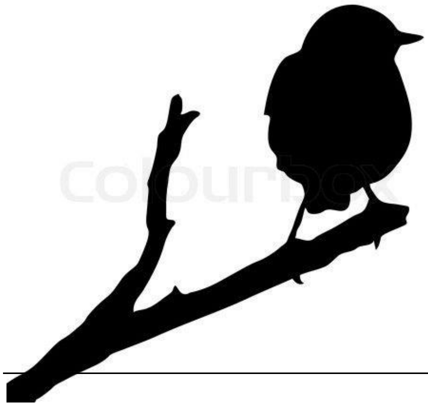
TESIS DOCTORAL

DIFERENCIACIÓN de POBLACIONES a pequeña escala

mecanismos ecológicos y evolutivos implicados



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DIFERENCIACIÓN DE POBLACIONES A PEQUEÑA ESCALA

Mecanismos ecológicos y evolutivos implicados

Carlos Camacho Olmedo

Tesis Doctoral

Universidad de Sevilla 2017

DIFERENCIACIÓN DE POBLACIONES A PEQUEÑA ESCALA

Mecanismos ecológicos y evolutivos implicados

Memoria presentada por el Ldo. ***Carlos Camacho Olmedo*** *para optar al título de Doctor por la Universidad de Sevilla*

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CERTIFICA

Que los trabajos de investigación incluidos en esta memoria han sido realizados en su totalidad por Carlos Camacho Olmedo y son aptos para ser presentados ante el tribunal designado para aspirar al grado de Doctor por la Universidad de Sevilla. Y para que así conste, extiendo el presente certificado en Sevilla, a 18 de septiembre de 2017.

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GENERAL INTRODUCTION

ABSTRACT

Dispersal and gene flow have traditionally been thought to counteract the effects of divergent selection and thus prevent evolutionary differentiation. However, recent research suggests that, if non-random, dispersal may promote population differentiation even at microgeographic scales. Examples of the eco-evolutionary consequences of non-random dispersal are still very scarce in the literature, possibly because long-term data from continuously monitored populations of marked individuals are needed. I document the effect of phenotype-dependent dispersal with respect to body size (tarsus length) on the evolutionary dynamics of a wild population of pied flycatchers (*Ficedula hypoleuca*) between 1988 and 2016. I characterized the natal dispersal patterns and phenotypic trajectories of dispersers and philopatric individuals originating from ecologically distinct (coniferous *vs.* deciduous), adjacent (1 km) habitats, and investigated the genetic architecture of, and patterns of selection on, tarsus length.

Pied flycatchers breeding in the oak and the pine forest showed strong divergence in tarsus length during the colonization phase of the pinewood, despite geographic proximity and extensive dispersal (ca. 25% of each cohort change habitats). However, the initial degree of differentiation was not sustained over the 29-year period. Differential dynamics of phenotype-dependent dispersal between habitats seem to be the key force shaping the phenotypic trajectories of the study populations. Males moving from the pine to the oak forest tended to be smaller over time, whereas those that remained in the pine forest tended to be larger. Dispersers from the oak to the pine forest also tended to be larger over time, and thus the positive trend of philopatric birds was furthermore reinforced. No clear evidence of phenotype-dependent dispersal was found in females but, importantly, an analysis of both sexes combined confirmed the patterns observed for males alone.

Temporal changes in breeding density and the level of nest-site competition could have provided the ecological mechanism for the habitat segregation of size classes. Breeding density increased in both habitats following nest-box addition. Nevertheless, due to the lack of natural cavities, nest-site competition –and therefore the relevance of body size in territorial contests– was probably magnified in the pinewood. Based on density and dispersal rates dynamics, it seems that the pine forest became progressively more attractive for pied flycatchers at the expense of a decrease in the attractiveness of the oakwood. Because large-dominant and small-subordinate males are generally sorted into preferred and non-preferred habitats, the steep increase in breeding density in the pinewood resulted in a shift in the patterns of phenotype-dependent dispersal. No evidence of adaptive habitat choice in relation to body size was found in this population possibly because, as demonstrated here, most pied flycatchers, regardless of their phenotype, return to the habitat they imprinted on as fledglings.

Quantitative genetic analyses revealed a substantial component of additive genetic variance underlying tarsus length, although there were substantial differences among pools of dispersers and philopatric birds. Specifically, individuals genetically predisposed to be larger preferentially dispersed into the oak forest. However, selection gradients on this group, unlike the other groups, were negative and, therefore, resulted in a negative response to selection. Dispersers to the oak forest actually showed a decline in tarsus length over time that, nevertheless, deviated from the lack of trend observed in the entire oak population. No such discrepancy was found in the pine forest, indicating a greater genetic contribution of dispersers to the pine forest in shaping the distribution of phenotypes within their recipient population. Examination of the breeding success of each group suggested that this unusual asymmetry resulted from uncoupled patterns of dispersal and gene flow in the oak forest, since the reproductive output of immigrants into the oakwood was lower than that of the other groups.

Overall, these results indicate that the effect of dispersal on microevolutionary processes may vary from being positive to non-existent depending on the habitat type, although the ecological factors responsible for such discrepancy remain to be elucidated. Collectively, the five chapters presented in this thesis represent a major step forward in our understanding of the specific role of gene flow on eco-evolutionary dynamics and phenotypic evolution.

RESUMEN

Los modelos evolutivos clásicos consideran que la dispersión y el flujo génico asociado contrarrestan los efectos de la selección natural y evitan la diferenciación evolutiva. Sin embargo, investigaciones recientes sugieren que, si se produce de forma no aleatoria, la dispersión puede promover la diferenciación de poblaciones, incluso a pequeña escala espacial. Los ejemplos de las consecuencias eco-evolutivas de la dispersión no aleatoria son todavía muy escasos en la literatura, posiblemente, porque es necesario contar con datos a nivel individual recogidos de forma continuada durante largos periodos de tiempo.

El objetivo de esta tesis es documentar los efectos de la dispersión dependiente del fenotipo –en este caso, el tamaño corporal, medido como la longitud del tarso– sobre la dinámica evolutiva de una población salvaje de papamoscas cerrojillo (*Ficedula hypoleuca*) entre 1988 y 2016. Para ello, se caracterizaron los patrones de dispersión natal y las trayectorias fenotípicas de los individuos dispersantes y filopátricos procedentes de dos hábitats ecológicamente distintos (pinar y robledal) pero geográficamente próximos (1 km) y se investigó la arquitectura genética y los patrones de selección sobre la longitud del tarso.

Los papamoscas nidificantes en el pinar y el robledal mostraron una fuerte divergencia en la longitud del tarso durante la fase de colonización del pinar, a pesar de la proximidad geográfica y las elevadas tasas de dispersión entre hábitats (aproximadamente, 25%). No obstante, el elevado grado inicial de diferenciación poblacional decreció gradualmente a lo largo de los 29 años de estudio. La dispersión dependiente del fenotipo parece ser la principal fuerza modeladora de las trayectorias fenotípicas de estas poblaciones. El tamaño medio de los machos dispersantes del pinar al robledal decreció progresivamente con el tiempo, mientras que, tanto los residentes del pinar, como los dispersantes del robledal al pinar, mostraron una tendencia temporal positiva. No se encontraron evidencias sólidas de dispersión dependiente del fenotipo en hembras, aunque el análisis de ambos sexos combinados confirmó los patrones observados para los machos.

Los cambios temporales en la densidad reproductiva y los niveles de competencia por el acceso a cavidades de nidificación parecen conformar el mecanismo ecológico que subyace a la segregación por tamaños. La colocación de cajas nido provocó un aumento en el número de parejas en ambos hábitats. Sin embargo, debido a la falta de cavidades naturales, la competencia por los nidales artificiales y, por tanto, la importancia del tamaño corporal en las disputas territoriales, posiblemente se magnificaron en el pinar. De acuerdo con los cambios temporales en el tamaño de población y las tasas de dispersión, parece que el atractivo del pinar aumentó notablemente a lo largo del periodo de estudio, en detrimento del robledal. Debido a que los machos más grandes y dominantes generalmente excluyen a los más pequeños de los hábitats preferidos, el acusado incremento en la densidad de parejas del pinar provocó una reversión temporal en los patrones de dispersión dependiente del fenotipo. Los

movimientos de dispersión dependientes del tamaño en esta especie no parecen estar guiados por los posibles beneficios de fitness, probablemente porque, como demuestra esta tesis, la mayoría de los dispersantes, independientemente de su fenotipo, regresan al hábitat de nacimiento como consecuencia de un proceso de impronta.

Los análisis de genética cuantitativa revelaron un fuerte componente de varianza genética aditiva subyacente a la longitud del tarso, aunque se encontraron diferencias importantes entre individuos dispersantes y filopátricos. Específicamente, aquellos individuos genéticamente predispuestos a ser más grandes se dispersaron preferentemente del pinar al robledal. Sin embargo, los gradientes de selección en este grupo, a diferencia de los otros grupos, fueron negativos. Esto se tradujo en una respuesta negativa a la selección. Como cabría esperar, los dispersantes del pinar mostraron una reducción temporal en la longitud media del tarso que, sin embargo, se desvió de la ausencia total de tendencia observada en el conjunto de la población del robledal. No se encontraron, sin embargo, discrepancias semejantes en la población del pinar, lo que sugiere que la contribución genética de los dispersantes al conjunto de la población es mayor en el pinar que en robledal. Esta asimetría entre hábitats podría obedecer a una falta de equivalencia entre la dispersión y el flujo génico en el robledal, ya que el éxito reproductor de los inmigrantes a este hábitat es menor que el del resto de los grupos.

En conjunto, estos resultados indican que los efectos de la dispersión en los procesos microevolutivos pueden variar de ser positivos a inexistentes dependiendo del tipo de hábitat de destino, aunque todavía desconocemos los factores ecológicos responsables de dicha discrepancia. Colectivamente, los cinco capítulos presentados en esta tesis representan un gran paso adelante en nuestra comprensión del papel del flujo génico en las dinámicas eco-evolutivas y la evolución fenotípica de poblaciones salvajes.

GENERAL INTRODUCTION

New challenges in evolutionary biology

Understanding the mechanisms underlying population divergence has been a central issue in evolutionary biology since Darwin and Wallace proposed his theory of evolution by natural selection (Darwin & Wallace 1858; Darwin 1859). Most evolutionary models assume that population divergence, considered as the first step toward speciation (Schluter 2000), results from the combined effect of stochastic (genetic drift and mutation) and deterministic (natural selection and gene flow) processes (Lande 1976; Slatkin 1987; Lenormand 2002). Natural selection and gene flow have traditionally been viewed as opposing evolutionary forces, as the homogenizing effects of gene flow would typically counteract the diversifying effects of selection (Endler 1986; Slatkin 1987; Garcia-Ramos & Kirkpatrick 1997; Lenormand 2002). However, a growing body of theoretical and empirical work indicates that a complete lack of gene flow is not required for divergence and speciation to occur (Garant et al. 2007).

Barriers to dispersal or large geographic distances limiting gene exchange between populations are no longer considered an essential prerequisite for population divergence in the modern literature (Richardson et al. 2014; Fitzpatrick et al. 2015). Conversely, recent evidence from a variety of taxa –typically birds and fish– suggests that fine-scale population divergence might be more common than previously thought, even between closely adjacent populations of vagile organisms (Chan & Arcese 2003; Garant et al. 2005; Blondel et al. 2006; Bolnick et al. 2009; Milá et al. 2010; Bertrand et al. 2014; Arnoux et al. 2014; García-Navas et al 2014; Fitzpatrick et al. 2015; Camacho et al. 2016a; Szulkin et al. 2016; Izen et al. 2016).

Identifying the drivers of fine-scale divergence

Exploring geographic variation in genetic structure is a common approach to assess population differentiation. By characterizing the spatial distribution of allele frequencies, population sub-structuring can be detected and local populations identified (Garroway et al. 2013). However, understanding the drivers of local differentiation still requires an understanding of phenotypic change (Carrol et al. 2007; Lehtonen et al. 2009), as illustrated by the increasingly reported cases of fine-scale phenotypic divergence in different organisms, from plants to fish and mammals (Shine et al. 2012; Camacho et al. 2013; McDevitt et al. 2013; Arnoux et al. 2014; García-Navas et al. 2014; Langin et al. 2015; Izen et al. 2016; Herrera et al. 2017).

Several non-mutually exclusive mechanisms have been proposed in the literature as potential drivers of population differentiation over short geographic distances. Many studies have shown that some animals may be reluctant to move away from their birthplace despite high dispersal potential, suggesting that localized dispersal is a common factor contributing to the genetic isolation and subsequent differentiation of neighbouring populations (Schluter 1998; Garroway et al. 2013). Behavioural restrictions on dispersal are often observed in some island birds (Komdeur et al. 2004; Bertrand et al. 2014; Langin et al. 2015) and habitat specialists, unable to overcome non-physical barriers to dispersal in fragmented landscapes (Harris & Reed 2002; Lindsay et al. 2008; Woltmann et al. 2012). Furthermore, population differentiation can be reinforced due to divergent mating patterns, leading to non-random mate choice (Jiang et al. 2013; Langin et al. 2015).

GLOSSARY I – POPULATION DIVERGENCE: CONCEPTS & MECHANISMS

Adaptive divergence: the evolution of differences between populations as a result of adaptation to different environmental conditions and divergent natural selection.

Divergent selection: selection that favours opposite extremes of a trait in different habitats or populations and, therefore, acts against intermediate phenotypes.

Genetic drift: stochastic changes in allele frequencies across generations due to random sampling effects of parental genotypes.

Local adaptation: the evolution of traits in a population that results in a better average performance of native individuals in the home environment relative to immigrants.

Maladaptation: the phenotypic deviation of a population or organism from the optimal adaptive peak within a particular habitat.

Mating preference: a bias during mate choice that leads to a skew towards mating with individuals exhibiting certain phenotypic attributes.

Microgeographic adaptation: local adaptation of a population occurring within the normal dispersal radius of the focal organism.

Microgeographic divergence: differences in trait distributions between samples of individuals within the dispersal radius of the focal organism. Note that microgeographic divergence involves no presumption about the adaptive value of the trait divergence.

Migration-selection balance: an equilibrium level reflecting the divergent effect of natural selection and the homogenizing effect of random gene flow.

Population differentiation can also occur under extensive dispersal through two different mechanisms. First, the movement of individuals may not always be equivalent to the movement of genes, especially in spatially heterogeneous environments, where local selection pressures often lead to reduced survival and reproductive success of immigrants compared with locally adapted residents (Pärt 1991; Hendry 2004; Nosil et al. 2005; Garant et al. 2007). Major discrepancies between dispersal and realized gene flow due to local maladaptation and selection against immigrants may thus allow for population differentiation even when geographic proximity facilitates population exchange (Blondel et al. 1999, 2006; Postma & van Noordwijk 2005; Porlier et al. 2012).

Second, population divergence may be facilitated by gene flow itself, as suggested by recent outstanding studies showing that dispersal and gene flow are typically non-random (Garant et al. 2005; 2007; Edelaar & Bolnick 2012). It is now recognized that individuals tend to choose breeding habitats that resemble those they encountered early in life (i.e. natal habitat preference induction; Davis & Stamps 2004) or those that best match their phenotypes (i.e. matching habitat choice; Edelaar et al. 2008), leading to non-random dispersal. Contrary to the common belief, growing evidence indicates that non-random dispersal may reinforce, rather than counteract, evolutionary differentiation (Garant et al. 2005; Edelaar et al. 2008; Bolnick & Otto 2013). Dispersal therefore provides an important, but largely neglected, mechanism by which population divergence may evolve even at surprisingly small spatial scales (see below).

Dispersal: a key life-history trait

Dispersal is considered a key life-history trait, with important implications for the genetic structure and the demographic dynamics of natural populations (Greenwood & Harvey 1982; Johnson & Gaines 1990; Bélichon et al. 1996; Clobert et al. 2001; Bowler & Benton 2005). Dispersal movements can be classified into two main categories: breeding dispersal, by which already established adults change breeding territories between successive years; and natal dispersal, by which young move from their natal site to their first breeding site. Because of its higher frequency (i.e. the proportion of individuals dispersing) and magnitude (i.e. the dispersal distances), the effects of natal dispersal on populations are much greater than those of breeding dispersal (Greenwood & Harvey 1982; Clobert et al. 2001).

Natal dispersal can be broken down into three stages: departure from the natal site, transience, and settlement to breed (Bowler & Benton 2005), each of which can entail substantial costs (Massot & Clobert 2000; Clobert et al. 2001; Ims & Hjermann 2001; Bonte et al. 2012). First, moving through unknown and/or unsuitable habitats entails intrinsic risks and costs due to increased energetic expenditure or predation risk (Greenwood 1980; Clobert et al. 2001; Stamps 2001). Second, dispersers may suffer reduced survival or reproductive success compared to non-dispersers due to maladaptation to the new breeding environment, thus resulting in a mismatch between dispersal and realized gene flow (Hendry 2004; Nosil et al. 2005). Dispersers may also experience a stronger intra-specific competition than local individuals owing to reduced social familiarity and genetic relatedness with their neighbours (Ekman et al. 2000; Dickinson et al. 2014).

However, despite dispersal may sometimes be unsuccessful in terms of fitness, several beneficial factors may select for increased dispersal and promote its persistence in natural populations. First, dispersal can increase average fitness when environmental quality varies in time and space, because dispersers can avoid poor conditions at local sites (Olivieri et al. 1995). Second, dispersal can reduce kin competition and thereby increase inclusive fitness (Hamilton & May 1977; Cote & Clobert 2010). Dispersal can also reduce inbreeding and its associated costs (Greenwood et al. 1978; Perrin & Mazalov 1999). Finally, dispersers originating from an unsuitable environment for their particular phenotype may increase their fitness prospects by settling in more optimal locations (Edelaar et al. 2008; see below).

Dispersal decisions should typically reflect the balance among the various costs and benefits of each stage of dispersal (Clobert et al 2001; Lenormand 2002). Decisions during any of the three stages can be independent from each other, or occur simultaneously when multiple habitats can be assessed at the same time. Recent research suggests that variation in dispersal patterns at the individual and population levels results from the interplay between a suite of external (e.g. information about the surroundings) and internal (e.g. the phenotype of dispersers) factors (Benard & McCauley 2008; Clobert et al. 2009). Optimizing dispersal decisions according to environmental conditions and individual phenotype apparently requires a high behavioural flexibility. However, there is evidence for significant genetic variation for dispersal in different taxa, from bacteria to plants and animals (reviewed by Saastamoinen et al. 2017). Dispersal has been shown to have a detectable genetic basis in plants and insects (reviewed by Saastamoinen et al. 2017), and some efforts have been made to quantify the heritability of the propensity to disperse (Hansson et al. 2003; Doligez et al. 2009; Duckworth & Kruuk 2009), and of dispersal distance (Greenwood et al. 1979; Doligez et al. 2009; Kosrten et al. 2013) in avian models. However, evidence for the genetic basis of dispersal remains ambiguous (see Charmantier et al. 2011).

GLOSSARY II – DISPERSAL AND GENE FLOW

Breeding dispersal: the movement between two successive breeding sites.

Dispersal: the displacement of offspring away from their natal site. Dispersal does not necessarily imply gene flow, unless dispersers change allele frequencies in the recipient population (i.e. effective gene flow).

Dispersal radius: the geographic area within which individuals regularly move and interact, estimated as the median natal dispersal distance of a population.

Gene flow: the exchange of genes and alleles between populations that subsequently contributes to the future gene pool of the recipient population.

Matching habitat choice: when individuals use some measure of local performance to establish a preference for settlement in the habitats that increase their expected fitness. If different phenotypes choose different optimal habitats, such habitat choice causes individuals to disperse in a manner that results in phenotype-environment correlations.

Natal dispersal: the movement of individuals from their birthplace to their first breeding site.

Natal philopatry: animals returning to their native habitat for reproduction.

Phenotype-dependent dispersal: correlation between dispersal behaviour and individual morphological, behavioural, physiological and life-history traits. Such correlations can affect either the departure and settlement decisions of individuals, so that dispersers are phenotypically differentiated from the source population.

Random dispersal: the movement of individuals independently of their phenotype. Due to the homogenizing effects of random dispersal, population differentiation will only occur under strong local adaptation.

Realized gene flow: the movement and establishment of novel genes in the recipient population as a result of successful reproduction by migrants.

External and internal factors influencing dispersal

Dispersing individuals can gather information about ecological and demographic conditions at potential settling sites by assessing environmental parameters before or during dispersal. For example, individuals might get direct information from their habitat, such as parasite abundance (Brown & Brown 1992), or use their own internal condition or reproductive success as cues for habitat quality (Ims & Hjermann 2001). Dispersal decisions can also be influenced by early experience at the natal site, which can shape habitat preferences in adulthood and thus lead to biased dispersal towards natal-like habitats (Davis & Stamps 2004; Stamps et al. 2001). Dispersing individuals may also use information from other individuals of the same cohort to assess habitat quality (Ims & Hjermann 2001; Nicolaus et al. 2012). Public information, such as conspecific density or reproductive success, can be used to assess habitat quality and adjust dispersal decisions before the first reproduction (Greenwood et al. 1979; Doligez et al. 1999; Doligez et al. 2004; Hénaux et al. 2007). Maternal effects have also been recognized as an important determinant of natal dispersal, and evidence is accumulating that maternal exposure to particular conditions (e.g. conspecific density, nest-site availability or predation risk) may modulate offspring's dispersal behaviour even prior to birth (Tschirren et al. 2006; Bitume et al. 2014; Bestion et al. 2014; Duckworth et al. 2015).

Environmental characteristics are not the only determinants of dispersal costs and benefits. Dispersal may be strongly influenced by phenotypic attributes, so that dispersers might not be a random sample of the source population (Bowler & Benton 2005; Clobert et al. 2009; Edelaar & Bolnick 2012). For example, attributes such as age (Ims & Hjermann, 2001), body size and condition (Senar et al. 2002; Garant et al. 2005; Camacho et al. 2013), tegument colouration (Saino et al. 2014), or personality (Dingemanse et al. 2003; Fraser et al. 2001; Nicolaus et al. 2012) may affect individuals' propensity to disperse and thus lead to phenotype-dependent dispersal. Moreover, the effect of phenotype on dispersal and habitat selection often differs between the sexes; first, because in many species the critical decision on where to settle lies exclusively on one sex (Hendry et al. 2001; Dreiss et al. 2012), and second, because the territorial sex is usually more sensitive to competition, and will thus be more likely to disperse at high density or when in poor body condition (Doligez et al. 1999; Hardouin et al. 2012).

Dispersers have been repeatedly reported to be more aggressive, larger or in better condition than the average resident, since all of these traits may facilitate settlement and reduce the costs of dispersal (Barbraud et al. 2003; Duckworth 2006; Camacho et al. 2013). Especially under high-density conditions, dispersal out of the natal patch may be motivated by avoidance of competition for nesting sites or food by individuals with less social tolerance or with lower competitive abilities, resulting in phenotype-dependent dispersal (Cote & Clobert 2007). Low quality individuals (e.g. smaller or in poorer condition) might perform badly in or be competitively excluded from high-density patches, even when other habitat characteristics are favourable (Duckworth 2008) and, therefore, settle in lower density and/or lower quality habitats than dominant individuals (Verhulst et al. 1997; Doligez et al. 2004, 2008; Stamps 2006).

However, phenotype-dependent dispersal may not necessarily imply a despotic distribution of individuals (Fretwell 1972). Recent studies have suggested that individuals born in unsuitable environments (e.g. where maladapted phenotypes are counter-selected) would benefit from phenotype-dependent dispersal by shifting to habitats that better match their phenotype, a process known as matching habitat choice (Holt & Barfield 2008; Edelaar et al. 2008). Matching habitat choice might increase dispersers' fitness beyond that expected under either phenotype-independent dispersal or non-adaptive forms of phenotype-dependent dispersal (e.g. triggered by the competitive exclusion of low competitive phenotypes) and, therefore, their presumed evolutionary implications should be greater (Edelaar et al. 2008; Edelaar & Bolnick 2012; Bolnick & Otto 2013). Surprisingly, only a few studies have tested for this form of dispersal or evaluated its potential consequences (see Bolnick & Otto 2013 for a theoretical outline) and its relative occurrence in the wild therefore remains to be determined.

Model organism

European flycatchers in the genus *Ficedula* (Family Muscicapidae) comprise three closely related species: collared flycatcher (*F. albicollis*), semicollared flycatcher (*F. semitorquata*), and pied flycatcher (*F. hypoleuca*), all of which have been extensively studied (e.g. von Haartman 1949; Lundberg & Alatalo 1992; Price 2008; Both & Visser 2001). Specifically, the pied flycatcher was used as a model in this thesis (see below for the rationale). The pied flycatcher is a small (11−13 g), insectivorous, migratory passerine that breeds in temperate forests across Europe and overwinters in dry tropical forests of western Africa (Lundberg & Alatalo 1992; Ouwehand et al. 2016; Fig. 1a). It nests in natural tree cavities and readily uses nest boxes when provided (Lundberg & Alatalo 1992), which makes individual monitoring and manipulation, and sample collection relatively easy. For this and other reasons (see below), the pied flycatcher has long been a popular model organism for ecological and evolutionary research (e.g. Harvey et al. 1984; Alatalo & Lundberg 1986; Both & Visser 2001; Lehtonen et al. 2009; Potti & Canal 2011).

During the breeding season, males exhibit a conspicuous black-and-white plumage, with prominent forehead and wing patches, whereas females are grayish-brown (Lehtonen et al. 2009; Potti et al. 2014; Fig. 2ab). Males and females also show slight size dimorphism (females > males; Potti 1999). Males arrive at the breeding grounds between late April and early May, search for a suitable cavity for nesting and announce themselves to females by singing actively (Lundberg & Alatalo 1992; Potti 1998). Females arrive approximately a week after males and select a breeding territory based on male and territory characteristics (Sirkiä & Laaksonen 2009; Canal et al. 2011). Pied flycatchers can be found in a wide variety of woodland, ranging from pure deciduous to pure coniferous forests (Lundberg and Alatalo 1992; Mäntylä et al. 2015), but they usually prefer to breed in deciduous forest types due to the scarcity of tree cavities in coniferous habitats (Alatalo et al. 1985; Sanz 1995).

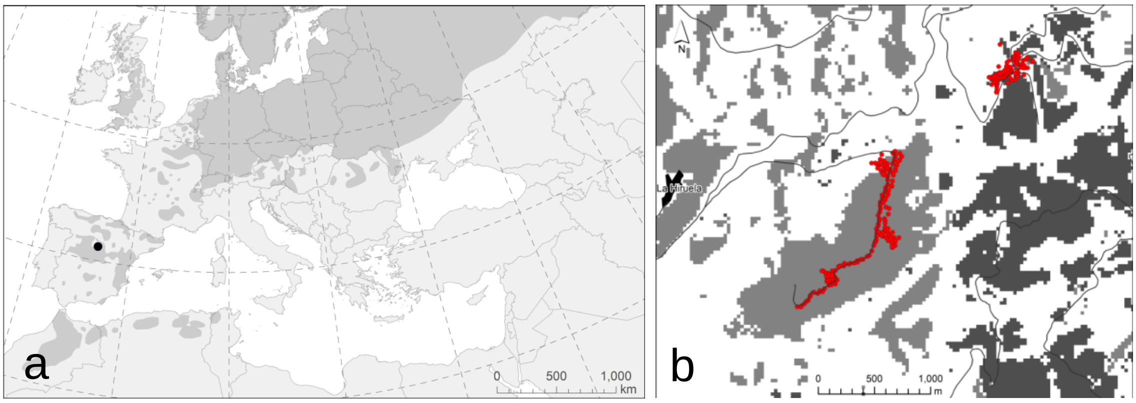

Figure 1. (a) Distribution range of the pied flycatcher (*Ficedula hypoleuca*), including its three accepted subspecies: *speculigera* (distributed across North Africa, from Tunisia to Morocco), *iberiae* (restricted to the Iberian Peninsula) and *hypoleuca* (distributed across the rest of Europe). The location of the study area is also shown (black dot). **(b)** 30-m resolution map showing the spatial configuration of deciduous habitats (light grey), coniferous habitats (dark grey), and unsuitable habitats for pied flycatchers across the study area (white area), including rock outcrops, mountain shrublands and riverside vegetation. La Hiruela, the closest urban area to the study site, is also shown (black area). Nest boxes are represented by red dots.

Pied flycatchers may postpone breeding until their second or, more rarely, third year of life (Potti & Montalvo 1991a; Montalvo & Potti 1992). Natal philopatry is widespread in the species (Sanz 2001). Males, and to a lesser degree females, show limited dispersal (median dispersal distance, males: 445 m, females: 600 m; Potti & Montalvo 1991a; Fig. 3). Breeding site fidelity is also very high, especially in older individuals, which rarely move from their breeding site (Harvey et al. 1984; Montalvo & Potti 1992).

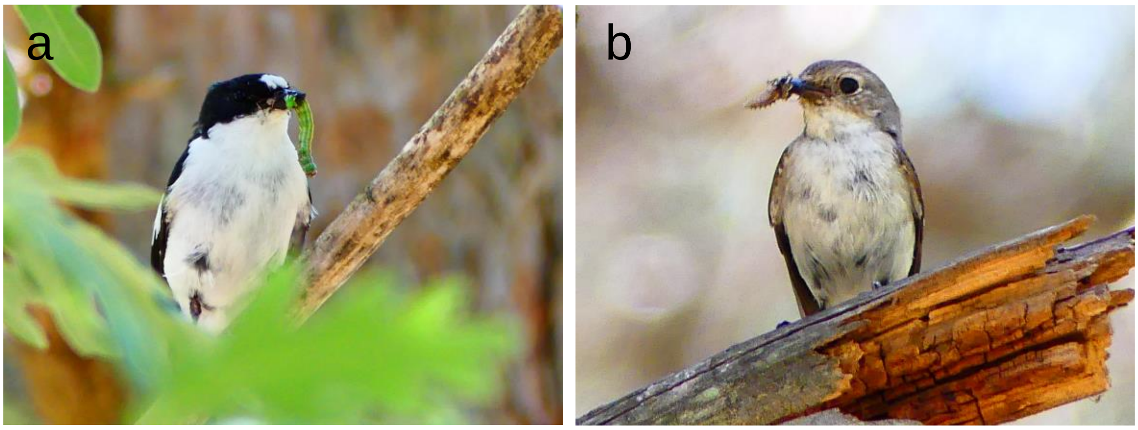

Figure 2ab. Male (left) and female (right) pied flycatcher of the subspecies *F. hypoleuca iberiae.* Andrés Robledo©.

Females produce a clutch of four to six eggs in a single brood each year, which she incubates alone for 12–13 days (Lundberg & Alatalo 1992). Pied flycatchers are predominantly socially monogamous, but some males succeed in becoming socially polygamous (Lundberg & Alatalo 1992; Canal et al. 2011, 2012). Males and females may provision nestlings at similar rates, although the degree of male assistance decreases from monogamous females to primary and secondary females (Dale et al. 1996). Fledglings usually leave the nest between 15 and 19 days after hatching and remain in its vicinity for several days. Before departure to winter quarters, young perform exploratory trips around their natal site, with important implications for future settlement decisions (Chernetsov et al. 2006; Camacho et al. 2016b). Mean distance of post-fledging dispersal has been estimated between 0.6–1.4 km, depending on the study population (van Balen 1979; Vysotsky et al. 1990).

Pied flycatchers feed on small invertebrates and, as in many other forest passerines, caterpillar is usually the most important prey during reproduction. Caterpillars form a substantial part of the nestling diet of pied flycatchers breeding in oak-dominated forests across Europe, followed by flies and beetles (Sanz 1998). However, both the diet composition and the average prey size of pied flycatchers may vary widely between forest types (Sanz 1998). Specifically, in mixed and coniferous forests, the main food sources typically consist of flies, beetles, spiders and wasps (Sanz 1998; Burger et al. 2012). Because of the high-quality nutritional profile of caterpillars (Arnold et al. 2010), it is often assumed that a higher proportion of this prey in the diet should result in better nestling condition. However, although several studies on tits (*Parus spp.*) have found support for this prediction (Wilkin et al. 2009; García-Navas et al. 2011; but see Serrano-Davies & Sanz 2017), there is some controversy as to whether this relationship also applies to pied flycatchers (Eeva et al. 2005; Burger et al. 2012). Thus, the long-standing assumption that deciduous forests are of higher quality than other forest types –especially coniferous forests– for pied flycatchers and other insectivorous birds is now being questioned (Visser et al. 2004; Both et al. 2006; Camacho et al. 2015; Serrano-Davies & Sanz 2017).

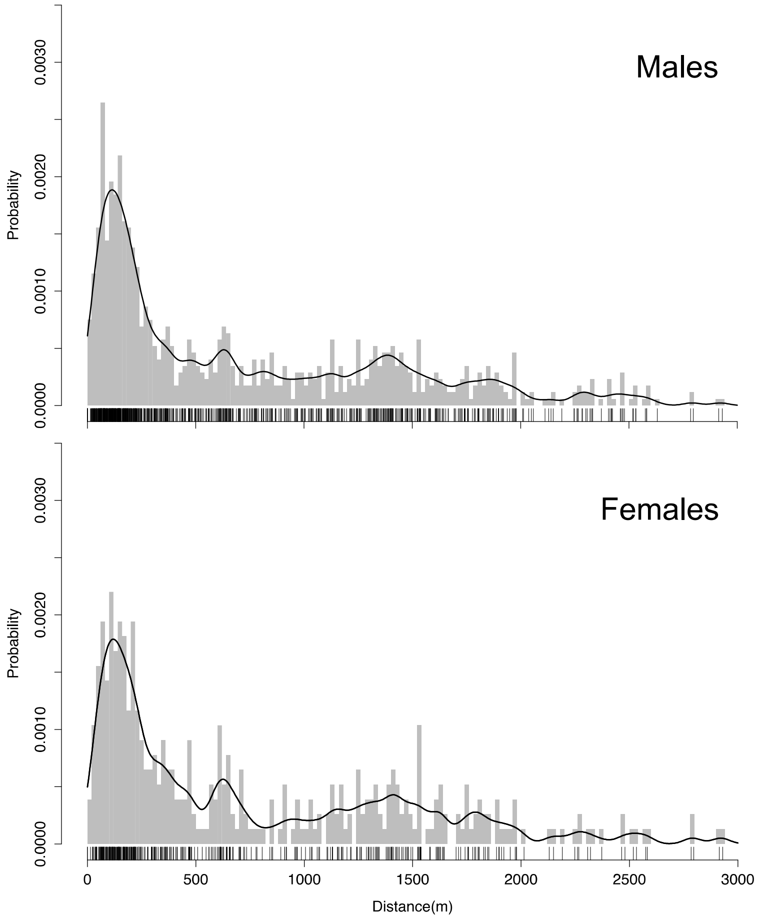

Figure 3. Frequency distributions of natal dispersal distances in male (top panel) and female (bottom panel) pied flycatchers, expressed as the probability density function of observed dispersal distances in the study population. Black vertical lines below the x-axis represent the actual dispersal events, and the black line is a (non-parametric) smoothing spline fit to the empirical distance distributions.

Study system

The study area consists of a two-patch system comprising a mature oak forest of 9.3 ha near La Hiruela (Madrid, 41°04'N 3°27'W, 1,250 masl) and a pine plantation of 4.8 ha near Colmenar de la Sierra (Castilla La Mancha, 40°40'N 4°8'W, 1,300 masl). Both forests are separated by a 1-km wide strip of unsuitable habitat for hole-nesting passerines (Fig. 1b).

Figure 4. Mature oak forest (a) and mixed pine plantation (b) where pied flycatchers have been extensively studied since 1984 and 1988, respectively. Carlos Camacho®.

The two study sites differ markedly in vegetation structure and composition, as well as in macroinvertebrate diversity. Pyrenean oaks (*Quercus pyrenaica*) at a mean density of 460 trees ha^{-1} dominate the deciduous habitat (Fig. 4a). Forest exploitation in the oak patch ceased some 50 years ago and, although cattle-raising is still a common activity, a dense ground cover and understory of oak saplings, *Erica arborea*, *Cistus laurifolius*, and *Crataegus monogyna* re-evolved (0.5−3 m high; mean cover 80%). Caterpillars are abundant in the oakwood and constitute the bulk of prey for pied flycatchers breeding in this habitat, as revealed by the non-systematic analysis of video recordings inside nest boxes when nestlings were 10 days old (unpubl. data). The pine forest is characterized by a monoculture of Scots pine (*Pinus sylvestris*) at a mean density of 200 trees ha^{-1} and scattered *Cupressus arizonica* and *Pinus pinaster* trees (Fig. 4b). Sparse shrub cover of *Cistus laurifolius* and *Crataegus monogyna* is restricted to open areas (0.5−2 m high; mean cover 5%). Caterpillar abundance in the pine forest is comparatively small, and examination of video recordings and of prey left in the nest indicate that parents in the pinewood rely more on spiders, coleoptera larvae, and grasshoppers to feed nestlings.

Before the onset of the study, a small breeding population of pied flycatchers was already present in the oak forest, although bird numbers further increased after the addition of wooden nest boxes in 1984 (Potti & Montalvo 1990; Camacho et al. 2013). By contrast, pied flycatchers did not breed in the pine plantation before the study began due to the lack of natural cavities, although they soon established after nest boxes were provided in 1988. Nest boxes (156 and 81 in the oak and the pine forest, respectively) were installed on trees 2.5−4 m above the ground, at a mean distance of 20 m (SD 9.2) (Fig. 1b). Nest-box numbers and location have remained constant throughout the study, unless a minor move was necessitated by tree fall.

Many aspects of the ecology of pied flycatchers have been studied in the study area over the past three decades (e.g. Potti & Montalvo 1990; Potti et al. 2013; Camacho et al. 2013, 2016). Birds breeding in the oak and the pine forest differ in key life history and morphological traits, such as laying date (pine > oak), clutch size (oak > pine), and body size and weight (oak > pine), and there are also important differences in reproductive performance, measured as the number of hatchlings, fledglings, and recruits (pine > oak) (Camacho 2011). On average, 50% of breeding individuals each year are immigrants of unknown origin and over 25% of native individuals change habitats during natal dispersal (Camacho et al. 2013), indicating that both study populations are far from being isolated. Note that thorough this thesis the term 'population' is used in the simple ecological and practical sense to indicate groups of birds that are spatially more or less contiguous in the same habitat type within a landscape (Blondel et al. 2006).

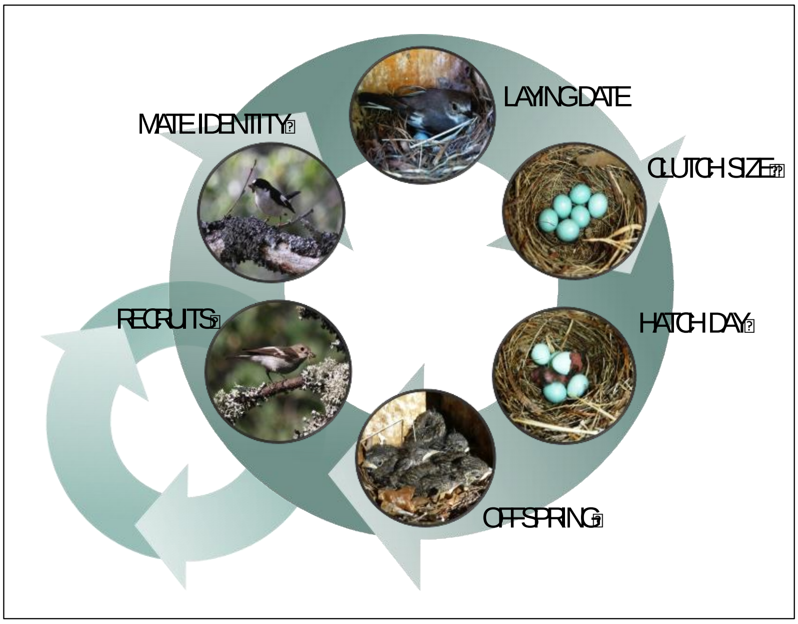

Figure 5. Lifelong monitoring of individually marked pied flycatchers provides information on basic life history parameters, morphological traits, and individual relatedness, which is essential for investigating microevolutionary dynamics in the wild.

This two-patch system is ideal for investigating the factors promoting fine-scale population divergence despite extensive dispersal for a number of reasons:

(i) Local habitat heterogeneity might exert strong habitat-specific selection that could affect phenotype distributions on a scale smaller than the scale of dispersal and potential gene flow (Blondel et al. 2006).

(ii) Nestlings can be marked for individual recognition and, except some polygynous males that left their secondary females unattended, all adults are captured and identified in subsequent years (Camacho et al. 2017).

(iii) Every year, all captured individuals are measured for standard morphological traits and their basic life history parameters, including breeding success, are determined (e.g. Potti et al. 2013; Camacho et al. 2015).

(iv) Local recruitment rates in this population are among the highest reported in the literature on the species (7–13% depending on the year; Potti & Montalvo 1991a) and, therefore, the origin and dispersal behaviour of a large number of individuals is known.

(v) Dispersal movements out of the study area are extremely rare, suggesting that virtually all survivors return to breed into the study plots.

(vi) Natal dispersal between habitats is common and has been found to be biologically relevant in previous studies on this population (see e.g. Camacho et al. 2013, 2016).

(vii) Less than 2% of adults move from the forest patch where they first bred, so breeding dispersal between habitats can be considered negligible (Camacho et al. 2013).

Tarsus length as the focal trait

Tarsus length, a proxy of structural size in passerines (Senar & Pascual 1997), was used as the focal trait in this thesis. Tarsus length has been shown to be under natural and sexual selection in the pied flycatcher, and is considered to be an ecologically relevant trait (Lundberg & Alatalo 1992). For example, structural size determines the ability of individuals to compete for and defend nest-sites during male-male interactions (Lundberg & Alatalo 1992). Body size plays an important role in determining the outcome of these interactions and is therefore predicted to influence the departure and settlement decisions of pied flycatchers across deciduous and coniferous habitats (Lundberg et al. 1981; Alatalo et al. 1985; Sirkiä & Laaksonen 2009; Camacho et al. 2013). In Iberian populations, tarsus length also plays an important reproductive role, since male success in extra-pair reproduction is positively related to tarsus length (Canal et al. 2011).

Dispersal propensity of males and, to a lesser degree, females, has been shown to be influenced by tarsus length, although this relationship may largely depend on the natal habitat type (Camacho et al. 2013). Pied flycatchers typically have longer tarsi in deciduous than in coniferous forests, suggesting a tendency for dominant birds to occupy broad-leaved habitats (Alatalo et al. 1985; Camacho et al. 2013). In addition, the habitat segregation of large and small individuals might reflect a differential ability to exploit different foraging niches and/or food resources. Although the functional significance of tarsus length has not been explicitly evaluated, dietary studies suggest that individuals of different size might specialize on different habitats or prey species and perform differently in coniferous and deciduous forests (Lundberg et al. 1981; Sanz 1998).

Tarsus length has proven a suitable trait for microevolutionary studies in other forest passerines (Merilä 1997; Kruuk et al. 2001; Charmantier et al. 2004a). Specifically, in the pied flycatcher, tarsus length is heritable (Alatalo & Lundberg 1986; Potti & Merino 1994) and has been shown to be under apparent stabilising selection in a northern population (Alatalo & Lundberg 1986). Importantly, the tarsus is fully grown by the time the nestlings leave the nest, and its length remains constant throughout adult life (Lundberg & Alatalo 1992; Potti & Merino 1994). For all these reasons, tarsus length represents an ideal trait to investigate the evolutionary dynamics of bird populations exposed to distinct breeding environments.

OBJECTIVES AND STRUCTURE

The main objective of this thesis is to understand the ecological and evolutionary mechanisms underlying population differentiation over short geographic distances. To address this issue, we used as a model system a long-term (1988–2016) monitored population of pied flycatchers (*Ficedula hypoleuca*) breeding in two ecologically different (pine and oak) but geographically close (1 km) habitats. The thesis consists of 5 chapters, each of which aims to find the answers to the questions raised by the previous chapter:

Chapter I examines microgeographic patterns of genetic (neutral markers) and phenotypic (tarsus length) variation between pied flycatchers exposed to different breeding environments and evaluates changes in the degree of phenotypic divergence over time. Moreover, we review previous research conducted in this population to see if there is any indication of the most common requirements of small-scale divergence models, including habitat-specific divergent selection, assortative mating and non-random dispersal.

Chapter II challenges the traditional view of dispersal as a force opposing microgeographic divergence by providing evidence that non-random dispersal regarding body size (measured as tarsus length) can result in population structuring. First, we assess the degree of population exchange using immigration-emigration rates and then examine the dynamic effect of body size and habitat of origin on the dispersal decisions of individuals.

Chapter III explores the relationship between body size and breeding performance in the two habitats to test whether the observed patterns of size-dependent dispersal and settlement translate into fitness benefits (i.e. matching habitat choice).

Chapter IV investigates the relative role of early experience and genetic background in determining the settlement decisions of pied flycatchers in order to understand why most individuals return to their natal patch and not to the patch that best suits their particular morphology. For this, we conducted an ambitious cross-fostering experiment both within and between habitats and followed individuals until recruitment and subsequent settlement.

Chapter V focuses on the evolutionary potential of dispersers and philopatric birds of each habitat (four groups). Based on a multigenerational pedigree, we estimated the additive genetic variance of tarsus length and also calculated selection gradients on this heritable trait in order to examine the role of phenotype-dependent dispersal in microevolutionary processes. Finally, we check whether the observed phenotypic trajectories of each of the four groups are consistent with the predicted evolutionary response to selection.

CHAPTER I - Microgeographic population structure despite extensive dispersal in the pied flycatcher (*Ficedula hypoleuca*)

ABSTRACT Exploring geographic variation in genetic structure and morphological characters is essential for understanding population differentiation. In a pied flycatcher (*Ficedula hypoleuca*) population monitored between 1988 and 2016, we investigated genetic (neutral markers) and phenotypic (tarsus length) patterns of divergence between the natural habitat of the species (oakwood) and a newly created habitat (pinewood) located 1 km apart. Pied flycatchers breeding in the oak and the pine forest showed strong divergence in tarsus length despite geographic proximity and extensive dispersal (ca. 25% of each cohort change habitats). However, the patterns of phenotypic change over the 29 years of study differed between sexes. Males were on average larger in the oak than in the pine forest, but this difference was greatest immediately after the pine population was founded. From then on, the degree of phenotypic differentiation decreased gradually as a result of contrasting temporal trends in tarsus length in the two habitats. No phenotypic change was detected in the oak forest, whereas males in the pine forest showed a gradual increase in body size, thus attenuating the phenotypic difference between the two populations. Females also tended to have large tarsi in the oak forest compared with the pine forest but, unlike males, their body size increased slightly over time in both habitats. Based on 15 microsatellite loci, we found evidence of low but significant genetic differentiation between the two populations, suggesting congruence between genetic and phenotypic patterns of variation. However, molecular data were not available for the entire study period and, therefore, it remains unclear whether genotypic and phenotypic trends are coupled in this population. Our results are surprising given the remarkably small geographic distance between sites, the absence of barriers to dispersal, and the short time since pied flycatchers colonized the newly created, coniferous habitat.

Keywords: dispersal, genetic structure, microgeographic variation, microsatellites, morphological differentiation.

This chapter is based on the unpublished manuscript: Camacho, C., Canal, D., & Potti, J. Microgeographic population structure despite extensive dispersal in the pied flycatcher (Ficedula hypoleuca).

INTRODUCTION

Barriers to dispersal and/or large geographical distances limiting gene exchange between populations have long been considered as prerequisites for population structuring (Slatkin 1987; Price 2010). However, although recent empirical studies still provide support to this idea (e.g. Kekkonen et al. 2011; Procházka et al. 2011), a growing body of theoretical and empirical work suggests that population differentiation may occur even in closely adjacent populations of mobile organisms (Senar et al. 2006; Garant et al. 2005; Postma & van Noordwijk 2005; Blondel et al. 2006; Milá et al. 2010; Arnoux et al. 2014; Langin et al. 2015; Izen et al. 2016; Morinha et al. 2017).

Several factors can promote population divergence at small spatial scales. For example, behavioural restrictions on dispersal in mobile animals can contribute to the genetic isolation and subsequent differentiation of neighbouring populations (Schluter 1998; Garroway et al. 2013). Localized dispersal despite high dispersal potential has been reported, for instance, in some island bird populations, where individuals are reluctant to move away from their birthplace (Komdeur et al. 2004; Bertrand et al. 2014; Langin et al. 2015), as well as in fragmented landscapes, where habitat specialists are often unable to overcome non-physical barriers to dispersal (Harris & Reed 2002; Lindsay et al. 2008; Woltmann et al. 2012). Furthermore, population differentiation can be reinforced due to divergent mating patterns, leading to non-random mate choice (Jiang et al. 2013; Langin et al. 2015).

Population differentiation can also occur even when geographic proximity allows for the dispersal of individuals. For example, in spatially heterogeneous environments, strong local adaptation of populations to their local habitat can lead to reduced survival and reproductive success of immigrants and thus cause major discrepancies between dispersal and realized gene flow (Hendry 2004; Nosil et al. 2005; Garant et al. 2007). Local maladaptation and selection against immigrants may thus limit gene flow between neighbouring populations and allow genetic and phenotypic differentiation to be maintained (Blondel et al. 1999, 2006; Postma & van Noordwijk 2005; Porlier et al. 2012). Lastly, two distinct, but not mutually exclusive, forms of non-random dispersal can facilitate population differentiation at microgeographic scales, namely the tendency of individuals to choose breeding habitats that resemble those they encountered early in life (i.e. natal habitat preference induction; Davis & Stamps 2004) or those that best match their phenotypes and maximize their fitness (i.e. matching habitat choice; Edelaar et al. 2008).

Most studies testing for microgeographic divergence have been conducted at spatial scales that exceed the dispersal radius of the focal organism (reviewed by Richardson et al. 2014), possibly because, as stated by Izen et al. (2016), "we tend not to look for divergence where we do not expect to find it". However, emerging evidence of fine-scale population structure in a variety of taxa –typically birds– suggests that microgeographic divergence might be more common than previously thought, even in vagile species (Chan & Arcese 2003; Garant et al. 2005; Bertrand et al. 2014; Arnoux et al. 2014; García-Navas et al 2014; Camacho et al. 2016a; Szulkin et al. 2016). Further studies examining the genetic and phenotypic structure of populations over short distances are nevertheless needed to establish the minimum spatial scale at which evolutionary divergence can occur.

Here we document the evolution of differences in tarsus length (a proxy for structural body size in birds; Senar & Pascual 1997) within a population of pied flycatchers (*Fidedula hypoleuca*) exposed to different (oak and pine) breeding environments over a 29-year period. Pied flycatchers have been extensively studied in the oak and the pine forest since nest boxes were provided in 1984 and 1988, respectively (e.g. Potti et al. 2013; Camacho et al., 2013, 2015). Before the onset of the study, a small breeding population of pied flycatchers was already present in the oak forest, while the first settlers into the pine forest did not arrive until nest boxes were installed (Potti & Montalvo 1990). Birds breeding in the two habitats, as reported in other spatially structured populations of the species (Lundberg et al. 1981), differ in key life history traits, such as laying date (pine > oak) and clutch size (oak > pine), and there are also important differences in reproductive performance (pine > oak) (Camacho 2011). On average, 50% of breeding individuals each year are immigrants from outside the study area and over 25% of native individuals change habitats during natal dispersal (Camacho et al. 2013), indicating that the study population is far from being genetically isolated. Following the rationale of Langin et al. (2015), the spatial configuration of the oak and the pine forest may be considered as a case of sympatry —rather than parapatry— because the distance separating both habitats (1 km) is well within the potential dispersal radius of the species (Potti & Montalvo 1991a; Camacho et al. 2016b).

The main objective of this study was to test for morphological differentiation between the two habitats and to identify microgeographic patterns of genetic divergence. More specifically, (1) we used tarsus length measurements collected over the 29-year period to compare the phenotypic trajectories of pied flycatchers breeding in the oak and the pine forest and to examine changes in the frequency distributions from the beginning to the end of the study period; (2) we tested for genetic population structure in 2005−2006 based on neutral molecular markers; and (3) we reviewed research conducted in this population to see if there is any indication of differential habitat selection, assortative mating or non-random dispersal, considered as common requirements of small-scale divergence models.

METHODS

Field procedures

Fieldwork was conducted between 1988 and 2016 in a two-patch system formed by a mature oak forest and an adjacent pine plantation where pied flycatchers breed in nest boxes (see Camacho et al. 2015 for a detailed description of the study site). Nest boxes were checked every 1−3 days to record basic life history parameters, such as laying and hatching dates, clutch size and number of offspring. Nestlings were individually marked with numbered metal rings and measured for tarsus length (± 0.05 mm) on day 13 (hatch day = 1), when they have already reached the final adult size (Potti & Merino 1994). Breeding adults were captured at the nest box for identification and marking of unringed birds and measured for standard morphological traits.

Phenotypic differentiation

To examine phenotypic differences between the two habitats, males and females were treated separately because (1) they differ in ecologically important traits (e.g. skeletal body size, dispersal distance, arrival date, habitat choice and mate choice decisions) that may ultimately affect their spatial distribution (Potti & Montalvo 1991a, b; Sirkiä & Laaksonen 2009); (2) the strength and direction of selection pressures may differ between the sexes depending on the habitat type, thus leading to distinct phenotypes (Dreiss et al. 2012); and (3) exploratory analyses showed sex differences in body size variation over time across the two study sites (see 'Results').

First, we tested for differences in tarsus length between the two habitats and then compared the patterns of change over time. Second, we compared the frequency distribution of body sizes at the beginning and end of the study period, both within and between habitats. Bird numbers in the pine forest increased steeply since the first pair established in 1988, but it was not until 1993 that breeding density became comparable to that in the oak forest (see Fig. 3 in Chapter 2). Based on this, we defined the period between 1988 and 1993 as the colonization phase and, therefore, chose an equally long period of time (six years) at the end of the study period (2011–2016) for temporal comparisons.

Finally, we used a recently developed approach to estimate the 'exchangeability' of individuals between populations (Hendry et al. 2013). Briefly, this method uses the full distribution of a particular trait or group of traits to estimate the probability that a given individual originating from a given population is correctly classified to that population or, in other words, to infer how well the phenotypes of individuals from one population would fit into other populations (Hendry et al. 2013). Females were not considered in the comparison between frequency distributions or in the classification analysis given the lack of phenotypic differences between the two habitats (see 'Results').

Genetic analysis

Blood samples, taken from the brachial vein, were only available for a limited number of years, so we selected 2005 and 2006 based on the high degree of phenotypic divergence between the two habitats at that time (mean tarsus length: 19.50 ± 0.04 (SE) and 19.34 ± 0.06 (SE) in the oak and the pine forest, respectively; LM: habitat: $F_{1,295} = 5.97$, $P = 0.015$; sex: $F_{1,294} = 2.11$, $P = 0.148$; habitat x sex: $F_{1,293} = 0.02$, $P = 0.888$; see Fig 1). Males and females breeding in the study area in these years were genotyped at 15 microsatellite loci (see Canal et al. 2014). Our final data set (i.e. after excluding data from repeated individuals and/or from the same families) consisted of 243 birds in the oak forest and 108 birds in the pine forest.

To examine genetic differences between pied flycatchers breeding in the two study habitats we followed the rationale described by Postma et al. (2009). First, we tested for linkage disequilibrium using GENEPOP 4.0 (Raymond et al. 1995) and subsequently adjusted it with Bonferroni corrections for multiples tests. We also used GENEPOP to test for significant deviations from Hardy-Weinberg equilibrium across all loci (FIS, estimated following Weir &

Cockerham, 1984; Markov chain parameters were: 10,000 dememorisations, 1000 batches and 10,000 iterations per batch). Second, we quantified the level of genetic diversity within each population across loci using FSTAT (Goudet 1995) and compared them using a Wilcoxon signed-rank test. To estimate the extent of genetic differentiation (F_{ST}) between habitats we used GENETIX (5000 permutations; Belkhir et al. 2004). Further, we tested for genetic population differentiation in GENEPOP ("exact G test" option; Markov chain parameters were: 10,000 dememorisations, 1000 batches and 10,000 iterations per batch). Finally, we used a Bayesian approach as implemented in the program STRUCTURE (Pritchard et al. 2000) to infer the number of genetic clusters (K) in the population. Simulations were run assuming the admixture model with correlated allele frequencies. Four independent runs (K = 4), with twenty replicates for each K, were performed with a 10^5 burning period followed by 10^6 MCMC repeats after burning. To obtain the true value of K based on the method described by Evanno et al. (2005) we used the STRUCTURE HARVESTER web (Earl & VonHoldt 2012).

Data analyses

All statistical analyses were performed using R version 3.3.1 (http://www.R-project.org). To examine phenotypic differences between the two habitats, we fitted Generalized Linear Mixed Models (GLMM, normal error distribution and identity link functions) including tarsus length as the response variable, and habitat type, year and their interaction as explanatory variables. In these models, year was treated as a continuous predictor to formally test for temporal trends in the average tarsus length. Individual identity was fitted as a random effect to account for repeated measures of the same individuals in different years. Local birds (i.e. individuals ringed as nestlings) and immigrants (i.e. previously unringed adults) were pooled together for the analyses. GLMMs were fitted using the R package 'lmerTest' (Kuznetsova et al. 2016). Model selection was carried out by stepwise removal of non-significant terms from a saturated model (i.e. containing all main effects and interactions). Before interpreting the output of the GLMMs, we systematically performed model diagnostics statistics to avoid misleading conclusions based on statistical artefacts. To this end, we checked assumptions about the distribution of residuals through diagnostics plots and examined collinearity and the presence of influential cases. These analyses did not show any obvious deviation from GLMM assumptions or any collinearity problems.

Pairwise comparisons of the frequency distributions of body size were made using Kolmogorov-Smirnov (KS) two-sample tests, as implemented using function *ks.boot* (10,000 simulations) in the R package 'Matching' (Sekhon 2011). To evaluate exchangeability between populations based on tarsus length measurements we used discriminant function analyses (DFAs) and then compared the rates of correct assignment at the beginning and end of the study period using a chi-square test.

RESULTS

Phenotypic structure

Between 1988 and 2016, a total of 1,508 males and 1,557 females were measured for tarsus length (2,752 and 2,908 measurements, respectively). Mean tarsus length of adult pied flycatchers changed over time and across the two study sites depending on sex (year x habitat x sex: $F_{1,\,4016.2}$ = 11.21, P = 0.0008). Therefore, we ran separate models for males and females.

Males breeding in the oak forest were, on average, larger than those in the pine forest (mean ± SE: 19.44 ± 0.02 and 19.36 ± 0.03, respectively), this difference being greatest (3.9%) at the beginning of the study period. However, the degree of phenotypic differentiation between populations was gradually reduced as a result of contrasting temporal trends in the body size of males in the two habitats. Specifically, males in the pine forest showed a significant increase in mean tarsus length over time (b = 0.017 ± 0.003, t = 5.43, P < 0.001), whereas no phenotypic change was detected in the oak forest (b = 0.001 ± 0.002, t = 0.59, P = 0.59), the difference between slopes being significant (year x habitat: $F_{1,\,1928.1}$ = 23.7, P < 0.001; Fig. 1).

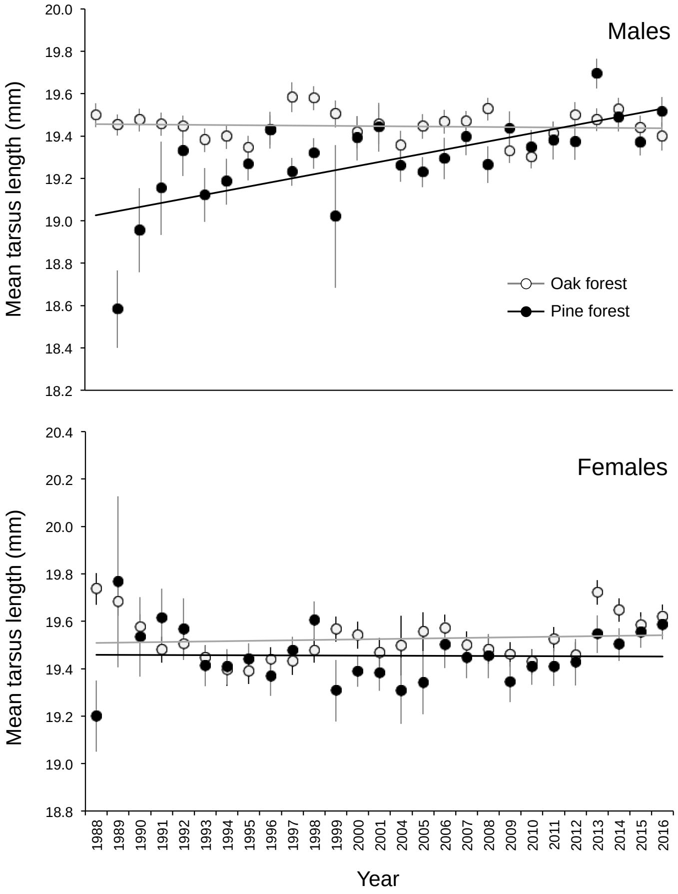

Figure 1. Temporal trends in mean (± SE) tarsus length of male and female pied flycatchers breeding in the oak (grey line, open circles) and the pine forest (black line, filled circles) over the study period. Note that the year 1988 is omitted in the top panel because the only one male breeding in the pine forest was not measured.

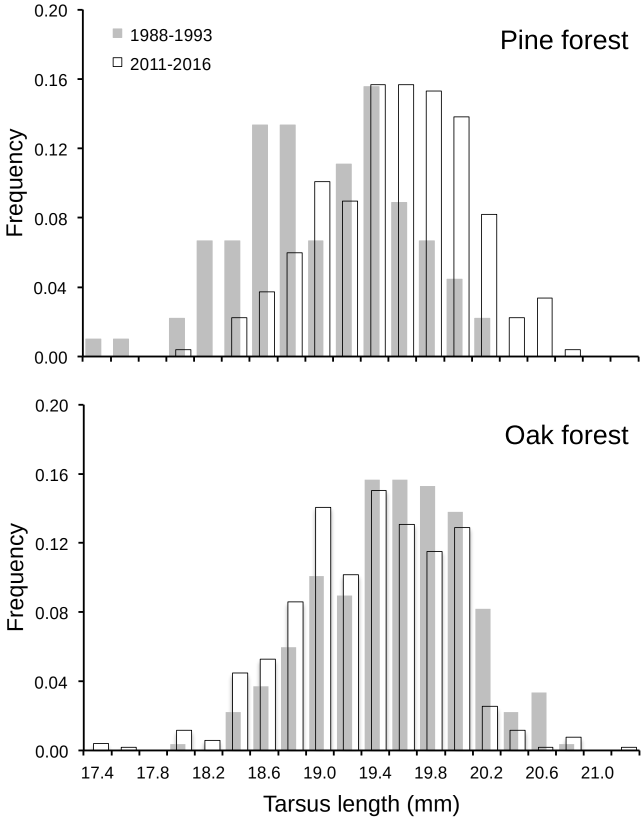

Figure 2. Frequency distribution of tarsus length of male pied flycatchers breeding in the pine and the oak forest at the beginning (1988–1993) and end (2011–2016) of the study period.

Females also tended to be larger in the oak forest compared with the pine forest (19.51 ± 0.02 and 19.48 ± 0.03 respectively), although the difference did not reach statistical significance ($b = -0.054 \pm 0.028$, $t = -1.88$, $P = 0.059$). Moreover, in contrast to males, mean tarsus length of females increased slightly over time in both habitats ($b = 0.004 \pm 0.001$, $t = 2.510$, $P = 0.012$), after excluding the interaction between year and habitat (Fig 1). Further comparisons (size distributions and misclassification analyses) between habitats were restricted to males due to the lack of differences in female size.

Results of the KS-tests showed significant differences in the body size distributions of males between the pine and the oak forest at the beginning of the study ($D = 0.269$, $P = 0.009$). However, this difference was no longer evident in more recent years ($D = 0.054$, $P = 0.655$; Fig. 2). Consistent with a phenotypic change over time, the KS-tests showed that the size distribution of males breeding in the pine forest differed markedly between the beginning and end of the study period ($D = 0.258$, $P = 0.012$), whereas no change over time was detected within the oak population ($D = 0.102$, $P = 0.071$; Fig. 2).

Exchangeability analyses for tarsus length provided further evidence for a reduction in the degree of phenotypic differentiation over time, as classification to the population of origin dropped from 83.6% (1.7 times the random expectation) at the beginning of the study to 66.2% (1.3 times the random expectation) at the end, the difference being statistically significant ($\chi^2 = 27.37$, *d.f.* = 1, $P < 0.0001$).

Genetic structure

F_{ST} values revealed a low but statistically significant genetic differentiation between the two populations (F_{ST} = 0.002, P < 0.001), which was corroborated by the exact test of genotypic differentiation (χ^2 = 53.99, *d.f.* = 30, P = 0.004). No evidence for departure from H-W equilibrium was found in the pine population (χ^2 = 22.82, *d.f.* = 30, P = 0.82), whereas a significant deviation from H-W expectations was observed in the oak population (χ^2 = 43.9, *d.f.* = 30, P = 0.05) due to an excess of homozygotes in three of the markers (Fhy 301, Fhy 339 and Fhy 401; Table 1). Further analyses showed that the excess of homozygotes in those loci was due to rare, private alleles appearing at low frequencies. To assess the impact of outlier loci on the F_{ST} estimates, we repeated the analysis after excluding individuals carrying the rare alleles in the deciduous patch, and found that the two populations showed no evidence for H-W disequilibrium (oak forest: χ^2 = 37.79, *d.f.* = 30, P = 0.15; pine forest: χ^2 = 25.6, *d.f.* = 30, P = 0.69), had similar genetic diversity (Z = 0.78, P = 0.43) and, although marginally non-significant, the level of genetic differentiation was similar to that found using the entire data set (F_{ST} = 0.001, P = 0.06). These results suggest that the rare alleles found in some individuals of the oak population likely contributed to, but were not fully responsible for, the genetic differentiation between sites. Not surprisingly, the popular Bayesian clustering approach STRUCTURE failed to characterise discrete genetic groups, because as shown by simulations (Kalinowski 2011), individual-based clustering methods may not appropriately summarize population structure in scenarios with a low number of genetic clusters (n = 2) and a low F_{ST} value, as occurs in our study system.

Table 1. Number of alleles (*Na*), expected (*He*) and observed (*Ho*) heterozygosity of 15 microsatellite loci within the two study populations of pied flycatchers. Numbers of individuals sampled are shown in parentheses.

Locus	Oak forest (227)			Pine forest (98)		
	N_a	H_e	H_o	N_a	H_e	H_o
Fhy 216	7	0.522	0.524	6	0.487	0.429
Fhy 237	6	0.414	0.432	6	0.442	0.378
Fhy 301	14	0.857	0.859	12	0.841	0.857
Fhy 304	9	0.774	0.806	9	0.789	0.806
Fhy310	13	0.875	0.885	12	0.871	0.867
Fhy 329	8	0.711	0.696	8	0.678	0.724
Fhy 339	11	0.831	0.819	10	0.831	0.806
Fhy 356	12	0.838	0.841	12	0.835	0.857
Fhy 361	6	0.536	0.533	6	0.617	0.633
Fhy 401	13	0.815	0.819	11	0.755	0.776
Fhy444	14	0.877	0.855	13	0.870	0.918
Fhy 466	12	0.829	0.859	11	0.830	0.878
Fhy 236	25	0.896	0.907	18	0.893	0.847
f3–60CR	35	0.956	0.965	29	0.942	0.990
f1–25CR	7	0.737	0.775	6	0.763	0.837

DISCUSSION

Our results confirm earlier evidence of phenotypic differentiation between adjacent habitats (Camacho et al. 2013) and also reveal striking patterns of phenotypic change over time. Males, but not females, showed marked differences in size between the oak and the pine forest, but the magnitude of this difference decreased over the 29 years of study. Examination of the phenotypic trajectories in each habitat revealed contrasting patterns of change over time and, as a result, males in the pine forest eventually became almost indistinguishable from their oakwood conspecifics.

Morphological differentiation between adjacent populations has been extensively documented in sedentary bird species (e.g. Blondel et al. 1999; Senar et al. 2002; Chan & Arcese 2003; Garant et al. 2005; Bertrand et al. 2014; Garroway et al. 2013; Arnoux et al. 2014; García-Navas et al. 2014; Langin et al. 2015) and, more rarely, also in migratory species (Walsh et al. 2012; Camacho et al. 2016a). However, to our knowledge, a difference in body size close to that of pied flycatchers during the colonization phase of the pinewood (3.9%) has never been reported at spatial scales comparable to that of our study system in either sedentary or migratory birds (<10 km, 0.52–1.1% difference; Senar et al. 2002; García-Navas et al. 2014; Camacho et al. 2016a).

Remarkably, there was significant genetic differentiation between the two populations. Although the low F_{ST} value (0.002) found for the pied flycatcher might be considered a lower bound in the context of molecular population genetics (e.g. $F_{ST} < 0.003$; Lemoine et al 2016), we suggest that the level of genetic differentiation within the study population is exceptionally high considering the short distance and extensive dispersal between habitats. For example, even when comparing the magnitude of genetic differentiation found in different studies is not devoid of problems (Meirmans & Hedrick 2011), our estimate is of the same order of magnitude as those found over much larger regions in other songbirds, such as the house sparrow (*Passer domesticus*) across Finland ($F_{\mathrm{ST}} = 0.004$; Kekkonen et al. 2011) or the great tit (*Parus major*) across Europe ($F_{\mathrm{ST}} = 0.008$; Lemoine et al. 2016). Further support for the biological relevance of our F_{ST} estimate comes from some studies showing strong population divergence in phenotypic and life-history traits despite low levels of genetic differentiation, such as those found between immigrants and residents in an island population of great tits in the Netherlands ($F_{\mathrm{ST}} = 0.007$; Postma & van Noordwijk 2005; Postma et al. 2009) or among isolated populations of blue tits (*Cyanistes caeruleus*) located 20 km apart ($F_{\mathrm{ST}} = 0.005$–0.008; Ortego et al. 2011).

Several non-mutually exclusive mechanisms may account for population structuring at the scale of our study system. Localized dispersal is often proposed as responsible for fine-scale population structure within bird populations, although this only applies to species that display a behavioural reluctance to move away from its natal site (Ortego et al. 2011; Bertrand et al. 2014; Woltmann et al. 2012; Langin et al. 2015). For example, the small dispersal ranges of blue tits on the island of Corsica have been shown to increase their potential for local adaptation to distinct habitats and thus promote population divergence at a microgeographic scale (Blondel et al. 1999; Porlier et al. 2012). However, in our study population, extensive dispersal between habitats argues against this scenario.

Local ecological divergence has also been shown to drive fine-scale population divergence in several bird species (e.g. Chan & Arcese 2003; Blondel et al. 2006; Garant et al. 2005; Postma & van Noordwijk 2006; Porlier et al. 2012), and might also underlie the size differentiation of pied flycatchers. For example, the average prey size of pied flycatchers is known to be larger in deciduous habitats as compared with coniferous habitats (Lundberg et al. 1981; Sanz 1988), suggesting that individuals of different size might be locally adapted to different habitats. However, although we have found some evidence for selection against 'wrong' phenotypes in the study population (Camacho et al. 2015), the rapid emergence of morphological differences between the two populations suggests that they have not diverged exclusively in response to habitat-specific divergent selection.

Non-random dispersal with respect to phenotype may provide a more plausible explanation for the rapid emergence of population structure within this pied flycatcher population. Following nest box addition, there was a massive colonization of the newly created, coniferous habitat, which is reminiscent of the effects of a founder event (Reznick & Ghalambor 2001). However, a comparison to the main source population (i.e. oak forest) revealed that, while settlers into the pine forest during the colonization phase were indistinguishable from residents of the oak forest, the latter habitat attracted larger-than-average immigrants from the pine forest, resulting in the habitat segregation of size classes (Camacho et al. 2013). Likewise, size-dependent dispersal was found to be responsible for the rapid morphological differentiation between two populations of citril finches (*Serinus citrinella*) separated by less than 5 km (Senar et al. 2002, 2006) and between great tits inhabiting ecologically distinct sectors of woodland separated by 4 km (Garant et al. 2015). Together with our study, these examples illustrate that phenotype-dependent dispersal can have important consequences for population divergence over very short distances (Edelaar & Bolnick 2012).

Interestingly, we found that the magnitude of population divergence decreased over the study period; therefore, other factors limiting the potential of phenotype-dependent dispersal to maintain population structure may have been operating. First, the strong tendency of pied flycatchers to return to their natal habitat, but not to the habitat that would best match their phenotype, might have prevented local adaptation and ultimately eliminate the initial differences between habitats (Edelaar et al. 2008; Camacho et al. 2015; 2016). Second, the survival and/or breeding success of dispersers might vary depending on the destination habitat, so the contribution of phenotype-dependent dispersal to population differentiation might differ between the oak and the pine forest. Moreover, random mating with respect to tarsus length has certainly not contributed to reinforce population structure either (see Suppl. Mat. 1). Finally, size-dependent dispersal in the pied flycatcher is exclusive to males (Camacho et al. 2013) and, therefore, the random movement of females could also have contributed to swamp the overall size differences between populations.

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SUPPLEMENTARY MATERIAL 1: Between-habitat variation in mating preferences with respect to body size.

To address a possible role of size-based assortative mating in population differentiation between the two study habitats, we tested the correlations between male and female tarsus length across mated pairs in the pine and oak forest. Mate fidelity is uncommon in the pied flycatcher, but a small proportion of individuals retained the same mates between years in the study population. Specifically, 2.7% of the pairs (*n* = 2,807) were repeatedly encountered during 2–3 years and subsequently excluded from the analysis to avoid pseudoreplication. In total, our data set comprised 2,732 pairs.

Mean tarsus length of females gradually increased over time in both habitats, whereas in the case of males, tarsus length only increased in the pine forest, resulting in contrasting trends in the two sexes (see main text). Coherently, males and females did not mate assortatively in relation to body size in either habitat (oak forest: *r* = 0.043, *P* = 0.067, *n* = 1038 pairs; pine forest: *r* = 0.048, *P* = 0.195; *n* = 794 pairs), confirming previous results obtained with a lower number of pairs (Potti 2000).

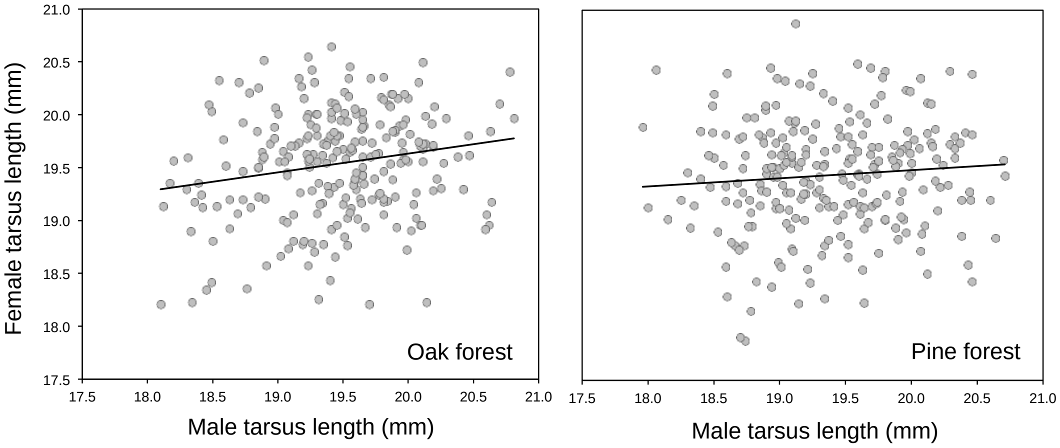

Figure SM1. Female mating preferences with respect to body size, measured as tarsus length. Each point represents a pair of breeding birds. For clarity, a randomly selected subset of 250 pairs is included in each panel.

Reference: Potti, J. 2000. Causes and consequences of age-assortative pairing in pied flycatchers (*Ficedula hypoleuca*). Etología 8:29–36.

CHAPTER II - Temporal dynamics of phenotype-dependent dispersal and fine-scale population divergence: a case study in a Mediterranean songbird

ABSTRACT Dispersal and gene flow have traditionally been thought to reduce the potential for local specialization and thus prevent evolutionary differentiation. However, if non-random, dispersal can actually promote local adaptation and population divergence even at microgeographic scales. Between 1988 and 2016, we investigated dispersal patterns of pied flycatchers (Ficedula hypoleuca) breeding in an old oak forest and an adjacent (1 km) pine plantation and examined its potential influence on fine-scale population differentiation. Natal dispersers moved between habitats according to their size (tarsus length), although this pattern was much more pronounced in males than in females. Furthermore, patterns of phenotype-dependent dispersal changed over time depending on males' dispersal status and origin. Males dispersing from the pine to the oak forest were, on average, larger than those moving the other way round and also than philopatric individuals of either habitat, but in contrast to the latter, they showed a slight decrease in size over the study period. Males breeding in the pine forest showed a significant increase in size regardless of their origin, and no phenotypic change was detected in residents of the oak forest. Estimates of breeding densities at the two study sites suggest that the relative attractiveness of the oak patch to high-quality phenotypes (i.e. large individuals) could have diminished over time as a result of a temporal increase in the levels of nest-site competition in this habitat, which in turn might explain the observed temporal changes in the phenotype of birds. Differential patterns of phenotype-dependent dispersal may have played a critical role in shaping population differentiation soon after the pine population was founded, and might also underlie the observed phenotypic trajectories of both populations, although other mechanisms (e.g. natural selection, genetic drift) could also have played a role. Our findings provide important empirical support for theoretical studies suggesting new ways in which phenotype-dependent dispersal can promote population differentiation.

Keywords: competition, demography, dispersal, habitat preferences, long-term trends, microevolution, phenotypic divergence, population structure.

This chapter is partially based on the article: Camacho, C., Canal, D., Potti, J. 2013. Non–random dispersal drives phenotypic divergence within a bird population. Ecol. Evol. 3:4841–4848. However, the present manuscript includes previously unnoticed –and yet unpublished– patterns that became apparent during the preparation of Chapter 5, when we shifted from a static snapshot to a dynamic assessments of phenotypic trends using an enlarged data set.

INTRODUCTION

Understanding the causes of population divergence is a central issue in evolutionary biology (Schluter 2000). Most evolutionary models suggest that population divergence reflects a balance between divergent selection and the homogenizing effects of gene flow (Slatkin 1987; Garcia-Ramos & Kirkpatrick 1997; Lenormand 2002). Microgeographic divergence, understood as differences in trait distributions within the dispersal radius of an organism (Richardson et al. 2014), is therefore predicted to be rare because dispersal is assumed to homogenize gene pools among neighbouring populations (Hendry & Taylor 2004; Nosil & Crespi 2004; but see Garant et al. 2007). However, recent research has challenged the traditional view of dispersal as a force opposing evolutionary differentiation and reconsidered the role of non-random dispersal in the adaptive evolution of wild populations (e.g. Garant et al. 2005; Postma & van Noordwijk 2005; Edelaar & Bolnick 2012). Evidence has since accumulated that phenotypic differentiation can evolve despite genetic exchange between populations (e.g. Senar et al. 2006; Bolnick et al. 2009; Porlier et al. 2009, 2012; McDevitt et al. 2013; Fitzpatrick et al. 2015), but comprehensive, long-term studies investigating the complex role of dispersal in shaping the evolutionary trajectories of populations are badly needed.

Most previous work has assumed that dispersal is random with respect to phenotype. However, there is growing appreciation that dispersers may not be a random sample of the source population (Bowler & Benton 2005; Clobert et al. 2009; Edelaar & Bolnick 2012). For example, attributes such as dominance rank, habitat familiarity, body size, tegument colouration, physiological state, biomechanical dispersal capacities, or personality have been shown to influence the departure and settlement decisions of individuals (Senar et al. 2002; Bolnick et al. 2009; Cote et al. 2010; Saino et al. 2014; Camacho et al. 2013; Scandolara et al. 2013). In addition, the effect of phenotype on dispersal and habitat selection may differ between the sexes because, in many species, the critical decision on where to settle lies exclusively on one sex (Hendry et al. 2001; Dreiss et al. 2012). Finally, environmental conditions may interact with phenotypic traits and lead to phenotype-dependent variation in dispersal decisions (Bowler and Benton 2005; Benard & McCauley 2008; Clobert et al. 2009).

Habitat quality, including intrinsic attributes and density-dependent competition for limiting resources, has traditionally been identified as the major environmental driver of dispersal decisions (Senar et al. 2002; Massot et al. 2002; Matthysen; 2005; Doligez et al. 2008; Delgado et al. 2010; Scandolara et al. 2013). Experimental studies provide evidence that, in spatially and temporally heterogeneous breeding environments, individuals track variation in habitat quality to adjust dispersal and settlement decisions, so that dominant individuals are more likely to settle in high-quality habitats (Verhulst et al. 1997; Stamps 2006; Doligez et al. 2008; Clobert et al. 2009). Nevertheless, phenotype-dependent dispersal may not necessarily imply a despotic distribution of individuals (Fretwell 1972). Instead, an ideal free distribution (Fretwell and Lucas 1970) may result from phenotype-dependent dispersal if individuals select the habitat that best match their phenotype to maximize fitness (i.e. matching habitat choice; Holt & Barfield 2008; Edelaar et al. 2008). Either form of phenotype-dependent dispersal will contribute to a greater or lesser extent to the habitat segregation of phenotypes and thus may be presumed to have important implications for population differentiation (Edelaar et al. 2008; Edelaar & Bolnick 2012; Bolnick & Otto 2013).

Most studies examining the consequences of phenotype-dependent dispersal for spatial population structure at microgeographic scales have found evidence for population differentiation even in the absence of major spatial differences in selection (e.g. Garant et al. 2005; Postma & van Noordwijk 2005; Senar et al. 2006; Bolnick et al. 2009; McDevitt et al. 2013). However, most of these studies cover a relatively short period of time (but see Garant et al. 2005 and Postma & van Noordwijk 2005) and more evidence from longitudinal field studies is therefore needed to understand, on one hand, the mechanisms underlying the emergence and evolution of phenotype-dependent dispersal and, on the other hand, its long-term consequences for population differentiation.

Here, we document the temporal patterns of phenotype-dependent dispersal in a spatially structured population of pied flycatchers (*Ficedula hypoleuca*) breeding in an oak forest and a pine plantation separated by only 1 km. The pied flycatcher is a small (11−13 g), migratory passerine that breeds in tree cavities and readily uses nest boxes when provided (Lundberg & Alatalo 1992). Females have larger body size than males, showing sexual size dimorphism (Potti 1999). Male pied flycatchers arrive from spring migration before than females, choose a territory (i.e. nest cavity), females choose males for breeding, and males defend their territories by aggressive intra-sexual interactions (Lundberg et al. 1981; Dale & Slagsvold 1990; Lundberg & Alatalo 1992). Body size plays an important role in determining the outcome of male-male interactions and, therefore, is predicted to influence the settlement patterns of males after a nest-site shortage (Lundberg et al. 1981; Alatalo et al. 1985; Sirkiä & Laaksonen 2009; Camacho et al. 2013). Pied flycatchers can be found in a wide variety of woodland, ranging from pure deciduous to pure coniferous forests (Lundberg and Alatalo 1992; Mäntylä et al. 2015), but they are widely thought to prefer deciduous forests as breeding habitats due to the scarcity of tree cavities in coniferous sites (Alatalo et al. 1985; Sanz 1995). However, the addition of nest boxes can substantially improve the quality and attractiveness of coniferous patches and thus potentially attenuate the effects of nest-site competition in the preferred, broad-leaved habitats (Lundberg et al. 1981). Long-term data from continuously monitored, adjacent populations of marked pied flycatchers offer a unique opportunity to investigate the mechanisms behind the emergence and evolution of phenotype-dependent dispersal between contrasting environments.

First, we hypothesized that, if size-dependent dispersal is responsible for the observed emergence of size differences between habitats (oak > pine) during the colonization phase of the pine forest (Camacho et al. 2013), there would be an association between body size and dispersal propensity between the two study sites. Second, we hypothesized that the patterns of phenotype-dependent dispersal would be more evident in males than in females because, unlike males, females do not need to assert physical dominance to acquire a nest site but rather compete for established males. Finally, if pied flycatchers are able to track variation in the quality of potential breeding habitats, we hypothesized that phenotypic differences between dispersers and non-dispersers would not be consistent over time, as there are reasons to think that habitat quality has changed throughout the nearly three decades of study (Camacho et al. 2013).

METHODS

Study system and general procedures

Data were obtained between 1988 and 2016 from a spatially structured population of pied flycatchers breeding in nest boxes in two different habitats located in Central Spain: an old deciduous forest dominated by oaks (*Quercus pyrenaica*) and a mixed coniferous plantation dominated by *Pinus sylvestris*. Both forests are separated by only 1 km, and there are no physical barriers to dispersal between them (see Camacho et al. 2015 for a detailed description of the study area).

Before nest boxes were provided, a small breeding population of pied flycatchers was already present in the oak forest, whereas they did not breed in the pine plantation due to the lack of natural cavities (Potti & Montalvo 1990). In 1988, nest boxes were erected in the pinewood and the first pied flycatcher pair soon established. From that moment, we followed their descendants and those from the oak forest over a period of 29 years (Potti & Montalvo 1991a, b; Potti et al. 2013; Camacho et al. 2015).

Nest boxes (*n* = 156 and 81 in the oak and the pine forest, respectively) were regularly checked soon after the arrival of the first individuals from spring migration to determine breeding phenology and reproductive success of all breeding pairs (see e.g. Potti et al. 2013; Camacho et al. 2015). Breeding individuals were captured while incubating (females) or feeding nestlings (both sexes) using a spring trap located inside the nest box (Camacho et al. 2017). Birds were individually marked with colour and metal rings, measured for tarsus length (± 0.05 mm) and aged as either one year or older following the criteria of Karlsson et al. (1986) and Potti & Montalvo (1991a). Based on the patterns of age at first breeding in birds of exactly known age, unringed birds aged as older when breeding for the first time were assigned an age of two years (Potti and Montalvo 1991b). During 2002–2003, sampling intensity was limited, and these years were not considered (see Potti et al. 2013; Camacho et al. 2015).

Dispersal patterns

Natal dispersal, defined as the movement of individuals from their birthplace to their first breeding site, is here considered as a change of habitat patch irrespective of the distance between the natal nest box and the first breeding nest box. Breeding dispersal is likewise defined as a change of patch after the first reproduction. Based on the habitat of origin and the direction of natal dispersal, locally born individuals were classified into four groups: dispersers from the oak to the pine forest, dispersers from the pine to the oak forest, philopatric birds of the oak forest, and philopatric birds of the pine forest. Dispersal distances of juveniles or adults in this population rarely exceed 1.5 km (Potti & Montalvo 1991a) and explorations of peripheral areas within the dispersal range of the species indicate that anecdotal dispersal events outside the study plots are unlikely to bias the results of this study.

Natal dispersal rates, defined as the proportion of individuals emigrating from one habitat patch to the other, were calculated by dividing the number of recruits that changed habitats throughout the study period by the total number of recruits of all cohorts that returned to the study area. In addition, natal dispersal rates were calculated separately for each year using the annual numbers of recruits. Dispersal rates after the first reproduction were estimated as the proportion of adults that changed the habitat where they bred for the first time. Unringed birds that were first caught as breeding adults were defined as being immigrants from outside the study area. Between-habitat differences in the annual rates of 'foreign' immigration, expressed as the number of unringed birds relative to the total number of individuals, were also determined.

To test for phenotype-dependent dispersal we focused on natal dispersal, because the proportion of adults changing habitats later in life is negligible (Camacho et al. 2013). We tested for phenotype-dependent dispersal based on tarsus length, which is considered a good proxy for body size in passerine birds (Senar & Pascual 1997). Specifically, we compared the body size of philopatric birds and dispersers originating from the oak and the pine forest and explored the temporal trends in mean body size of each of the four groups.

Breeding density estimates

Local breeding density of pied flycatchers was estimated as the annual number of nest boxes occupied (i.e. where a complete clutch was laid and incubation began) by the species relative to the total surface area of each site (Blondel et al. 1999; Garant et al. 2005). Breeding density is a good predictor of habitat quality for hole-nesting birds and, although there is no specific information for the pied flycatcher (but see Alatalo et al. 1982), it is a confirmed cue for breeding habitat selection in its sibling species, the collared flycatcher (Doligez et al. 2004). Because direct assessment of the relative attractiveness of different habitat patches to new breeders is often difficult to perform in the field, we used local breeding density as an index of current patch attractiveness, assuming that pied flycatchers are attracted by conspecifics (Alatalo et al. 1982). Natal dispersal rates were used as an additional indirect measurement of patch attractiveness to new breeders, assuming a negative association between departure rates and patch attractiveness (Doligez et al. 2004; Robertson & Hutto 2006).

Data analysis

All statistical analyses were performed using R version 3.3.1 (http://www.R-project.org). To formally test for phenotype-dependent dispersal in locally born individuals, we used a Generalized Linear Model (GLM, binomial error structure and logit link function) including individuals' dispersal behaviour (return to natal patch = 0; disperse to adjacent patch = 1) as the response variable, and natal habitat (pine or oak), morphology (tarsus length), year of recruitment and their pairwise interactions as independent variables. A significant interaction between morphology and habitat of origin would indicate that dispersal is phenotype dependent, as shown by Bolnick et al. (2009); a habitat effect alone would represent asymmetric dispersal between habitats, and a morphology effect would suggest that certain phenotypes are more likely to disperse than others regardless of their origin.

In addition, we used linear models (LM) to test for differences in the phenotypic (tarsus length) trends of the four groups of dispersers in order to gain further insight into the temporal patterns of phenotype-dependent dispersal. Dispersal category (class variable; 0 = remain in natal patch, 1 = disperse to adjacent patch), year (treated as a continuous variable) and their interaction were included in the model as fixed effects. The across-years repeatability of tarsus length is high in both sexes (males: R = 0.78, females; R= 0.75; Camacho et al. 2013), so measurements were averaged when individuals were recaptured ≥2 years to minimize measurement error and to avoid pseudoreplication.

Males and females were treated separately in all analyses because (1) they differ in morphological (see above) and life-history traits (dispersal distance, arrival date and habitat selection decisions), among others (Potti & Montalvo 1991a, b; Sirkiä & Laaksonen 2009), that might ultimately affect their dispersal behaviour (Clobert et al. 2009); (2) the settlement patterns of females are determined by where they can find males that have already found a suitable cavity for nesting (Lundberg & Alatalo 1992); and (3) in contrast to males, female dispersal and habitat selection are unaffected by body size in our study system (see 'Results').

To test for overall differences between habitats in natal and breeding dispersal rates we used chi-square tests, whereas we used Wilcoxon signed-rank tests to compare annual dispersal rates and local breeding densities throughout the study period. To determine the significance of the interactions, we compared models with and without the interaction term using the function *anova* with the argument test set to 'Chisq' for the GLMs (i.e. likelihood ratio test; Dobson 2002). Diagnostic plots (e.g. distribution of residuals, influential data points) were systematically performed when fitting the models to avoid misleading conclusions based on statistical artefacts. These analyses did not show any obvious deviations from the assumptions of linear models.

RESULTS

Dispersal rates

Most pied flycatchers (73%), regardless of sex, returned to their natal habitat patch to breed, suggesting strong natal philopatry. However, individuals originating from the oak and the pine forest between 1988 and 2016 apparently exhibited different levels of natal philopatry, since the proportions of males and females leaving the pine forest to breed in the oakwood significantly exceeded that of birds moving the other way round (Fig. 1). Examination of temporal (annual) variation in dispersal rates confirmed this result (Wilcoxon signed-rank test, T = 288, P = 0.0008) and further revealed that differential dispersal was most pronounced during the 1990s due to massive emigration from the pine to the oak forest (Fig 2a).

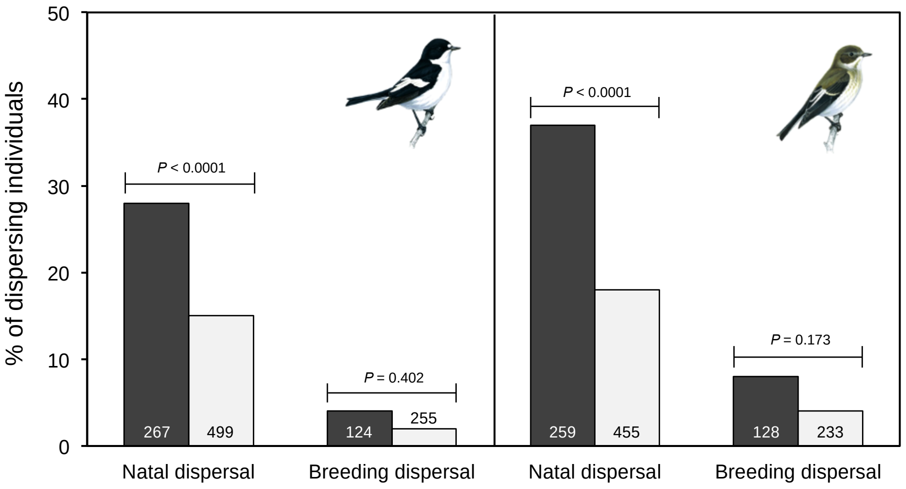

Figure 1. Natal and breeding dispersal rates of male (left) and female pied flycatchers between the coniferous (dark bars) and the deciduous forest (light bars). Figures within/beside bars are total numbers of individuals.

Once settled, most males and females (96%) remained in the same patch in the following years, indicating high breeding site fidelity. However, as for natal dispersal, the (few) adult males that changed the habitat of first reproduction tended to move from the pine to the oak forest rather than the other way round, although this difference was not significant (Fig. 1).

Mean immigration rates from areas other than our study plots were rather similar in the oak and the pine forest (52.4% ± 2.02 SE and 46.6% ± 3.63 SE, respectively), and there was also no difference between habitats in the annual immigration rates (Wilcoxon signed-rank test, $T = 258.5$, $P = 0.097$).

Temporal variation in local breeding density

Local breeding density of pied flycatchers increased in both forest patches after the addition of nest boxes, but a comparison of the annual number of pairs relative to the total surface area of each plot revealed significant differences in the dynamics of both populations (Wilcoxon signed-rank test, $T = 293$, $P = 0.011$; Fig. 2b). No cases of breeding had been recorded in the pine forest before the first pair established in 1988 and, as a result, the initial breeding density was 20-fold lower than in the oak patch. However, the number of breeding pairs in the pine forest showed a comparatively steeper increase over time, so that the annual difference in breeding density between the two habitats even changed from being negative to positive in some years of the second half of the study period (Fig. 2b).

Temporal trends in phenotype-dependent dispersal

Males' propensity to disperse between habitats depended on the interaction between body size, habitat of origin and year (LRT, $\chi^2 = 25.404$, *d.f.* = 3, $P = 0.0001$). Further analyses showed that male size changed over time, although the magnitude and direction of this change differed among the four groups of dispersers (LM, dispersal category x year: $F_{3,741}$ = 6.642, P = 0.0002). In particular, males from the pine forest showed opposite trends depending on dispersal behaviour (dispersal behaviour x year: $F_{1,349}$ = 10.198, P = 0.002; Fig 3a). Post-hoc comparisons showed that, while the body size of dispersers from the pine forest decreased over time (estimate ± SE: −0.022 ± 0.008, P = 0.009; Fig 3a), philopatric males showed a temporal increase in body size (0.016 ± 0.005, P = 0.003; Fig 3a). Males originating from the oak forest also showed slightly different phenotypic trends depending on dispersal behaviour (dispersers: 0.016 ± 0.008, P = 0.047; philopatric: 0.001 ± 0.003, P = 0.871; Fig 3b). However, unlike the case in the pine forest, the slopes did not differ significantly (dispersal behaviour x year: $F_{1,554}$ = 1.896, P = 0.169).

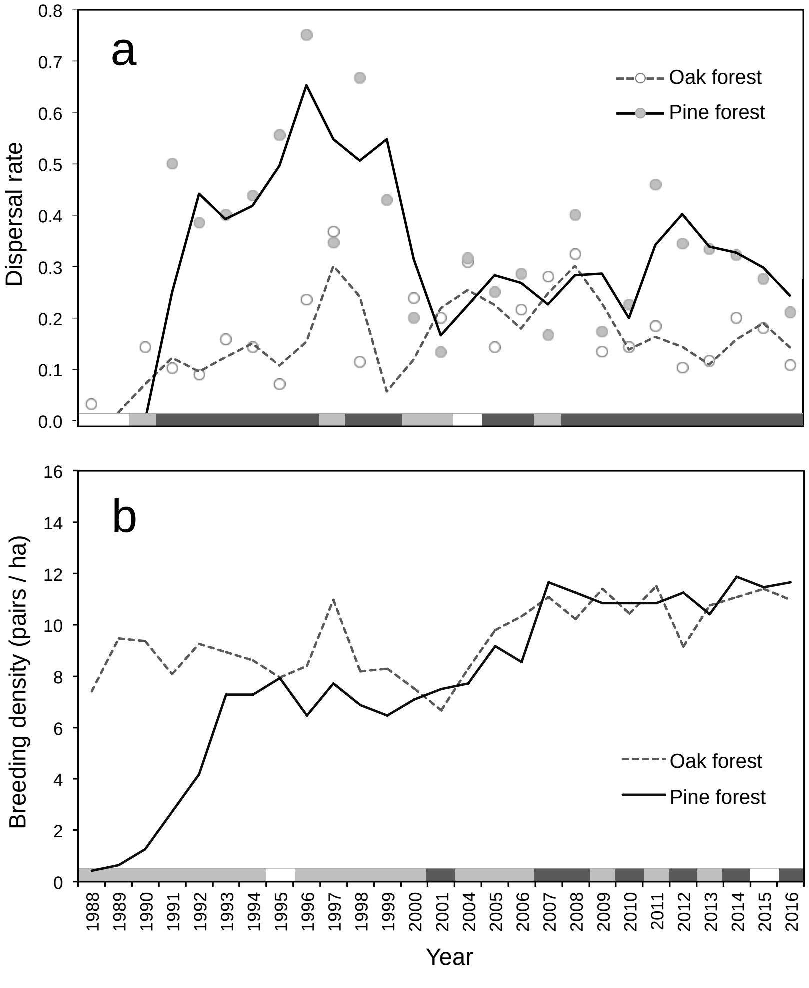

Figure 2a. Natal dispersal rates in the coniferous and the deciduous forest throughout the study period. Dots are raw data. Because of substantial between-year fluctuations, a smoothed curve fitted to a 2-year moving average was plotted to express more clearly the trends in dispersal rates. **2b.** Breeding density of pied flycatchers in the coniferous and the deciduous forest. In the upper and lower panel, blocks beside the x-axis indicate whether the difference in annual dispersal rate or breeding density between the pine and the oak forest was positive (dark grey), negative (light grey) or zero (white).

Body size had a comparatively little influence on female dispersal decisions, as indicated by the lack of statistical support for phenotype-dependent dispersal in either habitat (GLM, all interactions $P > 0.19$; Fig 3c, d). Temporal trends in mean body size of each group of dispersing females were rather similar to those of males, although differences among groups did not reach statistical significance (LM, dispersal category: $P = 0.54$; year: $P = 0.64$; dispersal category x year: $F_{3,669} = 1.804$, $P = 0.145$).

Immigrants from outside the study area that settled in the pine and the oak forest did not differ in body size in either males or females (LM, both $P > 0.30$) and, although they both showed a slight increase in tarsus length over time (males: 0.006 ± 0.003, $P = 0.014$; females: 0.005 ± 0.002, $P = 0.02$), the temporal trends did not differ significantly between habitats (year x habitat: $F_{3,710} = 3.577$, $P = 0.059$).

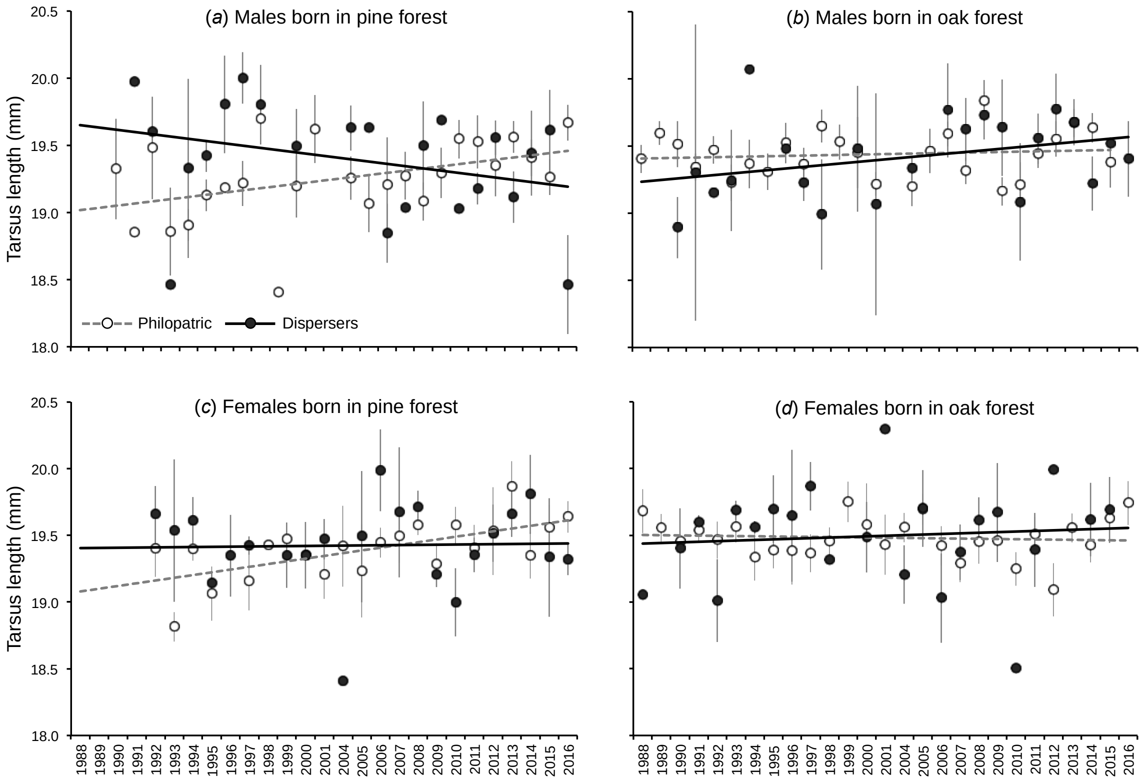

Figure 3. Temporal trends in mean (± SE) tarsus length of natal dispersers returning to the oak and the pine forest over the study period. Separate trends are shown for males and females depending on the habitat of origin (deciduous or coniferous) and the dispersal strategy (philopatric or disperser). Note that the difference between the trends of dispersers and philopatric males of the pine forest (left upper panel) remained significant after removing the year 2016.

DISCUSSION

Our results confirm earlier evidence of phenotype-dependent dispersal between the oak and the pine forest in locally born males and, to a lesser degree, females, but not in immigrants of either sex (Camacho et al. 2013). Here, we have uncovered a remarkable shift in the patterns of phenotype-dependent dispersal, as demonstrated by the reversed body size trends of dispersers and philopatric birds born in the pine forest. Body size is known to influence the departure settlement decisions of pied flycatchers moving between oak and pine habitats in northern latitudes (Lundberg et al. 1981; Alatalo et al. 1985) but, as shown here, the direction and strength of this effect depend on several interacting factors, including sex, habitat of origin and time. Complex natal dispersal patterns have been previously reported in different taxa, from insects to birds and mammals (reviewed by Clobert et al. 2009). However, the dynamic interaction between individuals' phenotype and local environmental conditions has rarely been examined empirically in wild populations (Garant et al. 2005; Tarwater & Beissinger 2012). Here, we combined individual-based information on dispersal behaviour and morphology with long-term data on population dynamics to elucidate the complex dispersal patterns of pied flycatchers and potential underlying mechanisms.

To our knowledge, only two other studies have examined annual variation in the phenotype (e.g. body size or condition) of dispersers over prolonged time frames. In a 22-year study of a green-rumped parrotlet (*Forpus passerinus*) metapopulation, Tarwater & Beissinger (2012) showed that phenotypic differences between dispersers and non-dispersers were not consistent between years due to annual variation in environmental conditions (e.g. nest-site competition and rainfall) between two adjacent patches. Likewise, Garant et al. (2005) analysed body size trends over a 36-year period in a spatially structured population of great tits that more closely resembles our study system in terms of species requirements and size-based social dominance hierarchy. They found that dispersers to low-density areas tended to be increasingly larger as a result of dominant individuals moving from crowded areas to avoid intraspecific competition (Garant et al. 2005). Similarly, in our study we observed a gradual change overt time in the body size of dispersers to both habitats, suggesting that, although still poorly understood, spatio-temporal variation in phenotype-dependent dispersal is not unique to a particular taxon or species guild.

Long-term data on population trends suggest that temporal changes in local breeding density may provide the ecological mechanism responsible for the observed segregation of phenotypes. Local population density (either in the year of birth or in the year of first breeding) has been identified as a major environmental determinant of dispersal in different bird species (Doligez et al. 2004; Fletcher 2007; Delgado et al. 2010; Scandolara et al. 2013) and it might also have influenced the departure decisions of pied flycatchers. Breeding density in the pine forest soon after our study began was still very low; therefore, the oak forest was probably regarded as a better-quality habitat, as judged by lower dispersal rates (Doligez et al. 2004). Males dispersing into the oak forest were actually larger than those that remained in the pine forest, possibly as a result of the displacement of small, subordinate males from the preferred broad-leaved patch (Lundberg et al. 1981; Camacho et al. 2013). However, it seems that the provision of nest boxes drastically improved the non-preferred habitat at the expense of a decrease in the attractiveness of the oakwood. Based on the link between breeding density and the distribution of dominant individuals, we suggest that, like other weakly territorial species, pied flycatchers might use the presence of conspecifics as a cue for habitat selection (Doligez et al. 2004; Fletcher 2007; Scandolara et al. 2013).

Nest-site competition in the pine forest could mediate the dynamic interplay between population density and phenotype-dependent dispersal. Because of the lack of natural cavities, the intensity of competition for nest sites under high-density conditions might be magnified in the pine forest compared to the oak forest (Semel & Sherman 2001) and this, in turn, might have enhanced the relevance of body size in male-male contests in the pinewood (Alatalo et al. 1985; Sirkiä & Laaksonen 2009). Experimental support for the role of nest-site competition in the spatial segregation of phenotypes has been found, for instance, in a bluebird (*Sialia mexicana*) population in which more aggressive males compete more effectively for territories with a high density of nest boxes and, as a consequence, aggressive and non-aggressive males are sorted into distinct breeding habitats (Duckworth 2006). Alternatively, large and small males could be locally adapted to different habitats and settle accordingly to maximize fitness (i.e. matching habitat choice; Edelaar et al. 2008). However, no empirical evidence supporting optimal habitat choice in relation to body size has been found in this population (Camacho et al. 2015). Nest-site competition is therefore likely to be the primary explanation for the observed increase in the body size of males settling in the pine forest.

No clear evidence of phenotype-dependent dispersal was found in females, in agreement with a previous experimental study demonstrating random female settlement (Dale & Slagsvold 1990). Females do not compete for nest sites, but choose a male or the resources defended by the male. Hence, their departure and settlement decisions are unlikely to be influenced by their competitive ability, but should be determined primarily by where they can find males that have already acquired a suitable nest site (Dale & Slagsvold 1990; Lundberg & Alatalo 1992).

Although female dispersal is mostly random with respect to tarsus length, thus causing a re-assortment of phenotypes each generation with respect to this heritable trait (Alatalo & Lundberg 1986, Potti & Merino 1994; see Chapter V), size-dependent dispersal of males alone might have contributed to shape the observed phenotypic trajectories of birds breeding in the oak and the pine forest. Examination of temporal trends in the size of dispersers revealed a close parallelism between dispersers from the oak to the pine forest and the entire male population in the recipient habitat, suggesting that the shift in phenotype-dependent dispersal may have attenuated the degree of phenotypic differentiation between populations (Garant et al. 2005; Bolnick et al. 2009). Males dispersing from the pine to the oak forest nevertheless showed a gradual decline in body size that deviates from the lack of trend recorded in the entire male population in this habitat (Camacho et al 2013). Local maladaptation of immigrants (Postma & van Noordwijk 2005; Nosil et al. 2005) and selection against immigrants (Hendry 2004) may be invoked as the most likely explanations for this discrepancy, since the reproductive output of immigrants into the oak forest tends to be inferior to that of the other groups (see Chapter 5).

In conclusion, our results suggest that the patterns of phenotype-dependent dispersal in our population have changed over time primarily as a result of the steep population growth after the rapid colonization of the newly created, coniferous habitat. In our study system, the departure and settlement decisions of male pied flycatchers reflect a dynamic balance between dispersal motivation, determined by relative breeding density in the source and destination patches, and their ability to acquire a nest site in densely populated areas, determined by body size. On a more general level, our results indicate that size-dependent dispersal by relatively few non-philopatric males is strong enough to promote population differentiation at short spatio-temporal scales. However, the diversifying effects of size-dependent dispersal alone might not be sufficient to maintain phenotypic variation within populations over long time periods.

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CHAPTER III - Testing the matching habitat choice hypothesis in nature: phenotype-environment correlation and fitness in a songbird population

ABSTRACT The matching habitat choice hypothesis holds that individuals with different phenotypes actively select the habitats to which they are best adapted, thus maximizing fitness. Despite the potential implications of matching habitat choice for many ecological and evolutionary processes, very few studies have tested its predictions. Here, we use a 26-year data set on a spatially structured population of pied flycatchers (Ficedula hypoleuca) to test whether phenotype-dependent dispersal and settlement translate into increased fitness, measured by recruitment success in the first breeding attempt. Males at the upper and lower extremes of the body size distribution tend to disperse into deciduous and coniferous habitats, respectively. If matching habitat choice has evolved in this population, the fitness of large males is expected to be higher in the deciduous habitat, where males are on average larger in size than in the coniferous habitat. However, the reverse would be true for small-sized males, which are more frequent in the coniferous forest. We found that the recruitment success of first-time breeders in the coniferous forest increased non-linearly with body size, with males at the middle of the size distribution having higher recruitment success than both large and small individuals. However, contrary to expectations, no clear trend was observed in the deciduous forest, where males of either size had similar recruitment success. Based on these results, and after discarding other important processes potentially confounding matching habitat choice, as genotype- and body condition-dependent dispersal, competitive exclusion remains the most likely force shaping the non-random distribution of male pied flycatchers with respect to body size. Hence, a conclusive demonstration of the operation and occurrence of matching habitat choice in nature remains to be done.

Keywords: body size, Ficedula hypoleuca, local adaptation, non-random dispersal, pied flycatcher, population divergence.

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INTRODUCTION

Habitat selection and preceding dispersal decisions can strongly influence individuals' survival and reproductive success, and therefore have important consequences for many ecological and evolutionary processes (Fretwell & Lucas 1970; Cody 1984; Morris 2003). Evidence is accumulating that individuals disperse non-randomly with respect to genotype and morphological, physiological or behavioural traits (Edelaar & Bolnick 2012). Specifically, dispersal can be affected by attributes such as age, sex, dominance rank, body size and condition, physiological/biomechanical dispersal capacities, or personality, thereby leading to phenotype-dependent dispersal (reviewed in Clobert et al. 2009).

Many empirical studies have revealed an adaptive relationship between the phenotype of individuals and the location of settlement, and a few of them have also shown that organisms may move non-randomly across different environments to settle in the habitats that may enhance individual performance (e.g. Blondel et al. 1999; Garant et al. 2005; Dreiss et al. 2012). Along this line, the matching habitat choice hypothesis, unlike other forms of phenotype-dependent dispersal (e.g. size-dependent dispersal driven by competitive exclusion of less competitive phenotypes towards less appreciated habitats, or increased dispersal propensity or distance in bold, explorative animals), states that individuals −assumed to be able to assess fitness prospects in each habitat prior to settlement− modify habitat selection according to their phenotype, such that they settle in the habitats with the highest fitness prospects (Holt & Barfield 2008; Edelaar et al. 2008). Under the hypothesis' predictions, similar phenotypes are expected to have similar habitat preferences and concentrate in the environments they are best suited to (Edelaar et al. 2008). Thus, matching habitat choice may contribute to the spatial sorting of phenotypes through phenotype-dependent dispersal, thereby leading to population differentiation in functional traits and even potentially driving sympatric speciation (Edelaar et al. 2008; Holt & Barfield 2008).

Despite its likely ecological and evolutionary implications (see Edelaar et al. 2008), to the best of our knowledge only three studies have examined the matching habitat choice hypothesis or evaluated its evolutionary consequences (but see Bolnick & Otto 2013 for a theoretical outline). The evidence from two experimental studies has so far provided positive support for the hypothesis' predictions by showing that manipulation of colour phenotype in the pygmy grasshopper (*Tetrix subulata*) influences microhabitat choice and affects subsequent fitness (Wennersten et al. 2012; Karpestam et al. 2012). In addition, a field study has shown that female barn owls (*Tyto alba*) showing reddish and whitish phenotypes settle in alternative habitats, and that such colour-dependent habitat choice apparently increases fitness and reinforces local adaptation (Dreiss et al. 2012). However, to our knowledge, the hypothesis' predictions remain to be comprehensively tested under field conditions.

A possible explanation for the scarcity of studies is that testing for matching habitat choice may be challenging, particularly in the wild, because: (1) an extensive effort is required, as individuals' traits need to be measured and then linked to departure and settlement decisions; (2) its effects are typically subtle and may not be readily detectable in natural environments; (3) convincing tests to demonstrate that it actually occurs, such as phenotype or environment manipulation or habitat translocations, are not easy to perform due to logistic constraints; and (4) it may be hard to distinguish from several additional processes that also may result in the assortment of phenotypes to different habitats (e.g. selective mortality and phenotypic plasticity; Garant et al. 2005; Edelaar et al. 2008). In addition, providing evidence that differential habitat choice translates into enhanced fitness would be required. Consequently, matching habitat choice remains to be unequivocally demonstrated in the field, and accumulating evidence from empirical studies is needed to determine its relative occurrence in nature.

The pied flycatcher (*Fidedula hypoleuca*) is a long-distance migrant passerine using cavities in temperate forests as breeding sites. Although the species is able to reproduce in a wide array of forest habitats, the first males arriving from wintering quarters settle preferentially in deciduous forests (Alatalo et al. 1985; Lundberg & Alatalo 1992) possibly because they may find it easier to find suitable nesting sites or food. In Central Spain, however, most pied flycatchers return to their birthplace and the ones that move in a different habitat do it according to their body size, although this pattern is more pronounced in males than in females (Camacho et al. 2013, 2016). By following individuals stemming from cross-fostered nests between an oak and a pine forest until their first breeding attempt, we have shown that the habitat preferences of pied flycatchers are not genetically determined, but influenced by early experience in the natal patch (Camacho et al. 2016b). In addition, we have found that natal habitat preference may act concurrently with phenotype-dependent dispersal, since large individuals changing habitats during natal dispersal tend to settle in the deciduous forest, the reverse being true for the smaller males, more often found in the coniferous site (Camacho et al. 2013).

Several studies, from the community to the individual level, have also reported that large and small birds are generally found in deciduous and coniferous forests, respectively (Gaston 1974; Price 1991; Forstmeier et al. 2001; Korner-Nievergelt & Leisler 2004). In an attempt to link individual morphology to function and, ultimately, performance, some authors have suggested that larger individuals may prefer larger prey items and, therefore, use the habitats holding a higher proportion of large arthropods (i.e. deciduous forests; Price 1991; Forstmeier et al. 2001). In contrast, small individuals may preferentially use coniferous habitats because their small body size could enable them to forage most efficiently in the outermost twigs of coniferous trees (Gaston 1974; Korner-Nievergelt & Leisler 2004). In the pied flycatcher, the average prey size is smaller in coniferous than in deciduous forests (Lundberg et al. 1981) and, although adaptive size-related dietary or microhabitat shifts have not been reported, individuals of different size might specialize on different habitats or prey species and perform differently in coniferous and deciduous forests, as occurs in other songbirds (Forstmeier et al. 2001).

Here we examine the fitness consequences of breeding habitat choice of male pied flycatchers to test whether small and large-sized individuals are suited to different habitats and, therefore, as predicted by the matching habitat choice hypothesis, settle accordingly to maximize fitness. The coniferous/deciduous study plots occupied by pied flycatchers for more nearly three decades provides us with a good opportunity to address matching habitat choice, as (1) it represents a pseudoexperimental setting in which, since artificial nest boxes for pied flycatchers were provided in the coniferous forest, all individuals are assumed to be able to assess the same set of environments (Camacho et al. 2016b); (2) a (dynamic) association between male size, a highly heritable trait (Lundberg & Alatalo 1986; Potti & Merino 1994), and breeding habitat exists, (3) it results from size-dependent dispersal and settlement (Camacho et al. 2013), and (4) annual data on individual reproductive success are available.

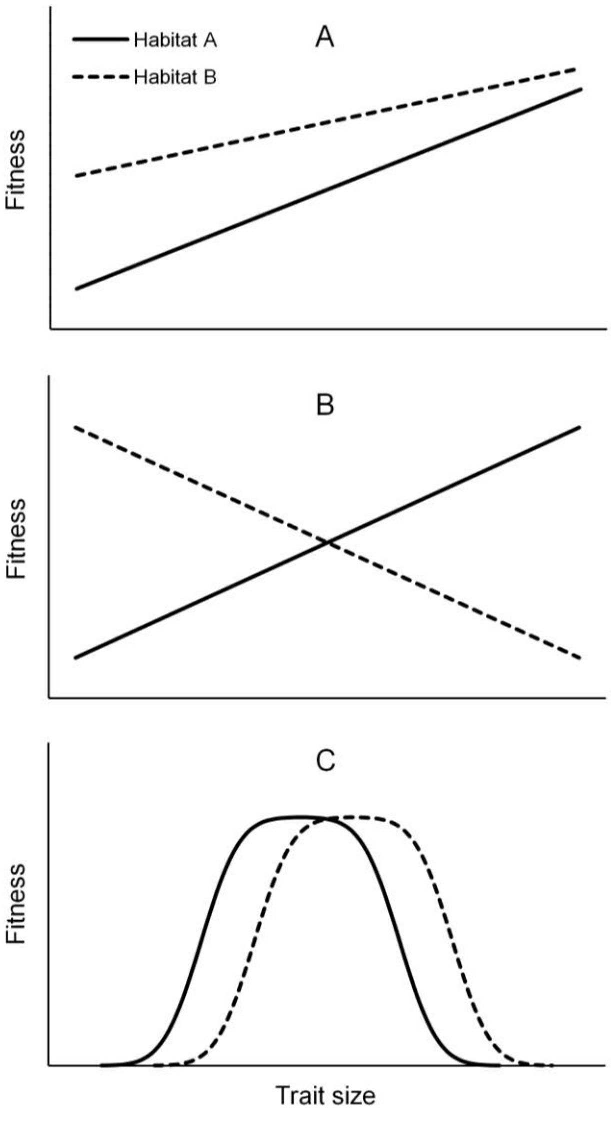

Figure 1. Example of three possible scenarios of phenotype-environment interaction in fitness, illustrating how individuals' decisions on where to settle may be influenced by the habitat features that best match their particular phenotype to maximize fitness. (**a**) Fitness increases linearly with trait size in habitats A and B. However, individuals at the lower extreme of the trait distribution will only maximize fitness by settling in habitat A, while fitness of individuals at the upper extreme of the distribution will be equally high in either habitat. (**b**) Fitness increases linearly with trait size in habitat A, but so decreases in habitat B. As a result, large phenotypes will maximize fitness in habitat A, while the opposite is true in habitat B. (**c**) Fitness increases non-linearly with trait size in habitat A and B, which are both suboptimal for the phenotypes at the upper and lower extremes of the trait distribution. However, the optimal phenotype varies depending on the habitat type, as medium-small and medium-large phenotypes best match habitats A and B, respectively.

If pied flycatchers exert matching habitat choice, we would expect that the fitness of large males would be higher in the deciduous than in the coniferous forest, whereas the opposite would be true for smaller individuals. Despite the distribution of phenotypes between the two habitats changed over time, we expect to find distinct relationships between body size and fitness even when data of all years are combined, as the average size of males settling in the oak forest was larger than that of males settling in the pine forest. To evaluate the above mentioned expectations, we followed locally born individuals until recruitment to investigate whether body size-dependent habitat choice of males had an effect on the recruitment success of their offspring. In addition, we assessed the influence of alternative processes potentially confounding matching habitat choice, such as genotype- and body condition-dependent dispersal (Edelaar & Bolnick 2012).

METHODS

Study system and data collection

Breeding success and morphometric data were obtained during a long-term study (1988–2013) of a pied flycatcher population in central Spain. Here, pied flycatchers breed in nest boxes located in a deciduous and a coniferous forest plot separated by 1 km (see Camacho et al. 2013). The deciduous forest (DF) is a highly heterogeneous and structurally complex habitat of 9.3 ha dominated by old oaks (*Quercus pyrenaica*) at a mean density of 460 trees ha^{-1}. Resource exploitation ceased over 50 years ago, and a dense ground cover and understory re-evolved, mostly oak saplings, *Erica arborea*, *Cistus laurifolius* and *Crataegus monogyna* (0.5–3 m high; mean cover 80%). The coniferous forest (CF) represents a more homogeneous habitat of 4.8 ha characterized by a monoculture of Scots pine (*Pinus sylvestris*) at a mean density of 200 trees ha^{-1} and scattered *Cupressus arizonica*, *Pinus pinaster* trees. Sparse shrub cover of *Cistus laurifolius* and *Crataegus monogyna* is restricted to open areas (0.5–2 m high; mean cover 5%).

Before the onset of the study, pied flycatchers were confined to natural tree holes in DF (Potti & Montalvo 1990) and no cases of breeding had been observed in CF due to the absence of natural cavities. In 1984 (DF) and 1988 (CF), wooden nest boxes (156 and 81, respectively) at a mean distance of 20 m (SD 9.2) were provided and pied flycatcher densities gradually increased in both habitats (Camacho et al. 2013).

Nest boxes were regularly checked from when the first males arrived from spring migration to determine exact laying dates, clutch sizes, hatching dates and numbers of fledged young. Nestlings surviving from hatching to day 13 were then marked with numbered metal ring, and their ectoparasite loads were recorded (Merino & Potti 1995). Parents were captured while incubating (females) or feeding nestlings (both sexes) using a nest box trap installed inside the nest. All adults were marked with a unique combination of metal and colour-coded rings and measured for body mass (to the nearest 0.1 g) and tarsus length (to the nearest 0.05 mm) as a proxy for body size (Senar & Pascual 1997). Each male was ascribed to the habitat in which it settled to breed, regardless of his origin. Breeding densities were determined by quantifying nest box occupancies by pied flycatchers (Blondel et al. 1999; Garant et al. 2005).

Fitness measurement

To characterize fitness, measured as the annual number of recruits relative to that of non-recruiting fledglings (i.e. recruitment success), we used a longitudinal data set containing all the information on each individual (annual data on reproductive success are shown in Table SM1). In contrast to males, the settlement patterns of female pied flycatchers are determined by where they can find males that have already found a suitable cavity for nesting (Lundberg & Alatalo 1992). Hence, female assortment across habitats would be more a reflection of male distribution than of female size-based settlement decisions. Indeed, as expected, female dispersal and habitat selection is not significantly affected by body size in our study system (Camacho et al. 2013). Given that the occurrence of phenotype-dependent dispersal is the main prerequisite of the matching habitat choice hypothesis, females were not further considered.

Habitat familiarity, previous breeding experience and age-related improvements in individual performance may influence reproductive output and subsequent fitness (e.g. Doligez et al. 1999; Brown et al. 2008; Limmer & Becker 2010) and thus potentially confound matching habitat choice (e.g. potentially masking a fitness mismatch between phenotype and environment). Therefore, only first-time breeders of known origin were considered, whether they move from their natal habitat to a new one (dispersers) or remain in their natal habitat to breed (philopatric). All nests that were known replacement clutches (i.e. laid after failure (1.7%) or predation (10.1%) of the first one) were omitted from analyses. Breeding date is likely the most important determinant of breeding success and recruitment in *Ficedula* flycatchers (Lundberg & Alatalo 1992; Potti et al. 2002) and thus variation in success of replacement clutches may likely reflect the effects of extremely delayed timing, rather than a possible mismatch between phenotype and habitat type. All nests subjected to experimental manipulations (e.g. cross-fostering) were also omitted.

Because of discontinuities in the intensity of fieldwork, the years 2002 and 2003 were removed from the data set. As recruits may postpone their first reproduction until the second or, more rarely, third year of life (Potti & Montalvo 1991a), all nests of males breeding between 1999 and 2003 were excluded from analyses to avoid underestimating their true recruitment success (Potti et al. 2013). For the same reason, all nests of males breeding beyond 2010 were also excluded. Dispersal outside the study plots is an extremely rare event (pers. obs. from non-systematic explorations of surrounding areas); therefore, we are confident that the spatial scale of the study area is not a source of bias in recruitment rates. Overall, for fitness analyses we used data from the first reproductive attempts by 304 individuals (202 and 102 males in DF and CF, including 27 and 29 dispersers, and 175 and 73 philopatric birds) belonging to 23 cohorts (see also Table 1).

Familial resemblance in dispersal patterns and effect of body condition

Genetic-based variation in dispersal or the body condition of the first-time breeders could lead to individual variation in the propensity to exchange habitats, thus potentially confounding matching habitat choice (Edelaar & Bolnick 2012). Therefore, we examined if there is detectable familial resemblance and influence of body condition in the propensity to 'stay' (i.e. returning to the natal forest at the first breeding attempt) or exchange habitats (oak *vs.* pine or *vice versa*), and also examined the similarity in dispersal movements of full sibs. We are aware that these tests may be considered as crude approximations to familial resemblance in dispersal propensity due to the local scale of our sampling. To assess differences in body condition between dispersers and philopatric individuals, we also used data from first-time breeders, as pied flycatchers rarely exchange habitats after natal dispersal (Camacho et al. 2013).

Data analyses

All statistical analyses were performed in R 2.14.0 (http://www.R-project.org). To investigate matching habitat choice, we fitted a Generalized Linear Mixed Model (GLMM; binomial errors, logit link function) jointly including the number of recruits and the number of non-recruiting fledglings as the response variable. Tarsus length and its quadratic term (to test for linear and non-linear relationships respectively; Fig. 1), breeding habitat and their interactions were included as predictor variables. The interaction between phenotype and habitat is considered to be an important test of the hypothesis (Karpestam et al. 2012; Fig. 1), as it would indicate whether males of a particular size perform differently in a particular habitat than do others. Furthermore, its shape will reveal whether males preferentially settle in the habitat in which their particular phenotype performs best, thereby supporting matching habitat choice. Breeding date was included as a covariate in the model, as it is one of the most important determinants of breeding success and recruitment in *Ficedula* flycatchers (Potti & Montalvo 1991a; Lundberg & Alatalo 1992; Potti et al. 2002). Nest box and female identity were included in the model to account for territory quality and mate quality variation, respectively, and we also included year as a random effect to account for annual heterogeneity in breeding performance. The GLMM was fitted using the function *lmer* of the R package 'lme4' (Bates et al. 2011). Model selection was carried out by sequentially dropping non-significant terms from fully saturated models (containing all main effects and interactions), in a hierarchical way, starting with the least significant order terms.

Exploratory analyses showed no significant influence of factors potentially affecting fitness, as mating status (i.e. primary or secondary broods of bigamous males; Lundberg & Alatalo 1992), ectoparasite loads in nests (i.e. numbers of blowfly larvae and prevalence of blood-sucking mites; Merino & Potti 1995), breeding density, or male age at first breeding (results from the GLMMs are shown in Table SM2); these terms were hence not further considered in the models. Other potentially important factors, such as natal habitat, dispersal behaviour (i.e. disperser *vs.* philopatric) and their interaction also dropped from exploratory models. On the other hand, dispersers are often more likely to produce dispersing young than philopatric individuals (Bélichon et al. 1996), which may lead to underestimation of dispersers' fitness (Doligez & Pärt 2008). However, exploratory analyses on recruitment success showed no differences in local recruitment between dispersers and philopatric males breeding in each study plot (authors' unpubl. data).

To test whether body condition affects the propensity of males to exchange habitats irrespective of their origin we fitted a linear model (normal distribution, identity link function) including the body mass of first-time breeders as the response variable. Tarsus length was included as a covariate, and dispersal behaviour (disperser *vs.* philopatric) as a fixed effect. To test for familial resemblance in the propensity to stay or exchange habitats we fitted a GLMM (binomial errors, logit link function) as explained above with son dispersal behaviour coded as a binary response variable (0 = stay in natal habitat, 1 = disperse from natal habitat) and father dispersal behaviour (0, 1) as the explanatory variable. As male movement between forests is non-random with respect to body size and directionality (Camacho et al. 2013), we included in the model son tarsus length and natal habitat as a covariate and a fixed effect, respectively. Family identity and son cohort were also included as random effects. Similarity in dispersal movements of full sibs was examined by means of a chi-square test with Yates continuity correction. Sample sizes differ among analyses because not all data were known for all individuals.

RESULTS

Male size, habitat choice and fitness

Local males breeding in CF from the beginning of the study were, on average, smaller than those in DF (mean ± SE: 19.24 ± 0.04 *vs.* 19.37 ± 0.03; GLM: $t_{1,303} = 2.09$, $P = 0.038$). Linear and non-linear interactions between male size and forest type (Fig. 2) had a significant influence on recruitment success, after controlling for the significant effect of breeding date (Table 1).

Table 1. Effect of tarsus length, breeding habitat type and their interaction on the recruitment success of male pied flycatchers. Number of males = 304; number of nest boxes = 220; number of mates = 273; number of years = 18.

	Estimate	SE	*Z*	*P*
Intercept	−784.37	290.52	−2.70	0.0069
Tarsus length	83.98	30.33	2.70	0.0069
Breeding habitat	807.89	305.41	2.64	0.0082
Breeding date	−0.05	0.01	−3.76	0.0002
Tarsus length x Breeding habitat	−84.49	31.86	−2.65	0.0080
Tarsus length^2	−2.14	0.79	−2.71	0.0068
Tarsus length^2 x Breeding habitat	2.21	0.83	2.66	0.0079

Even though the interaction terms indicate that male fitness varied spatially according to body size, fitness variation was not in line with matching habitat choice' predictions. Recruitment success in CF increased non-linearly with body size, with males at the very middle of the size range having higher fitness than both large and small-sized males. However, no clear trend was observed in DF, where males of either size had similar fitness (Fig. 2). A test of the relationship between recruitment success and body size including only the recruits remaining in the natal plot showed similar results to those obtained by including also the dispersers from the nearby plot (details not shown). Fitness effects of habitat matching could have been mediated via laying date (e.g. advantage of larger males in DF if they would breed earlier therein than in CF, and *vice versa* for smaller males). However, results remained unaltered after excluding the laying date from the GLMM.

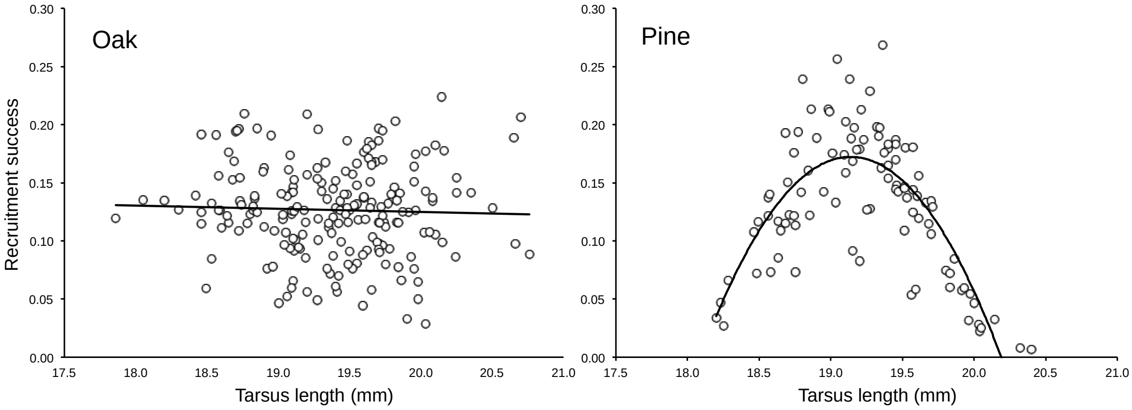

Figure 2. Relationship between body size and male fitness in the deciduous and the coniferous forest. Shown are the values predicted by the model.

Familial resemblance in dispersal patterns and effect of body condition

Results of the GLMM (number of males: 238; number of families: 164; number of cohorts: 21; see Table 2) showed no influence of father dispersal behaviour on the dispersal propensities of their sons (estimate ± SE: 0.12 ± 0.38, $P = 0.75$), after controlling for the significant effect of son origin (0.88 ± 0.34, $P = 0.009$) and removing the non-significant effect of son tarsus length (0.48 ± 0.31, $P = 0.12$) from the final model. There was also no association between full sibs in their dispersal behaviour (29 sibling pairs stayed in their natal forest *vs.* 15 dyads differig in their direction of movement: $\chi^2 = 1.83$, *d.f.* = 1, $P = 0.18$). The propensity of males to exchange habitats was not dependent on body condition, as dispersers ($n = 92$) and philopatric birds ($n = 342$) had similar body masses ($t_{1,463} = -0.16$, $P = 0.88$) after controlling for the significant effect of tarsus length ($t_{1,463} = 6.66$, $P < 0.0001$).

Table 2. Father-son comparison of the propensity to change habitats as part of natal dispersal from the hatching habitat to that of first breeding. Note that if fathers disperse from A to B, their prospective dispersing sons 'only may' move from B to A. Therefore, for more meaningful father-son comparisons, we grouped together 'resident' *vs.* 'dispersing' individuals in both the parents and sons classes in a 2-way table. Figures are numbers of families.

Father dispersal	Son dispersal			
	Stay in natal plot	Oak to pine	Pine to oak	Total
Stay in natal plot	104	12	12	128
Oak to pine	13	-	4	17
Pine to oak	16	3	-	19
Total	133	15	16	164

DISCUSSION

Male pied flycatchers move between coniferous and deciduous forests according to body size (Camacho et al. 2013) and, as shown here, their size influenced recruitment success differently in each forest type. Males are therefore assumed to have the opportunity to improve their fitness by moving between habitats. Nevertheless, recruitment success following habitat choice was not in line with the observed movement propensities of males in relation to size, suggesting that habitat matching is little or not influential for the spatial sorting of pied flycatcher phenotypes. We have also shown that the dispersal propensity of males do not seem to be affected by individual quality (i.e. body condition) or influenced by their genetic background. Therefore, potentially confounding effects of matching habitat choice in relation to individual variation in dispersal propensities have been discarded.

Male pied flycatchers of different size tend to occupy different habitats (Camacho et al. 2013) so that the central prerequisite of the matching habitat choice hypothesis is fulfilled (Edelaar et al. 2008). Nonetheless, it should be noted that associations between phenotype and environment are not unique to matching habitat choice, but may also result from some alternative mechanisms (e.g. selective mortality, phenotypic plasticity; Edelaar et al. 2008). However, those processes do not appear to be responsible for the non-random distribution of pied flycatchers since, as we have shown previously, the habitat segregation of size classes actually results from differential dispersal and habitat selection (Camacho et al. 2013).

Under matching habitat choice, similar-sized individuals should be more likely to disperse into the forest patch affording them the highest fitness given their body size (Edelaar et al. 2008). Nevertheless, as shown here, habitat preferences and subsequent fitness in the pied flycatcher are apparently decoupled. Large males are more often found in the deciduous forest, but their fitness is not greater than that of smaller males breeding there. Neither is the fitness of small-sized males breeding in the coniferous forest greater than that of small-sized males breeding in the deciduous site.

Several hypotheses can be proposed to explain the observed mismatch between habitat preferences and fitness. For example, compatibility of mates in terms of size has been implied in increased reproductive success in other species (Selander 1966; Gosler 1987; but see Wiggins & Pärt 1995) and could also influence pied flycatcher fitness, because pairs in the pine forest tend to be more dimorphic than those in the oak forest. However, the degree of intra-pair similarity did not influence recruitment success in either habitat (Camacho 2011). Another possibility is that underestimation of dispersers' recruitment relative to that of philopatric individuals might have masked or attenuated differences in recruitment success (Doligez & Pärt 2008). However, we found no effect of natal habitat or dispersal behaviour on recruitment success, suggesting that our estimates of recruitment success were not confounded by male origin or movement. In addition, the fitness of pied flycatchers could be influenced by other ecologically important traits not considered here (e.g. temperament; Duckworth 2006).

Regarding the particular fitness trends we observed, one could easily notice that matching habitat choice fails to explain, for example, why the recruitment success of large, and presumably higher quality, males in the forest type they preferentially settle (i.e. oak forest) is comparable to that of smaller males. On one hand, habitat preferences of high quality phenotypes may not match the actual quality of selected sites for several reasons (Hollander et al. 2011). For instance, the observed increase over time in the intensity of interspecific competition in the oakwood may have affected habitat quality for pied flycatchers (Camacho et al. 2013) and thus obscured any form of habitat matching in terms of fitness. On the other hand, in contrast to the pine monoculture, the highly heterogeneous and structurally complex oak forest could provide individuals with no previous breeding experience with multiple microhabitat and foraging opportunities (Lundberg et al. 1981), so that the breeding success of all pied flycatchers may be similar regardless of their morphology.

Matching habitat choice also fails to explain the general trend for small males to reproduce in the coniferous habitat. Empirical evidence from other songbirds indicate that small body size allows more efficient use of foraging techniques in coniferous forests (Gaston 1974; Forstmeier et al. 2001), where the average size of pied flycatcher prey is smaller than in broadleaved forests (Lundberg et al. 1981). Not surprisingly, the fitness of males at the upper extreme of the body size distribution was reduced in the oak forest, but unexpectedly, the fitness of small males was also very low despite reproducing in an apparently promising habitat. A plausible explanation is that, due to habitat simplicity, the coniferous forest might be most suitable for a particular phenotype (e.g. males in the middle of the size distribution). In addition, food resources may be often limited in the scarcely productive pine monoculture, so that the smallest males might be unable to compete for nest holes (i.e. territories) and/or rich food patches with medium-sized, potentially dominant individuals (Lundberg et al. 1981).

A review of the scant literature attempting to test the matching habitat choice hypothesis reveals that we are still far from a conclusive demonstration of its operation in nature. In the experiments with grasshoppers, phenotype manipulations did not completely recapitulate the natural tendencies of non-manipulated individuals, possibly because a mismatch between the complex natural phenotype of individuals and their novel appearance hindered grasshoppers from making colour-matched habitat choices. (Karpestam et al. 2012; Wennersten et al. 2012). It is also possible that grasshoppers' behaviour reflects additional influential processes (e.g. competitive exclusion; Edelaar et al. 2008) that are seldom reproduced, or hard to control for, in experimental settings. Another potential source of bias is that grasshoppers involved in the experiments aiming to provide evidence for the fitness benefits of matching habitat choice were actually unable to choose, but they were instead subjected to particular environments with no appropriate habitat to choose. Finally, although findings from free-ranging barn owls (Dreiss et al. 2012) are consistent with matching habitat choice, the authors recognized that other concurrent ecological processes could be operating (see also Bolnick et al. 2009). In this case, it was proposed that individual movements to new breeding sites might not result from active habitat choice, but instead reflect differences in competitiveness, for example, if some colour morphs are more aggressive and able to settle in the preferred habitats.

Competitive exclusion during settlement in the deciduous forest could have also influenced the non-random dispersal and subsequent distribution of pied flycatchers, although underlying –but hard to detect– matching habitat choice cannot be ruled out. Pied flycatchers first explore the preferred deciduous habitats before searching for alternative breeding sites (Alatalo et al. 1985; Lundberg & Alatalo 1992). Nonetheless, their ability to succeed in settling in the preferred territories is generally determined by the size-based competitive ability of individuals (Alatalo et al. 1985; Sirkïa & Laaksonen 2009). It may be due to this reason that most of the smallest, subordinate males are likely 'relegated' to the coniferous site by large, dominant males (Camacho et al. 2013). Note that a small fraction of small males also occurs in the deciduous forest, possibly as a result of natal habitat preferences (Camacho et al. 2016b), or perhaps because they benefited from an early arrival from spring migration or an extremely aggressive behaviour that compensated for their presumably reduced competitive abilities.

In summary, matching habitat choice alone fails to explain the fitness accrued by pied flycatchers exerting differential dispersal and habitat selection decisions according to body size. Several potentially confounding processes commonly present in nature (e.g. natal habitat preference and male-male competition) might act additively to matching habitat choice, swamp its presumably subtle effects, and thus make it difficult to detect and demonstrate.

How to test for matching habitat choice in the wild?

We will conclude by suggesting some ways in which the matching habitat choice hypothesis can be tested in natural settings. Comparisons of the settlement patterns and fitness of naturally different phenotypes across spatially heterogeneous environments may be a valuable means to determine the actual occurrence of matching habitat choice in nature. For example, as a general rule, small individuals cool faster than large ones (Schmidt-Nielsen 1984), and thus individuals of different size are expected to use thermal environments differentially. Dispersal responses of individuals to thermal gradients in some taxa (e.g. grasshoppers, Ahnesjö & Forsman 2006; nightjars, Camacho 2013) provide ample opportunities for testing whether naturally distinct phenotypes move between 'thermal patches' differently and whether those movements translate into direct fitness benefits.

Experimental alteration of the phenotype by altering, for instance, food supply to young individuals through brood manipulations (Gustafsson & Sutherland 1988) or by hormonal ‘phenotypic engineering’ (Ketterson et al. 1996) may help researchers to determine whether manipulated or naturally different phenotypes 'match' the habitats where they would predictably disperse and whether those dispersal decisions influence their fitness prospects. Likewise, translocation experiments between contrasting environments (see Burger & Both 2011) or modification of the future fitness expectations of individuals by modifying, for example, parental brood size or competitive regimes via manipulations of fledgling sex ratio (Nicolaus et al. 2012) would also be also helpful.

Because additional processes leading to phenotype-dependent dispersal may operate concurrently with matching habitat choice, disentangling its effects from those of other processes is a major challenge for testing the hypothesis under field conditions. For example, as shown here and in Garant et al.’s (2005) work, dominance rank appears to be a major determinant of the direction of dispersal in spatially heterogeneous environments. In such cases, perhaps researchers should rather focus on functionally relevant phenotypic traits that are not involved in competitive interactions but, at the same time, likely related to their dispersal decisions. Potential candidates are, for example, background-matching colour pattern (Merilaita & Lind 2005) or anatomical adaptations to resource exploitation *vs.* predator avoidance (Moore et al. 2015) that might determine the dispersal decisions of phenotypically different individuals experiencing spatial variability in resource availability or predation pressure.

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SUPPLEMENTARY MATERIAL 1: Frequency distributions of tarsus lengths for male and female pied flycatchers breeding in the oak and the pine forest between 1988 and 2013.

To test for differences in the body size (tarsus length) distribution of pied flycatchers breeding in the oakwood and the pinewood over the entire study period, we performed separate Kolmogorov-Smirnov (KS) two-sample tests for males and females, as implemented using function *ks.boot* (10,000 simulations) in the R-package 'Matching' (Sekhon 2011).

Results of the KS-test showed that body size distributions differed significantly between both forest types in males (*D* = 0.11, *P* = 0.02), but not in females (*D* = 0.07, *P* = 0.2), as illustrated by the right-skewed distribution of male sizes in the coniferous forest in relation to that in the deciduous forest (Figure SM1).

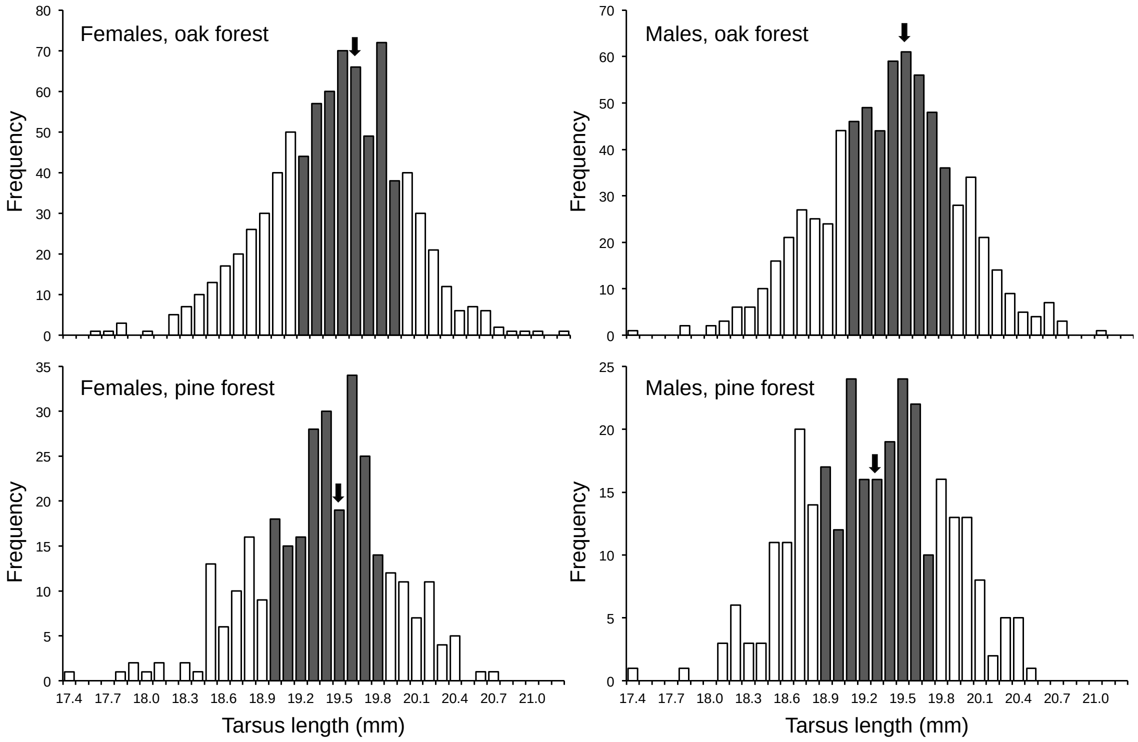

Figure SM1. Body size (tarsus length) distributions of pied flycatchers breeding in the coniferous and the deciduous forest. Black arrows denote median values and shaded bars represent the Q1-Q3 range.

Reference: Sekhon, J.S. 2011. Multivariate and propensity score matching software with automated balance optimization: the matching package for R. J Stat Softw 42:1–52.

SUPPLEMENTARY MATERIAL 2

Table SM1. Number of pied flycatcher nests producing offspring and mean number of fledglings produced per nest (mean ± SD) in the deciduous and the coniferous forest between 1988 and 2013. A rough index of "year quality" is also shown.

	Deciduous forest			**Coniferous forest**		
Year	No. Nests	Brood size	Quality index[1]	No. Nests	Brood size	Quality
1988	68	3.6 ± 1.7	3.8	2	2 ± 2.8	4.0
1989	88	3.9 ± 2	4.5	3	5 ± 0	5.0
1990	88	1.6 ± 2.4	2.9	6	4.3 ± 0.8	4.5
1991	75	4.2 ± 2	4.5	13	5.3 ± 0.5	4.8
1992	86	4.1 ± 1.8	4.4	20	4.5 ± 1.2	4.7
1993	81	3.7 ± 1.9	3.8	35	4.5 ± 1.5	4.4
1994	80	3.2 ± 2.3	3.6	35	4.2 ± 1.6	4.2
1995	72	3 ± 2.2	3.9	38	4.6 ± 2.2	4.6
1996	78	3.9 ± 1.9	3.9	4	2 ± 0.8	2.0
1997	104	2.6 ± 2.2	4.1	37	1 ± 1.7	3.4
1998	64	4.5 ± 1.4	4.5	31	4.8 ± 1	4.8
1999	72	3.2 ± 1.6	3.5	7	3.6 ± 1.5	3.6
2000	72	2.3 ± 2.2	2.8	34	4.1 ± 1.8	4.4
2001	61	2.4 ± 2	3.3	36	4 ± 1.5	4.1
2004	75	4.5 ± 1.6	4.6	36	4.8 ± 0.8	4.8
2005	89	4.5 ± 1.6	4.5	43	4 ± 1.4	3.9
2006	94	4.9 ± 1.3	4.9	41	4.6 ± 1.6	4.7
2007	98	4.4 ± 1.6	4.6	50	4.9 ± 1.2	5.0
2008	98	4.2 ± 1.7	4.4	49	3.3 ± 1.9	3.4
2009	104	4.4 ± 1.7	4.6	52	4.3 ± 1.4	4.4
2010	90	4.9 ± 1.2	5.0	48	4.4 ± 1.6	4.4
2011	108	3.9 ± 2.3	4.7	52	4.4 ± 1.4	4.5
2012	84	3.9 ± 1.8	4.6	52	4.3 ± 1.3	4.5
2013	97	4.1 ± 1.5	4.3	46	3.8 ± 1.7	4.1

[1] year quality, estimated as the total average fledgling number, excluding predated nests (see Bouwhuis et al. 2010).

Reference: Bouwhuis, S., Charmantier, A., Verhulst, S., Sheldon, B.C. 2010. Trans-generational effects on ageing in a wild bird population. J Evol Biol 23:636–642.

SUPPLEMENTARY MATERIAL 3

Table SM2. Results from exploratory GLMMs evaluating the effect of potentially influential factors for the recruitment success of male pied flycatchers. Number of males = 304; number of mates = 273; number of years = 18. Estimates and p-values of non-significant and hence removed variables are from when they were added alone to the final model.

	Estimate	SE	*Z*	*P*
Intercept	−795.80	281.96	−2.82	0.005
Tarsus length	83.18	29.44	2.83	0.005
Breeding habitat	824.87	295.93	2.79	0.005
Breeding date	−0.05	0.01	−3.78	<0.001
Tarsus length x Breeding habitat	−86.24	30.87	−2.79	0.005
Tarsus length^2	−2.17	0.77	−2.83	0.005
Tarsus length^2 x Breeding habitat	2.25	0.80	2.80	0.005
Age at first breeding	−0.71	0.01	−0.01	0.991
Mating status	−0.12	0.42	−0.27	0.785
Prevalence of blood-sucking mites	0.13	0.21	0.64	0.520
Prevalence of blowfly larvae	0.00	0.24	0.01	0.990
Natal habitat	0.09	0.23	0.39	0.698
Dispersal behaviour	0.35	0.23	0.15	0.140
Breeding density	−0.01	0.01	−1.64	0.102

CHAPTER IV - Natal habitat imprinting counteracts the diversifying effects of phenotype-dependent dispersal in a spatially structured population

ABSTRACT Habitat selection may have profound evolutionary consequences, but they strongly depend on the underlying preference mechanism, including genetically determined, natal habitat and phenotype-dependent preferences. It is known that different mechanisms may operate at the same time, yet their relative contribution to population differentiation remains largely unexplored empirically mainly because of the difficulty of finding suitable study systems. Here, we investigate the role of early experience and genetic background in determining the outcome of settlement by pied flycatchers (Ficedula hypoleuca) breeding in two habitat patches between which dispersal and subsequent reproductive performance is influenced by phenotype (body size). For this, we conducted a cross-fostering experiment in a two-patch system: an oakwood and a conifer plantation separated by only 1 km. Experimental birds mostly returned to breed in the forest patch where they were raised, whether it was that of their genetic or their foster parents, indicating that decisions on where to settle are determined by individuals' experience in their natal site, rather than by their genetic background. Nevertheless, nearly a third (27.6%) moved away from the rearing habitat and, as previously observed in unmanipulated individuals, dispersal between habitats was phenotype-dependent. Pied flycatchers breeding in the oak and the pine forests are differentiated by body size, and analyses of genetic variation at microsatellite loci now provide evidence of subtle genetic differentiation between the two populations. This suggests that phenotype-dependent dispersal may contribute to population structure despite the short distance and widespread exchange of birds between the study plots. Taken together, the current and previous findings that pied flycatchers do not always settle in the habitat to which they are best suited suggest that the strong tendency of most individuals –regardless of their phenotype– to return to the natal site might trigger maladaptive settlement behaviours and thus constrain the potential of phenotype-dependent dispersal to promote microgeographic adaptation.

Keywords: cross-fostering, *Ficedula hypoleuca*, local adaptation, natal habitat preference induction, matching habitat choice, non-random dispersal, pied flycatcher.

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INTRODUCTION

Selection of breeding environments is an important determinant of individual fitness and has therefore been a topic of considerable study in the realm of the ecology and evolution of dispersal (Johnson & Gaines 1990; Szulkin & Sheldon 2008; Duckworth & Badyaev 2007; Duckworth & Kruuk 2009; Clobert et al. 2012). Recent literature indicates that dispersal is typically non-random, and there is accumulating evidence that individuals often display a preference for a specific habitat type (Clobert et al. 2009; Edelaar & Bolnick 2012). Habitat preferences can have different underlying causes that include both genetic and environmental factors (e.g. Massot & Clobert 2000; Cote & Clobert 2007; Duckworth 2009). First, experience in the natal patch can shape habitat preferences in adulthood, so that individuals tend to return to their birthplace or to other breeding habitats that resemble those they encountered at an early age, a phenomenon called natal habitat preference induction (Davis & Stamps 2004; Stamps et al. 2009). Second, habitat preferences can be genetically determined (Jaenike & Holt 1991; Doligez et al. 2009). Finally, individuals may modify habitat selection according to their particular phenotype to settle in the habitats they are best suited to (Edelaar et al. 2008).

Numerous theoretical studies suggest that individual variation in habitat preferences can play an important role in population differentiation and ultimately also sympatric speciation (reviewed by Edelaar & Bolnick 2012). Nevertheless, recent simulation-based studies of the evolutionary consequences of habitat preferences suggest that these may be largely contingent on the underlying preference mechanism (Beltman & Metz 2005; Berner & Thibert-Plante 2015). For example, natal habitat preference induction could promote population differentiation because the offspring of dispersers settling in a new, previously unused habitat may become reproductively isolated very quickly from the original source population. However, when the imprinting mechanism entails a substantial cost (e.g. energetic or nutritional costs of information processing and storing; Dukas 1999), population differentiation is more likely to occur through genetically determined preferences (Beltman & Metz 2005). It should be noted that different preference mechanisms might act redundantly in nature, producing similar dispersal patterns, and even operate synergistically to promote population differentiation (Bolnick et al. 2009). For example, phenotype-dependent and natal habitat preferences may reinforce each other, since the former can facilitate local adaptation while the latter typically contributes to reproductive isolation (Edelaar et al. 2008; Bolnick et al. 2009).

Different mechanisms generating habitat preference and non-random dispersal have been well characterized from a theoretical standpoint (Clobert et al. 2009; Edelaar & Bolnick 2012; Davis & Stamps 2004). In addition, some efforts have been made to quantify the heritability of the propensity to disperse (Doligez et al. 2009; Hansson et al. 2003) and of dispersal distance (Doligez et al. 2009; Greenwood et al. 1979; but see van Noordwijk 1984), and to assess the influence of early life experience (Alonso et al. 1998; Ekman et al. 2002; Cote et al. 2010; Piper et al. 2013) and phenotypic traits (Garant et al. 2005; Camacho et al. 2013) on dispersal and settlement decisions. However, almost no studies have been conducted to determine the relative contribution of different preference mechanisms to the expression of breeding site selection within a biologically realistic framework (but see Bolnick et al. 2009).

In this study, we tested the role of early life experience and genetic background in the settlement patterns of pied flycatchers (*Ficedula hypoleuca*) breeding in two contrasting habitats: a coniferous forest and a nearby deciduous forest separated by only 1 km. Males, and to a lesser degree, females breeding in the deciduous forest are morphologically different from those breeding in the nearby coniferous forest, the latter being smaller in size (Camacho et al. 2013, 29). Over 25% of pied flycatchers returning to breed for the first time in the study area change habitats through dispersal and, from that moment, they rarely change the patch where they first bred (Camacho et al. 2013). Male pied flycatchers disperse between the two forests according to body size, so that individuals moving from the coniferous to the deciduous patch are larger than those moving the other way round, and also than those that remain in the coniferous patch. We have also shown that, contrary to the deciduous forest, where male size does not determine fitness, the latter increases non-linearly with body size in the coniferous forest. Nevertheless, the observed patterns of phenotype-dependent dispersal and settlement do not translate into clear fitness benefits (Camacho et al. 2015). Neither the proportion of individuals that return to their natal patch is what one would expect based on nest-site availability in each patch (Camacho et al. 2013), suggesting that additional preference mechanisms (e.g. natal habitat preference induction or genetically-determined preference) must be operating.

Cross-fostering experiments between alternative habitats provide an essential tool to assess whether individuals settle to breed in one forest type or another owing to their genetic background or either mostly on the basis of the environment they experienced during sensitive periods along their ontogeny (Merilä & Sheldon 2001; Stinchcombe 2014). Here, we capitalize on this approach to separate these two types of effects. Note that the terms "natal patch" and "natal habitat" are used interchangeably in the context of this work, because only one patch per habitat type is considered. For the same reason, no specific assessment is made to separate the potential natal habitat effects on settlement decisions from those attributable to the specific features of each patch. We integrate our findings with earlier work on the same system concerning size-dependent dispersal and fitness differences among individuals of different size and discuss the relative importance of concurrent preference mechanisms in the spatial assortment of phenotypes.

METHODS

Study system

Fieldwork was conducted between 2006 and 2015 in a two-patch system: a mature oak (*Quercus pyrenaica*) forest of 9.3 ha, and a nearby (1.1 km) mixed pine (mostly *Pinus sylvestris*) plantation of 4.8 ha separated by unsuitable breeding habitat for pied flycatchers (i.e. a mixture of rock outcrops and riverside vegetation holding few suitable holes). Many aspects of the ecology of pied flycatchers have been studied in the deciduous and the coniferous forest since wooden nest boxes (156 and 81) were provided in 1984 and 1988, respectively (e.g. Camacho et al. 2015). Pied flycatchers are small (ca. 12 g) long-distance migrants overwintering south of the Sahara and breeding across Europe (Lundberg & Alatalo 1992). Males often arrive from spring migration before females, search for a suitable cavity for nesting and announce themselves to females by singing actively (Lundberg & Alatalo 1992; Potti 1998). Pied flycatchers exhibit strong natal and breeding site fidelity (Montalvo & Potti 1992), and local recruitment rates are the highest reported in the literature on the species (Potti & Montalvo 1991a; Canal et al. 2014). Males, and to a lesser degree females, may postpone breeding until their second or, more rarely, third year of life (Potti & Montalvo 1991a).

Field procedures

After arrival of the earliest males from spring migration, nest boxes were checked at 1–3-day intervals to determine exact laying dates, clutch sizes, hatching dates and fledging success. Breeding birds were caught on day 8 (hatch day = 1) using a nest box trap, and measured for tarsus length (to the nearest 0.05 mm) and body mass (to the nearest 0.1 g). Fledgling mass and tarsus length were measured on day 13, when nestlings have already attained the definitive adult size (Alatalo & Lundberg 1986; Potti & Merino 1994). Both adults and fledglings were individually marked with numbered metal rings. Nestling mortality was controlled from hatching to fledging by considering both the number of young surviving at day 13 and those found dead in the nest 18–20 days after hatching.

Experimental procedures

Cross-fostering was conducted in 2006–2009 (complete clutches and half broods; 2–3 nestlings) and 2012–2013 (half broods; 2–3 nestlings). Note that recruits stemming from complete and half clutches or broods swapped between nests are equally suitable for the purpose of this study. We cross-fostered birds both within and between habitats in order to test, on one hand, how likely it is for a bird raised in its habitat of origin to return there to breed (i.e. control group) and, on the other hand, how likely it is for a bird experimentally raised in a habitat other than its habitat of origin to move back to its habitat of origin (i.e. experimental group). Cross-fostering was carried out 2–3 days after the onset of incubation (clutches) or on day 2–3 after hatching (broods). All transfers were performed between nests of similar phenology (i.e. matching hatching dates, and a difference of ± 1 day for eggs). Nestlings were individually marked for individual identification with a unique combination of colour markings on underparts (legs and wings) made with non-toxic felt pens and repainted every 2–3 days. To avoid skewing the masses of experimental nestlings in any direction during partial cross-fostering and thus minimize non-deliberate effects of competition between native and foster birds, all nestlings were weighed, ranked for weight, and then sequentially assigned to be either cross-fostered or remaining in the source nest.

Experimental manipulations had no apparent effect on recruitment rates (see 'results'). For all recruits stemming from cross-fostering experiments that were subsequently captured as first-time breeders, we recorded whether they returned to breed in their rearing patch or, conversely, moved into the adjacent one. It should be noted that, because the two habitat patches sampled in this study are isolated from other patches by unsuitable habitat, pied flycatchers returning to breed in the study area must choose between settling in their natal or the adjacent plot, or risk not breeding at all (Potti & Montalvo 1991a).

Data analyses

To investigate the roles of early experience and genetic variation in determining selection of either habitat by adult pied flycatchers in the two-patch system, we fitted a generalized linear mixed model (GLMM; binomial error distribution and logit link function) including birds' propensity to return to the rearing patch (0 = return to breed in the forest patch of their genetic parents; 1 = return to breed in the forest patch of their foster parents) as the response variable. As fixed effects, we included sex, the type of experimental treatment (cross-fostering within or between patches) as a control term, and the life stage of cross-fostering (egg or nestling) to account for possible ontogenetic effects on imprinting. Laying date at first reproduction, which is an accurate proxy for the arrival date in our study population (Potti & Montalvo 1991b), was also included as a covariate to account for the potential effect of arrival time (e.g. decreasing nest-site availability as the season progresses) on bird distribution across the two forests (Smith & Moore 2005). First-time breeders aged 2–3 years could have visited the study area in one or two seasons before being detected (Becker et al. 2008). However, when restricting the analysis to birds breeding for the first time at age 1 (*n* = 43), results were exactly the same (details not shown), suggesting that the age at first breeding does not affect the settlement pattern of pied flycatchers.

It is possible that some fledglings had visited (and possibly imprinted on) the adjacent forest prior to departure to the wintering areas, since post-fledging explorations may exceed the minimum distance separating the two plots (see van Balen 1979; Vysotsky et al. 1990). As the direction of exploratory trips is assumed to be random (Chernetsov et al. 2006), the likelihood of visiting the adjacent plot should increase with proximity of the latter to the rearing nest, thus obscuring the interpretation of our results. Accordingly, we used GPS coordinates to calculate the minimum linear distance between the rearing nest and the adjacent forest and included it in the model as a covariate.

Migratory birds may rely on a host of environmental cues and constraints to guide their settlement decisions after arrival, such as nest-site (Mänd et al. 2005) and food availability (Marshall & Cooper 2004), predation risk (Morosinotto et al. 2010) or conspecific attraction (Doligez et al. 1999). Nest-site availability, measured as the annual number of nest boxes not occupied by other hole-nesting species, differs between the two study patches and, furthermore, the magnitude of such differences may change over time (Camacho et al. 2013). Likewise, it is likely that other settlement cues, although not measured in this population, may vary over time and space, and this could severely bias the settlement decisions of pied flycatchers. To account for all these factors (i.e. between-year variability in habitat heterogeneity), we included in the model return year as a random effect. Nest identity was also fitted as a random effect to account for repeated measurements of experimental nests that produced ≥2 recruits (17%, *n* = 99).

Finally, we tested the interaction between tarsus length and rearing patch according to the notion by (Bolnick et al. 2009) that, when phenotype-dependent dispersal occurs, an individual's propensity to disperse into a non-natal habitat may depend on the interaction between its morphology and its rearing habitat. GLMMs were fitted in R 2.14.0 (http://www.R-project.org) using the function *lmer* in the package 'lme4' (Bates et al. 2011). Selection of the final model (containing only statistically significant terms) was carried out by dropping non-significant terms from a fully saturated model (containing all main effects and interactions) in a hierarchical way, starting with the least significant order terms.

RESULTS

Between 2006 and 2013, a total of 445 individuals from 116 nests (58 dyads) and 496 individuals from 120 nests (60 dyads) were cross-fostered between and within patches, respectively. Overall recruitment rates of cross-fostered young from experimental and control nests were, respectively, 14.7% (57 recruits from 389 confirmed fledged offspring) and 11.8% (48 recruits from 406 confirmed fledged offspring), the difference being not statistically significant (Pearson's Chi-squared test with Yates' continuity correction, $\chi^2 = 1.152$, *d.f.* = 1, $P = 0.28$). Recruitment rates did not differ between cross-fostered and non-manipulated fledglings either (respectively, 13.2% ($n = 795$) and 11.7% ($n = 1658$); $\chi^2 = 1.003$, *d.f.* = 1, $P = 0.32$), indicating that cross-fostering had no effect on the natural recruitment rates of pied flycatchers. Nests subjected to egg or nestling translocations showed similar recruitments rates (respectively, 13% ($n = 502$ eggs) and 13.7% ($n = 293$ nestlings); $\chi^2 = 0.030$, *d.f.* = 1, $P = 0.83$), indicating that the life stage at which the experiment was carried out did not bias our estimates. Overall recruitment rates did not differ either between cross-fostered males and females (respectively, 38 out of 335 *vs*. 44 out of 286 genetically-sexed nestlings; $\chi^2 = 1.860$, *d.f.* = 1, $P = 0.17$).

Table 1. Results of the GLMM (binomial error distribution and logit link function) analysing the effects of the experimental treatment (cross-fostering within and between habitats), life stage of cross-fostering (egg and nestling), sex, proximity to the adjacent habitat patch, breeding date, rearing patch, body size (tarsus length) and the interaction between rearing patch and body size on birds' propensity to return to the habitat where they had been raised (0 = return to breed in the non-rearing patch; 1 = return to breed in the rearing patch).

	Estimate	SE	*Z*	*P*
Intercept	0.963	0.218	4. 414	<0.001
Experimental treatment	0.243	0.442	0.550	0.582
Life stage of cross-fostering	0.214	0.456	0.470	0.638
Sex	−0.602	0.447	−1.345	0.179
Breeding date	0.012	0.055	0.228	0.819
Proximity to adjacent habitat	0.000	0.001	1.083	0.279
Tarsus length	0.129	0.453	0.286	0.775
Rearing habitat	−0.036	0.437	−0.083	0.934
Tarsus length x Rearing habitat	−0.893	1.006	−0.888	0.375

Number of returning birds = 105; Number of years = 8; Number of nests = 83. Estimates and P-values of non-significant (removed) variables are from when they were added alone to a null model containing only the random effects.

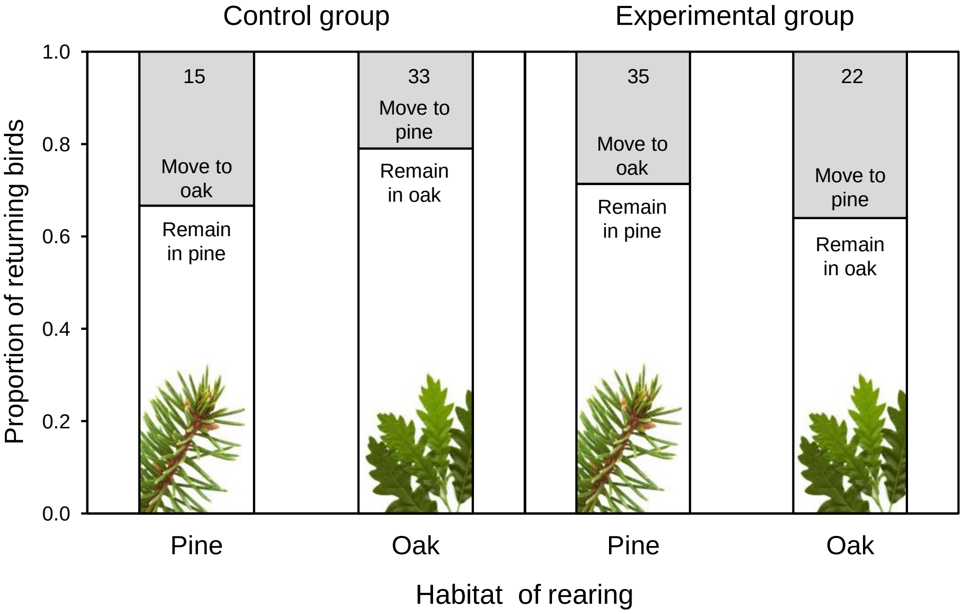

Figure 1. Proportions of pied flycatcher nestlings raised by foster parents that returned to breed in the same habitat where they fledged (open area) or in the alternate habitat type (shaded area) after being cross-fostered within (control group) or between (experimental group) habitats. Figures inside bars are the total number of recruits (both sexes combined).

DISCUSSION

By following individuals that had been cross-fostered to the same or a different habitat patch type, we provided evidence that pied flycatchers returned mostly to breed as adults in the area where they had fledged, regardless of their sex and origin. This is consistent with the hypothesis that individuals' decisions on where to settle are based on the environment they experienced at an early age, rather than on their genetic background. Birds breeding in the coniferous and the deciduous forest show phenotypic and genetic differences (Camacho et al. 2013; Chapters 1 and 2). Combined with our previous work demonstrating that bird movement between the study plots is affected by body size (Camacho et al. 2013), our results suggest that phenotype-dependent dispersal may act concurrently with natal site fidelity and thus contribute to the observed population differentiation of pied flycatchers over a very small geographic scale.

Spatial and temporal scale of imprinting

Our study supports earlier evidence from experimental (Berndt & Winkel 1979; Vallin & Qvarnström 2011) and observational studies (Chernetsov et al. 2006) showing that pied flycatchers tend to return as adults to their natal patch, and that this is not genetically pre-programmed. However, it is not known whether the returning birds seek for a natal-like habitat or simply for their birthplace, as they only had one patch of each habitat type to choose between but, according to other studies on this species (Berndt & Winkel 1979; Vallin &

Qvarnström 2011), the former seems more likely. On the other hand, the observed propensity of pied flycatchers to settle in their natal patch could alternatively be interpreted in terms of spatial constraints –rather than choice– if, for example, nest-site availability away from the natal site is limited (Smith & Moore 2005) or if it is easier to return to the same site after migration (Pärt 1995). Nevertheless, it is unlikely that pied flycatchers are constrained to choose between the two study plots since, on one hand, the distance between them is extremely short, thus facilitating bird exchange between both sites and, on the other hand, nest-site availability does not yet seem to be a limiting factor for pied flycatchers in the study area (Camacho et al. 2013, 2015).

It is known that experience in the natal site can strongly influence later habitat choice in the pied flycatcher (Chernetsov et al. 2006; Vallin & Qvarnström 2011), but evidence from translocation experiments suggests that imprinting may also occur well beyond fledging. (Berndt & Winkel 1979) transferred caged 5–6 weeks old fledglings 250 km away from their birthplace in northern Germany and found that all recruits returned to the area where they had been released, not to their natal patch, indicating that imprinting may extend over the fledgling stage. However, such tendency to return to the site of release after translocation might alternatively result from the typically high costs of finding the way home (Pärt 1995).

Besides the duration of the imprinting process, the spatial scale of post-fledging exploration may affect settlement patterns in the subsequent years. Male pied flycatchers commonly settle closer to their natal sites than females, although both sexes seem to imprint on a similarly small area (i.e. several kilometres in diameter; Chernetsov et al. 2006). Mean distance of post-fledging dispersal has been estimated between 0.6–1.4 km, depending on the study population (van Balen 1979; Vysotsky et al. 1990). Exploratory trips by fledglings that extend beyond the distance separating the two forests (1.1 km) might facilitate imprinting on the patch adjacent to their natal patch, and thus determine future habitat shifts. However, even though detailed data on prospecting behaviour by fledglings are not available for the study area, this seems unlikely to bias our results, as the likelihood of changing habitats was apparently not determined by proximity of the rearing nest to the neighbour forest.

Some individuals could have visited the study area in one or more seasons before reproducing for the first time and explored future territories away from the area they explored as a fledgling (Becker et al. 2008). But if this occurred, it certainly did not confound our estimates, as there were no differences between first-year and older first breeders in the propensity to change habitats. In addition, the timing of arrival from spring migration, which is known to have a profound effect on the breeding phenology and success of pied flycatchers (Potti 1998), might influence territory acquisition. Late migrants could be time-constrained to choose their breeding site and thus occupy the remaining free territories, whatever the habitat type (Smith & Moore 2005). This may be particularly true for first-year breeders, which usually show delayed arrival dates (Potti 1998; Potti & Montalvo 1991b). However, we found no phenological or age effects on birds' propensity to return to their rearing patch. Finally, an additional potentially confounding factor is that some individuals could perceive certain forest patches as offering better foraging and nest-site opportunities than others based on e.g. asymmetry in breeding density and presence of competitors, and settle accordingly (Garant et al. 2005). However, we think this is unlikely to bias our estimates for two main reasons. First, as shown here, males and females are equally likely to return to breed in the area where they were raised, despite the fact that only the former compete for nest sites (Lundberg & Alatalo 1992). Second, earlier results on the same system indicate that nest-site availability and population density do not play an important role in the local distribution of pied flycatchers (Camacho et al. 2013, 2015).

Natal habitat imprinting vs. phenotype-dependent dispersal

So far, few studies have attempted to tease apart the relative effects of natal patch preferences and phenotype-dependent dispersal on population differentiation. (Bolnick et al. 2009) also reported a strong tendency in lake and stream sticklebacks to return to their natal area and, as in pied flycatchers, their movement between habitats was phenotype-dependent. In our study population, around a quarter of the (unmanipulated) natal dispersers do not return to the forest patch they presumably imprinted on. Conversely, they move naturally into the adjacent habitat patch according to their body size, so that individuals dispersing from the coniferous to the deciduous forest are larger than those moving the other way round (respectively, 19.47 ± 0.07 mm and 19.39 ± 0.08 mm; mean ± SE; Camacho et al. 2013). Likewise, we observed qualitatively similar differences between cross-fostered individuals moving from the coniferous to the deciduous forest and those moving the other way round (respectively, 19.52 ± 0.08 mm and 19.28 ± 0.15 mm; mean ± SE). Even though this difference (1.1%) might seem modest, it is well within the range reported in other studies made at small spatial scales (5–10 km; 0.9–1.2% difference; Senar et al. 2006; García-Navas et al. 2014; Camacho et al. 2016a). Nevertheless, in contrast to the large sample of unmanipulated individuals, the effect of the interaction between body size and rearing patch on the propensity to change habitats did not reach significance in our experimental birds (see 'results'), possibly because the sample size of 'non-philopatric' recruits stemming from the experiment is very limited compared with the observational study (29 *vs.* 115). Indeed, a power analysis based on the observed differences between the two groups of dispersers (effect size $d = 0.52$) showed that, for $\alpha = 0.05$, an overall sample size of $n = 120$ would be required to have 80% power, which means that, according to the actual recruitment rate (13.2%) and proportion of recruits moving between habitats (27.6%), over 3,200 individuals would need to be cross-fostered. Still, taken together, the results of this experiment and our previous studies (Camacho et al. 2013, 2015) give support to the notion by Bolnick et al. (2009) that phenotype-dependent dispersal and natal site fidelity may act concurrently.

More pied flycatchers disperse between the two habitats compared with the sticklebacks study (30% *vs*. 10%) but, unlike in the stickleback populations, phenotype-dependent dispersal and natal habitat preferences of pied flycatchers do not appear to act synergistically but partially cancel out each other. Bolnick et al. (2009) showed that morphologically different sticklebacks preferentially settle in the habitat conferring a fitness advantage (ultimately promoting microgeographic adaptation). By contrast, decisions on where to settle and fitness appear to be decoupled in our study population (Camacho et al. 2015), possibly because of the strong tendency to return to the natal habitat patch, not to that in which their particular phenotype performs best (see Piper et al. 2013; Fletcher et al. 2015; but see Stamps et al. 2009). Thus, the strong (maladaptive) reluctance of the majority of birds to move away from either of the two habitat patches might constrain, rather than promote, microgeographic adaptation and population divergence.

Conclusions

Early experience in the natal patch may play a crucial role in determining subsequent dispersal and settlement in the pied flycatcher (Chernetsov et al. 2006; Burger & Both 2011; Berndt & Winkel 1979; this study) and possibly also in a broad range of animals (Davis & Stamps 2004). Taken together, the results presented herein support previous evidence indicating that phenotype-dependent dispersal between the two plots might contribute to the observed morphological and genetic differentiation of pied flycatchers (Camacho et al. 2013; Chapter 1). However, the potential of phenotype-dependent dispersal to increase the magnitude of divergence between these two populations might be constrained by the strong natal site fidelity, as individual performance in each forest patch is strongly determined by morphology (Camacho et al. 2015). We suggest that the heretofore largely neglected −but likely widespread− interplay between early experience in the natal site and individual phenotype should be fully taken into account in future studies investigating the mechanisms underlying non-random dispersal and habitat selection.

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CHAPTER V - Differential dispersal drives the evolutionary dynamics of structural size within a wild bird population

ABSTRACT Examples of the eco-evolutionary consequences of phenotype-dependent dispersal are still very scarce in the literature, possibly because long-term data from continuously monitored populations of marked individuals are needed. Here we document over a 29-year period the effect of dispersal on the evolutionary dynamics of tarsus length in a wild population of pied flycatchers (*Ficedula hypoleuca*). We analysed the genetic architecture of, and patterns of selection on, tarsus length of dispersers and philopatric individuals originating from ecologically distinct (coniferous and deciduous) habitats. Quantitative genetic analyses revealed a substantial component of additive genetic variance underlying tarsus length, although the variance components of this trait varied between pools of individuals differing in natal dispersal behaviour. Specifically, individuals that were genetically predisposed to be larger preferentially dispersed into the oak forest. However, selection gradients on this group, unlike the other groups, were negative and therefore resulted in a negative response to selection. Dispersers to the oak forest actually showed a decline in tarsus length over time that, nevertheless, deviated from the lack of trend recorded in the entire oak population. No such discrepancy was found in the pine forest, suggesting a greater genetic contribution of dispersers to the pine forest in shaping the distribution of phenotypes within their recipient population. Examination of the breeding success of each group suggested that this asymmetry resulted from uncoupled patterns of dispersal and gene flow in the oak forest since, on average, the reproductive output of immigrants into the oakwood was lower than that of the other groups. Taken together, these results indicate that the effect of dispersal on microevolutionary processes may vary from being positive to non-existent depending on the habitat type, with important implications for eco-evolutionary dynamics of wild populations.

Keywords: additive genetic variance, *Ficedula hypoleuca*, local adaptation, natural selection, non-random dispersal, pied flycatcher, tarsus length.

This chapter is based on the manuscript: Camacho, C., Martínez-Padilla, J., Canal, C. & Potti, J. Differential dispersal drives the evolutionary dynamics of structural size within a wild bird population (submitted).

INTRODUCTION

> *The desirability of making testable evolutionary predictions from the measurement of selection in the wild contrasts with the rarity of the opportunity to do so* (Grant & Grant 1995).

Understanding the genetic basis and evolutionary dynamics of morphological traits is a major goal in evolutionary biology. Microevolution, defined as a change in gene frequencies over time, is mainly driven by deterministic mechanisms, including natural selection and gene flow (Slatkin 1987; Hendry & Kinnison 2001; Lenormand 2002). Most evolutionary models assume that gene flow counteracts gene frequency changes caused by natural selection (Slatkin 1987; Garcia-Ramos & Kirkpatrick 1997; Lenormand 2002). However, recent theoretical and empirical papers have shed new light into the long-standing debate on the interaction between diversifying selection and homogenizing gene flow and showed that the latter can positively influence the evolutionary dynamics of phenotypic traits under particular conditions (e.g. Garant et al. 2005; Postma & van Noordwijk 2005; Garant et al. 2007; Edelaar & Bolnick 2012; Bolnick & Otto 2013).

Dispersal is now largely recognized as a non-random process being influenced by phenotypic attributes, such as morphology, physiology and behaviour (Bowler & Benton 2005; Clobert et al. 2009). Natal dispersers are likely to experience reduced survival or reproductive success beyond the intrinsic costs and risks of movement (e.g. increased energy expenditure or predation risk; Bonte et al. 2012; Cote & Clobert 2010) due to maladaptation to novel breeding environments (Hendry 2004; Nosil et al. 2005). However, individuals settling in a new habitat might also benefit from dispersal, especially if born in an unsuitable environment where maladapted phenotypes are counter-selected. Recent studies have suggested that phenotype-dependent dispersal may confer survival and/or fecundity advantages to dispersers that select the habitats that best match their phenotype, a process known as matching habitat choice (Edelaar et al. 2008). From an evolutionary perspective, it would not be surprising that dispersal decisions according to the phenotypic specializations of individuals determine the direction and strength of selection on phenotypic traits, thus influencing the evolutionary dynamics of natural populations (see Bolnick & Otto 2013). Nevertheless, despite its potential importance, the role of phenotype-dependent dispersal in microevolution of quantitative traits has received surprisingly little empirical attention and remains poorly understood (Garant et al. 2005; Postma & van Noordwijk 2005).

Evolutionary dynamics of phenotypes occurs when there is a concomitant action of selection on, and additive genetic variation of, a given trait. Joint estimations of these two components allow calculation of the change in the frequency distribution of phenotypes within a population, i.e. response to selection (R) either using the breeder equation (Lande & Arnold 1983) or the Robertson-Price equation (Robertson 1966; Price 1970). Given that dispersal can be phenotype-dependent, a full understanding of the evolutionary dynamics of phenotypes within populations requires exploring the magnitude and direction of selection, but also the proportion of phenotypic variance that is additive between groups of resident and dispersers.

Here we use an extensive data set collected over a 29-year period from a wild population of pied flycatchers (*Ficedula hypoleuca*) to investigate the effect of dispersal on the evolutionary dynamics of tarsus length, a quantitative trait commonly used as a proxy for structural body size in passerine birds (Senar & Pascual 1997). For this, we analyse the genetic architecture of, and patterns of selection on, tarsus length of dispersers and philopatric individuals originating from ecologically distinct (coniferous and deciduous) habitats. Heritability analyses based on traditional methods (e.g. parent-offspring regressions) have suggested that tarsus length is a heritable character in the pied flycatcher (Alatalo & Lundberg 1986; Potti & Merino 1994). Furthermore, a previous study of this population suggests that individuals of different size may specialize on different habitats and thus perform differently in pure coniferous and deciduous habitats (Camacho et al. 2015).

Most pied flycatchers return to their natal patch to breed regardless of the habitat type, but over 25% of recruits change habitats. Remarkably, males and, to a lesser degree, females move between habitats according to tarsus length (Camacho et al. 2013, 2016). Mean tarsus length has increased over time in the pinewood, but has remained constant in the oakwood, so that the degree of phenotypic differentiation is currently smaller than it was at the beginning of the study (Chapter 1). Evidence from ecological studies conducted in this population suggests that phenotype-dependent dispersal might have contributed to shape the observed phenotypic trajectories of the two populations (Camacho et al. 2013; Chapter II). However, a detailed knowledge of selection acting on tarsus length and a decomposition of total phenotypic variance between additive and non-additive factors in relation to dispersal strategies is required for a comprehensive understanding of the evolution of tarsus length in this population. Based on the observed temporal trends in tarsus length of the four groups of dispersers (see ‘Results’), we expect that our predictions of the evolutionary response to selection derived from estimated selection and genetic parameters match the direction of the specific phenotypic trend of each group.

METHODS

Study system

The study was carried out between 1988 and 2016 in a Spanish population of pied flycatchers breeding in two different habitat patches: a structurally complex oak (*Quercus pyrenaica*) forest and a mixed pine (mostly *Pinus sylvestris*) plantation located 1 km apart (see Camacho et al. 2015 for a description of the study site). Pied flycatchers breeding in the oak and the pine forest have been monitored since nest boxes (156 and 81) were provided in 1984 and 1988, respectively (e.g. Canal et al. 2012; Potti el al. 2013; Camacho et al. 2016b). Despite geographic proximity and extensive dispersal between habitats, both populations show subtle ecological and genetic differences (Camacho et al. 2013, 2016) and their phenotypic trajectories with respect to tarsus length also differ (Chapter 1).

General field procedures

Field protocols have been described in detail elsewhere (Camacho et al. 2017). Briefly, nest boxes were checked every 1−3 days to record basic life history parameters: laying and hatching dates, clutch size and number of offspring. Nestlings were individually marked with numbered metal rings and measured for tarsus length (±0.05 mm) and body mass (± 0.1 g) on day 13 (hatch day =1), when they have already reached the final adult size (Potti & Merino 1994). Parents were captured while they were feeding 8-day-old nestlings with a nest-box trap, aged as yearling on older based on plumage characteristics (Karlsson et al. 1986), and measured for standard morphological traits.

Morphological measurement

Tarsus length, a proxy of structural body size, was used as the focal trait in this study. Body size influences phenotype-dependent dispersal and the subsequent distribution of pied flycatchers, which are typically larger in deciduous habitats than in coniferous habitats (Alatalo et al. 1985; Camacho et al. 2013). Body size may influence male mating success (Canal et al. 2011) and is considered a major determinant of fitness in this species, because it determines the ability of individuals to defend nest-sites and to exploit different foraging niches and food resources (Lundberg et al. 1981; Alatalo et al. 1985; Sirkïa & Laaksonen 2009).

Besides its ecological relevance, tarsus length is a suitable model trait for microevolutionary studies, because it is fixed throughout the life of individuals since the end of the growth period at the age of 13 days (Potti & Merino 1994), giving little room to individuals to adjust their phenotype to changing environmental conditions. Furthermore, in a northern population of pied flycatchers, tarsus length was apparently under stabilising selection, suggesting that evolution penalises individuals at the extremes of the body size distribution (Alatalo & Lundberg 1986). For all the reasons above, tarsus length represents an ideal trait to investigate the evolutionary dynamics of bird populations exposed to distinct breeding environments.

Dispersal behaviour

Natal dispersal is here defined as a change of forest patch irrespective of the distance between the natal nest box and the first breeding nest box. The treatment of dispersal as a habitat shift has been found to be biologically relevant in previous studies on this population, as it depended on both individual (e.g. sex, body size, natal experience) and environmental (e.g. local breeding density) factors (Camacho et al. 2013, 2016). In addition, the likelihood of changing habitats is not determined by proximity of the natal nest box to the adjacent habitat (Camacho et al. 2016b). Less than 2% of adults move away from the habitat where they first bred, so breeding dispersal between habitats can be considered negligible (Camacho et al. 2013). Based on the habitat they originated from and the direction of natal dispersal, individuals were classified into four groups: dispersers from the oak to the pine forest, dispersers from the pine to the oak forest, philopatric birds of the oak forest, and philopatric birds of the pine forest.

Data analyses

Phenotypic trends of dispersers and philopatric birds

All statistical analyses were performed using R version 3.3.1 (http://www.R-project.org). To examine differences among dispersal groups in the magnitude and direction of phenotypic change over time we used a linear model with tarsus length as the response variable. Year was treated as a continuous predictor variable to formally test for temporal trends in tarsus length. As fixed effects, we included sex, dispersal category, and the interaction between them and year. Males and females were considered together in this work despite slight differences in their body size trends since we are interested in evolutionary responses at the population level.

Breeding success and dispersal behaviour

To test for differences in breeding success between dispersers and philopatric birds of each habitat we used a Generalized Linear Mixed Model (GLMM; binomial errors, logit link function). The response variable was a two-column matrix generated using the 'cbind' function to combine the annual number of recruits and non-recruiting fledglings (i.e. recruitment success). GLMMs were fitted using the function *lmer* in the R package 'lmerTest' (Kuznetsova et al. 2016). Due to some gaps in the lifelong capture histories of a fraction (8.5%) of individuals, we used annual, rather than lifetime, data to maximize our sample size. Dispersal group (class variable), sex (class variable), and their interaction were included in the GLMM as fixed effects. To determine the significance of each fixed effect, we used likelihood ratio tests (LRT) testing the effects of single-term deletions from the full model using the function anova (Zuur et al. 2009). Post-hoc Tukey tests were used to evaluate differences in mean recruitment success among dispersal groups using the *glht* function in the R package 'multcomp' (Bretz et al. 2016). Ring number and year were fitted as random factors to account for repeated measures of the same individuals and temporal heterogeneity in breeding performance. Territory quality is unrelated to breeding date or individual phenotype in this population (see Canal et al. 2012 for further details). Moreover, exploratory analyses comparing models with and without nest-box identity as a random factor showed no effect on breeding success and, therefore, territory quality was not accounted for in the final models.

Because of discontinuities in the intensity of fieldwork, the years 2002 and 2003 were removed from the data set. As recruits may postpone their first reproduction until the second or, more rarely, third year of life (Potti & Montalvo 1991a), all nests of birds breeding between 1999 and 2003 were excluded from analyses to avoid underestimating their true recruitment success (Potti et al. 2013). For the same reason, all nests of birds breeding beyond 2013 were not considered either. Broods that were manipulated for experimental purposes (e.g. cross-fostering) were removed from the data set.

Quantitative genetics of tarsus length

We estimated additive and non-additive variance components of tarsus length through a mixed-model restricted-maximum likelihood (REML) in ASReml-R 3.0 (Butler 2009). We used the pedigree information to fit an individual 'animal model' (Kruuk 2004). The social pedigree of our pied flycatcher population included 3,184 marked individuals for 12 generations, 1,379 maternities, and 1,436 paternities. The rate of extra-pair paternity in our study population is 15% (Canal et al. 2012), below the threshold of 20% suggested to impact heritability estimates (Charmantier & Reale 2005). Moreover, there is no reason to think that the frequency of extra-pair paternity varies according to the dispersal status of individuals, so undetected extra-pair paternity is unlikely to confound our results. Moreover, an association between inbreeding and individual phenotype could affect our estimates (Reid et al. 2011), but this seems unlikely because we have previously shown that the levels of genetic relatedness are equally low in both habitats (Camacho 2011). We did not know the mother and father of the focal individual in all cases, so when only one parent was known, we assigned a "dummy" code to the missing parent to preserve sibship information. The phenotypic data set included 2,647 records (males, n = 1,352; females, n = 1,295) for which the habitat of origin and the natal dispersal behaviour were known.

To examine the genetic basis of tarsus length, the total phenotypic variation of this trait was partitioned into additive (V_A) and non-additive variation. Specifically, we first fit a univariate animal model where the tarsus length (Tl) of individual i was modelled as follows:

$$Tl_i = \mu + Ld + SEX + HABITAT + AGE + a_i + y_i + pe_i + e_i$$

In the above model, μ is the population mean phenotype. Laying date (Ld), age, breeding habitat and sex were included as fixed effects. As random effects, we included the additive genetic (a_i), individual-specific (permanent environment, pe_i) and year-specific (common environment, y_i) effects and the residual term (e_i). We also included pe_i to control for multiple measurements of the same individual (Kruuk 2004). The term y_i considers the temporal environmental heterogeneity throughout the study period.

To specifically test for the genetic (G) basis of tarsus length depending on the dispersal behaviour of individuals (D), we built a second model equivalent to the first one but only including the Genotype-by-Dispersal interaction (G x D) as a random term. In the presence of G x D, V_A is expected to change depending on the particular dispersal behaviour of individuals. To formally test for the significance of the interaction term, we used a LRT comparing the likelihood of the models with and without the interaction term with the function *anova*. Specifically, an LRT assumes 2 times the difference in REML log-likelihood scores between a model with and without the random effect of interest following a chi-square distribution with one degree of freedom (Pinheiro & Bates 2000). Thus, LRT = $-2(L2 - L1)$, where $L1$ is the log likelihood of the first model and $L2$ the log likelihood of the model including G x D.

We calculated h^2 as the relative contribution of V_A to the total phenotypic variance. Thus, $h^2=V_A/V_P$ and, according to the model described above, $h^2=V_A/(V_A+V_{PE}+V_Y+V_R)$. Finally, we calculated the evolutionary potential (I_A) and the coefficient of additive genetic variance (CV_A)

as proxies of the evolvability of tarsus length following García-González et al. (2012). In both indexes, V_A is scaled by the mean (García-González et al. 2012) to allow comparisons of estimates of evolvability among species and populations as follows:

$$I_A = \left(\frac{V_A}{\bar{x}^2}\right) \text{ and } CV_A = \frac{\sqrt{V_A}}{\bar{x}}$$

Selection analysis

Selection on tarsus length –both standardized directional (β) and nonlinear (γ) selection gradients– was estimated based on the standard methods of Lande & Arnold (1983). Prior to analyses, fitness measures were converted to yearly relative fitness by dividing individual values by the mean of the population in that year (ω). Tarsus length was standardized to a mean of zero and a standard deviation of one (z). Selection was then measured as β = cov(ω, tarsus length)/$V_{\text{tarsus length}}$. We used three different fitness measures: (1) offspring production (number of chicks fledged), (2) local recruitment (number of fledglings that later became recruits to the breeding population), and (3) recruitment success (recruits/fledglings produced per brood). We are confident that local recruitment can be used as a reliable proxy for individual fitness because (1) all pied flycatchers breeding in the two habitats during the study period –except some polygynous males that left their secondary females unattended– were captured for identification (Camacho et al. 2017); (2) dispersal movements out of the study area are extremely rare (pers. obs. from non-systematic explorations of surrounding areas); (3) local recruitment rates are already among the highest reported in the literature on the species (7–13% depending on the year; Potti & Montalvo 1991a), suggesting that virtually all survivors return to breed into the study plots.

Standardized selection gradients (β) were estimated as the slope of the regression of relative fitness on standardized trait values (Lande & Arnold 1983). Similarly, to estimate non-linear selection gradients (γ), we squared the standardized trait values. Both directional and non-directional selection gradients were estimated using linear mixed effect models (LMM) with individual identity as a random factor to account for repeated measures on the same individuals. In all models, we included sex as a fixed effect and year as a random effect. Breeding date (egg-laying date) and age were also included as covariates because these two parameters are tightly associated with breeding success and recruitment in *Ficedula* flycatchers (Potti & Montalvo 1991a, b; Lundberg & Alatalo 1992; Potti et al. 2013). Since we were interested in exploring selection depending of the dispersal behaviour (D), we estimated the statistical significance of the interaction between standardized trait values and D (z*D). By doing so, we specifically tested whether the slope of the relationship between relative fitness (ω) and standardized tarsus length (z) differed among dispersal groups.

For the same reasons as above (see “Breeding success”), all nests of individuals breeding in 1999–2003 and 2014–2016 were removed from the data set. Birds stemming from manipulated broods (e.g. cross-fostering) and nests subjected to experimental manipulations were also removed from the data set.

Response to selection

To calculate the predicted response to selection (R) suggested by Lande (1979), we used the estimates of additive genetic variance (V_a) and directional selection gradients (β), from the equation $R = V_a \times \beta$. We used Lande's equation instead of the Breeder's equation in the form of $R = h^2 \times S$ because selection gradients more accurately represent the response to selection than do selection differentials (S) when phenotypic variance changes depending on spatial and temporal heterogeneity in environmental conditions, as occurs in our system (see below) (Hereford et al. 2004). We provided the V_A, and β split by dispersal group, allowing examination of the response to selection for each dispersal group separately.

RESULTS

Phenotypic trends of dispersers and philopatric birds

Mean tarsus length of pied flycatchers (both sexes combined) breeding in the oak and the pine forest varied significantly over the study period, but the magnitude and direction of this change differed between dispersers and philopatric birds depending on their origin ($F_{1,3}$ = 6.21, P = 0.0003).

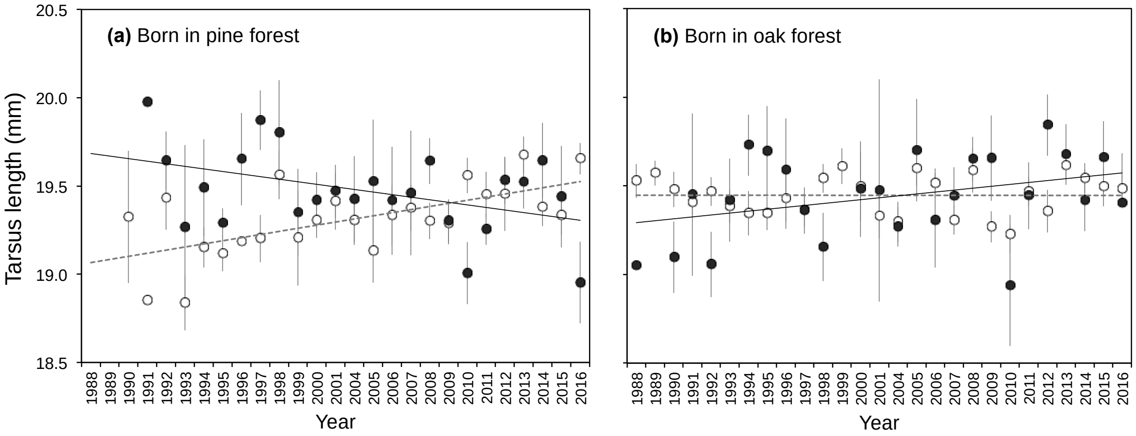

Figure 1. Temporal trends in mean (± SE) tarsus length of natal dispersers (males and females combined) returning to the oak and the pine forest over the study period. Separate trends are shown for philopatric birds and depending on the habitat of origin (deciduous or coniferous).

Separate models by dispersal category provided further insight into the individual phenotypic trajectories of each group. Specifically, pied flycatchers born in the pine forest showed opposite trends in mean tarsus length depending on whether they remained in their natal patch (b = 0.016 ± 0.004, t = 4.04, $P < 0.001$) or dispersed to the oak forest (b = −0.008 ± 0.005, t = −1.47, P = 0.143), the difference between the slopes being significant ($F_{1,502}$ = 13.12, P < 0.001; Fig. 1a). Birds born in the oak forest also showed slightly different phenotypic trends depending on the dispersal strategy, but in this case the difference between the slopes was not significant ($F_{1,910}$ = 2.55, P = 0.111; Fig. 1b). Individuals moving from the oak to the pine forest showed a slight, non-significant increase in tarsus length over time (b = 0.009 ± 0.006, t = 1.59, P = 0.114), whereas no phenotypic change was detected in philopatric birds of the oak forest (b = 0.001 ± 0.002, t = −0.22, P = 0.826).

Breeding success and dispersal behaviour

Overall, 1,416 reproductive attempts by 867 individuals belonging to 29 cohorts (1984–2012) were used in the analysis of breeding success. LRT showed significant differences in average recruitment success among the four dispersal groups (χ^2= 16.011, *d.f.* = 3, P = 0.001) irrespective of sex (χ^2= 0.337, *d.f.* = 1, P = 0.562). Dispersers to the pine forest and to the oakwood had the highest and the lowest recruitment success, respectively, the difference between them being statistically significant (Table 1; Fig. 2). Pairwise comparisons among groups showed no significant differences between immigrants and native birds of either habitat, suggesting that forest type was the main factor responsible for the observed differences among groups in average recruitment success (pine > oak, Table 1). However, it is important to note that immigrants tended to perform better on average than native birds in the pine forest, while the opposite was true in the oak forest (Fig. 2).

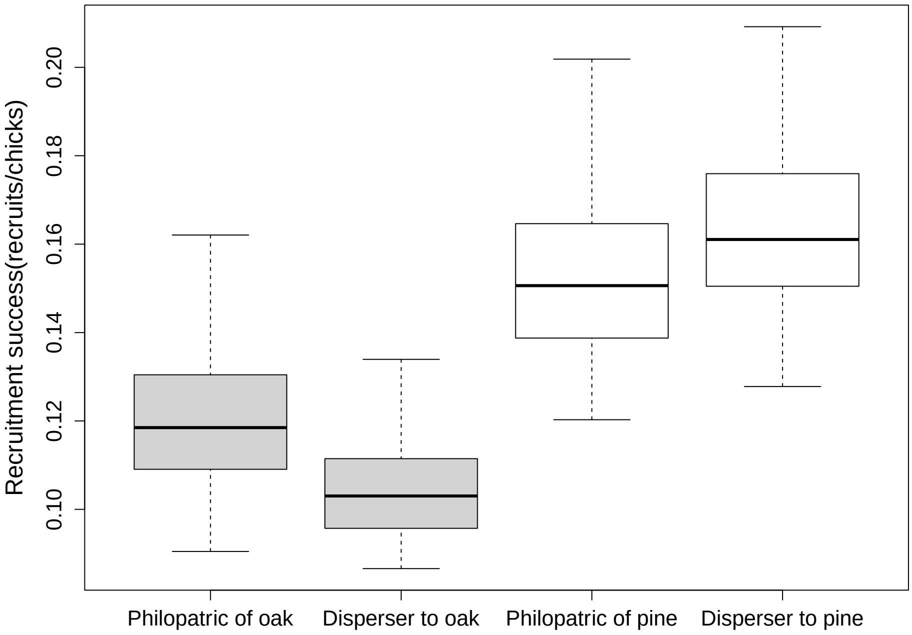

Figure 2. Recruitment success of male and female pied flycatchers breeding in the oak (shaded boxes) and the pine (open boxes) forest over the study period. Fitted values of the GLMM are shown separately for each dispersal group. Shown are the median (central line in box), the upper and lower hinges (edges of the box approximating the first and third quartiles) and the whiskers (defined as 1.5x the hinge spread).

Table 1. Pairwise comparisons of the average recruitment success of dispersers and philopatric birds breeding in the oak and the pine forest during the study period.

Comparisons	Estimate	SE	Z	P
Dispersers to pine - residents of oak	0.372	0.126	2.948	0.0157
Residents of pine - residents of oak	0.317	0.108	2.936	0.0165
Dispersers to oak - residents of oak	−0.107	0.158	−0.676	0.9028
Residents of pine - dispersers to pine	−0.055	0.141	−0.387	0.9795
Dispersers to oak - dispersers to pine	−0.478	0.183	−2.619	0.0416
Dispersers to oak - residents of pine	−0.424	0.167	−2.539	0.0515

Quantitative genetics of tarsus length

Overall, there was detectable additive genetic variance for tarsus length (Tables 2, 3). We found that the G x D interaction explained a significant proportion of the phenotypic variance of tarsus length compared to a model without this interaction term, suggesting that the additive genetic variance of tarsus length differed among dispersal groups (Table 2). Coefficients of additive genetic variation (CV_A) and mean-standardized additive genetic variances (I_A) were lowest for philopatric birds of the oak forest and highest for dispersers into this habitat, whereas birds breeding in the pine forest showed intermediate values (Table 3).

Table 2. Results from the univariate random regression analysis of tarsus length. Reported chi-square values are for comparisons with the previous model, and the P-values for the associated LRT. V_{PE} refers to permanent environment variance and V_A to additive genetic variance. G x D is the phenotypic variance–covariance matrix related to dispersal behaviour. Note that year was included as random factor, but see the 'Data analyses' section for further details.

Model	Variance components	LogL	χ^2	P
1	-	269.36		
2	Sex+LD+AGE+Habitat+V_Y+V_{PE}	843.36	1148.00	<0.001
3	Sex+LD+AGE+Habitat+V_Y+ V_{PE}+V_A	882.13	77.54	<0.001
4	Sex+LD+AGE+Habitat+V_Y+V_{PE}+V_A+GxD	884.23	4.18	**0.0407**

Table 3. Variance components of tarsus length evaluated for all individuals regardless of dispersal behavior (Full) and depending of the dispersal behavior. V_P is the sum of the variance components (phenotypic variance), V_A the additive genetic variance, V_{PE} the permanent environment variance component V_Y the year variance, and V_R the residual variance. CV_A and I_A are the mean standardized indexes of evolutionary potential. For all variance components, we show the Zratio and the statistical significance (***: p<0.001; **: p<0.01; *: p<0.05; ns: p>0.05).

Category (n_{ind}/n_{cap})	V_P (Zratio)	V_A (Zratio)	V_Y (Zratio)	V_{PE} (Zratio)	V_R (Zratio)	CV_A	I_A	h^2 (SE)
Full (1466/2649)	0.296	0.1166*** (6.576)	0.0031* (2.4476)	0.1024*** (5.9495)	0.0739*** (18.5165)	0.0176	0.0003	0.386 (0.051)
Philopatric of oak (795/1455)	0.291	0.1090*** (4.394)	0.0031* (2.042)	0.1179*** (5.5199)	0.0610*** (17.7083)	0.0170	0.0003	0.376 (0.077)
Dispersers to pine (149/261)	0.303	0.1368 ^{ns} (0.1240)	0.0048^{ns} (1.1378)	0.1080^{ns} (0.9119)	0.0535*** (7.0732)	0.0190	0.0004	0.434 (0.382)
Philopatric of pine (347/641)	0.318	0.1635*** (4.173)	0.0054^{ns} (1.5225)	0.0618* (2.0876)	0.0869*** (11.7837)	0.0209	0.0004	0.513 (0.101)
Dispersers to oak (169/289)	0.365	0.3015** (6.7978)	0.0045^{ns} (1.0519)	0.000006*** (7.3824)	0.0597*** (7.2824)	0.0282	0.0008	0.826 (0.034)

Table 4. Selection gradients and responses to selection on tarsus length of disperser and philopatric pied flycatchers of each habitat. Here we show selection gradients considering offspring ($\beta_{offspring}$), recruits ($\beta_{recruits}$), and recruitment rates ($\beta_{recruit\_rate}$) as proxies of fitness, as well as the response to selection for each fitness measure.

Category (n_{ind}/n_{cap})	$\beta_{offspring}$ (SE) [p-value]	$\beta_{recruits}$ (SE) [p-value]	$\beta_{recruit\_rate}$ (SE) [p-value]	$R_{offspring}$	$R_{recruits}$	$R_{recruit\_rate}$
Full (1466/2649)	0.0195 (0.0083) [0.0192]	0.0498 (0.0323) [0.1340]	0.0206 (0.0318) [0.517]	0.0023	0.0058	0.0024
Philopatric of oak (795/1455)	0.0155 (0.0124) [0.4904]	0.0346 (0.0439) [0.4290]	−0.0026 (0.0419) [0.949]	0.0017	0.0038	−0.0003
Dispersers to pine (149/261)	0.0154 (0.0224) [0.7920]	0.2018 (0.1091) [0.0660]	0.1404 (0.1047) [0.182]	0.0021	0.0276	0.0192
Philopatric of pine (347/641)	0.0177 (0.0128) [0.1692]	0.1037 (0.0741) [0.1626]	0.0848 (0.0712) [0.2340]	0.0029	0.0170	0.0139
Dispersers to oak (169/289)	0.0499 (0.0241) [0.0381]	−0.0928 (0.0977) [0.3430]	−0.1263 (0.0941) [0.183]	0.0150	−0.0280	−0.0381

Selection patterns

Univariate analyses including all years combined showed that selection on tarsus length varied in strength and direction depending on the fitness component considered, although only when offspring production was used were selection gradients statistically significant (Table 3). Nevertheless, there was no evidence for either directional or stabilizing selection on tarsus length when either the number of recruits or recruitment rates were used. No effect of sex was found for any of the fitness measures considered. Neither directional nor non-directional selection gradients were influenced by dispersal behaviour (z*D interaction) for any of the fitness variables (Table 4), indicating that the strength of selection on the focal trait did not differ among dispersal groups. However, it is important to note that, although not significant, selection gradients showed negative linear effects on tarsus length of dispersers from the pine to the oak forest, with important implications for the direction of the evolutionary response to selection (see below).

Table 4. Estimates of the interaction between standardized tarsus length (z) and dispersal behaviour (D) for all the three fitness measures considered. The z*D informs whether the slope of the relationship between relative fitness (ω) and standardized tarsus length differs among dispersal groups.

	Linear selection			Non-linear selection		
	d.f.	*F*	*P*	d.f.	*F*	*P*
Number of offspring	1349.63	1.349	0.2450	1850.56	0.906	0.3414
Number of recruits	1869.03	0.250	0.6174	1872.90	0.657	0.4177
Recruitment success	1727.07	0.162	0.6876	1734.68	1.142	0.2855

Response to selection

Non-philopatric birds, regardless of their origin, showed the highest responses to selection at the population level (Table 4). However, the response to selection of the dispersers to the pine forest did not have a significant genetic component (Table 3), and therefore, the magnitude of their response was rather limited compared to that of the dispersers to the oak forest (Table 4). Philopatric birds of the oak forest showed a very modest response to selection, whereas that of dispersers to this habitat was, depending on the fitness proxy considered, between 6.5 and 15.9 times the estimated response for the overall population, and between 2.7 and 5.2 the estimated response found for philopatric birds of the pine forest (Table 4). Remarkably, the direction of the responses to selection in dispersers and philopatric birds born in the pine forest were opposite, being negative in the former and positive in the latter. This suggests a shift in the frequency distribution of tarsus lengths in birds originating from the pine forest, but in opposite directions depending of the dispersal behaviour of individuals (Fig. SM1).

DISCUSSION

We have shown that the evolutionary dynamics of tarsus length can explain the phenotypic changes within a wild population of pied flycatchers monitored over a 29-year period. We have provided evidence for a substantial component of additive genetic variance underlying structural size, measured as tarsus length. Furthermore, we have shown that the variance components of tarsus length can vary between pools of individuals differing in natal dispersal behaviour. However, the differences among groups in selection patterns were comparatively smaller. Since the evolutionary response to selection on a particular trait is predicted to be the product of the level of genetic variation in the trait and the selection gradient (Falconer & Mackay 1996), the observed differences in the evolutionary response to selection should be attributable to differences in the amount of additive genetic variance between dispersers and philopatric birds, and not to differential selection pressures. Overall, these results suggest that differential dispersal is the most important factor influencing the evolutionary dynamics of body size within this pied flycatcher population.

The estimated response to selection for dispersers to the oak forest is at least twice as high as that of the other groups. Moreover, in contrast to the other groups, the negative selection gradients on dispersers to the oak forest resulted in a negative response to selection. Differential responses to selection on morphological traits in the absence of spatial variation in selection pressures have been previously found in a population of great tits (*Parus major*) occupying ecologically distinct sectors of woodland in Oxfordshire, UK (Garant et al. 2005). Habitat-specific differences in the expression of genetically based variation in great tit fledgling mass resulted in divergent phenotypic responses that, contrary to the general belief, seemed to be reinforced by phenotype-dependent dispersal between habitats (Garant et al. 2005). This also seems to be the case in the pine forest, where increasingly larger immigrants from the oakwood likely reinforced the positive trend of the native birds. By contrast, pied flycatcher size has remained unchanged in the oak forest despite immigration of increasingly smaller birds from the pinewood. Possibly, the relatively low reproductive success of dispersers into the oak forest could have 'muted' their genetic contribution to the overall population (i.e. dispersal > gene flow; Hendry 2004; Nosil et al. 2005). Perhaps, dispersers to the oak forest experienced a stronger intra-specific competition than local individuals owing to their average larger size (Ekman et al. 2000; Dickinson et al. 2014), although the exact reasons for breeding failure in this group remain unclear.

Our predictions of the direction of the evolutionary responses to selection are in good agreement with the phenotypic trends documented in the four dispersal groups. However, the predictive value of our estimates should be treated with caution, because most previous studies using long-term data to assess microevolutionary change in morphological traits in wild vertebrates have found substantial deviations from expectations (Merilä & Sheldon 2001; Merilä et al. 2001a; Brookfield 2016; but see Grant & Grant 1995). Discrepancies between observation and expectation have often been explained by changing environmental conditions over time (e.g. population density and intensity of competition or climate change; Cooch et al. 1991; Larsson et al. 1998; Merilä et al. 2001b; Garant et al. 2004), although other mechanisms might also account for the disagreement between observed and expected trends (see Merilä et al. 2001a; Kruuk et al. 2001; Charmantier & Garant 2005 for further discussion on this issue).

Our quantitative genetic analysis confirms earlier findings from other wild bird populations of significant additive genetic variation in tarsus length (e.g. Alatalo & Lundberg 1986; Larsson et al. 1998; Kruuk et al. 2001; Charmantier et al. 2004a; Poissant et al. 2016). Moreover, the estimated heritability of tarsus length in this pied flycatcher population is well within the range of values previously reported for this and closely related species (h^2 = 0.35–0.89; Alatalo et al. 1990; Potti & Merino 1994; Kruuk et al. 2001; Merilä & Sheldon 2001). Nonetheless, there were significant differences in the quantitative genetics of the four dispersal groups, as revealed by the genotype-by-dispersal interaction. Differences in the variance components of tarsus length have been previously found, for instance, between populations of blue tits (*Cyanistes caeruleus*) occupying different habitats (Charmantier et al. 2004a). When compared to this and other avian studies (Merilä & Sheldon 2001), the difference between some of the pools of birds examined in our study (e.g. philopatric individuals of the oak forest and dispersers from the pine to the oak forest) is striking given that they share the same breeding environment. Based on the relatively scant literature available, several mutually non-exclusive hypotheses can be formulated to explain this pattern.

First, differences in quantitative genetic parameters of morphological traits might reflect spatial and temporal variation in environmental quality due to e.g. density-dependent processes or differences in food availability (Merilä 1997; Merilä & Sheldon 2001; Garant et al. 2004). However, although a differential increase in breeding density and the intensity of competition for nest sites has been documented in the oak and the pine forest (Camacho et al. 2013), this is unlikely to explain the observed dissimilarities between groups, because the variance components of tarsus length differed markedly even between birds exposed to the same natal or breeding environment. Second, blood-sucking ectoparasites could also play a role, since they have been shown to affect the expression of additive genetic effects of tarsus length (Charmantier et al. 2004b) and the subsequent final adult size (Merino & Potti 1994). However, it is unlikely that variation in parasite prevalence at the nest level has induced differences between groups in additive genetic variance, because we have found no evidence of familiar resemblance in dispersal propensity among siblings from the same nests (Camacho et al. 2015).

Based on our estimates, the strength of selection on tarsus length within this population ranges from weak to non-existent depending on the fitness measure considered (see e.g. Kruuk et al. 2001 and Charmantier et al. 2004a for a comparison with other long-term filed studies on tarsus length; but see De Lisle & Svensson 2017). Dispersers and philopatric individuals appeared to be under qualitatively similar selection pressures –or more accurately, the lack thereof– despite being exposed to different breeding environments. These results are partially in disagreement with a previous study of this population where we showed that breeding success is associated with tarsus length in the pine but not in the oak forest (Camacho et al. 2015). However, unlike in this study, a formal selection analysis (*sensu* Lande & Arnold 1983) was not performed in Camacho et al. (2015) and, more importantly, only the first breeding attempt of males alone was considered.

Several explanations can be proposed to explain the apparent negligible selection on tarsus length in this pied flycatcher population. Estimates of selection gradients might vary across life stages (Millet et al. 2015; Bourret et al. 2017), as suggested by the slight discrepancy between estimates when different fitness components (e.g. offspring production or survival from fledging to adulthood) were used. Natural selection on tarsus length could thus be operating on differences in survival from fledging to adulthood, rather than on differences in reproductive output. Nevertheless, we cannot test this possibility because no dispersal category can be assigned to non-survivors and, therefore, potential differences in individual mortality depending on the dispersal strategy cannot be examined. Lastly, tarsus length might not be the true target of selection in the pied flycatcher so that, as found in the collared flycatcher (*F. albicollis*), the potential effects of selection on tarsus length might be masked by stronger selection on body condition (e.g. body mass corrected for skeletal size; Alatalo et al. 1990; Merilä et al. 2001b).

In summary, our results indicate that phenotype-dependent dispersal positively influences the evolutionary dynamics of tarsus length in the pine forest, whereas it seems to have a neutral effect in the oak forest, where dispersal might be substantially higher that gene flow. Taken together, the findings of this study suggest that the effects of natural selection on the evolutionary dynamics of a quantitative trait may be overwhelmed by differential dispersal, although other forces (e.g. genetic drift and mutation) cannot be ruled out. Further research is therefore needed to fully understand the proximate causes of the observed patterns.

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SUPPLEMENTARY MATERIAL 1: Phenotypic evolution of disperser and philopatric pied flycatchers of the oak and the pine forest.

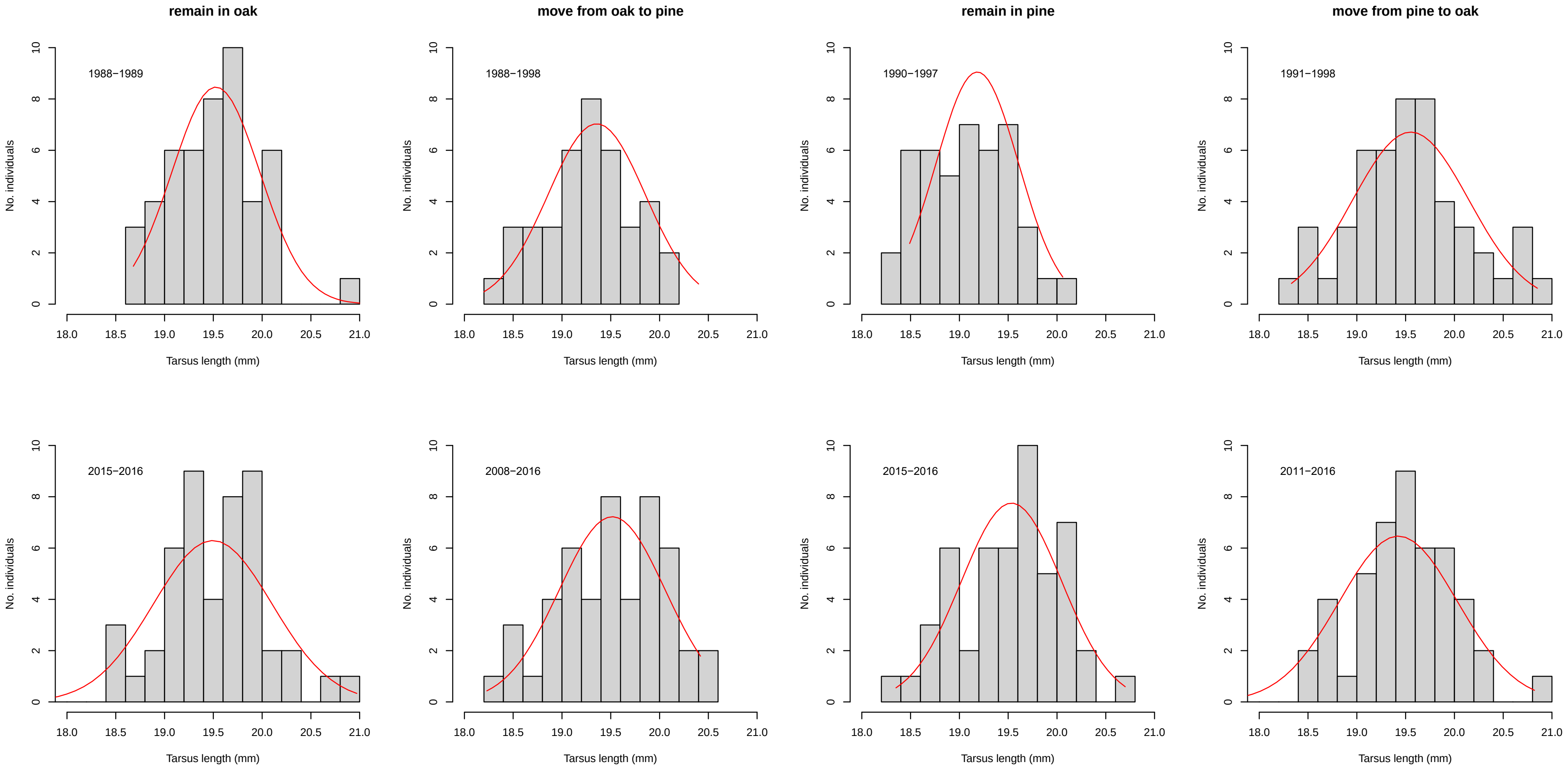

Figure SM1. Change in the frequency distributions of tarsus lengths of disperser and philopatric pied flycatchers of the oak and the pine forest between the beginning (upper panels) and end (bottom panels) of the study period. Note that time periods vary among plots because more years needed to be considered to reach a reasonable sample size (n = 50) of the less numerous groups.

DISCUSSION

This thesis documents the establishment, dynamics, and phenotypic evolution of a newly founded wild population of a migratory bird, the pied flycatcher, connected by dispersal to the natural ancestral population. Nest-box addition in the coniferous study site just three decades ago made an essential but previously unavailable resource available for pied flycatchers and, almost unintentionally, we witnessed an extremely rare phenomenon in the scientific literature: the establishment of the first individuals in a new area (Russell et al. 2005; Grant & Grant 2009). Since then, data on population size, dispersal rates, life-history and morphological traits of virtually all breeders and their descendants have been carefully collected on a yearly basis to document the eco-evolutionary dynamics of both populations. Each of the five chapters of this thesis already provides a thorough discussion of the relevant issues, so this final section represents a synthetic effort to integrate their main findings into an eco-evolutionary framework.

In 1988, a one-year-old male of unknown origin and a one-year-old female originating from the oak forest arrived at the pine forest to breed, thereby triggering the massive colonization of a novel environment. Less than a decade after the settlers' arrival, the number of breeding pairs in the pinewood reached values similar to those in the oakwood. **Chapter I** shows that phenotypic differences with respect to structural body size between the two populations arose immediately after the study began, which is reminiscent of the effects of a founder event (Reznick & Ghalambor 2001). However, new settlers into the pine forest did not differ significantly from residents of the oak forest, indicating that size-biased immigration into the pine population was not responsible for the rapid emergence of phenotypic structure. Examination of the individual phenotypes of the descendants of the new settlers in the pine forest nevertheless revealed visible differences depending on their natal dispersal behaviour (**Chapter II**). Pied flycatchers emigrating from the pine forest were larger than the average size of birds that remained there to breed, though this difference was more pronounced in males than in females. Differential patterns of phenotype-dependent dispersal in philopatric and non-philopatric birds of the pinewood therefore seem to be the major driver of microgeographic divergence within our study population. **Chapter II** challenges the general assumption that extensive dispersal and potential gene flow prevent microgeographic divergence (e.g. Hendry & Taylor 2004; Nosil & Crespi 2004; Moore et al. 2007), but rather add to recent studies suggesting that, if non-random, dispersal may contribute to the evolution of population divergence even at spatial scales of a few kilometres (Garant et al. 2005; Postma & van Noordwijk 2005).

Morphological differentiation at microgeographic scales has been extensively documented in sedentary and migratory birds, most often as a result of physical or environmental barriers to dispersal (Chan & Arcese 2003; Walsh et al. 2012; Bertrand et al. 2014; Garroway et al. 2013; Arnoux et al. 2014; García-Navas et al. 2014; Langin et al. 2015; Camacho et al. 2016a; Morinha et al. 2017). However, to our knowledge, a degree of morphological divergence close to that of pied flycatchers at the beginning of the study has never been reported at spatial scales comparable to ours.

Nevertheless, as shown in **Chapter I**, the initial degree of population differentiation was not sustained over time, mainly due to a gradual increase in mean body size of males breeding in the pine forest. Differential dynamics of phenotype-dependent dispersal in philopatric and non-philopatric birds of the pinewood seem to be the key force attenuating the size differences between populations. Males moving from the pine to the oak forest tended to be smaller over time, whereas those that remained in the pine forest tended to be larger. Dispersers from the oak to the pine forest also tended to be larger over time, and thus the positive trend of philopatric birds was furthermore reinforced (**Chapter II**). Most importantly, an analysis of both sexes combined confirmed the patterns observed for males alone, even when slight differences between males and females in the magnitude of phenotypic change over time might have attenuated the overall responses (**Chapter V**).

Besides the phenotypic trajectories of the dispersal groups, **Chapter II** investigates the ecological factors that may explain why the patterns of phenotype-dependent dispersal were reversed throughout the study period. Examination of commonly used predictors of habitat attractiveness for hole-nesting birds (e.g. breeding density, dispersal rates; Doligez et al. 2004; Robertson & Hutto 2006) suggests that the pine forest became progressively more attractive to new breeders at the expense of a decrease in the attractiveness of the oak forest. In the pied flycatcher, intrasexual competition by males is resolved by a size-based hierarchy, so that large and small males are generally sorted into preferred and non-preferred habitats respectively (Lundberg et al. 1981; Camacho et al. 2013). Based on the sharp increase in the number of pairs in the pine forest, it is not surprising that the attractiveness of this patch increased over time, especially because the presence of conspecifics is known to be a positive proximate cue for breeding habitat selection in *Ficedula* flycatchers (Alatalo et al. 1982; Doligez et al. 2004). Due to the lack of natural cavities, the intensity of competition for nest sites under high-density conditions was probably magnified in the pine forest compared to the oak forest, thereby enhancing the relevance of body size in territorial contests as the study progressed (Lundberg et al. 1981; Sirkiä & Laaksonen 2009). Non-random dispersal with respect to body size is therefore interpreted as the outcome of competitive interactions, in accordance with Fretwell's (1972) ideal despotic distribution model.

Chapter III provides further support for the role of competitive exclusion in the habitat segregation of phenotypes, since we found no conclusive evidence of adaptive habitat choice in relation to body size (i.e. matching habitat choice; Edelaar et al. 2008). By contrast, nearly all pied flycatchers return to their natal site to breed regardless of their phenotype, sometimes incurring severe fitness penalties. **Chapter IV** provides an explanation for this apparently maladaptive behaviour. By following cross-fostered individuals from fledging to recruitment, we demonstrated that the strongest force influencing the departure and settlement decisions of pied flycatchers is early experience in the natal site. Taken together, these results suggest that the potential of phenotype-dependent dispersal and matching habitat choice to increase the magnitude of divergence between the oak and the pine population might be constrained by the strong tendency of pied flycatchers to imprint on and return to their birthplace regardless of their morphology.

Quantitative genetic analyses presented in **Chapter V** demonstrates a genetic component of the phenotypic expression of tarsus length for all groups, except dispersers to the pine forest. Unexpectedly, the additive genetic variance of tarsus length differed markedly among individuals depending on their dispersal behaviour, an intriguing and largely unexplored pattern for which the limited current literature does not yet provide a satisfactory explanation (Charmantier et al. 2004b; Charmantier & Garant 2005). Our results indicate that individuals that are genetically predisposed to be larger preferentially dispersed into the oak forest. However, selection gradients on this group, unlike the other groups, were negative and therefore resulted in a negative response to selection (see Falconer & Mackay 1996). Remarkably, there was good agreement between the observed phenotypic changes and the evolutionary predictions, which is rarely the case in wild populations (reviewed by Merilä et al. 2001a and Brookfield 2016). Dispersers to the oak forest actually showed a gradual decline in tarsus length over time that, nevertheless, deviates from the lack of trend of the entire oak population. No such discrepancy was found in the pine forest, indicating a greater contribution of dispersers to the pine forest in shaping the distribution of phenotypes within their recipient population. Examination of the breeding success of each group suggests that this unusual asymmetry may result from uncoupled patterns of dispersal and gene flow in the oak forest. On average, the reproductive output of immigrants into the oakwood tends to be inferior to that of the other groups, and therefore, their genetic contribution to the overall population may vanish accordingly (Hendry 2004; Postma & van Noordwijk 2005; Nosil et al. 2005).

Overall, the findings of this thesis indicate that the effect of dispersal on microevolutionary processes may vary from being positive to non-existent depending on the habitat type, although the ecological factors responsible for such discrepancy (e.g. population density, food availability) remain to be elucidated. Dispersal and gene flow are obviously closely related, but our results suggest that the consequences of dispersal on gene flow may be habitat-specific and, therefore, confirm theoretical predictions that the strength of this relationship would ultimately shape the eco-evolutionary dynamics of wild populations. Examples of the eco-evolutionary consequences of phenotype-dependent dispersal are still very scarce in the literature (Garant et al. 2005; Postma & van Noordwijk 2005; Tarwater & Beissinger 2012), possibly because long-term data from continuously monitored populations of marked individuals are needed. Collectively, the five chapters presented in this thesis represent a major step forward in our understanding of the specific role of gene flow on eco-evolutionary dynamics and phenotypic evolution.

CONCLUSIONS

CONCLUSIONS

1. Pied flycatchers (*Ficedula hypoleuca*) breeding in different adjacent (1 km) habitats showed strong phenotypic variation in structural body size, indicating that population structure may evolve on a finer scale than is typically observed in mobile organisms.
2. Microsatellite analyses revealed a substantial degree of genetic differentiation given the absence of barriers to dispersal, suggesting congruence between genetic and phenotypic patterns of variation.
3. Microgeographic divergence was mainly driven by phenotype-dependent dispersal and the habitat segregation of size classes, although this pattern was much more pronounced in males than in females.
4. No clear evidence of adaptive dispersal in relation to habitat selection was found in the study species, possibly because most individuals, regardless of their morphology, imprint on and return to their natal site to breed.
5. Differential breeding density provides the ecological mechanism for phenotypic assortment. Pied flycatchers apparently use the presence of conspecifics as a cue for habitat selection but, under high-density conditions, larger males compete more effectively for nest boxes than smaller males. Consequently, individuals of different size tend to be sorted into distinct breeding habitats.
6. Based on local breeding densities and dispersal rates, it seems that the relative attractiveness of each habitat patch was reversed throughout the study and, as a result, the strength and direction of phenotype-dependent dispersal changed as well.
7. Birds breeding in different habitats showed contrasting phenotypic trajectories and, therefore, the magnitude of microgeographic divergence gradually decreased over time. Differential patterns of phenotype-dependent dispersal seemed to shape the phenotypic trajectories of both populations.
8. Quantitative genetic analyses provided evidence for a substantial component of additive genetic variance underlying structural body size, measured as tarsus length, although the variance components of this trait varied between pools of individuals differing in natal dispersal behaviour.
9. Differences in selection patterns between dispersers and philopatric individuals were comparatively smaller, suggesting that the observed differences in the evolutionary response to selection of each group should be attributable to the unequal amount of additive genetic variation.
10. Examination of the breeding success of dispersers into different habitats suggests that their genetic contribution to their recipient populations may be habitat-specific. Taken together, our results suggest that the strength of the relationship between dispersal and gene flow would ultimately shape the eco-evolutionary dynamics of quantitative traits in wild populations.

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AGRADECIMIENTOS

Es curioso. Me ha llevado mucho tiempo redactar las cien páginas anteriores y, sin embargo, estoy convencido de que la mayoría de los lectores sólo leerán ésta (Canal 2012). No es justo, casi nada lo es. Pero para compensar, trataré de serlo en estas líneas.

Si se trata de ser justos, me toca empezar por los principales damnificados. Mi familia y amigos merecen una disculpa por mis constantes y/o prolongadas ausencias. Jamás hicieron ninguna pregunta a pesar de no entender ni qué hacía ni por qué no dejé de hacerlo. Mis padres, además, merecerían un segundo volumen sólo para enumerar cada una de las ocasiones en que me tendieron la mano, o la guitarra... Esta tesis es tan vuestra como mía. La mayor parte de las ideas que figuran entre estas páginas maduraron al otro lado del oído de mi mujer, Alba. Ella solita ha soportado innumerables divagaciones en voz alta, la mayoría de las cuales desembocaron en un ejemplo sobre la gente que selecciona este bar y no el otro... En cualquier caso, su contribución va mucho más allá del papel de interlocutor y se resume en una sola palabra: equilibrio. Martín, por ahora tú sólo has sufrido mis ausencias, que seguramente ambos compensaremos con creces en apenas un mes.

Los primeros kilómetros del camino hasta llegar aquí resultaron casi impracticables aunque, de la mano de Sonia, se me hizo cuesta abajo. No sería justo no agradecer aquí todo el apoyo que recibí de tu parte cuando las cosas no eran sencillas. JuanPaco Beltrán, ahora mi tutor, siempre estuvo dispuesto a descolgar el teléfono para hacer una llamada, algo que siempre le agradeceré. Pedro Jordano es en buena medida responsable de esta tesis, pues movilizó cuantos recursos estuvieron en su mano para ofrecerme la posibilidad de trabajar haciendo Ciencia. Gracias, Pedro, tu gesto supuso un verdadero punto de inflexión y espero que puedas asistir de cerca de los que estén por llegar.

Hay un director para cada doctorando y, posiblemente también, un doctorando para cada director. Estoy seguro de que yo encontré el mío. Jaime Potti, al que le debo, sobre todo, respeto me ha dado, sobre todo, libertad. Jaime es fieramente humano, cercano, sabio y currante, y pese a ser mucho más grande que yo (no en talla), siempre me trató como a un igual. ¡Gracias! Fue un placer, jefe.

David Canal y Jesús Martínez-Padilla actuaron de directores en la sombra, por orden cronológico. Nunca podré agradeceros lo suficiente toda la ayuda prestada en lo personal y en lo profesional, respectivamente; y viceversa en tiempos recientes.

Loren Pérez-Rodríguez, Airam Rodríguez y Néstor Pérez, aunque pudieran parecer altamente consanguíneos, no lo son. Los tres, a los que admiro en lo humano y lo académico a partes iguales, han contribuido decisivamente a este trabajo, pues actuaron a la vez como sumidero de frustraciones y fuente de ideas y discusiones. Los compañeros de despacho

ayudaron cada uno en su medida, los que se fueron (una larga lista), los que llegaron (Quique y Sol) y los que no se van (Juanjo).

El campo es, sin duda, el alma de esta tesis. Desde que recluté en esta población, allá por 2009, han pasado por mis manos y las del resto varios miles de aves (casi 8000). Me gusta pensar que han sido muchas más sonrisas que pájaros. El grupo humano que cada primavera se reúne en La Hiruela (Madrid) para duplicar su modesta población se ha nutrido en los últimos años de personajes variopintos (variopinto es un eufemismo). María Cuenca, mi Perico, Carlos Marfil, Marta Guntiñas, Gerardo Jiménez, Gregorio Moreno-Rueda, Tomás Redondo, María Losada, Rocío Ruiz, Javi Manzano, Carlines, Loren, Pablo 'El inexperto', Julio Rabadán, Basti, Jesús M. Padilla, Justine Le Vaillant, Valeria Jennings y, por supuesto, Jaime Potti, han sufrido mi compañía en el campo mientras yo disfrutaba de la suya. David Ochoa y Fran Romero merecen una mención especial, pues hace ya tiempo que dejé de llamarlos compañeros para llamarlos amigos. Sin vosotros, esto no hubiera sido ni la mitad de divertido, y el dueño del único bar del pueblo sería la mitad de rico. Los vecinos de La Hiruela y el personal del Retén de Montesclaros siempre me hicieron sentir como en casa, aunque me declaro admirador y amigo del más hostil. La ventaja, Andrés, es que ya no necesito que haya papamoscas para querer subir a La Hiruela.

Esta tesis se ha realizado en detrimento de otra. Estoy seguro de que, todavía hoy, encontraré caras de sorpresa entre los lectores que intenten encontrar –sin éxito– la palabra chotacabras…

Triana, 18 de septiembre de 2017