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Research

A longitudinal analysis of the growth rate and mass of tail feathers in a great tit population: ontogeny, genetic effects and relationship between both traits

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Feathers have a diversity of functions in birds and are costly to produce, so their growth rate and mass can be reliable indicators of nutritional condition at the time of production. Despite the potential for feather metrics to advance our understanding of foraging, they are underused in avian ecology. One reason for this is the difficulty of interpreting whether individual variation is driven by ontogenetic, genetic or environmental effects, which is exacerbated by the fact that most analyses have been done on cross-sectional data. We addressed this deficit using a longitudinal dataset of tail feathers collected from great tits *Parus major* to test for ontogenetic and genetic effects on growth rate, mass and length, while controlling for body/feather size differences and other confounding factors. First, we found that the type of moult episode and experimentally-induced replacement differentially affected the length, mass and growth of feathers, providing evidence of an ontogenetic effect that should be considered when comparing these feather traits across individuals as a measure of condition. Second, we detected moderate to high repeatability and heritability values from parent–offspring regression for these three feather traits, which are suggestive of an underlying genetic component of variation. Third, we used a mean centring within-individual approach to test whether feather growth rate and feather mass (length-corrected) are indeed positively correlated with each other as overlapping indicators of body condition in birds, and found that this association, although positive, is weak and only significant between individuals. This suggests that both metrics are not so intimately linked as originally thought, and probably have different sensitivities to variation in foraging performance and ecological conditions. Together with the higher plasticity of feather growth rate compared to feather mass, our results support the idea that feather growth rate is better suited for examining short-term responses to environmental variation.

Keywords: adventitious replacement, growth bars, induced rectrix feather, Ireland, ptilochronology

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Introduction

Feathers fulfil multiple critical functions in birds, including flight, thermoregulation and social communication. Consequently birds invest heavily in their production and replacement through moult (Jenni and Winkler 2020a, b). Like many integumentary structures, fully-grown feathers are inert, and their physical (Dawson et al. 2000, Griggio et al. 2009) or chemical characteristics (Webster et al. 2002, Løseth et al. 2019) make it possible to reconstruct the conditions birds experienced during feather production in a relatively non-invasive manner. Many predominantly cross-sectional studies have used feather mass and metrics of feather growth rate obtained from the width of the naturally-occurring daily growth bars (i.e. ptilochronology, Fig. 1A; Grubb 1989, Brodin 1993) to infer foraging success and body condition during feather synthesis. This approach assumes that birds that are good at foraging and in good body condition will produce comparatively heavier feathers that will also grow relatively faster than those of birds in poor condition (Grubb 2006, Tellería et al. 2013). One advantage of measuring these feather traits is that they provide information about condition on a temporal scale of several days, or even weeks, unlike single-point morphometric indices (such as body mass or fat scores; Labocha and Hayes 2012), which are sensitive to temporal and environmental stochasticity. However, the potential association between feather metrics and body condition is likely influenced by ontogeny, genetics or energetic tradeoffs (Jenni and Winkler 2020b), and thus the influence of these factors needs to be better understood for a more reliable use of individual feather metrics as body condition indices.

Birds undergo different moult episodes during the course of their lives (sensu lato; Supporting information, Howell et al. 2003; but see Jenni and Winkler 2020b) and at specific times of the year, reflecting conditions at those specific times (Carlson 1998, De la Hera et al. 2009a). Feathers can also grow adventitiously when an original feather has been lost naturally or experimentally (Grubb 1989). This replacement most often occurs in winter (Grubb 2006), when birds normally struggle to acquire sufficient food. Many ptilochronological studies have ignored the moult episode that gave rise to each studied feather and assume that the growth rate and mass of feathers do not vary across episodes (Supporting information), which, if untrue, would lead to biased estimates (Murphy 1992, Talloen et al. 2008). Although cross sectional studies suggest systematic differences across moult episodes (reviewed in Jenni and Winkler 2020b), these studies do not account for ontogenetic and heritable effects (Van de Pol and Verhulst 2006). For example, the comparatively lighter mass of juvenile feathers (Jenni and Winkler 2020a) could be mediated by current environment conditions, or by a higher probability of heavy-feathered individuals surviving to older ages (i.e. a selective disappearance effect of light-feathered birds). Longitudinal datasets for the same feather position over different moult episodes are needed to help address these issues (Delhey and Kempenaers 2006).

Despite their ease of collection and storage, few studies have analysed longitudinal feather data in natural populations. Thus, the extent to which the feathers of individuals are truly plastic and thus their ability to reflect short-term environmental effects, such as changes in foraging success, is poorly known. Likewise, it remains unclear how heritable these traits are and whether they are potentially under selection within populations. Repeatability sets the upper limit of heritability (Dohm 2002) and a small number of studies suggest that feather growth rate and mass measurements are moderately repeatable (De la Hera et al. 2009b, Szép et al. 2019), reflecting intrinsic differences among individuals that have largely been ignored (Grubb 1989). Some or all of these intrinsic differences could be caused by permanent environmental effects (for life history trait examples, Quinn et al. 2009, Wilson et al. 2009), but they could also reflect additive genetic variation and therefore that the traits are heritable (Gienapp and Merilä 2010, De la Hera et al. 2013). Additional research on how feather traits differ between and
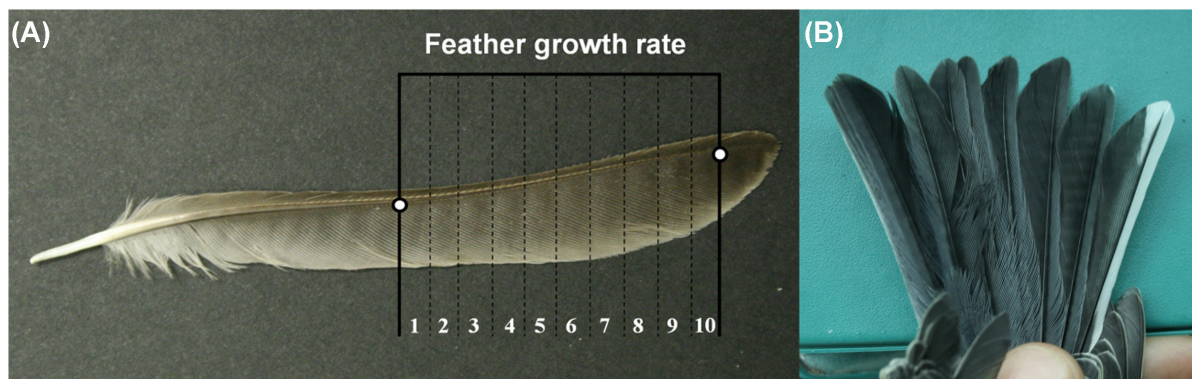

Figure 1. (A) An example of a tail feather (from a gray catbird *Dumetella carolinensis*) where 10 growth bars as they were considered in our study are delimited individually (Methods). Open circles in the figure represent the approximate positions of the two entomological pins to estimate feather growth rate (mm per 10 days). (B) Dorsal view of the tail of a great tit where the second outermost rectrix of bird's left side is adventitiously replaced after the original feather was plucked in a previous capture. Note that growth bars are narrower and particularly discernible in induced feathers compared to the rest of the rectrices.

within-individuals over time is required to better understand the potential population level consequences of this variation (Rowe and Houle 1996, Gienapp and Merilä 2010).

Feather mass is often overlooked in ptilochronological studies, and its relationship with feather growth rate has been rarely explored. This association is often assumed to be positive since both traits are considered overlapping indicators of body condition (Grubb 2006), i.e. feather growth rate reflects the condition of birds during 10 days of feather growth, and feather mass reflects condition during the whole process of feather synthesis. However, feather growth rate and feather mass can theoretically tradeoff against each other in some situations (Dawson et al. 2000, De la Hera et al. 2009a), predicting a negative relationship. Limited evidence to date suggest a negative association between feather mass and feather growth rate at the between-population level (De la Hera et al. 2009a), and a positive association among and within individuals of the same population (De la Hera et al. 2009a, Szép et al. 2019, Jenni and Winkler 2020b). The use of longitudinal feather data could help to clarify this question because the within-individual centring approach that is needed to detect what could be subtle effects (Van de Pol and Wright 2009) has never been applied directly on these two feather characteristics (see Szép et al. 2019 for an example analysing a combination of feather traits, but not feather mass alone). Moreover, given that long feathers are usually heavier and have wider growth bars than short feathers (Rohwer et al. 2009, De la Hera et al. 2011), a reliable analysis of the relationship between growth rate and mass would require controlling for feather size (typically feather length) to avoid spurious positive associations. Whether this precaution has been considered in previous research is not always clear (De la Hera et al. 2009b, but see Szép et al. 2019), and in general the relationship between feather mass and feather growth remains poorly understood.

In this study, we used a longitudinal dataset of tail-feather growth and mass measurements – consisting of original and experimentally induced feathers (four feather 'categories', Supporting information) collected from great tits *Parus major* over three years – to assess three different objectives. First, we examined the differences in feather growth rate, mass and length in the same 'feather position' grown during the four different episodes that can occur in great tits (i.e. three different moults plus adventitious replacement; Jenni and Winkler 2020a; Supporting information). Thus, our longitudinal dataset allowed us to test for ontogenetic within-individual changes in the metrics of the tail feather (Delhey and Kempenaers 2006), after controlling for other confounding factors (e.g. bird size, feather length, moult year, sex). Our second objective was to assess whether feather metrics of the same tail feather differed consistently among individuals, controlling for size, feather category and other sources of variation, pointing to intrinsic differences among individuals in these traits. For this purpose, we estimated adjusted repeatability values (Nakagawa and Schielzeth 2010) for the growth rate, mass and length of tail feathers. We combined this repeatability analysis with an estimation of the proportion of total phenotypic variance in these feather characteristics that can be explained by additive genetic variance (i.e. narrow-sense heritability; Falconer and Mackay 1996) using the regression slopes from a sub-sample of parent-offspring feather data, thus testing whether the observed intrinsic differences might indicate the potential for a response to natural selection on these traits (Edwards et al. 2017). Finally, for a subsample of individuals for whom we had multiple feathers, and for both 1) postbreeding and 2) induced tail feather categories, we used a within-individual centring approach to explore the association between feather mass and feather growth rate at the between and within-individual level. We predicted positive relationships between both traits if, as it has been traditionally assumed, both variables respond similarly to variation in environmental conditions (Grubb 2006).

Methods

Study area and feather collection

The study area comprised 12 woodland plots (area ranged from 11 to 25 ha) in the Bandon Valley, County Cork (Ireland), where nestboxes were installed for monitoring the breeding performance of hole nesting birds (tit species; Supporting information). Great tits were caught during the breeding season at nestboxes using clap-traps, or outside of the breeding season near previously installed feeders (typically between October and February) using mist-nets. Birds were fitted with unique-coded metal rings issued by the British Trust for Ornithology. Their age (first-year or older birds) and sex were determined according to plumage features. A digital calliper (resolution of 0.01 mm) was used to measure the distance between the inter-tarsal joint and the joint between tarsus and toes, with the latter bent at approximately 90° in relation to the tarsus (minimum tarsus; Demongin 2016).

As for many birds, great tits have a predictable moulting strategy involving a number of moulting episodes (Supporting information; Jenni and Winkler 2020a) as follows: 1) fledging period or prejuvenal moult (May–July in our population; O'Shea et al. 2018); 2) post-juvenile moult (July–September); and 3) postbreeding moult during the summer months (June–September). Which feathers are involved in each moult varies. During the fledging period (see the Supporting information for our working definition of this term) and the post-breeding moult the whole plumage is (re)grown, while the post-juvenile moult normally involves the replacement of all body feathers and a variable number of wing and tail feathers, where nearly 80 percent of juveniles in our population (n = 84) had the collected tail feather moulted (Jenni and Winkler 2020a). Great tits can also replace feathers that have been lost adventitiously, either naturally or experimentally (Willoughby et al. 2002), the latter normally leading to full regrowth within eight weeks (Carrascal et al. 1998, and own data in the Supporting information).

From November 2016 until June 2019, we sampled one fully-grown original tail feather from each captured bird

between the months of September and June. This was normally a rectrix, number 5 from the left (Jenni and Winkler 2020a), although the right-side rectrix 5 was sampled if the former was damaged, growing or if suspected to be a naturally induced feather (i.e. the original feather was not extracted by us, but lost by unknown causes). We collected, by plucking, 759 naturally moulted feather samples during the above-mentioned period and sites. In addition, we also plucked and stored 132 of the resulting induced feathers if fully grown when birds were recaptured. All induced feathers that were collected during the sampling period (September–June) were assigned to the same moult year (May–September) of the original feather they replaced. All feathers were stored in individual paper envelopes for later analyses.

Feather measurements

Feather category was determined with reference to the age of birds and whether feather loss had been induced. We obtained feather mass using a high resolution digital balance (Mettler Toledo AT201, resolution 0.01 mg). Feather growth rate was estimated using the pattern of alternate dark and light bands perpendicular to the feather shaft (Fig. 1A), a pair of which reflects one day's growth (Brodin 1993, Grubb 2006). These bands were visible to the naked eye in all of our tail feathers, except in 18 juvenile feathers. Feathers were placed on a black card over a thin cork sheet. Two entomological pins were inserted through the barbs and as close as possible to the inner side of the rachis, in order to delimit 10 growth bars centred around two thirds of feather length from its base (Fig. 1A). The distance between pins was measured with a digital calliper (0.1 mm resolution) after removing the feather, and used as our estimate of feather growth rate (mm/10 d). Feather growth rate was measured twice for all feathers (except for the juvenile group that was not measured). The growth rate estimate was highly repeatable across measurements, when the three feather categories were pooled together (intraclass correlation coefficient $R_i = 0.99$) and when they were analysed separately (postbreeding feathers: $R_i = 0.95$, n = 255; postjuvenile feathers: $R_i = 0.91$, n = 66; induced feathers: $R_i = 0.94$, n = 126; Lessells and Boag 1987). We used the mean values of these two measurements in subsequent longitudinal analyses. We also measured the overall length of each feather (from inferior umbilicus to the distal tip) using a digital calliper (resolution 0.1 mm) to control for differences in feather size in the analyses.

Final feather dataset analysed

To ensure our dataset was longitudinal, we only considered individuals that had feathers from at least two different feather categories within the same moult year (i.e. one induced feather and one of the three categories of original feathers), or two original feathers produced during different moult years. Data from two woodlands with a very limited number of feather samples (i.e. Farran and Piercetown with 5 and 4 feather samples, respectively) were removed to avoid problems

of convergence associated with site in the models. Moreover, we excluded one malformed feather and 8 feathers with wear levels that could affect the length and mass measurements (if wear was stronger than 'moderate', Baker 1993). Finally, four extremely short and slow-growing feathers (three induced feathers and one postbreeding feather that, very likely, was actually an induced feather too) were also discarded. This left a final sample size of 465 feathers from 179 different individuals (2–6 feathers per individual; Supporting information).

Statistical analyses

We used linear (Gaussian) mixed models to model feather length, feather mass and feather growth rate, with feather category, sex and their interaction as fixed effects, and with moult year (three levels: 2016, 2017 and 2018), individual identity (ring code) and site (ten woodlands) as random effects. The average of the different tarsus length measurements available for each individual was also included as an estimate of body size. For the feather mass and growth rate models, we also added feather length as a covariate to control for feather size, in order to determine which feather categories were heavier or more fast-growing regardless of their size. Finally, we repeated a similar analysis for feather growth rate, but using feather mass as a covariate instead of feather length, to confirm that results were the same regardless of which covariate was used (Jenni et al. 2020). We only considered random intercepts in our models because our data did not provide enough replicates within feather categories to conduct random slope analyses, which would have been a more appropriate analytical approach (Schielzeth and Forstmeier 2009). We used the lmerTest package in R ver. 3.6.3 (<www.r-project.org>) and obtained the minimal adequate model in each case using its *step* function, which performs a backward elimination of random-effects terms followed by a backward elimination of fixed-effects terms (Kuznetsova et al. 2017). This was considered appropriate over model averaging since few variables were involved.

We obtained the adjusted repeatability values (R) from the variance components of the minimal adequate model for each of the three feather traits detailed above. This was done by dividing the variance attributable to individual identity by the overall variance (Nakagawa and Schielzeth 2010). We also ran the minimal adequate models in the rptR package to obtain the 95% confidence intervals of R for the three feather traits (Nakagawa and Schielzeth 2010, Stoffel et al. 2017). We additionally used feather data from 19 nestlings that were recaptured as fully-grown birds later in life and their parents to estimate heritability of feather traits using parent–offspring regressions. Although obtaining reliable estimates of heritability would require a much larger sample size (n > 200; De Villemereuil et al. 2013), ideally in a cross generational pedigree to reduce the influence of shared environmental effects, we undertook these analyses to tentatively explore whether variation in heritability values between feather traits paralleled the results obtained in the repeatability analyses (Dohm 2002). We had genetic information (i.e.

microsatellite markers) for 15 of these 19 individuals and most of their parents (Reichert et al. 2021), which revealed one case in which the social and the biological father did not match (extra-pair paternity), but the latter could be identified and corrected in the final dataset. For the four individuals lacking genetic data, we assumed the social father was the biological father. There were four pairs of siblings among these 19 individuals, whose values were averaged, and two individuals that had the same mother but different fathers. Given that these two individuals cannot be considered independent data points, one of them was randomly excluded. In the end, we used 14 parent–offspring pairs for which we could calculate the average value between mother and father for most cases (n = 11). Feather data from only one parent was known in the remaining three cases, so we used this single value instead. We explicitly explored how the slopes of the regression between offspring and parent feather values (i.e. an estimate of the narrow-sense heritability; Falconer and Mackay 1996) varied between traits. The feather values used in these parent–offspring regressions were the residuals from linear (mixed) models for each feather trait that were similar to the models described previously (see the Supporting information for additional details) and, most importantly, accounting for body and feather size, for both parent and offspring. We had insufficient statistical power to take a multivariate approach to the parent–offspring regression. We averaged the residuals in individuals with more than one feather available. Individual feather trait values were standardized before estimating the regression slopes.

Finally, we tested explicitly whether feather mass and feather growth rate, both corrected for length, were positively correlated between and within individuals as ptilochronological studies assume (Grubb 2006). For this purpose, we used two different datasets: one of postbreeding feathers, and the second of induced feathers. In both cases, we restricted the sample to individuals with at least two different feathers of the same category. This provided 176 postbreeding feathers from 80 individuals for the first dataset, and 42 induced feathers from 19 individuals for the second. In both the postbreeding and induced feather mass analyses, we included the same predictors that were significant in the analysis of feather mass in Table 1, with the exception of feather category, since two independent datasets were created for each category of feather. Thus, we included feather mass as a dependent variable, individual identity and moult year as random factors, feather length as a covariate, sex as a fixed effect factor, the mean value of feather growth rate for each individual as the between-individual effect, and the value of growth rate for each feather minus the individual's mean value as the within-individual effect (Van de Pol and Wright 2009). The calculations for the between- and within-individual effects were obtained from standardized growth rate values corrected for the allometric effects of feather length (Lleonart et al. 2000), which allowed us to remove the effect of feather length on feather growth rate variation. Thus, the scaling relationship of growth rate on length was allometrically negative in both postbreeding (scaling coefficient = 0.56, n = 176) and induced feathers (scaling coefficient = 0.68, n = 42). After the

Table 1. Results of the minimal adequate models for (A) feather length (mm), (B) feather mass (mg) and (C) feather growth rate (mm/10 d; all from the tail). The effects removed during the backward elimination of non-significant effects by the *step* function in *lmerTest* (Methods) are identified by the term '*eliminated*' and in parentheses the p-value when dropped from the model. Note that juvenile feathers were excluded from the analysis of feather growth rate and are marked as not applicable, or '*NA*', in the table.

Random effects	(A) Feather length (mm) Variance ± SD			(B) Feather mass (mg) Variance ± SD			(C) Feather growth rate (mm/10 d) Variance ± SD		
Individual	1.96 ± 1.4 (465 obs., 179 id.)			0.103 ± 0.322 (465 obs., 179 id.)			0.518 ± 0.720 (447 obs., 179 id.)		
Site	0.21 ± 0.45			*eliminated (p = 1)*			*eliminated (p = 0.710)*		
Moult year	*eliminated (p = 0.509)*			0.023 ± 0.15			0.157 ± 0.397		
Residual	0.74 ± 0.858			0.024 ± 0.155			0.987 ± 0.994		
Fixed effects	Estimate ± SE	t-value	p-value	Estimate ± SE	t-value	p-value	Estimate ± SE	t-value	p-value
Intercept (female, postbreeding)	63.44 ± 0.22	285.52	< 0.001	−5.61 ± 0.58	−9.68	< 0.001	−0.19 ± 2.50	−0.08	0.939
Tarsus length	*eliminated (p = 0.596)*			*eliminated (p = 0.874)*			*eliminated (p = 0.822)*		
Feather length	NA			0.21 ± 0.01	22.80	< 0.001	0.44 ± 0.04	11.33	< 0.001
Sex (male)	3.75 ± 0.23	16.58	< 0.001	0.39 ± 0.06	6.21	< 0.001	−0.99 ± 0.21	−4.71	< 0.001
Feather category (postjuvenile)	−1.29 ± 0.14	−9.16	< 0.001	0.06 ± 0.04	−1.61	0.108	0.81 ± 0.17	4.72	< 0.001
Feather category (induced)	−3.25 ± 0.11	−30.59	< 0.001	−0.02 ± 0.04	−0.46	0.647	−5.48 ± 0.17	−31.64	< 0.001
Feather category (juvenile)	−3.19 ± 0.26	−12.30	< 0.001	−0.42 ± 0.07	−6.03	< 0.001	NA		
Sex:feather category (male, postjuvenile)	*eliminated (p = 0.387)*			−0.10 ± 0.05	−1.91	0.057	*eliminated (p = 0.814)*		
Sex:feather category (male, induced)				−0.12 ± 0.04	−3.16	0.002			
Sex:feather category (male, juvenile)				−0.31 ± 0.10	−3.17	0.002	NA		

transformation, the standardized values of feather growth rate and feather length were perfectly orthogonal.

Results

Factors affecting feather length and repeatability

The minimal adequate model for feather length included sex and feather category as fixed effects, and individual identity and site as random effects (Table 1A). After controlling for sex, because males had relatively longer tail feathers than females, and the other significant factors, the least squares means comparisons showed that postbreeding feathers were the longest category (Fig. 2A), differing significantly from postjuvenile, juvenile and induced feathers (Table 1A). Postjuvenile feathers were also significantly longer than juvenile (estimate 1.90 ± 0.29 mm, t=6.63, p < 0.001) or induced feathers (estimate=1.95 ± 0.15 mm, t=12.91, p < 0.001), whereas these latter two feather categories did not differ between each other in their length (estimate=0.05 ± 0.26 mm, t=0.21, p=0.836; Fig. 2A). The adjusted repeatability (R) for feather length after accounting for the significant effects in the model was 0.67 (CI 95%=0.57–0.76).

Factors affecting feather mass and repeatability

Feather mass increased with feather length, was comparatively greater in males than in females, and differed significantly among feather categories; there was also a significant interaction between feather category and sex (Table 1B). Thus, juvenile feathers were significantly lighter than any other feather category and did not exhibit the sex differences observed for postjuvenile, postbreeding and induced feathers (Fig. 2B). The best model also included individual identity and moult year as random effects. The adjusted repeatability (R) for feather mass was 0.69 (CI 95%=0.49–0.83).

Factors affecting feather growth rate and repeatability

Feather growth rate increased with feather length, and was greater in females when compared to males. After controlling for these length and sex effects, there were significant differences between feather categories (Table 1C, Fig. 2C). Thus, postjuvenile feathers grew faster than postbreeding feathers, whereas induced feathers showed the slowest growth rates and differed significantly from the other two feather categories analysed (postjuvenile estimate=6.29 ±0.19 mm/10 d, t=33.98, p < 0.001; Table 1C). Individual identity and moult year were also included in the final model for feather growth rate. Adjusted repeatability (R) of feather growth rate was 0.31 (CI 95%=0.21–0.43). All these results did not change qualitatively if feather growth rate was corrected for feather mass rather than feather length (R=0.32 [CI 95%=0.21–0.44], Supporting information).
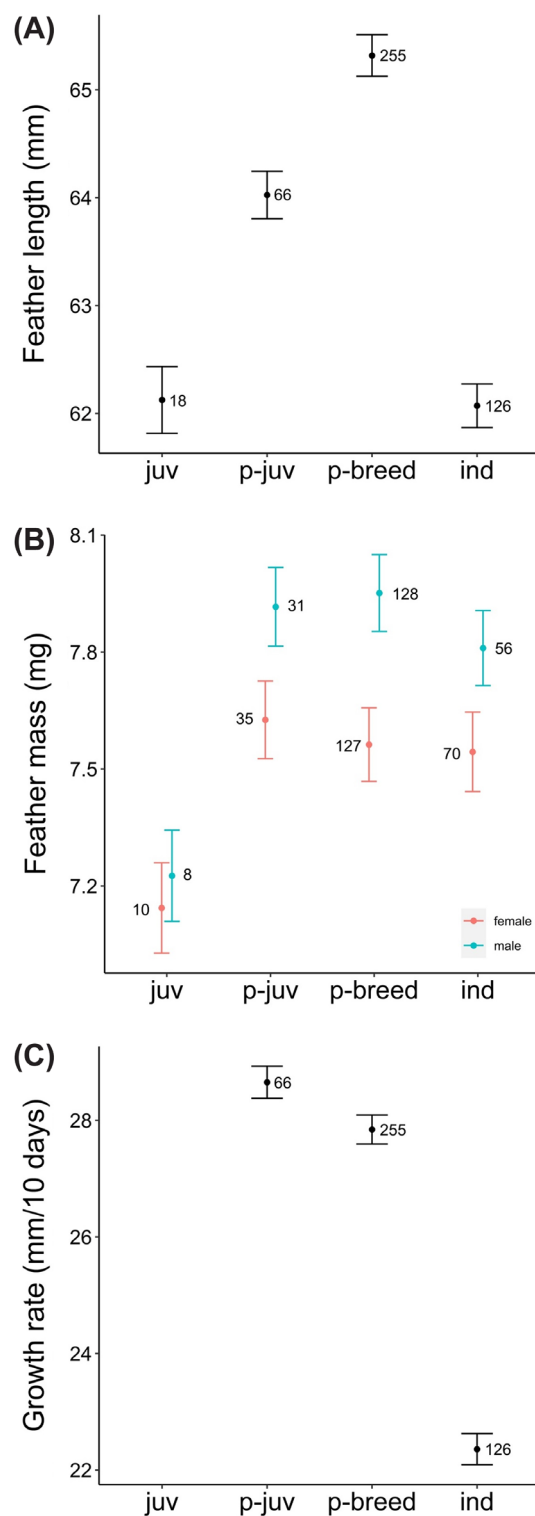

Figure 2. Variation in feather length (A), mass (B) and growth rate (C) between juvenile (juv), postjuvenile (p-juv), postbreeding (p-breed) and induced (ind) feathers. Feather trait variation is represented in the graphs as the least squares means and standard errors according to the models shown in Table 1. Note feather growth rate was not measured in juvenile feathers so there are no data shown for this feather category in the corresponding figure. Numbers beside the whiskers indicate sample sizes.

Offspring–parent regression to estimate heritability

The slope of the regression line between offspring and parents' values was high and significant for feather length (b = 0.69 ± 0.21SE, p = 0.006), high and marginally non-significant for feather mass (b = 0.51 ± 0.25SE, p = 0.061), and moderate and non-significant for feather growth rate (b = 0.33 ± 0.27SE, p = 0.253; Fig. 3).

Association between the growth rate and mass of feathers

In both the postbreeding and induced feather datasets, the relationship between feather mass and feather growth rate (length-corrected) was positive after controlling for feather length and other confounding factors, but this association was only significant for the between-individual effects in the induced feathers and not the within-individual effects (Table 2).

Discussion

We used different feather categories from the same birds, that is, samples of the same feather position produced during different moult episodes, to confirm size-independent systematic changes in feather trait values over individual lifetimes, providing evidence for ontogenetic variation in the length, mass and growth rate of tail feathers. Furthermore, we found

significant repeatability values for all feather traits, which were moderate to high for the structural traits (length and mass) and moderate to low for growth rate. After controlling for length-related variation, the association between feather growth rate and feather mass tended to be positive, but was only significant between individuals, suggesting that these two metrics are not so intimately linked as initially thought (Grubb 2006) and, hence, show different sensitivities to variation in environmental conditions and/or foraging performance. We found moderate to high associations between offspring and parents' feather characteristics, and coupled with the significant repeatabilities detected, this suggests that feather traits could be heritable.

Causes and consequences of variation among feather categories

Regardless of whether selective disappearance of individuals with certain feather characteristics occurs in our population (Rebke et al. 2010), our longitudinal dataset supports the idea that feather characteristics are, at least partly, ontogenetically determined, assuming the changes did not simply covary with some unknown environmental component over time. Feather characteristics varied systematically between feather categories within individuals over their lifetimes (Delhey and Kempenaers 2006, Pagani-Nunez and Senar 2012). Although fragmentary information exists from cross-sectional studies on how growth rate and mass vary between
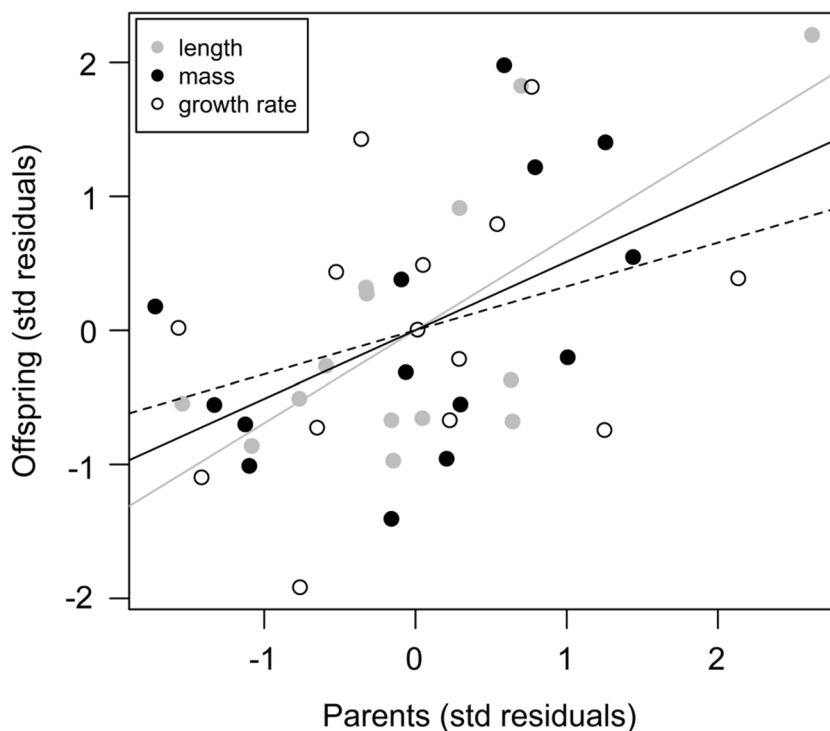

Figure 3. Relationship between offspring and parent values (n = 14) for feather length (grey points and line), mass (solid black points and line) and growth rate (white points and broken line). Values are the standardized residuals of the statistical models analysing feather trait variation that are shown in the Supporting information.

Table 2. Linear mixed models of postbreeding and induced feather mass (mg), exploring their relationships with (length-corrected) feather growth rate (mm/10 d) at the between-individual and at the within-individual levels (Methods; Van de Pol and Wright 2009), after controlling for feather length and other factors affecting feather mass (Table 1).

Random effects	Postbreeding feather mass (mg) Variance ± SD			Induced feather mass (mg) Variance ± SD		
Individual	0.11 ± 0.33 (176 obs., 80 id.)			0.06 ± 0.25 (42 obs., 19 id.)		
Moult year	0.03 ± 0.18			0.05 ± 0.23		
Residual	0.02 ± 0.13			0.03 ± 0.16		
Fixed effects	Estimate ± SE	t-value	p-value	Estimate ± SE	t-value	p-value
Intercept (female)	−4.75 ± 1.42	−3.36	0.001	−6.10 ± 3.18	−1.92	0.064
Feather length	0.16 ± 0.02	10.83	< 0.001	0.14 ± 0.04	3.98	< 0.001
Sex (male)	0.64 ± 0.10	6.59	< 0.001	0.63 ± 0.21	3.07	0.004
Between individual feather growth rate	0.06 ± 0.04	1.67	0.098	0.08 ± 0.04	2.32	0.031
Within individual feather growth rate	0.02 ± 0.02	1.07	0.289	0.12 ± 0.10	1.14	0.268

feather categories (Jenni and Winkler 2020a, b), our study is the first to simultaneously assess the differences in all feather categories that can occur in one bird species, emphasising the importance of classifying the correct feather category (Murphy and King 1991). This point is often overlooked (Talloen et al. 2008) but would be particularly important to consider in order to avoid biases when feathers are directly compared between groups of interest (Carbonell and Tellería 1999), or when they are used as a control for induced feather trait comparisons (Talloen et al. 2008).

Induced and juvenile feathers were significantly shorter than postbreeding and postjuvenile feathers, and induced feathers had by far the slowest growth rate. In the case of the juvenile feathers, although growth bars were not visible, such that feather growth rate could not be estimated, we know that the developmental speed of the juvenile plumage is typically very fast in passerines (Ricklefs 1968). This fact together with the simultaneous production of the whole plumage and other body structures in nestlings/fledglings would increase dramatically the energetic demands per unit time (Supporting information), thereby compromising the structural complexity and mass of juvenile feathers (Jenni and Winkler 2020a). In contrast, the energetic demands of replacing adventitiously a single induced tail feather are much lower than during natural moulting, and yet induced feathers were still comparatively short and slow-growing. One likely explanation for this is that the physiological processes that promote feather anabolism during normal moult (e.g. protein turnover and metabolism) are partially deactivated outside of these periods (Hoye and Buttemer 2011, Ben-Hamo et al. 2017, Jenni and Winkler 2020b). Other studies also reported that induced feathers were shorter, had different coloration or structural properties, and grew much slower than the original feathers they replaced (Talloen et al. 2008, De la Hera et al. 2010, Tonra et al. 2014). This last characteristic in particular is useful for distinguishing induced feathers from other feather categories (Fig. 1B). In our study, the growth rate of most induced feathers (range: 17.8–26.1 mm/10 d; n = 126 feathers) was lower than the growth rate of postbreeding (range: 24.1–32.8 mm/10 d; n = 255 feathers) or postjuvenile feathers (range: 24.9–30.8 mm/10 d; n = 66 feathers), so that feathers produced at less than 24 mm per 10 days in

great tits would most likely be induced and could be excluded to avoid potential bias in some analyses (Matysioková and Remeš 2010, De la Hera et al. 2011).

Postjuvenile and postbreeding feathers were both different from the juvenile and induced feather categories, but they also differed from one another in a number of respects. Postjuvenile feathers grew faster and were shorter than postbreeding feathers, but they did not differ in mass after controlling for feather length. These patterns reinforce the idea that individual birds take a longer time to produce similarly, or even more, complex feathers as they age, as has been shown also in sand martins *Riparia riparia* (Szép et al. 2019) and Eurasian blackcaps *Sylvia atricapilla* (De la Hera et al. 2009b). These two studies were based on longitudinal data that analysed the relationship between bird age in years and the characteristics of a single feather category (e.g. postbreeding or prebreeding feathers), but our study shows that this reduction in growth rate and the increase in feather length already occurs between the postjuvenile moult and the first postbreeding moult (Fig. 2).

Repeatability and heritability

Our study reaffirmed that structural traits are more repeatable than feather growth rate. Observed repeatability values in our study resemble the scores obtained in a study that analysed a small sample of postbreeding feathers in Eurasian blackcaps (R was 0.87, 0.79 and 0.29 for length, mass and growth rate, respectively; De la Hera et al. 2009b). These repeatability values suggest that these traits could be heritable, and therefore have the potential to respond to selection (Dohm 2002, Gienapp and Merilä 2010, De la Hera et al. 2013). For example, a fast feather growth rate could be favoured in populations that are temporally constrained by migration or predation pressures (Butler et al. 2008, Martin et al. 2018, Møller and Nielsen 2018), whereas heavier and longer feathers that typically have better mechanical properties and more resistance to becoming worn (Dawson et al. 2000, De la Hera et al. 2020) could be favoured in migrants or in birds living in habitats that promote feather degradation.

Repeat measures over different life stages also allow an estimate of phenotypic plasticity, since the proportion of total

variance that is within-individuals (i.e. $1 - R$) is attributable to a combination of phenotypic flexibility and measurement error (Dohm 2002). Our measurement error was very small, as shown by the repeatabilities from the same feather reported in the methods above, and therefore the within individual variance measured is primarily caused by plasticity. By using a large sample size and analysing the three feather traits on the same sample of tail feathers, our study confirms that feather growth rate is a more phenotypically flexible trait than feather mass and length, as previous evidence already suggested (De la Hera et al. 2009b, 2013, Gienapp and Merilä 2010).

Although the parent–offspring regression was significant only for feather length, the moderate to high positive slopes observed for feather growth rate and mass, respectively, provide additional support for the heritability of all these traits (Fig. 3), and it is likely that non significance was due to a type II error caused by small sample size specific to this analysis ($n = 14$). Results of these regressions paralleled the repeatability analyses: feather structural traits (length and mass) also had larger parent–offspring slopes than feather growth rate (Fig. 3). On the one hand, low local recruitment caused by small woodland sizes (O'Shea et al. 2018) meant that recapturing nestling great tits was challenging, limiting the number of parent–offspring comparisons, and preventing an animal model which theoretically should improve the heritability estimate (Wilson et al. 2009). On the other, this low recruitment also meant that our heritability estimate may well be relatively accurate because adults are unlikely to have been born in the same wood as the nestlings in the parent–offspring analysis, and therefore shared environment effects from the immediate local environment were unlikely to be strong. Once again, then, our results raise the possibility that feather metrics could be the target of selection in our study population (Takaki et al. 2001, Gienapp and Merilä 2010).

Association between the growth rate and mass of feathers

The parameter estimates in our results after accounting for other relevant factors, such as feather length, showed a positive relationship between feather growth rate and feather mass. In no case was this association negative, supporting the idea that these two traits do not tradeoff with each other, at either the between or the within-individual level within populations, but might do so between populations (De la Hera et al. 2009a, Jenni and Winkler 2020b). These observed positive values were only clearly significant between individuals in the induced feather dataset, while there was only a weak tendency towards significance in the post-breeding feather category (Table 2). At the within-individual level, there was no evidence at all of any correlation, even though 20 percent of the variance in the induced feather mass analysis was within individuals (the error component). This goes against the widely-accepted view that feathers that grow comparatively faster for their length are also comparatively heavier (Grubb 1992, De la Hera et al. 2009b, Szép et al. 2019). One possible reason for the discrepancy is that these metrics

estimate body condition on different temporal scales that might not necessarily be correlated. Thus, feather growth rate provides information on body condition over 10 days, whereas feather mass does so for the whole period of tail feather synthesis, which is estimated to average 24.7 days ($\pm$ 3SD) assuming feather growth rates are constant along the feather. More likely, the lack of correlation between both traits could be mediated by the lower plasticity of feather mass compared to feather growth rate, suggesting that the former might be less sensitive to variation in foraging performance and ecological conditions. Therefore, feather growth rate is likely to provide the best indication of between and within individual variation in body condition within populations, which could have important consequences for studies that are interested in monitoring short term responses to change. However, both feather growth rate and feather mass might not be useful for comparisons of body condition across populations (Jenni and Winkler 2020b), particularly if life-history traits (such as migratory behaviour) that can affect feather characteristics differ between populations (De la Hera et al. 2009a, Hernández-Téllez et al. 2021).

Concluding remarks

This study supports the idea that feather growth rate and feather mass are not so strongly linked to body condition or nutritional state as it was initially conceived, but these metrics can also be significantly affected by other factors that need to be taken into consideration, such as feather category, feather size or sex. After controlling for these confounding variables, we also confirmed that the expression of these feather traits had a significant individual, probably at least partially genetic, contribution (Bazzi et al. 2017), which was particularly true for feather mass, and this appeared independent of the well-known heritability of body size (tarsus length had no effect). Given that feathers are metabolically inert once their growth is completed, the structural configuration achieved during their production can affect some key feather functions and, hence, fitness. Consequently, it is reasonable to hypothesize that, at least in certain cases, feather growth rate and mass could be under selection and shaped by adaptive evolution. The selective pressures acting on these feather traits might also differ over the lifetime of birds, which could be an additional factor contributing to the within individual (ontogenetic) differences observed.

Permits – This study was conducted under licences from the Health Products Regulatory Authority (HPRA), The National Parks and Wildlife Services and the British Trust for Ornithology, and permission from Coillte Forestry and private landowners. The research project received ethics approval from the Animal Welfare Body at University College Cork and the HPRA (AE19130/P017), and was in accordance with the Guidelines for the Treatment of Animals in Behavioural Research and Teaching raised by the Association for the Study of Animal Behaviour.

Author contributions

Iván de la Hera: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Writing – original draft (lead). **Michael S. Reichert**: Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing – original draft (supporting). **Gabrielle L. Davidson**: Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing – original draft (supporting). **John L. Quinn**: Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Writing – original draft (supporting).

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Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.3xsj3txj3> (De la Hera et al. 2022).

Supporting information

The supporting information associated with this article is available from the online version.

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