or generations can be used to illustrate the evolutionary changes happening at the genetic level in a population, particularly when individuals in populations experience environmental variation over time. It is therefore possible to use the temporal trend in $PBVs$ to infer the evolutionary response of quantitative traits to natural selection (Réale et al., 2003; Wilson et al., 2007a) and to test for micro-evolution (Postma, 2006) and hence the genetic influence on phenotypic variation. With the pedigree information available, models also permit to test whether the genetic variation for a trait's plasticity influences heritability according to environmental variation. However, only a handful of studies that account for uncertainty in the predictions of breeding values have investigated genetic changes under a quantitative genetic framework in wild populations (Bonnet et al., 2019; Bonnet et al., 2017; Evans & Gustafsson, 2017; Gienapp & Merilä, 2014), along artificial selection experiments (Pigeon et al., 2016; Verhagen et al., 2019a). These studies mostly analysed morphological traits, thus our knowledge about the role of microevolution in wild populations is still poor, particularly for life-history traits.

Predicting evolutionary change in a natural population also depends on the environmental context and factors being considered. While the strength, form, and direction of selection can be easily quantified (Lande & Arnold, 1983), the environmental features imposing selection on phenotypes may not be that simple (van de Pol et al., 2016). However, controlling for environmental variables while conducting selection analyses may lead to different conclusions and environmental variables may not necessarily influence all components of selection in the same way. Yet, by choosing a priori a single environmental driver, one can miss important causes of the observed phenotypic change and predict inaccurate responses (Bourret & Garant, 2015; Charmantier & Gienapp, 2014; Le Vaillant et al., 2021; Merilä & Hendry, 2014). Multiple potential environmental drivers of the observed phenotypic changes are rarely studied exhaustively, despite the fact that more than one environmental factor may be facilitating or constraining the observed responses (Merilä & Hendry, 2014). Therefore, to obtain a more comprehensive picture of the evolutionary adaptation of wild populations quantifying the relative influence of multiple environmental factors influencing selection that act on life-history traits is still required. In addition, understanding the evolutionary mechanisms that result in local adaptation is compulsory to forecast population persistence under climate change scenarios (Both & Visser, 2005; Gienapp et al., 2014; Visser et al., 2015). Moreover, climatic change should be understood from a multidimensional perspective by considering various climatic factors to infer evolutionary response of life-history traits, particularly on those tightly linked to environmental variation and affected by climate change, like the time of breeding.

In a Spanish population of pied flycatchers (*Ficedula hypoleuca*), we explored the genetic variance of laying date in response to environmental variation over three decades. In this population, we observed variability in environmental conditions and in selection, but not a parallel shift in breeding date (Le Vaillant et al., 2021). With nearly three decades of monitoring during the reproductive period and the available pedigree, we can test for the genetic variation in the population over the years and the environmental conditions. Given the stasis in laying date (Le Vaillant et al., 2021), we do not expect to detect microevolutionary change. From response to selection's theory, we expect to find low heritability for laying date as previously found in others studies (see ESM Appendix A - Literature review). Our study offers important information to better understand the lack of phenotypic change in phenology observed in many wild populations (see Price et al., 1988 despite directional selection for early laying date.

Material methods

Study system

Data were obtained between 1987 and 2016 in a population of pied flycatchers breeding in nest-boxes in central Spain (La Hiruela, 41°04′ N, 3°27′ E). The population occupies an old deciduous forest dominated by oaks (*Quercus pyrenaica*) and a nearby (1 km) mixed coniferous plantation dominated by *Pinus sylvestris* (see Camacho et al., 2015 for a description of the study area). Individuals return from the wintering grounds from the third week of April (Potti, 1998a). Males are the first to arrive at the breeding site to hold a territory before the arrival of females about one week later on average (Potti & Montalvo, 1991b).

Nest-boxes (n=237) were inspected every 2-3 days from the beginning of the breeding season (first arrivals from spring migration) to record laying date (date of first laid egg), clutch size (typically 5-6 eggs), number of hatchlings (eggs hatched) and number of fledglings (chicks on day 13 post-hatching). Laying date was recorded for 1544 breeding attempts by 671 different females. Breeding males and females were captured using a nest-box trap, individually marked with colour (males) and metal (both sexes) rings, measured for tarsus length (±0.05 mm) and weighed (±0.1g). Many birds breeding in our nest boxes were of known age because they were first ringed as chicks. Unringed birds were aged as either one year or older following the criteria of Karlsson et al., 1986. All fledglings were ringed at the age of 13 days old, enabling us to follow their fate (return/no return) in the following years as a proxy of recruitment. Because of a reduced adult capture rate compared with the rest of the years, 2002 and 2003 were excluded from the analyses described below (see – statistical approach).

Environmental conditions

We focused on 9 climatic variables which have been shown to act on selection on laying date at the phenotypic level based on offspring recruitment in this population (Le Vaillant et al., 2021). Those variables act during the breeding period or a few weeks before laying of the first clutches: maximum and minimum values for temperature and rainfall indexes before the start of egg formation (from 10 to 3 days before laying date or pre-laying period see Potti, 1999 and Le Vaillant et al., 2022, during the laying stage (from 3 days before laying the first egg to the date of the last egg laid (Birkhead et al., 1997), and in April and May. Outside the breeding period only the North Atlantic Oscillation in winter (NAOw) influences selection. Indeed, conditions during the non-breeding season also determine selection on laying date in other pied flycatcher populations (Ahola et al., 2004; Both et al., 2006b), with positive values of NAO in winter being considered as adverse conditions (Przybylo et al., 2000).

Pedigree Structure

Relationships among individuals in the population (pedigree) were constructed from breeding females that were ringed as chicks. We assigned a mother and father to females from observational data on nest defence and attendance. Extra-pair paternity (*EPP*) may lead to inaccurate estimations of variances and covariances in the social pedigree due to misassigned paternities. However, a two-year study suggests that *EPP* rate in our population is not high, around 15% (Canal et al., 2012), below the threshold of 20% considered to impact estimates of additive genetic variance (Charmantier & Réale, 2005). In nests where brood manipulation (cross-foresting) experiments had been carried out, chicks or entire broods were removed from the data set to not confound the maternal effect due to the social parent rather than the genetic parent. If only one parent was known (2.35% from 3315 informative individuals), the missing parent was assigned a "dummy code" to avoid losing the information of the sibship into the pedigree. Overall, we had 14,973 records of 12 generations depth (see Appendix B - Pedigree) with 1,910 founders and with 25,531 full siblings for 12,956 maternities (mean maternal sibship size of 8.10) and 12,299 paternity records (mean paternal sibship size of 8.46).

Statistical approach

Yearly trends

We used linear models (LM) to assess the association between environmental factors and year. To explore the temporal trend in laying dates, we used Linear Mixed Models (LMM) with year (treated as a continuous variable), habitat (class: oak vs. pine) and bird age (class: one year vs. older) as explanatory terms. As random terms, we included female ID and year, treated as a categorical variable, to account for pseudo-replication and stochastic variation among years (see e.g. Evans and Gustafsson, 2017 for a similar approach). Models were fitted with the Packages 'lme4' (Bates et al., 2015) and 'lmerTest' (Kuznetsova et al., 2015) in `R` version 3.5.3 (R Core Team, 2017).

Testing for evolutionary (genetic) change

We fitted univariate "animal models", with egg-laying date as the phenotype of interest, to partition the total phenotypic variance (V_P) into genetic and environmental components, allowing us to calculate heritability and temporal variation in breeding values (see Kruuk, 2004). Animal models allow inclusion of fixed effects and random factors to account for known influences on the phenotype. Fixed effects in these models were factors that may influence laying date: habitat, to take into account potential consistent variation in the mean laying date between the oak and pine forests (unpublished data); mate's mating status, coded as "monogamous" and "primary/secondary female" (Canal et al., 2020); female age, coded as "one year old" or "older"; and, finally, female body mass, as representative of female size and condition. Bird origin (immigrant or locally born) was not included in the model since it did not explain much of the total variance in laying date (less than 1% - analyses not shown). The random effects split the variance non accounted for by the fixed effects and we considered the following: additive genetic variance (a); permanent environment effect, which considers the repeated measures of the same female over the years (p); mate (*male*) identity, variance associated with the year (y); and residual variance (ε). Thus, the model we fitted on laying date (z) for each female i was as follow:

$$z_i = Xb_i + Z_1 a_i + Z_2 p_i + Z_3 y_i + Z_4 mate_i + \varepsilon_i \tag{1}$$

Where z_i is the vector of individual trait values, X is the matrix of fixed predictors, b is a vector for fixed effects and Z_1, Z_2 and Z_3 are the design matrices that link fixed random effects to additive genetic variance (a_i), permanent environmental effects ($p\varepsilon_i$), year effect (y_i) and mate (*male*) identity ($mate_i$). ε_i is the error term or residual variance not explained by fixed or random factors. We calculated the narrow-sense heritability of laying date (h^2) as the proportion of additive genetic variance for laying date σ^2_A (z) divided by the total phenotypic variance ($V_P = V_A + V_{PE} + V_Y + V_{mate}$, see model (1)).

We inferred the yearly estimate of the rate of evolution (breeding value) and evolvability (variance of breeding values) through the univariate approach based on best linear unbiased predictors (*BLUPs*, Hadfield et al., 2010) from model (1). The *BLUPs* for the additive genetic effect (individual breeding values) were extracted from this model and were subsequently fitted for yearly estimates according to the number of individuals by years. As a result, we computed the posterior distribution of slopes of changes in mean breeding values across generations over the years. To test for true evolutionary change, we regressed the mean breeding value of each female breeder each year over time, which is the temporal change in annual mean breeding values for each 7450 iterations. The distribution of the regression coefficients was considered to be the posterior distribution of the genetic change. To reliably confirm any evolutionary change, we compared the temporal variation of breeding values with that expected by chance, i.e. by genetic drift (Hadfield et al., 2010). The potential contribution of genetic drift on evolutionary change was inferred by using 7450 neutral samples from the pedigree. Considering such neutral sampling, the expected distribution of slopes is 0. We simulated genetic drift down the pedigree of the population following Hadfield et al., 2010 and using the function `rbv()` in `MCMCglmm` of the R-package `pedantics` (Morrissey & Wilson, 2010) in `R` version 3.5.3 (R Core Team, 2017). Thus, the rate of evolutionary change in laying date was estimated by regressing the mean annual breeding value of female breeders against time for each of the sample iterations (2000). Annual mean breeding values (additive genetic variance) are then regressed against environmental conditions to test for the direction of response in a continuous environment.

Results

Evolutionary change

Laying date did not advance over the study period, nor did environmental conditions change; except for an increase of minimum temperatures in April, no other environmental index showed a clear trend across years (see Le Vaillant et al., 2021).

The models are based on independent estimates of the rate of evolution using best linear unbiased predictors ($BLUPs$) for laying date. Our results show that BLUPs for laying date do not change over time (Figure 1, r=-3.991e-3±SD 7.884e-3, t=-1.0.13, p=0.32, 26 df). The simulated means of breeding values (genetic drift) show no tendency overtime time (-2.846e-4±SD 5.101e-3, t=-0.056, p=0.956, df=26). The heritability of laying date was 0.114 (HPD95 interval from 0.034 to 0.186).

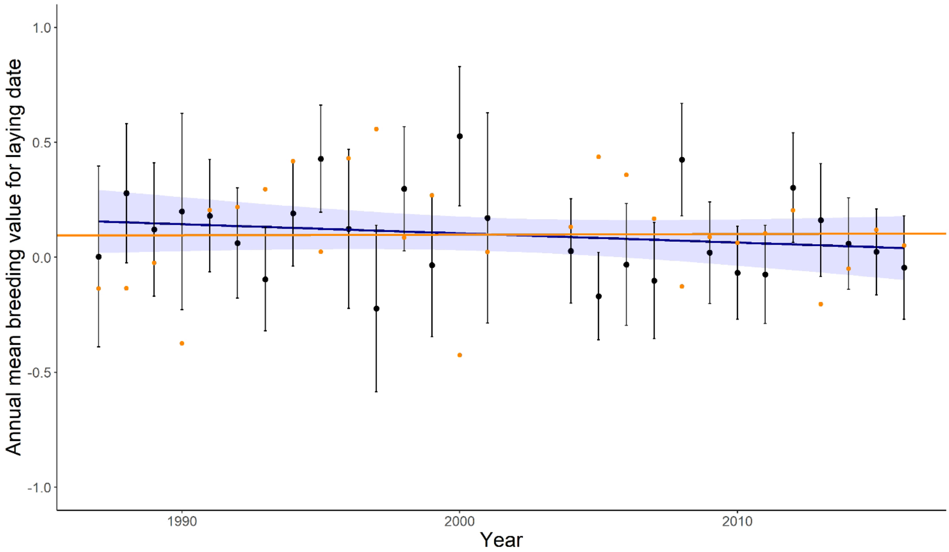


Figure 1: Change in the breeding value in time against genetic drift. Annual estimates of breeding value (±SD) and linear regression of breeding value with time. The orange points show the annual simulation of genetic drift with the orange line for the mean regression against time.

Estimates of evolutionary change of breeding values for laying date (Figure 2) show no shift related to genetic drift (mean=-4.559e-3, HPD95: -2.26e-2;1.921e-2) as the simulated genetic drift (mean=-9.012e-5, HPD95:-2.49e-2.371e-2) largely overlaps with the estimated posterior distribution of $BLUPS$. This means that a change in breeding values is unlikely to have been produced solely by genetic drift.

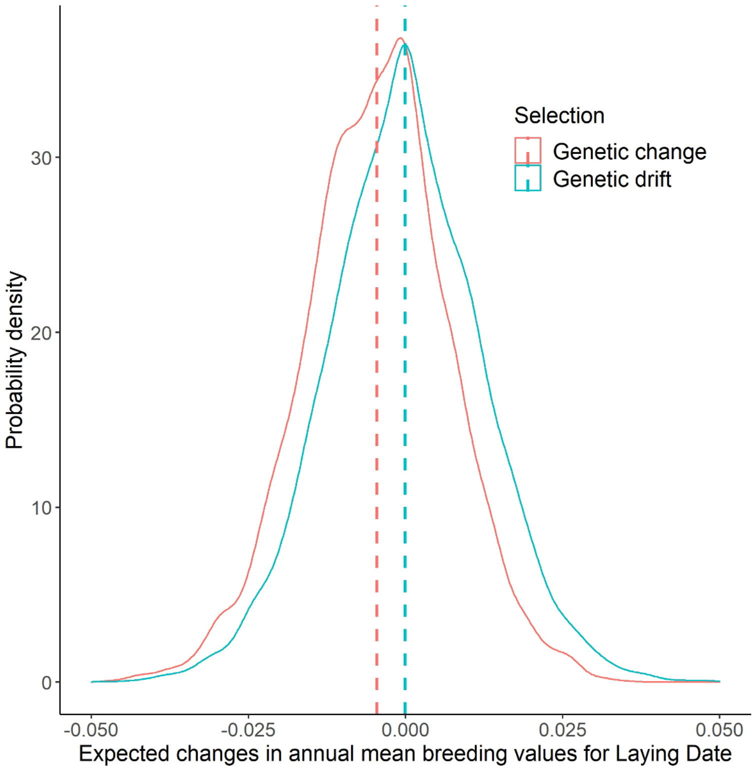


Figure 2: Estimation of the time trajectory of genetic change and evolutionary trajectories simulated when assuming genetic drift. The orange line shows the mode and 95% CI of the rate of evolution estimated with an animal model. The blue lines show 2000 simulations of genetic drift, based on the real population pedigree and on the posterior distribution of genetic variance for laying date estimated by the animal model. Dotted lines represented the mean for respective breeding value and genetic drift.

Expected response to selection

Overall, breeding values do not seem to be influenced by the multiple environmental factors tested over the years (see legend - Figure 3). There is only an exception, with a slight but significant trend observed with minimum temperatures in April (Figure 3, r=-3.501-2±SD 1.697e-2, t=-2.063, p=0.049, 26 df), which is the only environmental factors displaying a subtle increase along the decades (see Le Vaillant et al., 2021).

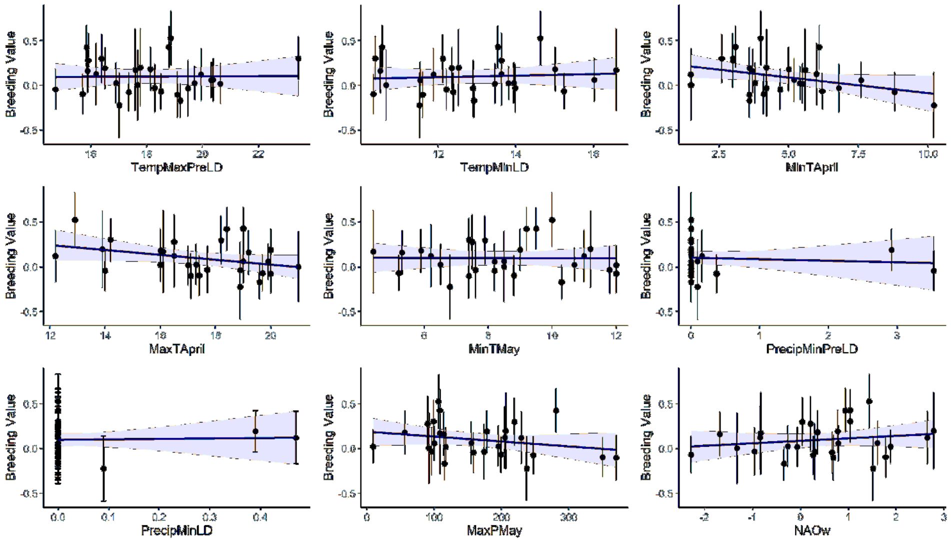


Figure 3: Linear regression between mean breeding value of laying date and various environmental conditions. TempMaxPreLD= Maximum temperature for the prelaying period; TempMinLD= Minimum temperature during laying; MinTApril/MinTMay=Minimum temperature in April/May, MaxTApril=Maximum of Temperature in April; PrecipMinPreLD= Minimum of precipitation for the prelaying period; PrecipMinLD= Minimum of precipitation during laying; MaxPMay=Maximum precipitation in May; NAOw=North Atlantic Oscillation in winter.
Linear regression for TempMaxPreLD: r=9.703-4±SD 1.894e-2, t=0.051, p=0.960, 26 df; TempMinLD: r=8.556-3±SD 2.114-2, t=0.400, p=0.693, 26 df; MinTApril: r=-3.501-2±SD 1.697e-2, t=-2.063, p=0.049, 26 df; MaxTApril: r=2.748-2±SD 1.478e-2, t=-1.859, p=0.074, 26 df; MinTMay: r=8.433-4±SD 1.717e-2, t=-0.049, p=0.961, 26 df; PrecipMinPreLD: r=1.688-2±SD 4.315e-2, t=-0.391, p=0.699, 26 df; PrecipiMinLD=: r=5.073-2±SD 3.220e-1, t=0.158, p=0.876, 26 df; MaxPMay: r=5.485-4±SD 4.204e-4, t=-1.305, p=0.203, 26 df; NAOw: r=2.766-2±SD 2.926e-2, t=0.945, p=0.353, 26 df

Discussion

A lack of phenotypic change in response to environmental variation is often observed in wild populations. In our study, the chances that a microevolutionary change cause a shift in breeding time are low, as also suggested by the low trait heritability. The low estimate of additive genetic variance could explain the lack of response to selection. However, the absence of consistent covariation between additive genetic variation for breeding time and the environmental gradient might explain the lack of evolution of breeding date, despite an expected change for early laying date in more adverse conditions for breeding.

Lack of microevolutionary change

Our estimate of heritability of laying date (ca. 11%) is similar to values found in other well studied songbirds (Gienapp et al., 2006; Sheldon et al., 2003) and slightly under the range reported for this life-history trait (between 0.15-0.30, but see ESM Appendix A - Literature review). The estimates of annual (predicted) breeding values did not change over time (Figure 1), indicating a lack of genetic change in the population. In addition, there is no possibility to tease apart the expected genetic change from a random evolutionary change simulating genetic drift (Figure 2). Our results do not support a genetic change of breeding time in our population, as inferred from overlapping temporal trend in $BLUPs$ to that expected from genetic drift (Hadfield et al., 2010). Nevertheless, we did not obtain conclusive evidence on whether the small magnitude of the genetic estimate alone can explain the observed lack of change in breeding time. A non-significant covariation between gradient of selection and additive genetic variance can constrain the relevant response of selection (Husby et al., 2011a). Therefore, a negative correlation between strength of selection and genetic variance can contribute to evolutionary stasis and explain the lack of response to selection often found in natural populations (Bonnet & Postma, 2018; Merilä et al., 2001b).

Minimum temperatures in May and April are the main drivers of selection on laying date at the phenotypic level (see Le Vaillant et al., 2021). The lack of temporal change in $BLUPs$ is therefore not surprising given the temporal variation in temperatures and, as a consequence, stasis in breeding time (Figure 11. In addition, environmental coupling of heritability and selection appears to be rare in wild populations and limits the evolutionary response (Merilä et al., 2001b; Ramakers et al., 2018b; Wilson et al., 2006). However, although temporal variation in environmental indexes is small, there is wide heterogeneity in the additive genetic values (Figure 3), suggesting that in our population there is raw genetic material that allow individuals to cope with changing environmental conditions. Despite selection for early laying date (see Le Vaillant et al., 2021), variation in the phenotype might rather be due to environmental than genetic effects. A possibility is that our population is adapting to environmental variation across years through plasticity rather than through a genotypic response (Teplitsky et al., 2008). As far as we are aware, there are no studies exploring the role of any environmental factor in the evolutionary change of breeding time in birds, perhaps due to the low chances of finding such a pattern for a phenotype highly influenced by environmental variation. There is only a recent study on parturition date in mammals (Bonnet et al., 2019) using the same comprehensive quantitative genetic models than those used here. Given the importance of environmental heterogeneity for the detection and interpretation of individual and genotypic variance in phenotypic traits, such a test is needed to fully comprehend how evolution acts on the expression and variance of traits highly linked to the environmental variation, particularly when the study aims to test other evolutionary mechanisms in action.

Expected response to selection

Predictions of the response to selection in natural populations are hard to validate, especially those stemming from the relatively few studies to date using the classical breeders equation (see (Kruuk et al., 2008; Merilä et al., 2001b)). Whatever the direction and the strength of selection, a relationship between genetic variance and selection should result in a small evolutionary response in the population (Wood & Brodie III, 2016). Moreover, few predictions in natural populations match the actual responses to selection (Pujol et al., 2018), as wild population have to face heterogeneous environments and developed mechanisms of adaptation in accordance with such heterogeneity to evolve. However, selection does not equal evolution (Gienapp & Merilä, 2014; Ozgul et al., 2009) and in our study, the intensity of negative selection increases (more negative), as minimum temperatures decreases (Le Vaillant et al., 2021). In our population, minimum temperatures increase overtime and additive genetic variation ($BLUPs$) decrease as minimum temperatures increases (Figure 3). Therefore, an increase in minimum temperature constrains evolutionary change of laying date. This limitation may reduce the potential response to selection of laying date and suggest a lack of potential adaptation in our population in response to this environmental variable. Two alternatives can emerge to explain the lack of phenotypic variation in our population, phenotypic plasticity and the complexity of environmental factors that an individual experience throughout its life.

First, environmental fluctuations underlie the temporal dynamic of adaptive evolutionary change (Siepielski et al., 2009) and might support here the presence of phenotypic plasticity in this songbird population (but see Le Vaillant et al., 2022). In addition, phenotypic plasticity can facilitate adaptive response for population, and besides it has been proved to evolve in the presence of genetic variance or genotype-by-environment interactions. However, the presence of genetic variance for plasticity necessarily means a change in the amount of the genetic variability (or additive genetic variance, V_a) with variable environmental conditions (Hoffmann & Merilä, 1999). Currently, our knowledge about the role of genetic versus phenotypic changes, via plasticity, to explain evolutionary adaptation of wild animal populations is as limited as contradictory (Bonnet et al., 2019; Bonnet et al., 2017; Kruuk, 2017). Therefore, it has been difficult to distinguish phenotypic response due to plasticity from genetic change in long term studies (Gienapp et al., 2008; Merilä, 2012; Merilä & Hendry, 2014). Secondly, our results illustrate the idea that there are multiple environmental factors that may influence selection at both phenotypic (Le Vaillant et al., 2021) and genetic level (this study). However, the multidimensional nature of the environmental conditions individuals experience both during breeding and wintering (Le Vaillant et al., 2021), preclude assigning to a single environmental factor the cause to evolution and local adaptation at least in a wild and open population. Instead, the concurrent action of multiple factors that change in intensity and selection overtime can explain the lack of evolutionary change in a life-history trait like breeding time.

Conclusions

The environmental influence on selection at genetic level in breeding time in our study population is small and insufficient to cause a shift of the mean phenotype. In addition, multiple environmental factors acting at the same time might complex an evolutionary response to selection. If plasticity can explain most of the observed trend, testing for adaptive plasticity is however seems a more complicated task (Ghalambor et al., 2007; Via et al., 1995).

Acknowledgments

We thank many volunteers, technicians and friends, too numerous to mention individually, that helped with data collection. J.L.V received financial support from the Spanish Ministry of Economy and Competitiveness (grant BES-2016-077694). CC received financial support from the Spanish Ministry of Science, Innovation and Universities (FJC2018-038412-I). DC was supported by a grant from the Hungarian Academy of Sciences (MTA Premium Postdoctoral Research Program; ID: 2019-353). J.M.-P. was supported by the Spanish Commission of Science and Technology (CICYT, grant number CGL2015-70639-P and PID2019-104835GB-I00) and is currently funded by ARAID foundation. Fieldwork was supported mainly by CICYT, most recently by projects CGL2014-55969-P and CGL2015-70639-P.

Supplementary Material

Appendix A - Literature review

We reviewed all published studies that quantified heritability of laying dates using the Web of Science bibliographic database in April 2021. We first conducted a literature search for the terms “laying date” AND “heritability”, which resulted in 70 references. Alternative search terms as ‘breeding date’ or ‘breeding time’ did not yield improved results over those with the term ‘laying date’. From all references obtained, we only considered those studies that were performed in wild bird populations and analyzed heritability of laying date under animal model framework (using MCMC glmm or ASRELM-R method, 22 references) as parent-offspring regression are supposed to be less accurate method for heritance estimates . In addition, we complemented our search with publications (n = 8) cited in other articles, but not sorted out by Web of Science, that also gave heritability estimates based on the animal model method.

Table 1: Review of publications that have studied heritability of laying date in avian populations. Sex (F: females, M: males). Observations is the number of observation of the model, N is the number of individuals and Years the numbers of study years. Error estimates for heritability when precise in the study, are presented as (±SE), [CI 95%] or (HDP).

References	**Species**	**Sexe**	**Observations**	**N**	**Years**	**h^2**
Vatka et al., 2020	*Poecile montanus*	F	3331	1950	43	0.13-0.2
	Parus major	F	3903	3187	49	0.24-0.43
Moiron et al., 2020b	*Sterna hirundo*	F	1106	209	26	0.12 (0.08)
Villemereuil et al., 2019	*Notiomystis cincta*	F	na	1207	17	0.02 (0.03)
	Notiomystis cincta	F	na	1207	17	0.03 (0.04)
Evans et al., 2020	*Parus major*	M	8189	5649	52	0.02 (0.01)
Sauve et al., 2019	*Cepphus grylle mandtii*	F	3782	954	42	0.03 [0;0.09]
	Cepphus grylle mandtii	F	3782	954	42	0.04 [0;0.11]
Verhagen et al., 2019a	*Parus major*	F	2045	120	20	0.42 (0.22)
Gienapp et al., 2019	*Parus major*	F	2015	2015	na	0.24 (0.07)
	Parus major	F	2015	2015	na	0.23 (0.21)
	Parus major	F	2015	2015	na	0.19 (0.06)
	Parus major	F	2015	2015	na	0.17 (0.06)
Gienapp et al., 2017	*Parus major*	F	4624	4032	na	0.14 (0.05)
	Parus major	F	3737	3019	na	0.38 (0.06)
	Parus major	F	4147	3532	na	0.41 (0.06)
Ouwehand et al., 2017	*Ficedula hypoleuca*	F	102	44	4	0.33
Dobson et al., 2017	*Sterna hirundo*	F	2787	2377	17	0.27 (0.09)
Germain et al., 2016	*Melospiza melodia*	F	1040	518	38	0.07 [0.04;0.10]
	Melospiza melodia	M	1040	483	38	0.02 [0.01;0.03]
Reed et al., 2016	*Parus major*	F	2714	1663	51	0.17 (HDP 0.12-0.29)
	Parus major	F	4062	2871	59	0.16 (HDP 0.01-0.20)
Visser et al., 2015	*Ficedula hypoleuca*	F	na	na	na	0.33 [0.25–0.39]
Gienapp et al., 2013b	*Parus major*	F	na	na	58	0.17 (0.09)
	Parus major	F	na	919	58	0.08 (0.02)
Kim et al., 2012	*Hydrobates pelagicus*	F	129	230	18	0.19 (0.11)
	Hydrobates pelagicus	F	70	132	18	0.33 (0.16)
	Hydrobates pelagicus	F	59	98	18	0.09 (0.16)
Liedvogel et al., 2012	*Cyanistes caeruleus*	F	3090	1158	7	0.02 (0.02)
Husby et al., 2011a	*Parus major*	F	3852	2394	32	0.10
Teplitsky et al., 2010	*Larus n.scopulinus*	F	611	207	29	<0.01 (0.03)
	Larus n. scopulinus	M	611	404	29	0.13 (0.03)
Husby et al., 2010	*Parus major*	F	3589	2243	33	0.09
	Parus major	F	7213	4698	33	0.13
Caro et al., 2009	*Cyanistes caeruleus*	F	454	47	14	0.43 (0.07)
	Cyanistes caeruleus	F	1128	276	29	0.20 (0.12)
Brommer and Rattiste, 2008	*Larus Canus*	F	11624	2262	37	0.11 (0.01)
Garant et al., 2008	*Parus major*	F	3357	2285	16	0.09 (0.03)
	Parus major	F	3575	2450	24	0.16 (0.03)
Brommer et al., 2008	*Larus canus*	F	10652	1916	37	0.15
Gienapp et al., 2006	*Parus major*	F	na	1314	29	0.17 (0.03)
Nussey et al., 2005c	*Parus major*	F	2195	833	31	0.30 (0.14)
Postma, 2005	*Parus major*	F	951	673	21	0.13 (0.11)
	Parus major	F	4819	2946	47	0.18 (0.03)
	Parus major	F	1593	1165	49	<0.01
	Parus major	F	1093	648	40	0.22 (0.05)
	Parus major	F	2613	1426	45	0.23 (0.04)
	Parus major	F	738	547	34	0.34 (0.08)
	Parus major	F	1023	724	18	0.24 (0.11)
	Parus major	F	820	565	11	0.11 (0.12)
	Parus major	F	na	na	na	0.19 (0.02)
Brommer et al., 2005	*Ficedula albicollis*	F	2726	1126	23	0.05 (0.01)
McCleery et al., 2004	*Parus major*	F	na	1777	39	0.16 (0.06)
Sheldon et al., 2003	*Ficedula albicollis*	F	7268	1523	20	0.19 (0.04)
Merilä et al., 2001b	*Ficedula albicollis*	F	na	na	na	0.19 (0.04)

```
####### PEDIGREE
library(pedantics)
##Built pedigree with individuals with phenotype
length(unique(ped.f$id))   #n=1607
length(unique(ped.f$dam))   #n=1600
length(unique(ped.f$sire))   #n=1455

# Only individuals that are informative (with phenotypic trait)
ped.a <-prunePed(ped = ped.f, keep = phen.f$animal)
ped.fix<-fixPedigree(ped.f, dat = NULL) #3132 observations

##Total pedigree
length(unique(ped.t$id)) #n=13266
length(unique(ped.t$sire))#n=1455

ped.fix<-fixPedigree(Ped=ped.t[, 1:3], dat = ped.t[, c(1,4:length(ped.t))
     ]) # 13973 observations
colnames(ped.fix)[4:length(ped.t)] <- colnames(ped.t)[4:length(ped.t)]
ped.stats <- pedigreeStats(Ped=ped.fix[, 1:3], lowMem=TRUE,
     graphicalReport = "n")

### Analyse pedigree
ped.stats$pedigreeDepth   #nb of generation = 13
Fdepth <- as.data.frame(ped.stats$pedigreeDepth, stringsAsFactors=FALSE)
Fdepth[,1]<-as.numeric(Fdepth[,1])   #1910 founders
ped.stats$totalFullSibs #25531
ped.stats$totalMaternalSibs - ped.stats$totalFullSibs #44630
ped.stats$totalPaternalSibs - ped.stats$totalFullSibs #44776
inbredC<-ped.stats$inbreedingCoefficients
mean(inbredC) # 0.000948452
mean(inbredC[inbredC>0]) # 0.0940475
max(inbredC) # 0.25

library(pedigree)
mean(countGen(ped.fix[,1:3])) #2.572297
f <- calcInbreeding(ped.fix[,1:3])
length(f[f>0])/length(f) #0.01008482
pedStatSummary(ped.stats)

##Phenotyped individuals: females (sex=2)
phenotyped <- ped.fix$id[ped.fix$sex==2]
ped.stats.pruned <- pedigreeStats(Ped=ped.fix[, 1:3], dat=phenotyped,
     lowMem=TRUE, graphicalReport = "n")
pedStatSummary(ped.stats, ped.stats.pruned)

#Representation Pedigree
drawPedigree(ped.fix[1:3], dots='y')
##with name cohort (=year)
ped.fix<-ped.fix[!is.na(ped.fix$YEAR), ]   ##!! 2003 missing cohort ->
     -1708 ind unknown
drawPedigree(ped.fix[1:3], dots='y', cohorts = ped.fix$YEAR,
     writeCohortLabels="y", sexColours=c('darkred','darkblue'))
##with only female (in grey, individual without phenotypic traits)
drawPedigree(ped.fix[1:3], dots='y', cohorts = ped.fix$YEAR,
     writeCohortLabels="y", dat=phenotyped)
#Individuals in full pedigree:13266
#Individuals in informative pedigree subset: 1089
```

Appendix B - Pedigree

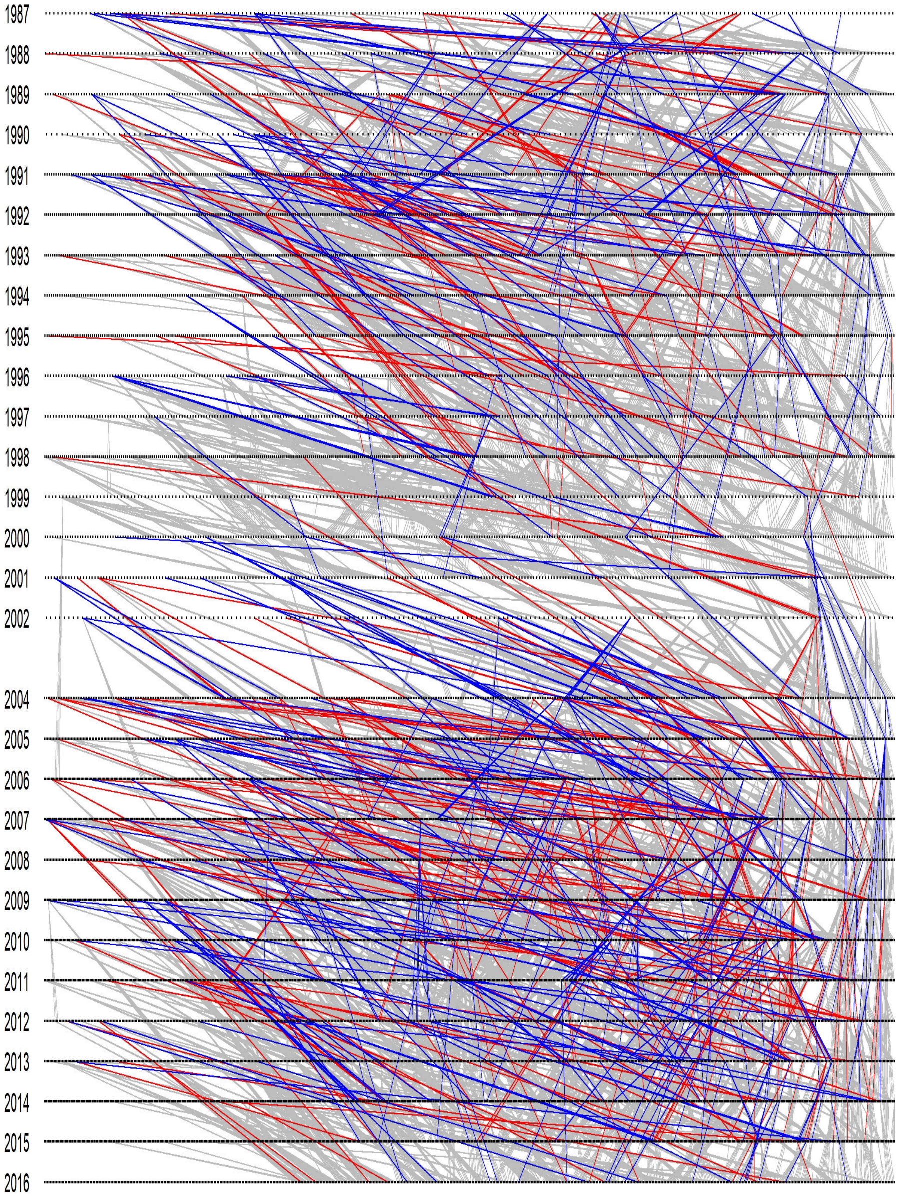


Figure 1: Social pedigree of the population: 13 generations of 1089 informative phenotypes or recruits females (coloured lines) from 13 266 offspring (coloured and grey lines) with 1 599 maternities (red) and 1 454 paternities (blue).

```
################# Genetic change analyses

####Model quantitative genetic
library(MCMCglmm)
prior1<-list(R=list(V = 1, nu = 0.002),
               G=list(
                 G1=list(V = 1, nu = 0.002),
                 G2=list(V = 1, nu = 0.002),
                 G3=list(V = 1, nu = 0.002),
                 G4=list(V = 1, nu = 0.002)))

modelGI<-MCMCglmm(LD~AGE+HBT+MASS+MATE,
                   random=~animal+ID+YEAR+MALE,
                   family="gaussian",
                   prior=prior1,
                   data=phen.f,
                   pedigree=ped.fix,
                   pr=TRUE,
                   burnin=100000,
                   nitt=15000000,
                   thin=2000)

autocorr.diag(modelGI$Sol)
autocorr.diag(modelGI$VCV)
mean(effectiveSize(modelGI$Sol))
effectiveSize(modelGI$VCV)
heidel.diag(modelGI$VCV)    # exceed 0.05
summary(modelGI)
modelGI$DIC    #9580.91

#########Heritability estimate
# VARIANCE ESTIMATES
Va.T <- modelGI$VCV[,"animal"]
posterior.mode(Va.T)
HPDinterval(Va.T)

Vpe.T <- modelGI$VCV[,"ID"]
posterior.mode(Vpe.T)
HPDinterval(Vpe.T)

Vy.T <- modelGI$VCV[,"YEAR"]
posterior.mode(Vy.T)
HPDinterval(Vy.T)

##ajouter random factors
Vm.T <- modelGI$VCV[,"MALE"]
posterior.mode(Vm.T)
HPDinterval(Vm.T)

Vr.T <- modelGI$VCV[,"units"]
posterior.mode(Vr.T)
HPDinterval(Vr.T)

Vp.T <- modelGI$VCV[,"animal"]+modelGI$VCV[,"ID"]+modelGI$VCV[,"YEAR"]+
    modelGI$VCV[,"units"]+modelGI$VCV[,"MALE"]
posterior.mode(Vp.T)
HPDinterval(Vp.T)

```

```
# As proportions of phenotypic variance (conditioned on fixed effects):
h2.T <- Va.T/Vp.T
posterior.mode(h2.T)
HPDinterval(h2.T)
plot(h2.T)
mean(h2.T)

################ INFERRING EVOLUTIONARY CHANGE
LD.blups <- colnames(modelGI$Sol) # Extract BLUPs
LD.blups.b <- substr(LD.blups[grep("animal",LD.blups)],8,18)  # Isolate BLUPs for additive genetic effect (=random effect in model)=#take ID females
LD.animal.indices <- match(phen.f$animal, LD.blups.b)

library(pedantics)
#Posterior
nbiter<-length(modelGI$Sol[,1]) # nb samples were taken from the MCMC chain  -> taking n of model.plot
##effective sample size >5000
LD.post <- 1:nbiter
LD.post.drift <- 1:nbiter

for(i in 1:nbiter){
  # Calculate annual mean breeding values for MCMC iteration i, as well as annual V(a) and annual sample size
  LD.annual.mean <- tapply(modelGI$Sol[i,][LD.animal.indices], phen.f$YEAR, mean)
  LD.annual.var <- tapply(modelGI$Sol[i,][LD.animal.indices], phen.f$YEAR, var)    #mean variance predicted BV per year ?
  LD.annual.n <- tapply(modelGI$Sol[i,][LD.animal.indices], phen.f$YEAR, length)

  LD.annual.mean.r <- tapply(modelGI$Sol[i,][LD.animal.indices], phen.f$SIREY, mean)
  LD.annual.var.r <- tapply(modelGI$Sol[i,][LD.animal.indices], phen.f$SIREY, var)
  LD.annual.n.r <- tapply(modelGI$Sol[i,][LD.animal.indices], phen.f$SIREY, length)

  repbv_fh <- rbv(ped = ped.fix, G = modelGI$VCV[,"animal"][i])

  # Simulate breeding values with V(a) = estimate at iteration i
  LD.simulation <- tapply(repbv_fh[match(phen.f$animal, ped.fix[,1])], phen.f$YEAR, mean)
  LD.simulation.var <- tapply(repbv_fh[match(phen.f$animal, ped.fix[,1])], phen.f$YEAR, var)    ### Variance of simulating BV - mean variance by year?
  # Regress means against year and save slope estimate
  LD.post[i] <- summary(lm(LD.annual.mean ~ as.numeric(names(LD.annual.mean))))$coef[2]
  # Regress simulated means against year and save slope estimate
  LD.post.drift[i] <- summary(lm(LD.simulation ~ as.numeric(names(LD.simulation))))$coef[2]
}

# Observed change in BLUPs
mean(LD.post) #or mean(d[which(d$Selection=="Genetic change"),"x"])
HPDinterval(mcmc(LD.post), 0.95)
table(LD.post<0)/nbiter  # Posterior probability that trend is positive
```

```
summary(mcmc(LD.post))
#Genetic drift
mean(LD.post.drift)
HPDinterval(mcmc(LD.post.drift), 0.95)
table(LD.post.drift<0)/nbiter  # Posterior probability that trend is negative
summary(mcmc(LD.post.drift))

##Compare two models simulation
mean(LD.post-LD.post.drift)  #distribution of difference
HPDinterval(mcmc(LD.post-LD.post.drift), 0.95)

d = data.frame(
  x = c(LD.post, LD.post.drift),
  Selection=rep(c("Genetic change", "Genetic drift"),
                c(length(LD.post), length(LD.post.drift))))

library(plyr)
library(ggplot2)

cdat <- ddply(d, "Selection", summarise, rating.mean=mean(x))   #expected shift
ggplot(d, aes(x=x, colour=Selection)) + geom_density() + theme_classic()+
  xlab("Expected changes in annual mean breeding values for Laying Date") + ylab("Probability density") +
  xlim(-0.05, 0.05)+#ylim(0, 150) +
  geom_vline(data=cdat, aes(xintercept=rating.mean, colour=Selection), linetype="dashed", size=1)+
  theme(legend.position = c(0.8, 0.8), legend.title=element_text(size=14), legend.text=element_text(size=14), axis.title.x = element_text(size=14), axis.text.x = element_text(size=12), axis.text.y = element_text(size=12), axis.title.y = element_text(size=14))

##
LDannual <- as.data.frame(LD.annual.mean)
LDannual$year<- as.numeric(names(LD.annual.mean))
gd.regression.LD<- lm(LD.simulation ~ as.numeric(names(LD.simulation)))
summary(lm(LD.simulation ~ as.numeric(names(LD.simulation)))) #trend genetic drift
summary(lm(LD.annual.mean ~ as.numeric(names(LD.annual.mean)))) #trend breeding value

##With mean breeding value
#ggplot(as.data.frame(LD.annual.mean),aes(x=as.numeric(names(LD.annual.mean)),y=LD.annual.mean))
ggplot(LDannual,aes(x=year, y=LD.annual.mean))+ylab("Annual mean breeding value for laying date")+xlab("Year")+theme_classic()+
  geom_point(size=2)+stat_smooth(method=lm, fill="blue",colour="darkblue", size=1,alpha = 0.1)+
  geom_errorbar(aes(ymin=LD.annual.mean-sqrt(LD.annual.var/LD.annual.n), ymax=LD.annual.mean+sqrt(LD.annual.var/LD.annual.n)), width=.1) +
  theme(legend.title=element_text(size=14), axis.title.x = element_text(size=18), axis.text.x = element_text(size=12), axis.text.y = element_text(size=12), axis.title.y = element_text(size=18))+
  ylim(-1,1) +

  #Add simulation drift
  geom_abline(intercept=gd.regression.LD$coef[1],slope=gd.regression.LD$coef[2], colour="darkorange", size=1) +
```

```r
  geom_point(aes(x=as.numeric(names(LD.simulation)),y=LD.simulation), colour="darkorange")   #+
#geom_errorbar(aes(ymin=LD.simulation-sqrt(LD.simulation.var/LD.annual.n), ymax=LD.simulation+sqrt(LD.simulation.var/LD.annual.n)), width=.1, colour="darkorange")

##With Va
LD.annual.var<- as.data.frame(LD.annual.var)
LD.annual.var$year<- as.numeric(rownames(LD.annual.var))
##With Va  (var breeding value by year)
summary(lm(LD.annual.var ~ as.numeric(names(LD.annual.var))))  #p-value: 0.7744
 ggplot(LD.annual.var,aes(x=year,y=LD.annual.var))+ylab("Variance additive")+xlab("Year")+
  geom_point(size=2)+theme_bw(base_size=18)+stat_smooth(method=lm, fill="blue",colour="darkblue",size=1,alpha = 0.1)

##With env conditions
BV<-as.data.frame(LD.annual.mean)
LD.annual.mean.SE<-as.data.frame(sqrt(LD.annual.var/LD.annual.n))
 Year<-as.data.frame(row.names(LD.annual.mean))
BV<-cbind(Year,BV,LD.annual.mean.SE,LD.annual.var)
colnames(BV)<-c("Year","Breeding_Value","Breeding_Value_SE","Va")
BV<-cbind(BV, DY[,c("LayingDate","Clutch","Fledge","Recruit","TempMaxPreLD","TempMinLD","MinTApril","MaxTApril",
 "MinTMay","PrecipMinPreLD","PrecipMinLD","MaxPMay","NAOw")])

############Trend BV env factors
##with all factors
 library(gridExtra)
 myPlot<-function(factor){
   ggplot(BV,aes(x=BV[,factor],y=Breeding_Value))+geom_point(size=2)+labs(x=factor, y="Breeding Value")+theme_classic()+stat_smooth(method=lm, fill="blue",colour="darkblue",size=1,alpha = 0.1)+
  geom_errorbar(aes(ymin=Breeding_Value-Breeding_Value_SE, ymax=Breeding_Value+Breeding_Value_SE, width=.01))
  }
do.call(grid.arrange,lapply(colnames(BV[,9:length(BV)]),myPlot))
```

Chapter 4

Genetic variation in phenotypic plasticity for breeding time in a small migratory songbird

Justine Le Vaillant, Jaime Potti, Carlos Camacho, David Canal, Jip Ramakers, Marcel Visser & Jesús Martínez-Padilla

A major goal in evolutionary biology is to understand how the interplay between natural selection and genetic variation results in local adaptation. Results about microevolutionary responses for advanced breeding date in birds are contrasting despite consistent directional selection for early breeding. However, if plasticity of avian breeding time have a genetic basis, might be an alternative mechanism favouring local adaptation in populations under fluctuating environmental conditions. In a previous chapter, using individual- based data from a long-term study (1987-2016) pied flycatcher (*Ficedula hypoleuca*) population in Spain, we showed that breeding time is phenotypically plastic to global and local environmental conditions, including the North Atlantic Oscillation in winter (NAOw) and accumulated temperature (Degree Day), respectively. Additionally, there is no clear temporal trend in these two environmental factors, but rather a high temporal variation. Here, we explore whether phenotypic plasticity of laying date has a genetic basis using advanced quantitative genetic models. Based on a social pedigree of 12 generations deep, we show that there is genetic variation in plasticity in breeding time. Our results suggest that Genotype-by-Environment (G×E) interactions can be a mechanism that determines local adaptation, considering climatic proxies of environmental variation during breeding and also during wintering. However, the robustness of G×E interactions in our population is not unequivocal and suggests that probably other mechanisms play a role in the evolutionary dynamics of breeding time. Further analyses are needed to explore the relative role of additional, perhaps not exclusive, evolutionary mechanisms, which may explain local adaptation in our population and clarify the role of different environmental factors in this process.

Unpublished Manuscript

Introduction

Environmental variation alters the form and strength of phenotypic selection (Grant & Grant, 1995; Siepielski et al., 2013; Siepielski et al., 2017; Wood & Brodie III, 2016), evolvability (Kruuk et al., 2008; Martínez-Padilla et al., 2017) and, ultimately, the mean and variance of phenotypes in populations (Garant et al., 2004). Evolutionary dynamics of phenotypes is therefore greatly influenced by how environmental factors change over time, either in a predictable or erratic fashion. However, in response to changing environmental conditions and apparent phenotypic selection, a lack of evolutionary change of phenotypes has often been reported in the literature (Charmantier & Gienapp, 2014; Kruuk, 2017). Despite phenotypic and genetic stasis of traits under changing environmental conditions, evolution can also occur by the interactive action of genotypes and environments (G×E), among other mechanisms, by which genotypes express different phenotypes depending on the environmental conditions (Pigliucci, 2005; Scheiner et al., 2019). But few studies have quantified the genetic covariance of G×E of labile traits in wild populations, mostly because of a lack of long-term data and statistical issues (Brommer, 2013; Gienapp, 2018; Hayward & Pemberton, 2018). A major goal for understanding plasticity role in adaptation is not only to detect genetics covariance (Bailey et al., 2021), but also the environmental conditions under which this mechanism can evolve and be selected for in wild populations.

Although it is generally assumed that both genetic (micro-evolution) and non-genetic (phenotypic plasticity) processes can determine the dynamics of phenotypes favouring local adaptation, environmental factors themselves may constrain our comprehension of these processes. Most of our knowledge on the role of phenotypic plasticity in adaption is based on local temperature-based indexes, but it is clear that other local and global environmental factors may influence selection and evolvability of phenotypes (Siepielski et al., 2017). The latter are particularly relevant for migratory species, where the environmental conditions that individuals experience throughout their annual cycle differ from the wintering to breeding areas (Charmantier & Gienapp, 2014). In fact, the environmental conditions that individuals of migratory species experience in their wintering grounds can have carry-over effects on multiple life-history or behavioural traits at the time of breeding (Harrison et al., 2011; O'Connor et al., 2014; Saino et al., 2004). Additionally, local environmental conditions do also have a deep impact on the same traits, as reinforced by studies describing that G×E interactions can be an evolutionary mechanism that shapes the evolution of phenotypes in local conditions (Charmantier & Garant, 2005; Husby et al., 2010). Therefore, under an unprecedented scenario of global change, it is compulsory to understand how the environmental conditions that individuals experience at both during winter and breeding grounds affect the evolution of phenotypes.

The comprehension that multiple environmental factors have on G×E interactions is as crucial as the phenotype under study (Gienapp, 2018). The degree of additive genetic variance or covariance of phenotypes can change across environments (Husby et al., 2011b; Ramakers et al., 2018a), but may also differ among traits (Postma, 2014). For instance, high evolvability is common for life-history traits (Charmantier & Garant, 2005; Postma, 2014), but life-history traits may also show heritable plasticity as reported in breeding date in birds (Brommer et al., 2008; Garant et al., 2008; Husby et al., 2010), and reproductive timing in red deer (Nussey et al., 2005b). However, the ubiquity of G×E is far from consistent in wild populations (Merilä & Hendry, 2014; Pelletier & Coltman, 2018), even for a single and widely studied life-history trait like reproductive timing (Brommer et al., 2005; Brommer et al., 2008; Ramakers et al., 2019– see Hayward and Pemberton, 2018 for a more comprehensive review). Therefore, the relative role of genetic and non-genetic changes in the local adaptation of wild populations is still unclear, particularly regarding life-history traits whose expression can be influenced by environmental conditions at both wintering and breeding grounds.

Phenological responses to environmental variation are well-documented under the current context of global climate change, where an advance in breeding time is often reported in animals (Menzel et al., 2006). For example, it is widely assumed that environmental conditions may mistime the mean breeding time and fitness optimum in wild temperate-zone bird populations, compromising their growth rates (Visser et al., 2004; Visser & Gienapp, 2019). However, breeding time has not advanced in all populations or, at least, not at the same pace (Ram et al., 2018; Shave et al., 2019), suggesting the action of different evolutionary mechanisms across different populations (Husby et al., 2010). Therefore, understanding the environmental factors that may act during the whole life cycle of migratory species is fundamental to understand the evolutionary dynamics of life-history traits.

In this study, we examine the role of G×E in the evolutionary dynamics of a life-history trait (laying date) using a long-term and individual-based data set of a wild population of pied flycatchers (*Ficedula hypoleuca*). Framed within advanced quantitative genetic models (*QGM*), we use the 'random regression animal model' approach to test fo G×E interactions (i.e *RRAM*, see Kruuk, 2004, as it uses covariance functions to fit variance components as a function of the environment. Phenological traits are often used in studies of response to environmental change, because changes in the component of phenotypic variation do not necessarily lead to micro-evolution (Charmantier & Gienapp, 2014; Merilä et al., 2001b). To explore G×E in our population, we used two different environmental factors. First, North Atlantic Oscillation in winter (NAOw), an index of a global climatic conditions. This variable has been used in migratory species to explore the influence of wintering conditions on behavioural, physiological or population dynamics derived traits (Both et al., 2006b; Møller, 2002). Second, we used Degree Days, an index that reflects the heat accumulated during a given period of time that is crucial for vegetation growth and development (Bonhomme, 2000). This index is a reliable proxy for insect emergence and therefore for the time at which food abundance reaches a peak (Sockman & Courter, 2018). In our population, we did not observe a net temporal change in laying date in relation to either of two environmental factors, North Atlantic Oscillation in winter and Degree Day, however, we did observe variability in environmental conditions and selection at the phenotypic level (see Le Vaillant et al., 2021 and below). Given that microevolution has not been detected in this population (see Chapter 3), we expect phenotypic plasticity to be an alternative mechanism of local adaptation when both environmental factors are considered.

Material methods

Study system

Data were obtained between 1987 and 2016 in a population of pied flycatchers breeding in nest-boxes in central Spain (La Hiruela, 41°04′ N, 3°27′ E). The population occupies an old deciduous forest dominated by oaks (*Quercus pyrenaica*) and a nearby (1 km) mixed coniferous plantation dominated by *Pinus sylvestris* (see Camacho et al., 2015 for a description of the study area). Individuals return from the wintering grounds from the third week of April (Potti, 1998a). Males are the first to arrive at the breeding site to hold a territory before the arrival of females about one week later (Potti & Montalvo, 1991a). Nest-boxes (n=237) were inspected every 2-3 days from the beginning of the breeding season (first arrivals from spring migration) to record laying date (date of first laid egg), clutch size (typically 5-6 eggs), number of hatchlings (eggs hatched) and number of fledglings (chicks on day 13 post-hatching). Laying date was recorded 1,544 times for 671 different females. Breeding males and females were captured during the nestling stage using a nest-box trap, individually marked with colour (males) and metal (both sexes) rings, measured for tarsus length (±0.05 mm) and weighed (±0.1g). All fledglings were ringed at the age of 13 days. Unringed birds were aged as either one year or older following the criteria of Karlsson et al., 1986. Because of reduced adult capture rates compared with the rest of years, 2002 and 2003 were excluded from the analyses described below (see – statistical approach).

Environmental conditions

We focused on two climatic variables: accumulation of temperature during a determined period of time, known as Growing Degree Days (Degree Day here after) and North Atlantic Oscillation in winter (NAOw). Degree Day has been found, using the package `ClimWin` (Bailey & van de Pol, 2016), to explain the variance in breeding date in the population and represented here the sum up of air temperature above 10.5°C from 24 April to 9 June (see Appendix A - `ClimWin` package). This factor, also known as Growing Degree Days, can be used to predict a phenology trait above a given threshold (Charmantier & Gienapp, 2014; Saino et al., 2011). NAOw is a key environmental factor that summarises the wintering conditions that individuals experience during the non-breeding period (January to March). Conditions during the non-breeding season determine selection on laying date in this (Le Vaillant et al., 2021) and other populations of pied flycatchers (Ahola et al., 2004; Both et al., 2006b). Large fluctuations between years and positive values of NAOw (high temperature and dry winter) are considered as adverse conditions (Marrot et al., 2018; Przybylo et al., 2000, but see Le Vaillant et al., 2021).

Pedigree Structure

Social relationships among individuals in the population (pedigree) were constructed from breeding females first captured (ringed) as chicks. We assigned a mother and father to females from observational data on nest defence and attendance. Extra-pair paternity (EPP) may lead to inaccurate estimations of variances and covariances in the social pedigree due to misassigned paternities. However, the EPP rates are not high in our population, around 15% (Canal, Jovani, Potti, 2012), below the 20% threshold considered to impact estimates of additive genetic variance (Charmantier & Réale, 2005; Firth et al., 2015). Cases where brood manipulation (cross-fostering) experiments had been carried out (Camacho et al., 2016; Potti & Canal, 2011) were removed from the data set. When one member of the pair, father or mother, was missing (2.35% from 3315 informative individuals) it was assigned a "dummy code" to avoid losing the information of the sibship into the pedigree. Overall, we had 14,973 records of 12 generations depth (see Appendix B - Pedigree) with 1,910 founders and with 25,531 full siblings for 12,956 maternities (mean maternal sibship size of 8.10) and 12,299 paternity records (mean paternal sibship size of 8.46).

Statistical approach

Temporal trends in breeding date and environmental factors

Linear Models (LM) were used to assess the association between annual NAOw indexes and Degree Day against year, the relationship between both variables and between laying date and environmental factors. To explore the temporal trend in laying dates, we used Linear Mixed Models (LMM) including year (treated as a continuous variable), habitat (class: oak vs. pine) and bird age (class: one year vs. older) as explanatory terms. As random terms, we included female identity (ID) and years (i.e., year, treated as a categorical variable), to account for pseudo-replication and stochastic variation among years (see e.g. Evans and Gustafsson, 2017 for a similar approach). Models were fitted with the Packages '`lme4`' (Bates et al., 2015) and '`lmerTest`' (Kuznetsova et al., 2015) in `R` version 3.5.3 (R Core Team, 2017).

Testing for the genetic basis of variation in plasticity

To explore patterns of variation in plasticity of breeding time, we used univariate 'random regression animal models' with genetic variance as a function of a continuously varying environment (Kruuk et al., 2001). Animal models allow inclusion of fixed effects and random factors to account for known influences on the phenotype. Fixed effects in these models were: habitat, which takes into account consistent differences in the mean laying date between the oak and pine forest; mating status, coded as "monogamous" and "primary/secondary female" (Canal et al., 2020); female age, coded as "one year old" or "older"; and, finally, female body mass, as a metric of female condition (Labocha & Hayes, 2012) which are known to be interrelate with laying date in passerines (Forslund & Pärt, 1995; Svensson & Nilsson, 1995; Verhulst & Nilsson, 2008). Bird origin (immigrant or locally born) was not included in the model since it did not explain much of the total variance in laying date (less than 1% - analyses not shown). The random effects split the variance non-accounted for by the fixed effects. Our analysis included the following random effects: additive genetic variance (a); permanent environment, which considers the repeated measures of the same female over the years (p); male identity ($male$), variance associated with the year (y); and residual variance (ε). Both environmental variables (NAOw and Degree Day) were scaled to a range of -1 to +1 and we only fitted polynomial (φ) functions of first order. Thus, we fitted the model on laying date (z) or each female i as follows:

$$z_i = Xb_i + Z_1\varphi(a_i, n_1, E) + Z_2\varphi(p_i, n_1, E) + Z_3y_i + Z_4male_i + \varepsilon_i \tag{1}$$

Where z_i is the vector of individual trait values, X is the matrix of fixed predictors, b is a vector for fixed effects. The term $Z_1\varphi(a_i, n_1E)$ is the random regression function of the additive genetic variance (V_a) of individual i on the environment (E). The term $Z_2\varphi(p_i, n_1, E)$ refers to the random regression function of order 1 of the permanent environmental effect of the individual i and environment E, and summarizes the effects conserved across the repeated records of the i individual. Finally, the terms z_3y_i and z_4male_i refer to the effect of year and the identity of the $male$ the female was mated with, respectively. ε_i is the error term or residual variance not explained by fixed or random factors. To properly split individual variation (I×E) into its genetic (G×E) and non-heritable component (PE×E) on total phenotypic variance in breeding date, we run the model sequentially, starting from the full model, then excluding or including G×E or PE×E interactions. The data set only included breeding females recorded at least in two different years.

All estimations provided are posterior modes from a Bayesian framework with `MCMCglmm` (Kingsolver et al., 2007). We fitted our models with a heterogeneous residual structure to account for potential variation in residual variance along the environmental factor (see Ramakers et al., 2019). For fixed effects, we used a weakly informative prior using standard inverse-Wishart priors, with parameters $V = diag(k)$ and $nu = 0.002$ for univariate models where k=6, for the number of environmental categories used. For the random effects in multivariate models, we used parameter-expanded priors ($V = diag(d)$, $nu = 1$, $alpha.mu = 0$, $alpha.V = diag(d) * 25^2$, where d is an identity matrix of dimension 2. All models were run for 5,500,000 iterations with a burning of 500,000 iterations and a thinning of 2,500 iterations. Models were compared using Deviance Information Criterion (DIC) with best models considered those that had a difference of more than 2 ΔDIC (Spiegelhalter et al., 2002). Confidence intervals (CI 95%) and pMCMC values were used to evaluate the significance of fixed effects.

Results

Laying date did not advance across years, neither the NAOw and Degree Day indices (see Chapter 2), but both showed large variation over the study period. Both factors were not correlated (r=0.005, n=27, P=0.296).

Random regression animal models

Animal random regression selection models supported that I×E interactions might arise both from genetic (G×E) and non-heritable components (PE×E) and that those interactions might be environmentally driven (Table 1). Specifically, individuals differ in their response to changing environmental conditions. The DIC values suggest that models including G×E interactions is the most robust one. However, the DIC of the model including G×E is lower than 5 in relation to the previous one, suggesting a non-conclusive support of G×E interactions (Table 1, $\Delta DIC < 2$). Our results point out to a small but heritable basis of variation in plasticity of laying date (G×E) in our population (Table 2). Contrary to habitat, mate and age, the fixed factors that explain variance on laying date, body mass do not seems to play a role on laying date in these models, since the posterior estimates are close to null (pMCMC >0.05) and the confidence interval crosses 0 in both models (Table 2).

Table 1: Quantitative genetic models to test for G×E and PE×E interactions on laying date. I represents the individual variance with G, the genetic variance, V_{PE} the variance of permanent effect and E denotes the environment with NAO in winter (NAOw) on the left side of the table and Degree Day on the right side of the table. The pedigree of the population is included as the G-matrix into the model. Results are sorted by decreasing DIC.

Models	DIC_{NAOw}	$\Delta\ DIC_{NAOw}$	$DIC_{DegreeDay}$	$\Delta\ DIC_{DegreeDay}$
null	9519.005	-7.695	9544.515	-9.159
V_I	9519.087	-7.777	9544.996	-9.640
$V_{PE}+V_A$	9518.853	-7.543	9545.090	-9.734
$V_{PE}+V_A+$ I×E	9514.565	-3.255	9539.076	-3.720
$V_{PE}+V_A+$ PE×E + G×E	**9511.310**	**0.00**	**9535.356**	**0.00**

Discussion

Our results suggest that genotype-by-environment (G×E) interactions can be considered as a potential mechanism to explain the evolutionary dynamics of phenotypes (Charmantier et al., 2008; Husby et al., 2011b; Yeh & Price, 2004). Overall, main inferences can be made from the quantitative genetic approach used in this pied flycatcher population. Although the heritable basis of plasticity (G×E) in natural populations is not widely described (Hayward & Pemberton, 2018; Ramakers et al., 2018b), our results suggest that genotype-by-environment interactions could play a role on local adaptation in natural populations, but perhaps married to other evolutionary mechanisms.

Table 2: Estimates of factors in the model using genotypes and individuals' environmental interactions with NAOw in winter (left) and Degree Day (right). Only the Fixed and random effect are shown. See Material and methods for further explanations on the variables input to the model. Post.mean denotes the posterior estimates of slope variance ; CI95: Confidence Interval 95% ; eff. samp : effective sample size

Factors	**NAOw**				**Degree Day**			
Fixed effect	post.mean	[CI95]	eff.samp	pMCMC	post.mean	[CI95]	eff.samp	pMCMC
Intercept	32.331	[24.110 ; 39.386]	2000	< 5e-04	32.096	[24.785 ; 40.403]	2000	< 5e-04
AGE	-4.295	[-4.997 ; -3.621]	2000	< 5e-04	-4.322	[-5.038 ; -3.634]	2000	< 5e-04
HBT	1.932	[1.281 ; 2.603]	2000	< 5e-04	1.817	[1.166 ; 2.428]]	2000	< 5e-04
MASS	-0.479	[-0.971 ; 0.053]	2000	0.065	-0.459	[-0.978 ; 0.057]	2000	0.089
MATE	0.891	[0.581 ; 1.216]	2000	< 5e-04	0.841	[0.518 ; 1.173]	2000	< 5e-04
E	-0.935	[-1.962 ; 0.233]	2130	0.088	-0.025	[-0.036 ; -0.0125]	1867	<5e-04
Random effect								
YEAR	12.210	[6.124 ; 19.74]	2000	-	6.886	[3.101 ; 11.390]	2000	-
MALE	0.635	[2.409e-09 ; 1.915]	2227	-	0.569	[2.314e-07 ;1.855]	2227	-
G × E	0.097	[4.110e-09 ;0.385]	2000	-	3.188e-05	[1.117e-11 ; 1.148e-04]	2632	-
PE × E	0.112	[2.592e-07 ; 0.406]	2278	-	3.556e-05	[1.059e-13 ; 1.287e-04]	2000	-
G × E+ PE × E	0.209	[1.055 e-04 ; 0.612]			6.745 e-05	[4.932e-08 ;1.751 e-04]		

Genetic basis of phenotypic plasticity

We tested for the existence of individual plastic responses (I×E) in our population by splitting it in genetic (G×E) and non-heritable components PE×E) of variance in laying date. When selection acts on the heritable part of plasticity (Nussey et al., 2005b; Nussey et al., 2005c; Scheiner, 2002), it can be indicative of the potential of phenotypic plasticity to evolve (Brommer et al., 2005; Nussey et al., 2005c; Nussey et al., 2007; Pigliucci, 2005). Under fluctuating environments, however, the plastic response at the phenotypic level is unlikely to be sufficient for a trait to evolve (Charmantier & Gienapp, 2014; Gienapp et al., 2014). Despite our best models being not totally conclusive (Table 1) and the low provided posterior means of the estimates of G×E (close to 0 – see Table 2), some interesting G×E patterns emerged with a non-fully conclusive support for the heritable basis of plasticity of laying date. Under fluctuating selection, phenotypic plastic might allow a rapid evolutionary response to environmental changes and provide a raw material for selection to act on.

In a temporally heterogeneous environment, plastic responses are expected to evolve to counteract the negative effects of unfavourable conditions, particularly when environmental conditions do not act in an opposite direction to the genetic response (Garant et al., 2004; Merilä et al., 2001b). However, consistency in G×E interactions in phenotypic traits in general, and life-history traits in particular, has been recently challenged. Specifically, 55% estimates (out of 93 published so far) of the association between either additive genetic variance or heritability and environmental conditions did not show any evolutionary change (reviewed in Hayward and Pemberton, 2018. Our results partially support the idea that G×E interactions play a role in local adaptation in our study population after using robust statistical procedures since the most robust model only differs in 3.2 and 3.7 units, for NAOw and Degree Days respectively, from the previous one that did not include G×E interactions. Several reasons may explain the lack of G×E in previous studies, among others, pedigree depth, temporal length of the data set, species-specific life-history traits, or the statistical approach itself. Among them, one commonly oversight reason is the choice of the focal environmental variable (Gienapp, 2018), which may cause disparities in the results even for the same species in different populations (discussed in Husby et al., 2010.

There are however potential caveats and limitations to our study. First of all, confidence intervals of both estimations of PE×E and G×E were close to 0. In the MCMCglmm package, the confidence intervals of random variables or interactions, unlike fixed effects, are constrained to be positive and the influence of a particular variable cannot be rejected based on whether zero is included within the posterior distribution margins of the random terms. Then, we used a powerful statistical approach when fitting the animal model by applying a Bayesian framework as considers a heterogeneous residual variance and splits I×E into PE×E and G×E simultaneously. Further, the full model including I×E and G×E was the most plausible one, with a difference greater than 3 DIC units in relation to other ones (see examples in Husby et al., 2010). Finally, since the confidence intervals of G×E interactions are close to 0, particularly when using Degree Day as environmental factor, our analyses do not unequivocally point towards phenotypic plasticity as a major factor shaping the evolutionary dynamics of breeding time in our population. In addition, we cannot rule out the possibility that other environmental variables play a role in G×E interactions in our population. Nevertheless, perhaps our results attest how evolution acts, not just by favouring phenotypic plasticity, but giving room for others evolutionary mechanism to operate (Bailey et al., 2021). Further analyses may tease apart the potential role that other mechanisms, like the environment-dependent effects that gene flow can play in the evolutionary dynamics of laying date in our population, or determine whether and how different mechanisms change their relative importance over time or under different environmental circumstances.

The importance of the environmental factor(s)

Detecting a significant influence of I×E interactions in wild populations for life-history traits is not common and has usually rendered contrasting results (see Hayward and Pemberton, 2018, particularly when splitting I×E into both G×E and PE×E. The apparent lack of interaction may be caused by choosing an incorrect environmental factor (Gienapp, 2018; Husby et al., 2010), by lack of statistical power (Husby et al., 2010), or by underestimating the influence of the heterogeneity of residual variance in the model outcomes (Nicolaus et al., 2013; Ramakers et al., 2020). By dealing with these issues, our statistical approach of PE×E implies that individuals show different reaction norms (Nussey et al., 2007), both in their intercepts and slopes, when facing different environmental conditions. This suggests that female pied flycatchers can cope with fluctuating environmental conditions by adjusting their breeding time. This combined influence of PE×E and G×E on the total phenotypic variance of laying date could explain the ability of the population to respond to a changing environment ((Nussey et al., 2007). However, establishing the link between individual level responses and local adaptation when considering G×E interactions is still a challenging task requiring further investigation.

Choosing the right environmental variable in local adaptation studies is a complex task and requires careful consideration depending of the species and location of the study population. We emphasize the importance of focusing on suitable environmental variables to explore the evolutionary dynamics of labile traits and, therefore, to detect G×E interactions in wild populations (Gienapp, 2018). We selected the environmental variables that can act as cues that can be used by females to adjust their laying date. In this Spanish pied flycatcher's population, it seems that local environmental conditions are the major factors acting on selection at phenotypic level. However, both Degree Day and NAOw are key cues that can facilitate the evolution of G×E interactions. Degree day represents the thermal conditions affecting the arthropod emergence on the breeding grounds and impacting so the phenology of reproduction of insectivorous birds (Saino et al., 2011). On the other hand, NAOw indexes summarize the wintering conditions experienced by pied flycatchers in sub-Saharan Africa and how dry the winter was at the global scale. Specifically, NAOw is the most reliable proxy of climatic conditions during the non-breeding season, which is a major determinant of behavioural and physiological traits during breeding (Møller, 2002). Our results suggest that evolution may favour those females that are able to flexibly adapt their timing of breeding depending on the conditions they experienced during winter. The mechanism behind this finding is unclear at present and multiple factors may be involved. We speculate that time of departure can be a key factor triggered by the environmental conditions that females experience during winter. It is possible that NAOw might impact the rate of fuel deposition and female condition at the time of departure to the breeding grounds (Ahola et al., 2004; Both et al., 2005; Lindström et al., 2019), thereby affecting the migration phenology and the individual condition at the timing of arrival (Both et al., 2016; Ouwehand & Both, 2017). Therefore, as an example, in years of harsh wintering conditions (high NAOw), females in better condition that adjust their departure date may benefit from breeding earlier and have a relative high fitness benefit in relation to other individuals in the population. Instead, such fitness benefit would be less important when environmental conditions during winter were good. Remarkably, our results emphasize the key role that overwinter environmental conditions, as opposed to breeding conditions, can have in the evolutionary dynamics of life-history traits (Møller, 2002). This is of crucial importance particularly for long-distance migratory species, where the evolution of key life-history traits affecting population dynamics can be determined in the wintering quarters.

Acknowledgments

We thank many volunteers, technicians and friends, too numerous to mention individually, that helped with data collection. All authors contributed to the drafts and gave final approval for publication. The authors declare no conflict of interest. J.L.V received financial support from the Spanish Ministry of Economy and Competitiveness (grant BES-2016-077694). CC received financial support from the Spanish Ministry of Science, Innovation and Universities (FJC2018-038412-I). DC was supported by a grant from the Hungarian Academy of Sciences (MTA Premium Postdoctoral Research Program; ID: 2019-353). J.M.-P. was supported by the Spanish Commission of Science and Technology (CICYT, grant number CGL2015-70639-P and PID2019-104835GB-I00) and is currently funded by ARAID foundation. Fieldwork was supported mainly by CICYT, most recently by projects CGL2014-55969-P and CGL2015-70639-P.

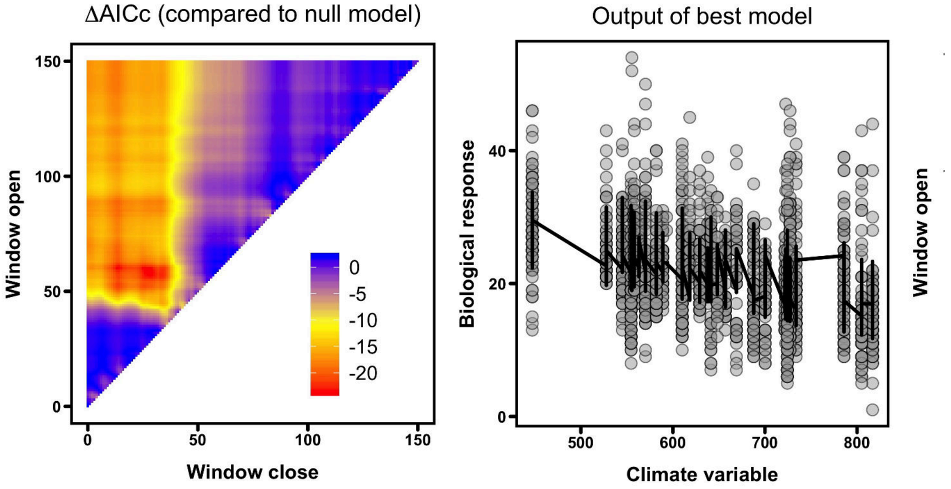
Supplementary Material

Appendix A - `ClimWin` package

Because the time period for selecting the best environmental agent is often subjective (van de Pol et al., 2016), we use the package `ClimWin` (Bailey & van de Pol, 2016) to select the best time window explaining most of the variability in the breeding date. By using a sliding window approach, the package tests and compares the effects of climate variables in different time windows (Bailey & van de Pol, 2016). With daily climatic variables (temperature, precipitation, NAO), diverse statistics (mean, slope, sum) with linear and quadratic functions, several models have been tested against laying date with individual identity as a random factor. We selected the best models according the $\Delta AICc$.

With used as a base model, a linear mixed models including age, habitat, body mass and mate status as fixed factors, and female identity, male identity and years as random terms (see Material & Method for more details). The best model sorted is a linear relation of sum up of temperature above 10.5°C (estimate =-0.029 ± SE 0.004, t=-6.454, p<0.001) in a window of 58 to 28 days before the last laying date (in our dataset the 23th of June), that is from the 24th of April to the 9th of June (Figure 1).

Figure 1: ClimWin window and effect of climatic factor. a (left): Comparison of best windows period for climatic effect on variability on laying date. b (right): Influence on laying date of the sum up temperature above 10.5° in the selected window.



```
### QUANTITATIVE GENETICS MODELS
# Model with environments divided into blocks of 5 (29/5 ~ 6 residual blocks)
###Only with NAO for demonstration
library(arules)
range(phen.f$sNAOw)
x <- discretize(phen.f$sNAOw, breaks=6,method="interval")
#method frequency to split according nb individuals
phen.f[which(phen.f$sNAOw >= -2.71 & phen.f$sNAOw < -1.87),"Block5"]<-"1"
phen.f[which(phen.f$sNAOw >= -1.87 & phen.f$sNAOw < -1.02),"Block5"]<-"2"
phen.f[which(phen.f$sNAOw >= -1.02 & phen.f$sNAOw < -0.18),"Block5"]<-"3"
phen.f[which(phen.f$sNAOw >= -0.18 & phen.f$sNAOw < 0.664),"Block5"]<-"4"
phen.f[which(phen.f$sNAOw >= 0.664 & phen.f$sNAOw < 1.51),"Block5"]<-"5"
phen.f[which(phen.f$sNAOw >= 1.51 & phen.f$sNAOw <= 2.36),"Block5"]<-"6"

###### Random Intercept Models
library(MCMCglmm)
Thin <- 2500
Burnin <- 500000
Nitt <- 1000*Thin+Burnin

## Model null : G
prior.G <- list(R = list(R1 = list(V=diag(6), nu=0.002)),
                G = list(G1 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                         G2 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                         G3 = list(V=1, nu=1, alpha.mu=0, alpha.V=625)))
m.G.I.sNAOw.b2<-MCMCglmm(LD~AGE+HBT+MASS+MATE,
                         random=~animal+YEAR+MALE,
                         rcov= ~idh(Block5):units,
                         prior= prior.G, pr=T,
                         data= phen.f, pedigree=ped.fix,family='gaussian',
                         thin=Thin, burnin=Burnin, nitt=Nitt, verbose=T)
m.G.I.sNAOw.b2$DIC # 9518.107

## Model VI : G+I
prior.GI <- list(R = list(R1 = list(V=diag(6), nu=0.002)),
                 G = list(G1 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                          G2 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                          G3 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                          G4 = list(V=1, nu=1, alpha.mu=0, alpha.V=625)))
m.G.I<-MCMCglmm(LD~AGE+HBT+MASS+MATE,
                        random=~animal+YEAR+MALE+ID,
                        rcov= ~idh(Block5):units,
                        prior= prior.GI, pr=T,
                        data= phen.f, pedigree=ped.fix,family='gaussian',
                        thin=Thin, burnin=Burnin, nitt=Nitt, verbose=T)
m.G.I$DIC  #9544.996

## Model VPE+VA :  G+I+E
prior.GIE <- list(R = list(R1 = list(V=diag(6), nu=0.002)),
                  G = list(G1 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                           G2 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                           G3 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                           G4 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                           G5 = list(V=1, nu=1, alpha.mu=0, alpha.V=625)))
m.G.I.sNAOw.b2.OK<-MCMCglmm(LD~AGE+HBT+MASS+MATE,
                        random=~animal+YEAR+MALE+ID+sNAOw,
                        rcov= ~idh(Block5):units,
```

```
                        prior= prior.GIE, pr=T,
                        data= phen.f, pedigree=ped.fix,family='gaussian',
                        thin=Thin, burnin=Burnin, nitt=Nitt, verbose=T)
m.G.I.sNAOw.b2.OK$DIC  # 9518.853

 ## Model VPE+VA + IXE :  G+I+E+IxE
 prior.GIE.IxE <- list(R = list(R1 = list(V=diag(6), nu=0.002)),
                 G = list(G1 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                          G2 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                          G3 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                          G4 = list(V=diag(2), nu=2, alpha.mu=c(0,0), alpha.V=diag(2)*625)))
m.G.IxE.sNAOw.b2<- MCMCglmm(LD~AGE+HBT+MASS+MATE+sNAOw,
                           random=~YEAR+MALE+animal + us(1+sNAOw):ID,
                           rcov= ~idh(Block5):units,
                           prior= prior.GIE.IxE, pr=T,
                           data= phen.f, pedigree=ped.fix, family='gaussian',
                           thin=Thin, burnin=Burnin, nitt=Nitt, verbose=T)

m.G.IxE.sNAOw.b2$DIC # 9514.565

 ## Model VPE+VA + PEXE + GXE : G+I+E+GxE+IxE
 prior.GIE.IxE.GxE <- list(R = list(R1 = list(V=diag(6), nu=0.002)),
               G = list(G1 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                        G2 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                        G3 = list(V=diag(2), nu=2, alpha.mu=c(0,0), alpha.V=diag(2)*625),
                        G4 = list(V=diag(2), nu=2, alpha.mu=c(0,0), alpha.V=diag(2)*625)))
m.GxE.IxE.sNAOw.b2<- MCMCglmm(LD~AGE+HBT+MASS+MATE+sNAOw,
                             random=~YEAR+MALE+us(1+sNAOw):animal + us(1+sNAOw):ID,
                             rcov= ~idh(Block5):units,
                             prior= prior.GIE.IxE.GxE, pr=T,
                             data= phen.f, pedigree=ped.fix, family='gaussian',
                             thin=Thin, burnin=Burnin, nitt=Nitt, verbose=T)
m.GxE.IxE.sNAOw.b2$DIC #9511.31
```

Chapter 5

Variability in reaction norms of breeding dates in response to global and local environmental variables

Justine Le Vaillant, Jaime Potti, Carlos Camacho, David Canal, Jip Ramakers, Marcel Visser & Jesús Martínez-Padilla

Variability of phenotypic traits is an indispensable requisite for microevolution and local adaptation to occur. In response to global change, phenotypic plasticity may allow individuals to change rapidly the expression of the phenotype facilitating the adaptation of populations to local conditions. However, the evidence for variability in plasticity and adaptive responses of phenological traits in wild populations is mixed. Here, we use a long-term (1987-2016) individual-based data set from a population of pied flycatchers, *Ficedula hypoleuca* to investigate variation in individual-by-environment interactions (I×E) of laying date in relation to two local and global climatic indexes. By using random regression mixed models, we tested for variation in reaction norms with Degree Day (local index) and NAO in winter (global index) as environmental climatic factors. We found support for I×E interactions for both climatic factors, and individual responses shaping population response, suggesting that the variation in plasticity in our population is driven by individual variation in the reaction norm. We found that individual variance differ depending on the environmental variable considered. We detected a higher but decreasing individual variance as NAOw values increases (worsen), and lower but constant individual variance for Degree Days. These results are suggestive that selection might favour plastic individuals when NAOw increases. These results suggest that individual variability in plasticity might be a relevant mechanism for adaptation to environmental change and the potential of individuals to respond and adapt to changing environmental conditions.

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Introduction

Understanding how populations can cope with rapid environmental change requires a full comprehension of how individuals differ in expressing a given phenotype in response to fluctuating conditions. Phenotypic plasticity is a short-term mechanism that allows individuals to adapt to rapid environmental changes, and might play a major role for the adaptation of populations to climate change (Gienapp et al., 2008; Scheiner et al., 2019). The study of labile trait expression through repeated measures of individuals allows quantifying the variation in plasticity in response to environmental changes. However, plasticity occurs at several levels (Brommer, 2013; Piersma & Drent, 2003) and, although the role of within-individual variation is recognized to drive phenotypic plasticity at the population level (Nussey et al., 2007; Sauve et al., 2019), it is also essential to partition the total phenotypic variance into its between and within individuals' components (Dingemanse et al., 2010; Westneat et al., 2015). This association is increasingly attracting more attention from evolutionary biologists aiming to understand whether individual plasticity might explain population-level response to climate change (Araya-Ajoy et al., 2015; Brommer, 2013; Westneat et al., 2015). In addition, individuals may differ in their degree of plasticity (individual-by-environment interaction e.g. I×E)). However, detecting variation in plasticity is not common (but see (Ramakers et al., 2020) and further work on plasticity in wild populations is needed to understand the mechanisms that might determine adaptive response to current climate change.

Phenotypic plasticity can be described by a reaction norm (Pigliucci, 2005; Via et al., 1995), measured by an intercept reflecting the individual's trait value in the average environment, and a slope that describes the change in the trait per unit change in the environment, i.e. the sensitivity of the trait to the environment. The interpretation of the association between intercepts of individual reaction norms (average phenotype) and slopes (levels of phenotypic plasticity) allows quantifying the individual response relative to the population, and testing whether a change in the mean phenotype of the population (mean reaction norms) is indeed a plastic response (Araya-Ajoy et al., 2015; Morrissey & Liefting, 2016). Random regression animal models (e.g.*RRAM*) are often used to model individual variation in plasticity (Brommer et al., 2012; Nussey et al., 2007). However, an appropriate sample size and study design are required to harnessing the power of these models (Martin et al., 2011; van de Pol, 2012). When I×E is detected, two major explanations are possible. First is that between-individual variation differ along an environmental gradient (I×E), or alternatively because residual (within-individual variation) changes along the chosen environmental variable. At the time that I×E is explored, it is required that both residuals and within-individual variances should be assumed to be heterogeneous along the environmental gradient. Otherwise, considering residuals of within-individual variance homogeneous over the environmental variable might inflate the estimated I×E variance. Thus, a proper consideration of residual variance is required to reliably estimate I×E interactions, accounting for heteroscedasticity in residual variances using *RRAMs* (Ramakers et al., 2020). A major challenge is therefore to quantify environmental heterogeneity and selecting the appropriate environmental variable in the measurement of individual's phenotypic responses. Long-term individual based data that accumulate repeated measures of labile traits in successive years and in different environment conditions are fundamental to estimate and compare the impact of environmental change on natural populations (Visser, 2008).

Using a 29-year study of breeding time in a population of pied flycatchers (*Ficedula hypoleuca*) in Central Spain, we quantified variation in plasticity of laying date in response to changing climate conditions. Laying date is a labile trait commonly studied in birds because of its strong impact on fitness (Dunn, 2004) and the ease of measuring individual reaction norms. Timing of breeding is under selection for early dates in many bird populations due to the global advance in spring onset as one of the consequence of climate change (see Dunn and Winkler, 2010. As many migrant passerine, the pied flycatcher is affected by both changes at global and local conditions, emphasizing the importance of plasticity in the adaptation to new local breeding conditions. Moreover, we have previously studied the non-conclusive influence of genotype-by-environment interactions in our population (Chapter 4), providing room to further whether I×E may explain total variance of laying date and whether it differs as climate conditions change. Here, we explore whether females differ in their laying date-environmental reaction norms to a global and a local environmental factor. We use to variables as proxy of cue environments that may drive trait expression, different than the variables that impact on selection (Gienapp & Brommer, 2014). We used Degree Day as a local index that reflects the heat accumulated in a given period of time and that is known to affect phenotypic variation in breeding dates in this population (see Chapter 4). In addition, we used the North Atlantic Oscillation Index in winter (NAOw) as a global factor previously demonstrated as being the an important environmental driver of selection on breeding time in pied flycatchers (see Le Vaillant et al., 2021). We expect to find differences in individual reaction norms depending on these factors, which would support the existence of variability in plasticity and selection for local adaptation at different levels.

Material methods

Study system

Data were collected between 1987 and 2016, excepting 2003, in a long-term population of pied flycatchers in central Spain (La Hiruela, 41°04′ N, 3°27′ E). The population breeds in nest boxes in an old oak forest and a nearby (1 km) mixed coniferous plantation (see Camacho et al., 2015 for a description of the study area). Nest boxes (n=237) were inspected every 2-3 days from the beginning of the breeding season (first arrivals from spring migration) to record laying date (date of first laid egg).

Breeding males and females were captured using a nest-box trap, individually marked with colour and metal rings, measured for their tarsus length (±0.05 mm), weighed (±0.1g) and aged as either one year or older following the criteria of

Karlsson et al., 1986. All fledglings were ringed at the age of 13 days, enabling us to follow their fate in the following years. In this study we considered data from individuals who bred at least twice along our study period, excluding the few known second (replacement) clutches in the same year. In total, we have 2103 records of laying date for 687 different females with an individual mean of 2.71, giving us enough power for the models to detect significant variation in plasticity using random regression (Martin et al., 2011; van de Pol, 2012).

Pedigree Structure

Genotypic variance is based here on the social pedigree, with "dummy code" assigned to a missing parent when only one parent was known. Such social pedigree can lead to unreliable or inaccurate estimations of variances and covariances due to misassigned paternities derived from extra-pair paternity and the probability of polygyny in this species (Canal et al., 2020). However, we do not expect our results to be influenced by potential EPP-related misassignments, as EPP rate in the study population is around 15% (Canal et al., 2012), below the 20% threshold considered to impact estimates of additive genetic variance (Charmantier & Réale, 2005; Firth et al., 2015). In summary, our data comprised 14973 records 12 generations depth with 1910 founders and 25531 full siblings for 12956 maternities (mean maternal sibship size of 8.10) and 12299 paternities records (mean paternal sibship size of 8.46).

Environmental factors

We selected the environmental factor explaining most of the variation of the phenotype according to the correct time period with the package `ClimWin` (Bailey & van de Pol, 2016). The sum up of temperature above 10.5°C from 24th April to 9th June has been found here to explain the variability in laying date in our population (see Chapter 4). This factor, known as Growing Degree Day, is the number of degrees needed to predict a phenological trait above a given threshold, here 10.5°C (in absence of know species-specific threshold for the development of invertebrate, we use here a general agriculture threshold, see (Bonhomme, 2000). For instance, a temperature of 11.5°C for a 10.5°C threshold equals 1 Degree-Day. All Degree-Day values were summed up across the focal period and averaged for obtaining the daily temperature (average air temperature). We also used the North Atlantic Oscillation in winter (NAOw) as the climatic variable influencing the wintering conditions experienced by pied flycatchers and inducing selection for early breeding dates (see Le Vaillant et al., 2021).

Statistical approach

To examine if females differed in their individual breeding date responses to environmental variation, we use a random regression mixed model, so-called 'animal model' (see Nussey et al., 2007). Individual laying date for each year is regressed against a global climatic index and temperature cues used by birds (i.e., respectively NAO in winter and the Degree Day of threshold 10.5 across the 24th

April to the 9th June) with individual reaction norm coefficients (slopes) fitted as random effects. Because some individuals have been recorded in some environments (years) not experienced by other individuals, we teased apart the effect of the environmental factor on laying date by a distinction into between-individual (bE_i) and within-individual variation (E_ij - E_i)(van de Pol & Wright, 2009). The laying date (z) of the i^th individual in the j^th year within the l^th "environmental block" was modelled as:

$$\begin{aligned} z_{ijl} =& \alpha z + a_i + b_i(E_{ij}\check{}E_i) + b_E i + age_{ij} + habitat_{ij} + mate_{ij} + mass_{ij} \\ &+ Z_1\varphi(p_i, n_1, E) + Z_2 a_i + Z_3 y_i + Z_4 male_i + \varepsilon_i \end{aligned} \tag{1}$$

where αz is the overall mean laying date (intercept), a_i and b_i are the individual intercept and slope, respectively, related to Degree Day or NAO in winter (E) and b is the population-level slope Fixed effects also included factors known to influence laying date in passerines (Verhulst & Nilsson, 2008) as: "age_{ij}"as the female's age (first-year or older), "$habitat_{ij}$" i.e. where the female bred in a given year (oak or pine forest), "$mate_{ij}$ ", the mating status of the females (monogamous and primary/secondary female) and finally, female body "$mass_{ij}$", as a metric of female condition. The random effects split the variance non-accounted for by the fixed effects by taking into account the repeated measures of the same female over the years : the term $Z_1\varphi(a_i, n_1 E)$ is the random regression function of order 1 of the effect of the individual i and environment E and $Z_2 a_i$, the additive genetic variance (VA) of individual i . Finally, the terms $Z_3 y_i$ and $Z_4 male_i$ refer to the effect of year and the identity of the male the female was mated with, respectively, and with (ε) the residual variance not explained by fixed or random factors.

To test for the individual interactions on total phenotypic variance, we include individual (I×E) in the random terms. All estimations provided are posterior modes from a Bayesian framework with `MCMCglmm` (Kingsolver et al., 2007) in `R` version 3.5.3 (R Core Team, 2017). To deal with the overestimation to detect the magnitude of variation in I×E (Brommer, 2013), we allow heterogeneity in the residual variance (heteroscedasticity) by grouping years with similar environment ("environmental block" divided in 9 residuals blocks) as suggested in Ramakers et al., 2020. For fixed effects, we used a weakly informative prior using a standard inverse-Wishart prior, with parameters $V = diag(k)$ and $nu = 0.002$ for univariate models where k=9, for the number of environmental categories used, and a parameter-expanded prior for the random effects in multivariate models ($V = diag(d)$, $nu = 1$, $alpha.mu = 0$, $alpha.V = diag(d) * 25^2$, where d is an identity matrix of dimension 2). All models were run for 20,100,000 iterations with a burning of 1,000,000 and a thinning of 10,000.

From these models, we constructed a 2 × 2 unstructured variance–covariance matrices for the intercept and the slope of individuals effects. We extracted then the individual-environment covariance estimates (estimates slopes variances) (see Ramakers et al., 2018b and calculated them along an environmental gradient for both factors considered. From these models, we also extracted the individual slope of the reaction norm and created four groups of females according to the level of response to visually show the differences in mean and variance of laying date along environmental gradients.

Results

Reaction norm

Between 1986 and 2016, the pied flycatcher shows plasticity in response to both Degree Day (Figure 1a) and NAOw variation (Figure 1b), at both population (solid lines) and individual levels (grey lines), with in average early breeding time to higher Degree Day and positive values of NAOw. The NAOw index and Degree Day showed no clear trend across years and neither accounts for variability in selection on laying date at phenotypic level (see Le Vaillant et al., 2021). Both factors were unrelated (r=0.005, n=27, P=0.296).

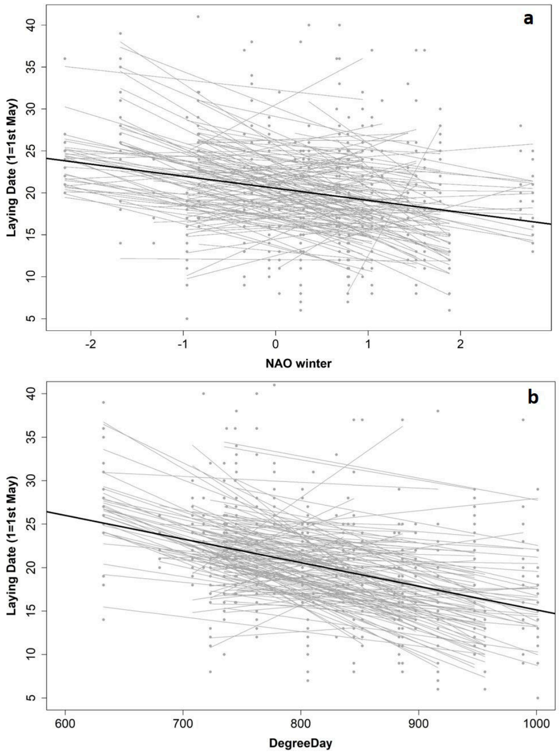


Figure 1: Observed reaction norms of laying dates against NAOw (a, above) and Degree Day (b, below). Greys dots represent an individual laying date expressed for a given environment in a specific year. Grey lines are individual reaction norm; black bold line is the mean reaction norm of the whole population. For visual clarity, only females breeding at least four times (n=140) are represented here although the full analyses includes females that bred at least twice (see Material and Method section).

Previous tests on several models allowing within-individual correlation with the environment found evidence for individual variation in the reaction norm (I×E, see Chapter 4). Models accounting additive genetic variance is the best explaining the total variance in laying date are detailed in Table 1.

Table 1: Estimates from the random regression mixed model of the laying date with NAOw (left) and Degree Day (right) between 1987 and 2016. Fixed and random effects are shown, with E as between-individual (e.g. bE_i) and $\overline{E}$ for within-individual variation (e.g.$(E_ij$ - $E_i)$). For explanation on the variables input to the models, see Material and Methods.

Factors	**NAOw**				**Degree Day**			
Fixed effect	post.mean	[CI95]	eff.samp	pMCMC	post.mean	[CI95]	eff.samp	pMCMC
Intercept	32.305	[25.120; 40.388]	2000	< 5e-04	56.526	[42.736; 69.676]	2000	< 5e-04
AGE	-4.278	[-4.987 ; -3.540]	2000	< 5e-04	-4.231	[-4.912 ; -3.534]	1873	< 5e-04
HBT	1.842	[1.173 ; 2.460]	2324	< 5e-04	1.861	[1.181 ; 2.498]	2000	< 5e-04
MASS	-0.443	[-0.976 ; 0.042]	2000	0.085	-0.513	[-1.080 ; -0.025]	2000	0.073
MATE	0.834	[0.551 ; 1.167]	2000	< 5e-04	0.902	[0.609 ; 1.201]	2158	< 5e-04
E	-1.003	[-2.117 ; 0.139]	2000	0.063	-0.025	[-0.035 ; -0.013]	2000	0.001
$\overline{E}$	-0.853	[-2.072 ; 0.309]	2000	0.142	-0.029	[-0.041 ; -0.016]	2000	< 5e-04
Random effect								
YEAR	11.98	[5.778 ;19.240]	2000	-	6.912	[3.378 ;11.38]	1439	-
MALE	0.754	[9.81e-07;2.217]	2000	-	0.449	[1.758e-07;1.532]	2160	-
G	4.500	[1.416;7.004]	1579	-	4.758	[1.859;7.198]	2000	-
I × E	0.828	[4.574e-06;1.709]	2000	-	2.971e-05	[1.145e-12;1.102e-04]	2000	-

The proportions of variance explained by differences between individuals and within individuals are similar in models with NAOw (Table 1, respectively: E = -1,003 HPD[-2.117; 0.139] and $\overline{E}$=-0.853 HPD [-2.072; 0.309]) whereas models with Degree Day are not fully conclusive (Table 1, E =-0.029 HPD[-0.035 ; -0.013] and $\overline{E}$= -0.029 HPD [-0.041; -0.016]). These results rely on the fact that the individuals' variation in reaction norm (plasticity) explains the mean variance of the population. The individual-environment covariance from these models (estimates slopes variances) is higher for positive NAO whereas it remains constant for higher Degree Day values, corresponding to adverse environmental conditions (Figure 2).

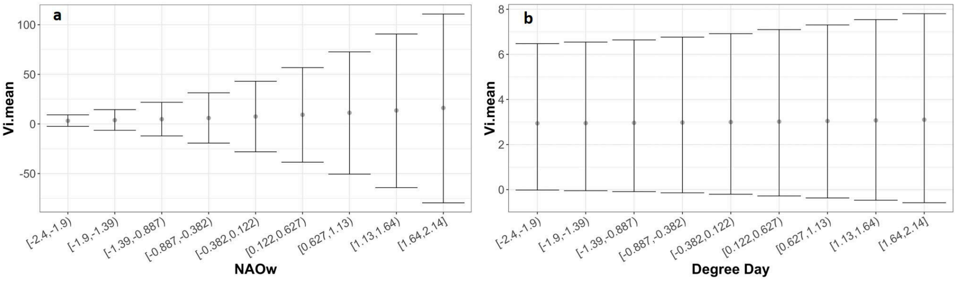


Figure 2: Association between individual variation Vi (mean ± HPD confidence interval) and environmental heterogeneity with NAOw (left, a) and Degree Day (right, b)

By comparing the individual slopes between laying date and i) environmental factors at the population level (Figure 3, solid line) and ii) four slope levels within the population (Figure 3, dashed line), we can extrapolate population response differences along the environmental gradient. For positive values of NAOw (Figure 3a) and higher Degree Day values (Figure 3b) the variance around the population mean is smaller, with the four among-population groups showing similar phenotypic response (early laying date) for adverse environmental conditions.

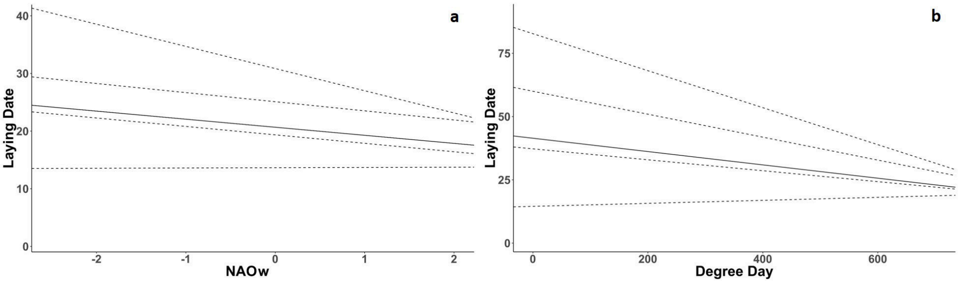


Figure 3: Slope of the average laying dates (1 = 1st May) in response to NAOw (left, a) and Degree Day (right, b). Solid line represents the average mean laying population reaction and dashed lines are examples for four groups of females according their level in response to the level factors

Discussion

Spanish pied flycatcher population starts breeding earlier as temperature accumulation increases (Degree day) and positive values of NAOw. However, our results suggest that female flycatchers differ in their reaction norm and show variable responses in relation to these two environmental factors. Variation between females in their plasticity (I×E) reflects within-individuals differences in their capacity to adjust laying date to environmental variation, while differences in mean reaction norm denote between-individual variations. Our study shows differences in reaction norms between individuals (Figure 1) also evidencing differences within individuals in their response (variation in I×E interactions, but see Chapter 4). The within- and between-variances are expected to be close to zero and nonsignificant when the within- and between-subjects effects are the same (van de Pol & Wright, 2009). Between- (bE_i) and within-individual variation ($((E_ij$ - $E_i))$) are factors explaining variation of laying date for NAOw, with however, less conclusive effect for Degree Day (Table 1). In all cases, in this population, the variation of breeding time seems to be explained at the individual and population levels, suggesting that phenotypic plasticity explains the variation of laying date in response to environmental variation (Sauve et al., 2019). While some studies have not detected differences in individual reaction norms in breeding phenology, despite plasticity being present in the studied populations (Bourret & Garant, 2015; Charmantier et al., 2008; Dobson et al., 2017; Froy et al., 2019; Przybylo et al., 2000), our results show that within-individual phenotypic plasticity can be sufficient to generate a phenotypic response at the population level (Nussey et al., 2007; Sauve et al., 2019). Therefore, we speculate that the effects at the individual level might alter the population response to the environmental factor explaining variation in laying date at the population level and so the capacity to track fluctuating conditions.

Temperature is considered a major cue that females use to start breeding, and indeed seems to explain most of the variation in breeding phenology in this pied flycatcher population (Le Vaillant et al., 2021). Indeed, females advanced their breeding time in response to higher Degree Day (Figure 1a), indicating hotter accumulated temperatures, but Degree Day showed no trend for an early temperature threshold accumulation over the study period, which help explaining the reason why breeding date has not advanced in the last three decades (Le Vaillant et al., 2021). Although birds are usually plastic in their population responses to spring average temperatures (Charmantier et al., 2008; Nussey et al., 2005c), the relationship between timing of breeding and mean temperatures is expected to be equivalent to that regarding heat accumulation (Charmantier & Gienapp, 2014). We therefore should expect an early breeding date with higher temperature accumulation. An increase in Degree Day at the time of laying could cause an increasing ecological mismatch with food abundance, therefore playing an important role in selection on timing of reproduction (Ramakers et al., 2018a). However, this assumption might not be applied in our population since diet of pied flycatchers may differ depending on habitats and temporal variation (Burger et al., 2012).

Evidence for I×E interactions is population-dependent and can show large differences even at small scales (Porlier et al., 2012). We found variance in the slopes of individual reaction norm suggesting that that there is variation in the degree of plasticity at individual level (Nussey et al., 2007). Here, females differed in elevation and slope of their laying date-temperature reaction norms (Figure 1). The difference in reaction norms may be due to the genetic structure of the population, as, in addition to the individual-by-environment interaction (I×E). Indeed, differences between family lines in their sensititivy to environmental change (slope of reaction norm) and their response (elevation of reaction norm), were partially confirmed here through the effect of genotype-by-environment interaction (G×E) (Chapter 4). Recently, in an experiment of genomic selection between early and late breedeers in great tits (*Parus major*), Verhagen et al., 2019a found genetic and phenotypic differences in responses in timing of breeding. Specifically, they found that artificial selection lead to differences in elevation, but not in the degree of plasticity between selection lines. These results also confirm the importance of considering the individual as a result of a selection lineage when looking at the between-individuals variance residing in the population.

The influence of environmental conditions on I×E

Detection of significant environmental interaction is highly related to the environmental variable under scrutiny and its relation with the actual driver of plasticity (Gienapp, 2018). In absence of informative environmental cues as predictors of plasticity, we used here two environmental factors as cues that might explain variation with a local and global influence (Table 1). However, degree of plasticity depends on the covariance of breeding time with the environmental factors. Hence, variance of individual plasticity is higher for NAOw than Degree Day, and only increases as Degree Days values rise (Figure 2), underlying the importance of NAOw in the plastic response. The NAOw index might impact selection on plasticity, while the Degree Day, by explaining the variability of the phenotypic trait (see Chapter 4), might impact selection on the phenotype. Degree Day might be a more relevant cue used by birds at laying time, whereas the NAOw might be an factor impacting the female during winter and return from migration and so its body condition at the time of breeding.

Positive values of NAOw are considered as adverse conditions for migrant birds, as much as high values of Degree Day index (Marrot et al., 2018; Przybylo et al., 2000; Saino et al., 2011). High covariance in slope among individual laying dates in poor environmental conditions (Figure 2) implies that selection might favour plastic individual in these conditions. Some evidence for selection on highly plastic individuals has been found in other studies in other short-lived songbirds (Brommer et al., 2005; Nussey et al., 2005c). In addition, an increasing population density and worsening weather conditions underlie directional selection for plasticity for calving date in an ungulate species (Nussey et al., 2005b) with an increase of variance in plasticity for offspring birth weight in the same population (Nussey et al., 2005a). On the contrary, similar individual responses to climate conditions have been found in a study of common guillemots, *Uria aalge* (Reed et al., 2006) who concluded there was stabilizing selection against plastic individuals. However, a general pattern of selection on plasticity cannot be extrapolated to all populations or species and could be partly explained by environmental heterogeneity (Porlier et al., 2012). This is because selection may vary depending on the environmental conditions and is stronger in favourable years, when individual reaction norms converge (Reed et al., 2009). We found a decrease in individual variance as Degree Days increase, and with positive values of NAOw (Figure 3). The decrease in variance in laying date response can therefore be explained with individuals expressing similar patterns of reaction (low between-individual variance) in poor environmental conditions (Figure 3), or either because the within-individual variance (heterogeneous residual variance) might decrease in stressful environments (Ghalambor et al., 2007; Nicolaus et al., 2013).

Whether plasticity is adaptive depends on the environment where it is expressed, because changes in the environmental values that an organism may encounter will alter the selective importance of plasticity (Ghalambor et al., 2007). When environmental variance is high, plasticity is variable and affects the performance in the average environment (elevation) and hence the intensity of selection on plasticity (Brommer et al., 2012; Brommer et al., 2005). As a consequence, to cope with climate change, some populations will need to adapt their trait sensitivity to the new environmental condition (slope of reaction norm) instead of towards the average phenotype in the population (elevation of reaction norm) (van Asch & Visser, 2007). Further studies on selection on phenotypic plasticity are required to fully understand response of reaction norm. However, the problem of detection of environmental variation due to variance in residuals (Ramakers et al., 2018a; Ramakers et al., 2020) has led to some mistakes in the past. Likewise, individual variation in plasticity (I×E) was not detected until heterogeneity was taken into account into the model (Charmantier et al., 2008; Husby et al., 2010). This shows the importance of environment heterogeneity for the detection and the interpretation of individual and genotype variance in phenotypic traits.

Conclusions

Individual plasticity plays a role in the response of populations to different environmental conditions and may favour local adaptation. Variability in plastic responses of individuals implies a potential for both selection (Le Vaillant et al., 2021) and evolvability of plasticity (Chapter 4) to favour evolutionary change (Nussey et al., 2007). The environmental fluctuations of the factors explaining variation of I×E interactions might explain the stasis of breeding time over the study period and may determine its rate of adaptation and selection on individuals in our population (Kruuk et al., 2003; Ramakers et al., 2018b).

Acknowledgments

We thank many volunteers, technicians and friends, too numerous to mention individually, that helped with data collection. All authors contributed to the drafts and gave final approval for publication. The authors declare no conflict of interest. J.L.V received financial support from the Spanish Ministry of Economy and Competitiveness (grant BES-2016-077694). CC received financial support from the Spanish Ministry of Science, Innovation and Universities (FJC2018-038412-I). DC was supported by a grant from the Hungarian Academy of Sciences (MTA Premium Postdoctoral Research Program; ID: 2019-353). J.M.-P. was supported by the Spanish Commission of Science and Technology (CICYT, grant number CGL2015-70639-P and PID2019-104835GB-I00) and is currently funded by ARAID foundation. Fieldwork was supported mainly by CICYT, most recently by projects CGL2014-55969-P and CGL2015-70639-P.

```
####Within- between- Individual variation models
##Only with NAO for demonstration
# - MNAOw: average value experienced by individual i (i.e. across different breeding seasons)
# - XMNAOw: individually centred value (across breeding seasons)

# Model with environments divided into blocks of 3(29/3 ~ 9 residual blocks)
hist(phen.f$XMNAOw, breaks=9)
library(arules)
x <- discretize(phen.f$XMNAOw, breaks=9,method="interval")
levels(x)  #-> 3 years by groups

phen.f[which(phen.f$XMNAOw >= -2.4& phen.f$XMNAOw < -1.9),"Block3"]<-"[-2.4,-1.9)"
phen.f[which(phen.f$XMNAOw >= -1.9& phen.f$XMNAOw < -1.39),"Block3"]<-"[-1.9,-1.39)"
phen.f[which(phen.f$XMNAOw >= -1.39& phen.f$XMNAOw < -0.887),"Block3"]<-"[-1.39,-0.887)"
phen.f[which(phen.f$XMNAOw >= -0.887& phen.f$XMNAOw < -0.382),"Block3"]<-"[-0.887,-0.382)"
phen.f[which(phen.f$XMNAOw >= -0.382& phen.f$XMNAOw < 0.122),"Block3"]<-"[-0.382,0.122)"
phen.f[which(phen.f$XMNAOw >= 0.122& phen.f$XMNAOw < 0.627),"Block3"]<-"[0.122,0.627)"
phen.f[which(phen.f$XMNAOw >= 0.627& phen.f$XMNAOw < 1.13),"Block3"]<-"[0.627,1.13)"
phen.f[which(phen.f$XMNAOw >= 1.13& phen.f$XMNAOw < 1.64),"Block3"]<-"[1.13,1.64)"
phen.f[which(phen.f$XMNAOw >= 1.64& phen.f$XMNAOw <= 2.14),"Block3"]<-"[1.64,2.14]"

###Model Quantitative genetic
require(MCMCglmm)
Thin <- 10000
Burnin <- 100000
Nitt <- 2000*Thin+Burnin

priorGIE <- list(R = list(R1 = list(V=diag(9), nu=0.002)),
                 G = list(G1 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                          G2 = list(V=1, nu=1, alpha.mu=0, alpha.V=625),
                          G3 = list(V=diag(2), nu=2, alpha.mu=c(0,0), alpha.V=diag(2)*625),
                          G4 = list(V=diag(2), nu=2, alpha.mu=c(0,0), alpha.V=diag(2)*625)))

mNAO<- MCMCglmm(LD~AGE+HBT+MASS+MATE+XMNAOw+MNAOw,
                random=~YEAR+MALE+animal+us(1+XMNAOw):ID,
                prior= priorGIE, pr=T,
                rcov= ~idh(Block3):units,
                data= phen.f, pedigree=ped.fix, family='gaussian',
                thin=Thin, burnin=Burnin, nitt=Nitt, verbose=T)
mNAO$DIC #  9312.229

Vsp <- mNAO$VCV[,"XMNAOw:XMNAOw.ID"]
mean(mNAO$VCV[,"(Intercept):(Intercept).ID"]) # VI 1.09914
mean(Vsp)    #0.576
posterior.mode(Vsp) #0.0069
```

```r
HPDinterval(Vsp)  #2.319e-07 1.52

###Individual variance-covariance matrix
Vint <- mean(mNAO$VCV[,"(Intercept):(Intercept).ID"])
Vsl <- mean(mNAO$VCV[,"XMNAOw:(Intercept).ID"])
Cov <- mean(mNAO$VCV[,"(Intercept):XMNAOw.ID"])
#Lower credible interval
Vint.cil <- HPDinterval(mNAO$VCV[,"(Intercept):(Intercept).ID"])[1]
Vsl.cil <- HPDinterval(mNAO$VCV[,"XMNAOw:(Intercept).ID"])[1]
Cov.cil <- HPDinterval(mNAO$VCV[,"(Intercept):XMNAOw.ID"])[1]
#Upper credible interval
Vint.ciu <- HPDinterval(mNAO$VCV[,"(Intercept):(Intercept).ID"])[2]
Vsl.ciu <- HPDinterval(mNAO$VCV[,"XMNAOw:(Intercept).ID"])[2]
Cov.ciu  <- HPDinterval(mNAO$VCV[,"(Intercept):XMNAOw.ID"])[2]
# Construct matrix  #G.mat Q matrix in G=zGz
Gmat.m <- matrix(c(Vint,Cov,Cov,Vsl), 2,2, byrow=T)
Gmat.cil <- matrix(c(Vint.cil,Cov.cil,Cov.cil,Vsl.cil), 2,2, byrow=T)
Gmat.ciu <- matrix(c(Vint.ciu,Cov.ciu,Cov.ciu,Vsl.ciu), 2,2, byrow=T)

## Get environment-specific variance through matrix multiplication
env<-data.frame(matrix(vector(), 9, 4,dimnames=list(c(), c("env","Vi.mean", "vi.cil", "vi.ciu"))))
x <- cbind(rep(1,dim(env)[1]),env$env) # z=creates vectors of 1 and the environment (here stored in object 'env')

 for (i in 1:length(env$env)){ # loop function to calculate variance in each environment
  env$vi.mean[i]<- matrix(x[i,],1,2)%*% gmat.m  %*%t(matrix(x[i,],1,2))  # gmat = g matrix
  env$vi.cil[i] <- matrix(x[i,],1,2)%*% gmat.cil%*%t(matrix(x[i,],1,2))  # gmat.cil = g matrix 0.025 quantile
  env$vi.ciu[i] <- matrix(x[i,],1,2)%*% gmat.ciu%*%t(matrix(x[i,],1,2))  # gmat.ciu = g matrix 0.975 quantile
 }
ggplot(env, aes(y=Va.mean, x=env))+geom_point(size=2, col="darkgrey")+theme_bw(base_size=12)+geom_errorbar(aes(ymin=va.cil, ymax=va.ciu))
```

General Discussion

Another curious aspect of the Theory of Evolution is that everybody thinks he understands it!, Jacques Monod 1974

The unifying goal of this PhD was testing the mechanisms that may drive the evolutionary dynamics of laying date in a context of current global change. By exploring the variability of the trait, the relative fitness benefits of laying earlier along the quantitative genetic and non-genetic changes in the population, I confronted the three main requisites needed for natural selection to occur. This PhD documents the crucial role that individual plasticity may have at shaping the complex and multidimensional environmental variation that this population of pied flycatcher *Ficedula hypoleuca iberiae* experiences from one year to the next. Although not fully conclusive, my results suggests that phenotypic plasticity may have some genetic basis and be a potential candidate to favour local adaptation in this population. As can be expected in a natural setting, perhaps the evolutionary potential of phenotypic plasticity might not be the only and unique mechanism that may drive local adaptation.

Evolutionary dynamics of laying date in heterogeneous environments

Breeding date is a labile trait related to reproductive performance and show variation to environmental factor(s). However depending of the breeding habitat and the local condition, laying date will vary annually. The Spanish population of pied flycatcher, *Ficedula hypoleuca*, displays a large flexibility in breeding phenology that may be caused by individual response to annual environmental variation (**Chapter I**). Current observed trends for change in breeding date and earlier phenology are best explained by individual adjustment to increasing temperatures (Both, 2000; Przybylo et al., 2000; Sheldon et al., 2003) and individual learning and experience (Grieco et al., 2002; Schiegg et al., 2002). Indeed, individuals that can adjust the expression of their phenotypes according to the prevailing environmental conditions by phenotypic plasticity, will more likely to prevail than individuals that remain inflexible to stochastic events. In our population, there is within-individual variance driven by stochastic and unpredictable environmental effects, known as predictability (see **Chapter I**), but it can differ between individuals and populations. In addition, within-individual changes in breeding date might mask patterns of reproductive senescence in reproductive success (van de Pol & Verhulst, 2006) and challenge the study of variation of plasticity in wild populations (Charmantier et al., 2014a; Nussey et al., 2013). Indeed, response to environmental conditions and expression of plasticity vary during the lifetime of individuals with generally younger and older individuals observed to be less plastic (Balbontín et al., 2009; Bonamour et al., 2020; Saino et al., 2004). Thus, results of this PhD suggest that individuals have the capacity to plastically respond to environmental variation, although other aspects related to senescence remain to be assessed to fully explore the within- and among-individual reaction norms in our population.

Individuals have the potential to respond to environmental changes with flexible trait expression, like breeding phenology in this PhD (Ahola et al., 2004; Both et al., 2004; Lehikoinen et al., 2004; **Chapter I**). However, stochastic environments impose challenges to drive evolutionary responses within an ecological framework. Hence, current increase in global temperature can also alter the frequency and intensity of unpredictable changes, as extreme weather events (IPCC, 2014) which may both influence the form and intensity of natural selection (Marrot et al., 2018; Marrot et al., 2017; Siepielski et al., 2017). Migratory birds are particularly sensitive to these climatic environmental fluctuations, as changes in local and global conditions will both influence selection on breeding time, particularly on extreme values as I have described in this PhD (**Chapter II**). In migratory birds the cues used by birds to migrate and start breeding are independent from the environmental factors on the breeding grounds (Both, 2000; Visser et al., 2004). Therefore, the specific environmental cues that drive phenological events (Simmonds et al., 2019) are distinct from the environmental factor driving natural selection during the breeding period in migratory birds (Pearce-Higgins et al., 2015). Phenological sensitivity and climate change exposure also vary between species and populations (Bailey et al., 2020; Thackeray et al., 2016). Therefore, considering the environmental fluctuations that populations experience, the phenology of birds is expected to increase in unpredictability (**Chapter I**) and a response to selection might be difficult to anticipate (**Chapter IV**). Indeed, spatial and temporal heterogeneity in climate change impose contrasting selection pressures on populations (de Villemereuil et al., 2020; Senner et al., 2017), with sometimes a temporal lag effect (Vatka et al., 2020), might constrain the adaptive potential of populations (Gienapp et al., 2014; Senner et al., 2018).

Rapid adaptive response to climate change with concomitant advances of breeding time are usually understood as local adaptations (Pulido & Berthold, 2004; Radchuk et al., 2019; Senner et al., 2017), but see Gienapp and Brommer, 2014. However, a lack of change does not mean that there is no response to environmental conditions or that evolution is not acting (see Hansen and Houle, 2004; Merilä et al., 2001b). In the pied flycatcher *Ficedula hypoleuca*, laying date has advanced over the past decades in many populations in Europe in contrast to the population studied here (Both et al., 2004; Both et al., 2006b; **Chapter II**. Populations differ in selection pressures for early laying date which constrain the adequate breeding responses to the local change in climatic conditions (Both, 2000) and may explain the difference in population responses in *Parus* species (Visser et al., 2003). Phenotypic selection on avian breeding date in response to environmental conditions has been detected in many studies (Charmantier et al., 2008; Marrot et al., 2018; Nussey et al., 2005c; Visser et al., 2006) and in the

Chapter II of this thesis. However, fluctuating phenotypic selection makes difficult to infer the direction of phenotypic variation (Bell, 2010; Chevin & Haller, 2014; de Villemereuil et al., 2020) and may hinder any trends for early laying date over time of populations facing any environmental change (Gienapp et al., 2006; Gienapp et al., 2014; Pujol et al., 2018; **Chapter II**; **Chapter III**).

Evolutionary change occurs when a change in the environment triggers a shift in the mean or variance of the genetic part of the phenotypic expression. Moreover, to demonstrate that adaptive evolution has occurred under natural selection, evidence is needed for genetic (Merilä & Hendry, 2014) or at least flexible genotypes depending of the environmental conditions (G×E interactions). I have not detected evolutionary change in laying date in the study population (**Chapter II**), despite quantifying phenotypic selection on laying date. This dissociated changes are called evolutionary stasis, and has been documented previously for other traits (Bonnet & Postma, 2018; Hansen & Houle, 2004; Merilä et al., 2001b). Breeding time in this population therefore showed a lack of evolutionary change, unsurprisingly because of its low additive genetic variance (Price et al., 1988). Such apparent evolutionary stasis might be interpreted as lack of local adaptation given the heterogeneous and temporally variable environment this population is experiencing. However, such local adaptation can be explained by phenotypic plasticity on breeding time, whereby different genotypes express different phenotypes along a gradient of given environmental factor. In this PhD I defend that within-individual plasticity of phenotypes can act as a buffer mechanism of adaptation, which does not necessarily require genetic changes.

Phenotypic plasticity might play a role in long-term strategy adaptation of population face to changing environmental conditions (Scheiner, 1993; Scheiner et al., 2019). Indeed, phenotypic plasticity has been hypothesized to precede and facilitate adaptation to novel environments (c.f. 'Baldwin effect'; 'plasticity-first' and 'genetic assimilation' theory, see also (Badyaev, 2009; Crispo, 2007; Lande, 2009; Waddington, 1953). Evolutionary theories underline the importance of phenotypic plasticity in driving genetic evolution (e.g. adaptive landscape, see Chevin et al., 2010; Price et al., 2003). However, plastic responses do not always result in changes in genetic variation (Wood & Brodie III, 2016), and although lab and field studies support that phenotypic plasticity influence evolutionary trajectories and promote adaptation (Schlichting & Wund, 2014; Schwander & Leimar, 2011), the contribution to evolution by phenotypic plasticity followed by genetic accommodation is still controversial (De Jong, 2005; Levis & Pfennig, 2016; Pigliucci et al., 2006; Via et al., 1995). Results found in this PhD (**Chapter IV** and **Chapter V**) certainly feed such controversy since we found non-conclusive evidences that G×E plays a clear role on local adaption, or specifically on I×E interactions. It is well established that phenotypic plasticity can evolve (Crispo et al., 2010) and my results partially agrees with it, however only few studies have so far demonstrated a genetic basis to plastic responses (Hayward & Pemberton, 2018; Ramakers et al., 2018b). In this thesis, I detected low genetic variation in the timing of breeding date (**Chapter IV**), supporting the hypothesis that phenotypic plasticity may have the potential to evolve and to promote evolutionary change in response to the selection detected early (**Chapter II**). However, fluctuation in environmental factors perhaps constrain the evolutionary dynamics of labile traits, explaining the few evidences of evolution of plasticity in phenological trait in response to climate change (Bradshaw and Holzapfel, 2008; Brommer et al., 2008; Charmantier et al., 2008; Gienapp and Brommer, 2014; Merilä and Hendry, 2014; Nussey et al., 2005c; Ramakers et al., 2018a; **Chapter III**).

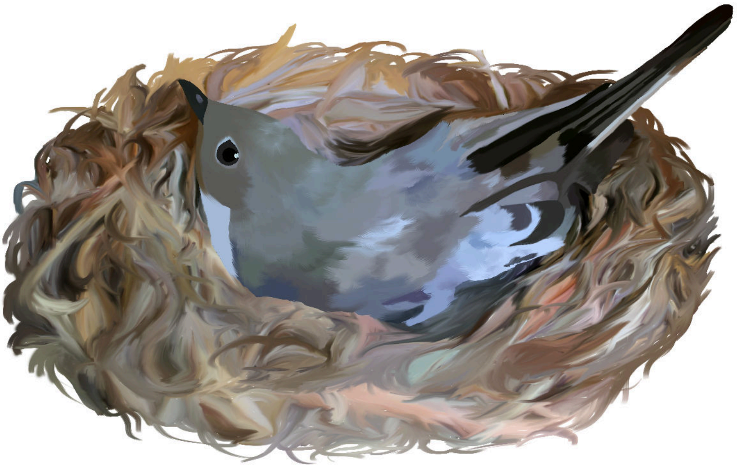
Despite that evolutionary responses to climate change is widespread, we know relatively few about the ecological consequence of climate change (Clutton-Brock & Sheldon, 2010; Parmesan, 2006; Samplonius et al., 2021) and the evolutionary mechanism involve in local adaptation is not yet no evident. By buffering the deleterious effects of novel environments, plasticity can allow populations to adaptively modifying the phenotype in response to novel selection pressures thus permitting organisms to maximise survival and reproduction (Chevin & Lande, 2010; Chevin et al., 2010). Changes in the timing of breeding might may be achieved by phenotypic adjustment with plastic response and genetic change. Both process can act together but at different time scales, although current evidences in wild populations are scarce (Charmantier & Gienapp, 2014; Gienapp et al., 2007; Gienapp et al., 2008; Merilä, 2012; Merilä & Hendry, 2014). My results suggest that the population might adapt faster to the environmental variation across years through plasticity (**Chapter IV**) than through genotypic change (**Chapter III**) in the trait itself. However, we cannot tell if plastic responses will be fast enough to adapt to unpredictable environmental fluctuation due to climate change.

Considerations about population dynamics

In an ecological context, environmental changes are concomitant with evolutionary changes during few generations and interact with population dynamics (Hendry, 2017; Pelletier et al., 2009; Schoener, 2011; Smallegange & Coulson, 2013). This eco-evolutionary dynamics perspective arise through the links between ecological interactions, contemporary evolution, and its fitness consequences on population growth rate and density (Fussmann et al., 2007; Hendry, 2017; Kinnison & Hairston, 2007). Without considering evolutionary adaptations of populations, it is nearly impossible to produce an accurate and reliable predictions of the demographic consequences of climate change on populations. Disentangling the influence of different evolutionary mechanisms shaping life-history traits is crucial to population persistence (Radchuk et al., 2019; van Benthem et al., 2017) and may unravel the links between individual- and population-level changes in populations (Bonnet et al., 2019). Although population can avoid extinction face to climate change through dispersal, plasticity or local adaptation (Garant et al., 2007a; Hoffmann & Sgrò, 2011), only the potential of rapid adaptive evolution can prevent population extinction (i.e. evolutionary rescue; Ashander et al., 2016; Bell, 2017; Bell and Gonzalez, 2009; Carlson et al., 2014). However, the capacity for population dynamic to promote rapid and local adaptation over extinction has barely been explored (Chown et al., 2010; Reznick & Ghalambor, 2001). This PhD certainly opens a challenging area where phenotypic plasticity and population dynamics should be married to understand and even predict the role of global change on population dynamics and persistence. Demographic buffering (carrying capacity) and adaptive plasticity may alleviate the negative impact of climate change and prevent populations from extinction (Chevin & Lande, 2010; Hilde et al., 2020). However, if plasticity is not sufficient , the response to novel environmental conditions will be maladaptive because of the demographic costs of selection (see Bell et al., 2021a). Moreover, if the size of the population is small, the amount of heritable variation and genetic diversity for adaptation may be limited (see Lanfear et al., 2014), abolishing the effect of selection which could be overwhelmed by random genetic drift (genetic bottleneck). Other population density-dependent factors may also constraining evolution, through demographic stochasticity, Allee effects and genetic limitation (e.g. inbreeding depression, loss of genetic variation, deleterious mutations and mutational meltdown). Demography may also play an underappreciated role in dispersal and its relative influences on gene flow and natural selection Hanski et al., 2011; Kinnison and Hairston, 2007; Postma and van Noordwijk, 2005. Predicting a phenotypic change and its population level consequences raise many unanswered questions on the mechanisms accounting for those changes and their long-term consequences for population persistence. Although it is well accepted that changes in population growth trajectories can have long-lasting effects, contrasting predictions can be made depending on the genetic and environmental contributions driving those changes. Forecasting the influence of environmental change and adaptive phenotypic plasticity on population should be on the first importance for dynamic model (Chevin et al., 2010; Hendry et al., 2008; Reed et al., 2010). The integration of evolutionary change and evolution of plasticity into demographic models can therefore help to predict and prevent extinction risk of natural population, but these models and assumptions remains challenging (Gienapp et al., 2013a; McLean et al., 2016; Reed et al., 2015; Visser & Gienapp, 2019).

Phenological asynchrony and shifts in phenology have an impact at the reproductive success of the whole population in a specific year, but also have an effect on the successive years. By affecting the growth rate of the population and the survival of the next generation, it might have severe consequences for stability and the demography of population (Plard et al., 2014; Simmonds et al., 2020). In response to warming climate and earlier spring, change on the breeding period have important consequence on the reproductive outcome and dynamic of the population and so on the evolutionary trajectory of this trait inside the population. Breeding populations of migratory birds are confronted with a significant evolutionary pressure to align their reproductive period with food availability (Both & Visser, 2005; Visser, 2008; Visser et al., 1998). Degree of asynchrony and preys availability have the potential to impact population size and growth (Samplonius et al., 2021; Shipley et al., 2020), however, phenological mismatch alone is unlikely to solely explain spatial and temporal variation in population trends (Bowler et al., 2019; Burgess et al., 2018; Senner et al., 2018).

For instance, the degradation of environmental conditions in a Finnish population of pied flycatchers is suspected to be a cause for the observed mismatch between phenology of the environment and breeding time, and therefore to be responsible in the decline in mean clutch size over time (Laaksonen et al., 2006). Phenotypic plasticity response in breeding time may alleviate this asynchrony between reproductive timing and food availability (Walker et al., 2019), as my results may suggest. However, a plastic response of birds population to environmental change does not often ensure a stable population (Charmantier et al., 2008), with populations with lower responses are declining (Both & Visser, 2001; Visser et al., 2006). Increasing evidences suggest that climate change affects the timing of breeding in birds, but the common assumption that such changes affect the population dynamics of birds is not always apparent (Dunn & Møller, 2014; Reed et al., 2013a; Reed et al., 2013b; Wilson & Arcese, 2003). Despite that changes in phenology have been related to species large scale population growth rates (Franks et al., 2018; Møller et al., 2008), how I×E and particularly G×E interactions for the timing of breeding relate to population growth rate is not well understood (McLean et al., 2016). I defend that phenotypic plasticity may have a role to avoid population extinction risk. Although I cannot ensure whether this evolutionary mechanism alone will be enough to prevent a decrease in the breeding population under events of future climate changes.



Summary

The main aim of this thesis was to investigate the evolutionary dynamics of a life-history trait in a Spanish population of wild pied flycatchers *Ficedula hypoleuca*.Since evolutionary change requires environmental variation to occur, this PhD tackles the role that environmental variation has on the evolutionary architecture of laying date. I developed a research agenda that explores the main pillars that natural selection lays on: sources of trait variance, phenotypic selection and the evolutionary potential.

Taking advantage of one of the longest-term monitored population embracing 29 years of data on breeding observations in a population of flycatchers, laying date has not statistically changed over the same period of time. Unsurprisingly, environmental conditions, either considering temperature- or precipitation-based variables, were not constant but did not show any trend overtime. Thus, considering that early breeders are favoured and that environmental conditions have changed from one year to the next in an unpredicted manner, it is expected that plasticity at individual or genetic level might play a major role on local adaptation. To formally explore this possibility, we tackled the main evolutionary mechanisms that explains variation on laying date in our population.

First, we initially explored the consistency in breeding phenology of females. The results points towards a lack of repeatability in their behaviour, mirroring the variability of laying date among females. As suggested in the **Chapter I**, phenotypic plasticity might play a role in the breeding phenology of pied flycatchers to optimise their reproductive success. However, flycatchers are migratory birds and its breeding cycle is affected by multiple local factors on their breeding ground as much as global factors along their migratory routes or wintering sites. From 1987 to 2016, the environmental conditions display large fluctuations, from precipitation to local temperature, and breeding females have followed these variations.

Identifying the relative role that multiple environmental factors causing a selection on breeding date is essential to understand these phenotypic variations. In the **Chapter II**, we explored nearly 30 local and global environmental factors to detect the main environmental factors that influence phenotypic selection on laying date. The results suggests that local factors like the minimum temperature in May and April shaped selection on laying date, suggesting that multiple environmental factors may constrain phenotypic change in this population of pied flycatchers.

To observe a response to selection in the long-term, the study trait has to be genetically transmittable (cf. heritable) from one generation to the next. However, as shown in the **Chapter III**, heritability of laying date is very low, due to the small proportion additive genetic variance in relation to total phenotypic variance. It suggests that environmental variation and not additive genetic variation may explain the variation in laying date in our population. Accordingly, our results did not find any support for a microevolutionary change in laying date in this population, inferred from a lack of temporal trend on breeding values.

Since microevolutionary changes did not explain adaptation to changing environmental conditions, we further explored whether genotype-by-environment (G×E) interactions. To do so, I first explored whether individual-by-environment (I×E) exists and then partitioned it in G×E and PE×E, accounting for the genetic and non-genetic variance of I×E interactions. The **Chapter IV** tested the potential evolutionary role of phenotypic plasticity (G×E). We found a non-conclusive support of G×E interactions, suggesting that it may explain local adaptation but not unequivocally. Given the non-conclusive influence of G×E, I further explored the role of I×E in this population.

In the **Chapter V**, I showed that variation in laying date in the population is driven by individual variation in response to two environmental factors. Breeding date expression might be associated with condition of individuals and highlights the need of taking into account individual variability of plasticity under environmental change scenarios. Overall, we found that laying date is a variable trait under stronger phenotypic selection when environmental conditions are not favourable. However, the evolutionary dynamics of this trait is better explained by non-genetic mechanisms, quite likely G×E interactions, rather than by a genetic shift. This PhD highlights the role and complexity that local and global environmental factors and the conditions that individuals experience at the time of breeding have on the evolutionary dynamics of life-history traits.

Resumen

El objetivo principal de esta tesis fue investigar la dinámica evolutiva de un rasgo de la historia de vida en una población española de papamoscas cerrojillo silvestres *Ficedula hypoleuca*. Dado que el cambio evolutivo requiere que ocurra una variación ambiental, este doctorado aborda el papel de la variación ambiental tiene sobre la arquitectura evolutiva de la fecha de puesta. Desarrollé una agenda de investigación que explora los principales pilares sobre los que se asienta la selección natural: las fuentes de variación de rasgos, la selección fenotípica y el potencial evolutivo.

Aprovechando una de las poblaciones monitoreadas a más largo plazo que abarca 29 años de datos sobre observaciones de reproducción en una población de papamoscas, la fecha de puesta no ha cambiado estadísticamente durante el mismo período de tiempo. Como era de esperar, las condiciones ambientales, ya sea considerando variables basadas en la temperatura o la precipitación, no fueron constantes pero no mostraron ninguna tendencia en el tiempo. Por lo tanto, considerando que se favorece a los reproductores tempranos y que las condiciones ambientales han cambiado de un año a otro de manera impredecible, se espera que la plasticidad a nivel individual o genético pueda jugar un papel importante en la adaptación local. Para explorar formalmente esta posibilidad, abordamos los principales mecanismos evolutivos que explican la variación en la fecha de puesta en nuestra población.

Primero, exploramos inicialmente la consistencia en la fenología reproductiva de las hembras. Los resultados apuntan hacia una falta de repetibilidad en su comportamiento, reflejando la variabilidad de la fecha de puesta entre las hembras. Como se sugiere en el **Capítulo I**, la plasticidad fenotípica podría jugar un papel en la fenología reproductiva de los papamoscas cerrojillos para optimizar su éxito reproductivo. Sin embargo, los papamoscas son aves migratorias y su ciclo de reproducción se ve afectado por múltiples factores locales en su zona de reproducción tanto como por factores globales a lo largo de sus rutas migratorias o sitios de invernada. De 1987 a 2016, las condiciones ambientales muestran grandes fluctuaciones, desde la precipitación hasta la temperatura local, y las hembras reproductoras han seguido estas variaciones.

Identificar el papel relativo que múltiples factores ambientales causan una selección en la fecha de reproducción es esencial para comprender estas variaciones fenotípicas. En el **Capítulo II**, exploramos casi 30 factores ambientales locales y globales para detectar los principales factores ambientales que influyen en la

selección fenotípica en la fecha de puesta. Los resultados sugieren que factores locales como la temperatura mínima en mayo y abril dieron forma a la selección en la fecha de puesta, lo que sugiere que múltiples factores ambientales pueden limitar el cambio fenotípico en esta población de papamoscas cerrojillo.

Para observar una respuesta a la selección a largo plazo, el rasgo de estudio debe ser genéticamente transmisible (cf. heredable) de una generación a la siguiente. Sin embargo, como se muestra en el **Capítulo III**, la heredabilidad de la fecha de puesta es muy baja, debido a la pequeña proporción de variación genética aditiva en relación con la variación fenotípica total. Sugiere que la variación ambiental y no la variación genética aditiva pueden explicar la variación en la fecha de puesta en nuestra población. En consecuencia, nuestros resultados no encontraron ningún apoyo para un cambio microevolutivo en la fecha de puesta en nuestra población, inferido de una falta de tendencia temporal en los valores genéticos en esta población.

Dado que los cambios microevolutivos no explicaron la adaptación a las condiciones ambientales cambiantes, exploramos más a fondo si las interacciones genotipo por ambiente (G×E). Para hacerlo, primero exploré si existe un individuo por entorno (I×E) y luego lo dividí en G×E y PE×E, teniendo en cuenta la varianza genética y no genética de I×E interacciones. El **Capítulo IV** probó el papel evolutivo potencial de la plasticidad fenotípica (G×E). Encontramos un apoyo no concluyente de las interacciones G×E, lo que sugiere que puede explicar la adaptación local, pero no de manera inequívoca. Dada la influencia no concluyente de G×E, exploré más a fondo el papel de I×E en este poblacione.

En el **Capítulo V**, mostré que la variación en la fecha de puesta en la población es impulsada por la variación individual en respuesta a dos factores ambientales. La expresión de la fecha de reproducción podría estar asociada con la condición de los individuos y destaca la necesidad de tener en cuenta la variabilidad individual de la plasticidad en escenarios de cambio ambiental. En general, encontramos que la fecha de puesta es un rasgo variable bajo una selección fenotípica más fuerte cuando las condiciones ambientales no son favorables. Sin embargo, la dinámica evolutiva de este rasgo se explica mejor por mecanismos no genéticos, muy probablemente interacciones G×E, en lugar de un cambio genético. Este doctorado destaca el papel y la complejidad que tienen los factores ambientales locales y globales y las condiciones que experimentan los individuos en el momento de la reproducción en la dinámica evolutiva de los rasgos de la historia de vida.

Resumé

L'objectif principal de cette thèse était d'étudier la dynamique évolutive d'un trait d'histoire de vie dans une population espagnole de gobe-mouches sauvages *Ficedula hypoleuca*. Étant donné que le changement évolutif nécessite une variation environnementale, cette thèse aborde le rôle de la variation environnementale sur l'architecture évolutive de la date de ponte. J'ai développé une ligne de recherche qui explore les principaux piliers sur lesquels repose la sélection naturelle : les sources de la variance des traits, la sélection phénotypique et le potentiel évolutif.

D'après le suivi à long terme, comprenant 29 ans de données d'observations dans une population reproductrice de gobe-mouches, la date de ponte n'a pas changé statistiquement au cours de la même période. Sans surprise, les conditions environnementales, que ce soit les variables basées sur la température ou les précipitations, n'étaient pas constantes mais n'ont montré aucune tendance au fil du temps. Ainsi, considérant que la reproduction précoce est favorisé et que les conditions environnementales ont changé d'une année à l'autre de manière imprévisible, on s'attend à ce que la plasticité au niveau individuel ou génétique puisse jouer un rôle majeur sur l'adaptation locale. Pour explorer formellement cette possibilité, nous avons abordé les principaux mécanismes évolutifs qui expliquent la variation de la date de ponte dans cette population.

Dans un premier temps, nous avons d'abord exploré la cohérence de la phénologie de reproduction des femelles. Les résultats indiquent un manque de répétabilité de leur comportement, reflétant la variabilité de la date de ponte chez les femelles. Comme suggéré dans le **Chapitre I**, la plasticité phénotypique pourrait jouer un rôle dans la phénologie de reproduction des gobe-mouches pour optimiser leur succès reproducteur. Cependant, les gobe-mouches sont des oiseaux migrateurs et leur cycle de reproduction est affecté autant par de multiples facteurs locaux sur leur lieu de reproduction, que par des facteurs globaux le long des routes migratoires ou des sites d'hivernage. De 1987 à 2016, les conditions environnementales présentent de grandes fluctuations, des précipitations à la température locale, et les femelles reproductrices ont suivi ces variations.

Identifier le rôle relatif que jouent de multiples facteurs environnementaux provoquant une sélection à la date de reproduction est essentiel pour comprendre ces variations phénotypiques. Dans le **Chapitre II**, nous avons exploré près de 30 facteurs environnementaux locaux et globaux pour détecter les principaux facteurs environnementaux qui influencent la sélection phénotypique à la date de ponte. Les résultats suggèrent que des facteurs locaux comme la température minimale en Mai et Avril ont façonné la sélection à la date de ponte, suggérant que de multiples facteurs environnementaux peuvent restreindre le changement phénotypique dans cette population de gobe-mouches noir.

Pour observer une réponse à la sélection à long terme, le trait d'étude doit être génétiquement transmissible (cf. héréditaire) d'une génération à l'autre. Cependant, comme le montre le **Chapitre III**, l'héritabilité de la date de ponte est très faible, en raison de la faible proportion de variance génétique additive par rapport à la variance phénotypique totale. Cela suggère que la variation environnementale et non la variation génétique additive peut expliquer la variation de la date de ponte dans notre population. En conséquence, et déduisant d'un manque de tendance temporelle sur les valeurs de reproduction dans cette population, nos résultats n'ont trouvé aucun support pour un changement micro-évolutif de la date de ponte dans cette population.

Étant donné que les changements microévolutifs n'expliquaient pas l'adaptation aux conditions environnementales changeantes, nous avons exploré davantage si les interactions génotype par environnement (G×E). Pour ce faire, j'ai d'abord exploré si l'interaction individu par environnement (I×E) existait, puis je l'ai partitionné en G×E et PE×E, en tenant compte de la variance génétique et non-génétique I×E. Le **Chapitre IV** a testé le rôle évolutif potentiel de la plasticité phénotypique (G×E). Nous avons trouvé un support, mais non concluant des interactions G×E, suggérant que cela peut expliquer l'adaptation locale, mais pas sans équivoque. Compte tenu de l'influence non concluante de G×E, j'ai exploré plus avant le rôle de I×E dans cette population.

Dans le **Chapitre V**, j'ai montré que la variation de la date de ponte dans la population est entraînée par la variation individuelle en réponse à deux facteurs environnementaux. L'expression de la date de reproduction pourrait être associée à l'état des individus et souligne la nécessité de prendre en compte la variabilité individuelle de la plasticité dans des scénarios de changement environnemental. Dans l'ensemble, nous avons constaté que la date de ponte est un trait variable sous une sélection phénotypique plus forte lorsque les conditions environnementales ne sont pas favorables. Cependant, la dynamique évolutive de ce trait est mieux expliquée par des mécanismes non génétiques, très probablement des interactions G×E, plutôt que par un changement génétique. Cette thèse met en évidence le rôle et la complexité que les facteurs environnementaux locaux et globaux et les conditions que les individus vivent au moment de la reproduction ont sur la dynamique évolutive des traits d'histoire de vie.

Conclusions

1. Despite the observed fluctuation of breeding phenology over the years, the study population of pied flycatchers breeding in Central Spain does not display any significant trend in laying date between 1987 and 2016.

2. Female pied flycatchers are highly flexible to change their breeding time with breeding dates not consistent among females on prelaying- period, laying dates, mating and hatching dates. These results suggest that phenotypic plasticity of breeding events may shape environmental variation.

3. There was fluctuation of both local and global environmental factors over the study period. However, only highest and lowest minimum temperatures in April and in May resulted in strongest negative selection on late breeders. This highlight the multi-factorial role that environmental factors may have on phenotypic selection and the importance of considering extreme values.

4. Despite having negative selection on late breeders, selection pattern was not parallel to an evolutionary change in this population. There was a lack of any temporal trend on breeding values similar to what can be expected by chance.

5. Given the environmental heterogeneity and individual plasticity of breeding events, the genetic bases of phenotypic plasticity in this population, as an evolutionary mechanism, may produce local adaptation. However, genotype-by-environment interactions were not fully conclusive and tone done the role this interactions may have in this population.

6. Individual-by-environment interactions explains a high proportion of variance of laying date. Results suggest that role of local factors at the time of breeding may play a stronger role than global factors on genotype-by-environment interactions.

7. The variation in plasticity in our population is driven by individual variation in plastic response. Moreover, this individual plasticity might be impacted by females condition altering the onset of egg laying.

8. The Spanish pied flycatcher population has the potential to respond to contemporary climate change however, we cannot ensure that this mechanism alone will be to explain phenotypic variation or evolutionary adaptation under foreseeable events of climate change.

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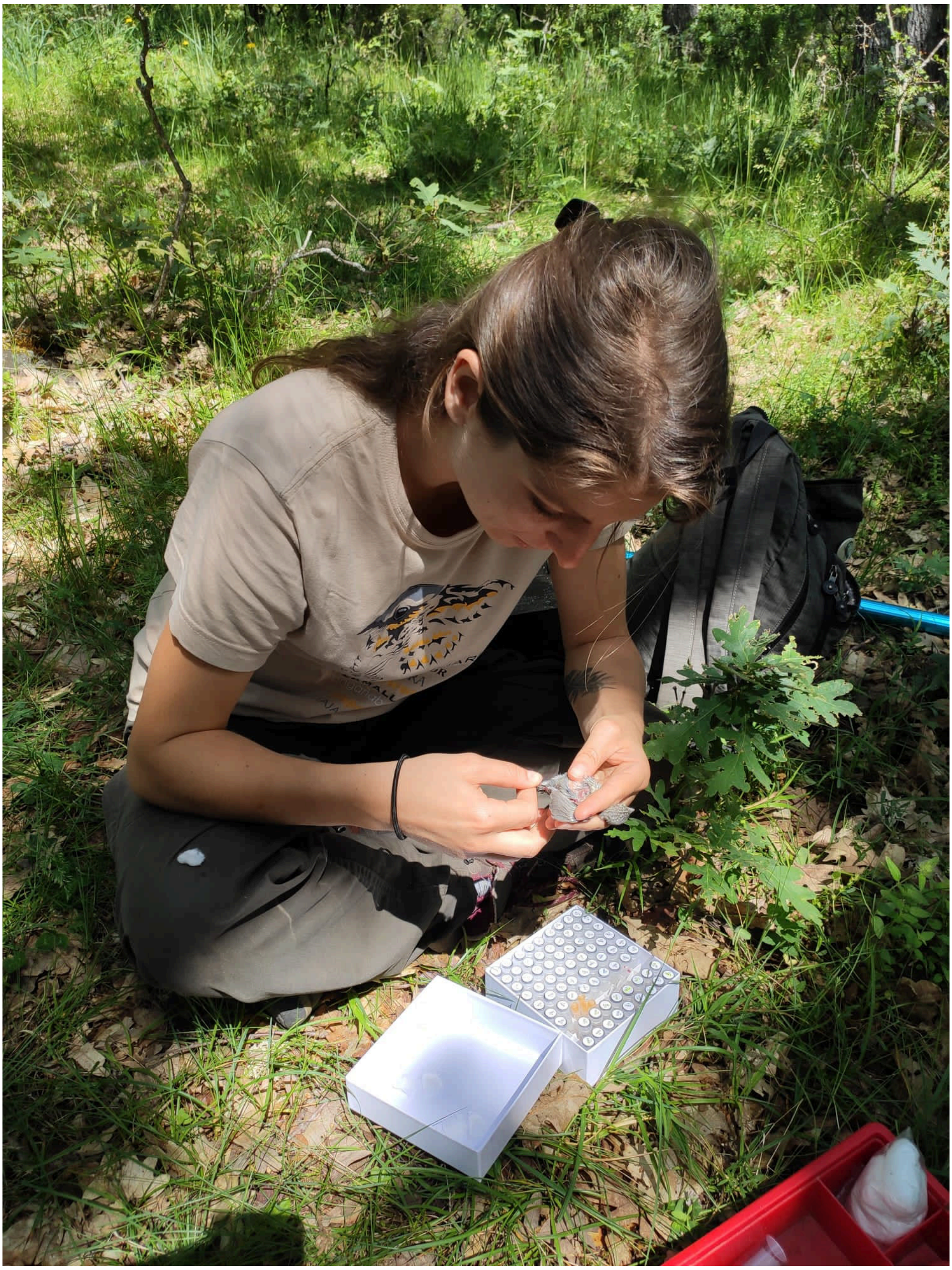


Curriculum vitae

Along the training and activities listed below, the candidate complete her education and set the requirement for the title of Doctor of Philosophy.

Congress

18-22 August 2018 **The Second Joint Congress of Evolutionary Biology**, *Evolution Montpellier 2018*, France.
Poster ; Symposium ’Rapid Evolutionary Responses to Global Change’ : ’The relative contribution of multiple agents on selection on laying date in a Mediterranean population of pied flycatchers’

4-8 September 2018 **International Congress of the Spanish Society of Ethology and Evolutionary Ecology**, *Mieres 2018*, Spain.
Talk : ’The relative contribution of multiple agents on selection on laying date in a Mediterranean population of pied flycatchers’

19-24 August 2019 **Congress of the European Society for Evolutionary Biology**, *ESEB 2019*, Turku, Finland.
Talk ; Symposium "Rapid Evolutionary Adaptation : Potential and Constraints" : ’Plasticity in evolutionary potential under environemental variation in a population of pied flycatchers, *Ficedula hypoleuca*’.

13-17 November 2019 **XXIV Congreso español y Iberico de Ornitologia**, *SEO BirdLife Congreso*, Cadiz, Spain.
Talk : ’Plasticity in evolutionary potential under environemental variation in a population of pied flycatchers, *Ficedula hypoleuca*’.

Internship

6th January 13th June 2020 **Netherlands Institute of Ecology (NIOO - KNAW)**, *Department of Animal Ecology*, Wageningen, Netherlands.

- Participation meetings of the Department (seminars, review publications and Journal Club)
- Join the Netherlands Annual Ecology Meeting 2020
- Assistant fieldwork in Hoge Veluwe

Formations

9-13 April 2018 **Workshop**, *Transmitting Science*, Barcelona, Spain.
’Introduction to Evolutionary Quantitative Genetics’

2017-2018 **Formación transversal doctorado**, *University of Oviedo*, Mieres, Spain.

- "Presentation in English" ; "Writing Scientific paper"
- VII Jornadas Internacionales de Doctorado (Award in Science)

November 2018 **Assistant professor**, *University of Oviedo*, Mieres, Spain.
Prácticas Laboratorio "Gestión de Espacios"

20-31 May 2019 **Course**, *Estacion Biologica de Doñana*, Andalousia, Spain.
IV Curso de Formación en Bienestar Animal para uso de Fauna Silvestre con Fines Científicos

2019-2021 **Formación doctorado**, *University of Sevilla*, Sevilla, Spain.

- Jornadas Doctorales 2021 (talk presentation)
- Assistance to seminars talks

List of Publications

2022 **Low repeatability of breeding events reflects flexibility in reproductive timing in the Pied Flycatcher**, *Ardeola*, 2022. 69(1) :21-39, Le Vaillant J., Potti J., Camacho C., Canal D., and Martínez-Padilla J.

2021 **Local and global climate conditions impose different selection on breeding date in migratory birds**, *Journal of Evolutionary Ecology*, 2021.00 :1–13, Le Vaillant J., Potti J., Camacho C., Canal D., and Martínez-Padilla J.

2020 **What’s your favourite Pokémon? Pocket monster popularity reflects interest in real-world Biology**, *Journal of Geeks Studies*, Vol. 7(1), Le Vaillant J.

Acknowledgements

First of all and foremost, I have to extend my gratitude to my project advisor and thesis director, Jesús Martínez-Padilla, who help me to conduct this PhD across the years and the several ups and down in my professional and personal life with a lot of understanding and humanity. Even if we spent most of time of the PhD in distinct laboratories, this work won't have be possible without your support.

A huge acknowledgement for all the *papamoscas* team for accepting to work with a french freak girl like me, along the thesis and on the fieldwork, and to sharing with me the working custom of the team:

Jaime Potti for sharing his huge scientific knowledge, expertise on flycatchers and his life experience with me, always with kindness, humor and enthusiasm.

Carlos Camacho and David Canal for their help and support along this work and to teach me how to deal with my PhD, my french accent and the scientific world. I hope I did honor to the flycatchers team and respected the tradition of good thesis work.

Of course I cannot forget all people from la Hiruela. Thanks to David Ochoa, Fran Romero, Jaime Muriel, David Idíaquez, María Losada, and all the people who also participate to the fieldwork along theses years.

Because this work have been also conducted in many different places, I would like to thank all the people I met during this thesis:

Thanks to Marcel Visser to welcome me in his department team at the NIOO, and allow me to work with his research group and on the fieldwork despite the complicate situation during the pandemic. Thanks to Barbara, Jip, Claire, Youri and many more people for the sharing moments and interesting and funny discussions about Science, flycatchers, birds and everything else.

I would like to thank all the people, PhD and master students in the UMIB with who I began to learn to speak Spanish and discover amazing places in Asturias. Thank you to Veronica and Erica, to be the nicest students working with me at the beginning of my PhD.

Thanks to all the amazing people I met at the EBD and in Sevilla. To Hyuen-Ji, *mi mujer*, for being such a friend to me; to Mar, for bringing me your support and friendship during all our moments together, and to all the others PhD students and workers at the EBD, with who I share unforgettable memories.

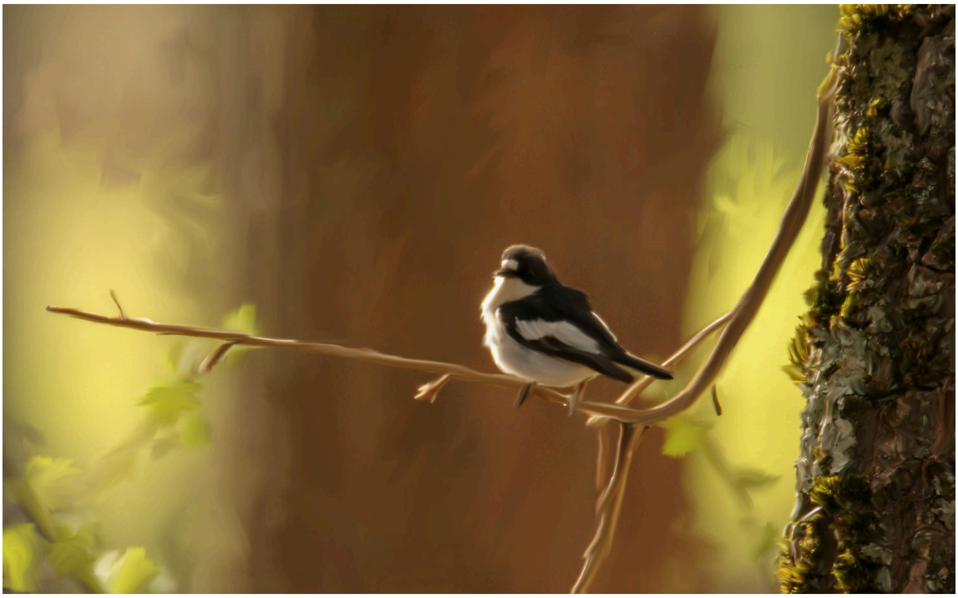
I do NOT thank the Spanish administration and the bureaucracy system of the country for making simple tasks harder and more difficult to complete than writing a thesis. *L'art de la torture est administratif.*

Muchas gracias a toda la gente del grupo Torquilla (Paloma, Vincente...), GO-SUR (Jesús, Alfonso...), SEO/BirdLife Asturias y a todos aquellos junto a los que he sacrificado horas de sueño para el anillamiento y la observación de aves. Un enorme gracias a Julio y Sarry que me han demostrado el arte de anillar, pajarear y de disfrutar de la vida entre amigos en Andalucía !

Arigato gozaimashita a mi sensei y mi compañeros de aikido de los dojos de Oviedo y Sevilla: Patricia, Desiré, Manuel, Alma, David... y quiero agradecer especialmente a Dani, quien me ha apoyado varias veces.

Un grand merci à tous mes amis qui m'ont suivi et soutenu depuis la France (Flo, Thomas, Luca, Yann, Pierre, Matthieu, Marco, Thibault...). Vous êtes trop nombreux pour que je vous cite tous mais vous êtes la preuve qu'une amitié au cours des années et malgrè la distance n'a pas de limite.
Puisse Son Appendice Nouillesque vous toucher.

Et pour finir, merci à toute ma famille qui m'ont toujours encouragé à poursuivre mes ambitions. Même si ces quelques mots sont sans doute la seule partie de cette thèse que vous allez comprendre, j'espère que vous réalisez l'importance que vous avez pour moi et votre influence lors de la réalisation de ce travail ...



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