Nestling Physiology Is Independent of Somatic Development in a Common Raptor, the American Kestrel (*Falco sparverius*)

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ABSTRACT

Although many studies have documented the developmental trajectory of somatic traits in birds, few measure physiological traits, and even fewer document individual variation in developmental trajectory across ecological context. Hematological traits underlying aerobic capacity can be predictive of nestling survival, fledgling flight ability, and ultimately recruitment. This study aimed to assess individual variation in the developmental trajectory of two physiological traits that underlie aerobic capacity, hematocrit and hemoglobin concentration, in relation to somatic development and ecological context. Our study species, the American kestrel (*Falco sparverius*), is sexually dimorphic and therefore likely to show sexual variation in developmental trajectory and nestling maturity. We used lay date, year, brood size, nestling sex ratio, and parental nest visit rate to assess ecological context. Although somatic traits showed similar trajectories across nestlings, developmental trajectory for hematocrit and hemoglobin concentration showed individual variation not previously documented. This individual variation in developmental change, or trajectory, for physiological traits could not be explained by somatic development, sex, parental nest visit rate, lay date, year, brood size, or nestling sex ratio. However, we did find higher final hemoglobin concentration in 2018 and in nests with earlier lay dates. These findings demonstrate the importance of assessing physiological traits that capture aspects of individual quality distinct from somatic traits. Future studies are needed to understand the causes of individual variation in developmental trajectory, which cannot be explained by the ecological variables presented here, and the potential fitness consequences of this variation.

Keywords: hematocrit, hemoglobin, development, ecological physiology, avian biology.

Introduction

Development in birds has been well studied in respect to embryonic development (Mueller et al. 2015), the altricial-precocial spectrum (Starck and Ricklefs 1998), and mass gain after hatch (Ricklefs 1973, 1979). However, the relationship between the development of somatic "structure" versus physiological machinery has received less attention, particularly in regard to individual variation. Several studies suggest that high postfledging mortality may be due to poor flight performance in fledglings (Sullivan 1989; Anders et al. 1997), which has been linked to both somatic (Verspoor et al. 2007; Chin et al. 2009) and physiological (Cornell et al. 2017) maturity of individuals. Species that show standard growth profiles (which have a fledging mass less than or similar to adult size) tend to show a linear increase in physiological traits associated with oxygen carrying capacity, such as hematocrit and hemoglobin concentration (table 1; O'Conner 1984). Species that show mass overshoot recession (MOR) profiles (which have a fledging mass that exceeds adult size and subsequently recedes) tend to show more complex patterns of development of hematocrit and hemoglobin concentration (table 1; O'Conner 1984). However, studies rarely take multiple repeated measures on the same individuals to document individual variation in trajectory of development and instead rely on representative, single-time-point measurements from individuals of different ages to understand broad developmental patterns (table 1). Although this allows for minimal disturbance of birds, it does not capture individual variation in trajectories of development, which can be even more sensitive than single-time-point measurements to ecological conditions such as food availability and parasite load (Merino and Potti 1998; Soler et al. 2003; Cornell and Williams 2017). This variation in trajectory of development can influence oxidative stress (reviewed in Metcalfe and Alonso-Alvarez 2010), which has been used to successfully predict recruitment of nestlings (Noguera et al. 2012) and longevity across avian species (Vágási et al. 2019).

Posthatching development of both somatic and physiological traits prepares chicks for the critical life-history transition

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Table 1: Studies documenting developmental trajectory of mass, hemoglobin concentration (Hb), and hematocrit (Hct) during the nestling period

Species	Mass growth profile	Hb pattern	Hct pattern	Repeated measures	Reference
Spotted turtledove (*Streptopelia chinensis*)	STD	Linear increase	Increase then plateau	No	Eklom and Lill 2006
Great tit (*Parus major*)	STD	Briefly flat then linear increase	Relatively linear increase	No	Kostelecka-Myrcha et al. 1973
Noisy miner (*Manorina melanocephala*)	STD	Linear increase	Linear increase	No	Bolton et al. 1999
European starling (*Sturnus vulgaris*)	STD	Linear increase	Linear increase	No	Kostelecka-Myrcha et al. 1972
Common sparrow (*Passer domesticus*)	STD	Linear increase	Linear increase	Not reported	Kostelecka-Myrcha et al. 1971
Tree sparrow (*Passer m. montanus*)	STD	Linear increase	Linear increase	No	Kostelecka-Myrcha et al. 1970
Gentoo penguin (*Pygoscelis papua*)	STD	Not reported	Rapid increase, plateau, rapid increase	Not reported	Kostelecka-Myrcha and Myrcha 1980
Chinstrap penguin (*Pygoscelis antarticus*)	STD	Not reported	Increase then plateau	Not reported	Kostelecka-Myrcha and Myrcha 1980
Adelie penguin (*Pygoscelis adeliae*)	STD	Not reported	Decrease (starts high) then increase	Not reported	Kostelecka-Myrcha and Myrcha 1980
Fairy martin (*Petrochelidon ariel*)	MOR	Increase then decrease	Increase then plateau	No	Simmons and Lill 2006
Welcome swallow (*Hirundo neoxena*)	MOR	Increase then decrease	Increase then plateau	No	Simmons and Lill 2006
Wilson's storm petrel (*Oceanites oceanicus*)	MOR	Decrease then increase	Increase, plateau, increase	No	Kostelecka-Myrcha and Myrcha 1989
Rainbow bee-eater (*Merops ornatus*)	MOR	Increase then plateau	Increase then plateau	For subset of individuals, but data not reported	Eklom and Lill 2006
Short-tailed shearwater (*Puffinus tenuirostris*)	MOR	Plateau then increase	Plateau then increase	No	Arnold et al. 1999
Gould's petrel (*Pterodroma leucoptera*)	MOR	Slight decrease, plateau, increase	Not measured	Yes, but data not reported	O'Dwyer 2004
American kestrel (*Falco sparverius*)	MOR	Individually variable	Individually variable	Yes	This study

Note. MOR = mass overshoot regression; STD = standard.

of fledging, which is marked by high mortality (e.g., Kersten and Brenninkmeijer 1995; Vernasco et al. 2018; Raybuck et al. 2019), especially in raptors (Newton et al. 2016). Although mass is often used to indicate individual quality, this metric has no correlation to postfledging survival in 10 of 32 species studied (reviewed in Magrath 1991; Schwagmeyer and Mock 2008). Nestling hematocrit has been correlated to survival during the nestling period (Nadolski et al. 2006; Kaliński et al. 2012; Glądalski et al. 2016) and after fledging (Bowers et al. 2014), although the developmental trajectory of this trait has been far understudied compared with body mass (e.g., Teather 1990; Becker and Wink 2003; Råberg et al. 2005; Mainwaring et al. 2009). Because heritability of hematological traits is low (Potti 2007; Morrison et al. 2009), ecological context is likely to have a strong influence. Indeed, hemoglobin concentration has been found to be a strong indicator of both ecological context and fitness-related traits in birds (reviewed in

Minias 2015), and European starling (*Sturnus vulgaris*) nestlings show more rapid development of hemoglobin concentration in nests initiated later in the breeding season (Cornell and Williams 2017). Trajectory of development is likely to be influenced by sex (Anderson et al. 1997; Hartley et al. 2000), sibling sex ratio (Becker and Wink 2003; Vedder et al. 2005), brood size (Nilsson and Gårdmark 2001; Miller 2010), phenology (Cordero et al. 2001; Cornell et al. 2017), and parental provisioning rate (Øyan and Anker-Nilssen 1996; Scheuerlein and Gwinner 2006). However, few studies take repeated measures on individuals throughout the nestling period (table 1) to test how ecological context relates to individual variation in developmental trajectory, and often only somatic traits are used to assess individual quality, which may or may not relate to physiological traits that predict recruitment.

We study the development of chicks throughout the nestling period in a wild common raptor, the American kestrel (*Falco sparverius*). Kestrels are a sexually dimorphic species with adult females that are 9% larger on average than males (Snyder and Wiley 1976). Our study had four major objectives: (1) to assess the individual variation and trajectory of development in somatic traits (tarsus length, mass, wing length), (2) to assess the individual variation and trajectory of development of physiological traits underlying aerobic capacity (hematocrit, hemoglobin concentration), (3) to investigate possible relationships between the development of physiological and somatic traits to identify possible correlations and/or trade-offs, and (4) to identify whether ecological context (lay date, year, brood size, nestling sex ratio, parental nest visit rate) relates to individual variation in developmental trajectory (change from day 7 to day 21) or maturity of late stage (21-d-old) nestlings.

Material and Methods

Field Methods

In 2018 and 2019, from May to July, we monitored a wild breeding population of American kestrels using nest boxes in southeastern Pennsylvania. Nest boxes were mounted on telephone poles, trees, or barns in rural areas. Of the 68 monitored nests, 19 were used in this study on the basis of ease of access for repeated measures (2018: $n = 9$ nests; 2019: $n = 10$ nests). Brood sizes ranged from one to five chicks in each nest, with an average of 3.5 ± 1.7 chicks. Active nests were checked for hatching, and hatch date was recorded for aging nestlings. If not observed, lay date of the first egg was recorded by back calculating 28 d for incubation and 1 d for each egg in the clutch. In 2018, 88 fledglings were produced from 25 successful nests, with a total nest failure rate of 26%, compared with 80 fledglings produced from 23 successful nests in 2019, with a 34% nest failure rate; however, these differences in nest failure rate (χ^2 test, $\chi^2 = 0.6$, df $= 1$, $P = 0.43$) and brood size at fledging (unpaired two-tailed t-test, $t_{60} = -0.9$, $P = 0.39$) were not significant. Mortality after hatching was uncommon for the 73 nestlings in our study, and any chicks that died before 21 d after hatch (6/73) were removed from analyses unless otherwise noted. Runts were not blood sampled on day 7 and were therefore also excluded from analyses (6/73 chicks, two of which did not survive to day 21).

Parental nest visit rate was measured on 6 d during the first 3 wk of chick development: days 5, 6, 12, 13, 19, and 20 (hatch day = day 0). In 2018, a camcorder (JVC Everio R) was set up on a tripod in a discrete location to film the box for 60 min on each of the 6 d. In 2019, a GoPro Hero7 Black camera was mounted to the nest box and used to film continuously for an average of 122 min (range: 60–253 min) on each of the 6 d. In 2019, two nests had only 5 d of recording as a result of equipment malfunction. A dummy GoPro camera was mounted to the box whenever not filming to avoid influencing natural behavior. All nest visit rates were calculated as per hour and per chick. In 2019, both camera types were used simultaneously during nine observations and compared to test whether the improved camera setup in 2019 led to significantly different data. However, no significant difference was found between camera setups in measuring parental nest visit rate (paired two-tailed t-test, $t_8 = 1$, $P = 0.35$).

Data on nestling development were collected between 8:00 a.m. and 12:00 p.m. at three ages: days 7, 14, and 21 after hatch. Nestling maturity refers to values collected on day 21, our last measurement of a typically 28-d nestling period. During sampling, we removed chicks from nest boxes and collected blood from the brachial vein (not more than 1% body mass following Cedar Crest College Institutional Animal Care and Use Committee permit 2018-01 and US Fish and Wildlife Service permit 22749), switching wings between sampling dates to reduce soreness. On average, chicks were sampled within 5 min (2018) or 4 min (2019) of removal from the nest box. Whole-blood samples were immediately placed on ice until laboratory analyses. After blood sampling on day 7, we marked individuals with unique color bands for individual identification (Darvic Wraparound 1FB, 5.5 mm, Avinet) and added federal metal bands on day 21. Chicks were sexed on day 21 by examining primary feather colors: blue for males and brown for females. Values for somatic traits were collected using a digital scale to obtain mass to the nearest 0.01 g, digital calipers to measure tarsus length to the nearest 0.01 mm, and a wing ruler to obtain flattened wing length to the nearest 1 mm. In 2019, adults of both sexes were captured during the nestling period to obtain adult values for the same traits.

Laboratory Methods

Hematocrit was measured by centrifuging the blood in a capillary tube at 13,000 rpm for 5 min. Packed cell volume and total volume were measured to the nearest 0.01 mm using digital calipers. Hemoglobin concentration (g/dL whole blood) was determined using the cyanmethemoglobin method (Drabkin and Austin 1932) with modifications for the use of a microplate spectrophotometer. A 5-μL aliquot of whole blood was diluted in 1.25 mL of Drabkin's reagent (Sigma Aldrich D5941), and absorbance values were measured in triplicate as a measure of intra-assay variation (coefficient of variation = 3% in 2018 and 5% in 2019) at 540 nm in a Fisher Multiskan FC 357 plate reader. Interassay variation of a pooled sample across plates was 8% (2018) and 5% (2019).

Statistical Methods

Developmental trajectory of each trait was calculated as percent change in relation to the earlier measurement. Nest ID encompassed both nest box number and year if the same nest box was used in multiple years. Brood size and nestling sex ratio metrics are based on day 21 values. Linear mixed effects models were used to control for the random effect of nest ID with all other variables run as fixed effects. Sex was always included as a fixed effect if significant. Interactions between fixed effects were tested and reported only if significant. If a significant effect of an ecological variable was found, we included it as a fixed effect in all other relevant models and noted this in the results. Because developmental trajectory of mass, tarsus length, and wing length were significantly related to each other (see "Results"), we used the first principal component of these variables to test the relationship between developmental trajectory of morphology and ecological variables. Although day 21 mass, tarsus length, and wing length were also related to each other (see "Results"), the first principal component for these explained <50% of the variation; thus, these metrics were tested individually in relation to ecological variables. All mean values are reported as ±standard deviation.

Results

Developmental Patterns of Somatic Traits

Mass showed an increase between days 7 and 14 (82% ± 36%) and a smaller increase on average between days 14 and 21 (9% ± 13%), with 19 of 63 individuals decreasing in mass (fig. 1). Females had higher mass than males on day 14 (females: 116.0 ± 11.3 g; males: 104.9 ± 11.0 g) and day 21 (females: 126.1 ± 14.0 g; males: 113.4 ± 13.0 g; linear mixed effects model, $F_{1,43} > 19.6$, $P < 0.01$) but not on day 7 (females: 64.1 ± 14.7 g; males: 62.4 ± 14.4 g; linear mixed effects model, $F_{1,43} = 0.5$, $P = 0.43$). Female chicks had significantly higher increases in mass from day 7 to day 21 (females: 62.0 ± 22.5 g; males: 50.9 ± 18.2 g; linear mixed effects model controlling for year as a fixed effect, $F_{1,43} = 12.0$, $P < 0.01$). On average, tarsus length increased by 35% ± 15% between days 7 and 14 and increased only slightly between days 14 and 21 (3% ± 3%; fig. 1). Female chicks had longer tarsus length on day 21 (linear mixed effects model, $F_{1,43} = 4.9$, $P = 0.03$) but not on days 7 and 14 (linear mixed effects model, $F_{1,42} < 1.9$, $P > 0.17$). However, the rate of tarsus development in any period was not significantly related to sex (linear mixed effects model controlling for year as a fixed effect, $F_{1,42} < 3.5$, $P > 0.06$). Wing length increased between days 7 and 14 (135% ± 33%) and between days 14 and 21 (47% ± 11%), but percent change was unrelated to sex in both periods (linear mixed effects model controlling for year as a fixed effect, $F_{1,43} < 1.2$, $P > 0.27$; fig. 1). Sex also did not relate to wing length on day 7, 14, or 21 (linear mixed effects model controlling for year as a fixed effect, $F_{1,43} < 1.8$, $P > 0.18$).

Developmental Patterns of Physiological Traits

The trajectory of development for hematocrit varied across individuals: between days 7 and 14, a total of 36 chicks increased in hematocrit (5%–220% increase) compared with 25 chicks that decreased (2%–34% decrease), and one chick changed by less than 1% (fig. 1). A higher proportion of chicks increased in hematocrit between days 14 and 21: a total of 45 chicks increased (1%–44% increase) compared with 16 chicks that decreased (1%–14% decrease), and one chick changed by less than 1% (fig. 1). The percent change in hematocrit between days 7 and 14 negatively related to percent change between days 14 and 21 (linear mixed effects model, $F_{1,42} = 5.1$, $P = 0.03$), and sex did not relate to percent change during either period (linear mixed effects model, $F_{1,42} < 2.4$, $P > 0.13$). Chicks with higher hematocrit on day 7 (closer to adult values) had lower percent change between days 7 and 14 (linear mixed effects model, $F_{1,42} = 144.8$, $P < 0.0001$) and between days 7 and 21 (linear mixed effects model, $F_{1,43} = 275.5$, $P < 0.0001$). The same effect was found for hematocrit at day 14 and the percent change from day 14 to day 21 (linear mixed effects model, $F_{1,42} = 46.0$, $P < 0.0001$). Sex was unrelated to hematocrit at days 7, 14, and 21 (linear mixed effects model, $F_{1,43} < 3.8$, $P > 0.05$).

Change in hemoglobin concentration also showed individual variation. Between days 7 and 14, a total of 30 chicks increased in hemoglobin concentration (2%–218% increase) compared with 26 chicks that decreased (2%–41% decrease), and two chicks changed by less than 1% (fig. 1). Similar to hematocrit, hemoglobin concentration increased in a higher proportion of chicks between days 14 and 21: a total of 38 chicks increased in hemoglobin concentration (2%–65% increase) compared with 19 chicks that decreased (1%–42% decrease), and one chick changed by less than 1% (fig. 1). Sex did not relate to percent change during either period (linear mixed effects model with year and lay date as fixed effects, $F_{1,40} < 0.1$, $P > 0.78$). The percent change in hemoglobin between days 7 and 14 negatively related to percent change between days 14 and 21 (linear mixed effects model with year and lay date as fixed effects, $F_{1,39} = 5.0$, $P = 0.03$). There was a significant relationship between hemoglobin concentration at day 7 and the percent change in concentration from day 7 to day 14, with chicks of higher hemoglobin concentration (closer to adult values) showing lower percent change (linear mixed effects model with year and lay date as fixed effects, $F_{1,39} = 147.1$, $P < 0.0001$). The same effect was found for the hemoglobin concentration at day 14 and the percent change from day 14 to day 21 (linear mixed effects model with year and lay date as fixed effects, $F_{1,39} = 27.3$, $P < 0.0001$). Sex was unrelated to hemoglobin concentration at days 7, 14, and 21 (linear mixed effects model with year and lay date as fixed effects, $F_{1,42} < 0.4$, $P > 0.54$).

Relationships between Quality Metrics

Percent changes in all somatic variables (tarsus length, wing length, mass) were significantly related to one another during every time period (table 2). The first principal component of percent change in these variables from day 7 to day 21 explained

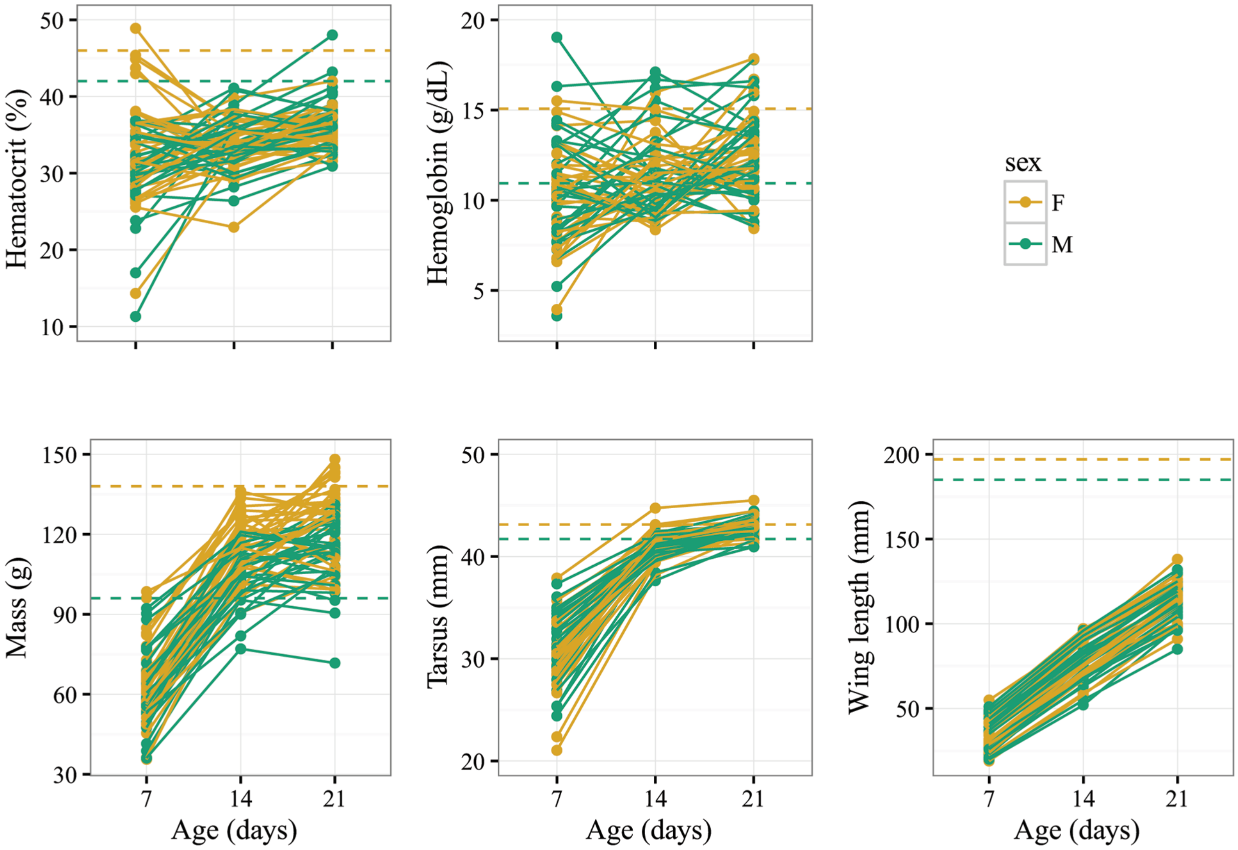

Figure 1. Development of physiological and somatic traits throughout the nestling period. Individual lines represent trajectories of individual nestlings. Adult values are given for context as dashed lines, and sex is denoted by line color.

90% of the variation with approximately equal loadings between wing length (0.56), mass (0.58), and tarsus length (0.59). There was no significant relationship between development of any of the somatic variables (tarsus length, wing length, mass) and development of either physiological variable (hematocrit or hemoglobin concentration; table 2). Percent changes of hematocrit and hemoglobin concentration between days 7 and 21 were significantly positively correlated, although changes between shorter time intervals were not (table 2). Consistent with percent change results, at day 21 mass was correlated with wing and tarsus lengths (table 2). However, no other maturity values at day 21 related to either hemoglobin concentration or hematocrit, including each other (table 2).

The Role of Ecological Context in Development and Nestling Maturity

Hemoglobin concentration at day 21 was significantly higher in chicks from earlier lay dates and was higher in chicks from 2018 (13.5 ± 2.5 g/dL) compared with chicks from 2019 (12.0 ± 1.9 g/dL; table 3; fig. 2). There was no significant interaction between year and lay date in the model ($F_{1,15} = 1.4$, $P = 0.26$). However, hematocrit at day 21 was not significantly related to any ecological variables (table 3). Developmental trajectory, or change, in both hematocrit and hemoglobin concentration between days 7 and 21 was unrelated to all ecological variables tested (table 4). Mass and tarsus length at day 21 were not significantly related to any ecological variables, controlling for sex as a significant fixed effect (table 3). Wing length was longer at day 21 in 2019 (118 ± 8 mm) compared with 2018 (108 ± 11 mm; table 3). There was a significant effect of year on somatic growth, with chicks growing more rapidly in 2018 compared with 2019 (table 4).

Discussion

We investigated the patterns of development for somatic and physiological traits in sexually dimorphic American kestrels. Although somatic traits showed similar trajectories across individuals, hematocrit and hemoglobin concentration showed surprising individual variation not previously documented. This individual variation in trajectory was unexplained by both the development of somatic traits and the ecological variables that we studied, although hemoglobin concentration at day 21 was correlated with lay date and annual variation. Collectively, our results suggest plasticity in the physiological development

Table 2: Results of linear mixed effects models between each individual quality metric

	Mass ~ tarsus	Mass ~ wing	Mass ~ Hct	Mass ~ Hb	Tarsus ~ wing	Tarsus ~ Hct	Tarsus ~ Hb	Wing ~ Hct	Wing ~ Hb	Hct ~ Hb
Days 7–14:										
df	1, 41	1, 42	1, 41	1, 39	1, 42	1, 41	1, 39	1, 42	1, 40	1, 39
F	209.3	21.5	1.6	.1	32.6	1.6	.1	1.8	<.1	3.9
Correlation coefficient	2.1	.6	.1	<.1	.3	<.1	<.1	.1	<.1	.2
P	<.0001*	<.0001*	.21	.80	<.0001*	.22	.70	.19	.91	.05
Days 14–21:										
df	1, 41	1, 42	1, 41	1, 39	1, 42	1, 41	1, 39	1, 42	1, 40	1, 39
F	26.0	21.0	.2	2.0	11.3	3.4	1.5	1.7	2.3	.1
Correlation coefficient	1.4	.4	−.1	.1	.1	−.1	<.1	−.1	.1	<.1
P	<.0001*	<.0001*	.62	.17	<.01*	.07	.23	.20	.14	.75
Days 7–21:										
df	1, 42	1, 42	1, 42	1, 42	1, 43	1, 43	1, 43	1, 43	1, 43	1, 43
F	297.3	59.4	.6	.2	80.8	.2	.2	1.0	3.1	5.0
Correlation coefficient	2.9	.7	.1	<.1	.2	<.1	<.1	.2	.2	.2
P	<.0001*	<.0001*	.44	.68	<.0001*	.66	.64	.32	.09	.03*
Maturity day 21:										
df	1, 42	1, 42	1, 42	1, 42	1, 42	1, 42	1, 42	1, 42	1, 42	1, 42
F	4.5	5.3	2.1	2.9	1.8	2.9	1.8	.3	<.1	.2
Correlation coefficient	1.8	.3	.4	.7	<.1	<.1	.1	.2	<.1	.1
P	.04*	.03*	.16	.10	.19	.10	.18	.59	.93	.69

Note. Hct = hematocrit; Hb = hemoglobin concentration.

*Statistically significant.

Table 3: Results of linear mixed effects models for ecological variables and maturity at day 21

Metric (day 21)	Mass	Tarsus	Wing length	Hct	Hb
Year:					
df	1, 17	1, 17	1, 17	1, 17	1, 16
F	1.1	1.8	5.5	<.1	6.2
P	.31	.20	.03*	.82	.02*
Lay date:					
df	1, 42	1, 42	1, 43	1, 43	1, 43
F	.3	.3	2.3	.2	4.5
P	.56	.61	.14	.63	.04*
Brood size:					
df	1, 17	1, 17	1, 16	1, 17	1, 15
F	3.4	<.1	<.1	.3	.3
P	.08	.86	.80	.61	.61
Sibling sex ratio:					
df	1, 17	1, 17	1, 16	1, 17	1, 15
F	.5	<.1	.2	3.9	.1
P	.49	.78	.66	.06	.72
Parent visit rate per chick:					
df	1, 17	1, 17	1, 16	1, 17	1, 15
F	<.1	.5	.6	<.1	.5
P	.72	.49	.47	.81	.50

Note. Hct = hematocrit; Hb = hemoglobin concentration.
*Statistically significant.

of traits that have a known fitness consequence, but unrelated to the commonly studied ecological variables discussed here.

Developmental Patterns of Somatic and Physiological Traits

For somatic traits, we found patterns of development consistent with other species that show the MOR growth pattern, with 95% of nestlings reaching the adult range for body mass by day 21 of a typically 28-d nestling period. However, for hematocrit and hemoglobin concentration, our data show an individually variable trajectory for development, with most chicks increasing toward adult values by day 21, although not linearly. In fact, 77% of chicks reached day 21 with hemoglobin concentration in the adult range. The individual variation in the developmental trajectory of physiological traits was striking compared with the essentially parallel trajectories of somatic traits. Because most other studies do not use repeated measures on the same individual (table 1), we are unsure of how common this is in other systems. Our understanding of how physiological traits develop could surely be advanced if more studies tracked individual variation.

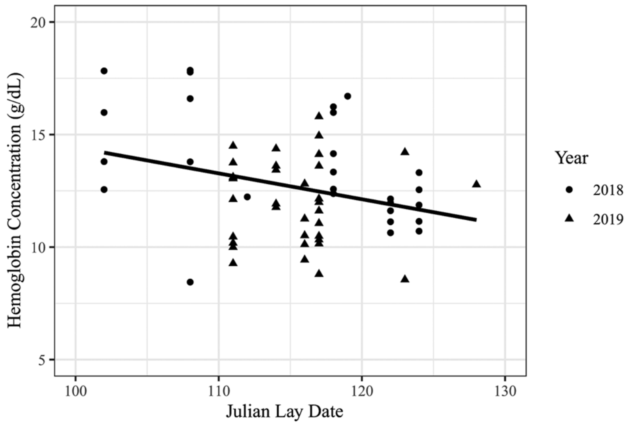

Figure 2. Relationship between lay date of the first egg in the nest and individual nestling hemoglobin concentration at day 21 with linear regression. Circles and triangles indicate individuals hatched in 2018 and 2019, respectively.

Despite sexual dimorphism in adults, sex differences in nestling development were found only for mass and tarsus length. In some systems, such as blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*; Kaliński et al. 2012), chicks of the larger sex have shown higher hemoglobin concentrations. In our baseline adult measurements, females had on average 27% higher hemoglobin concentration and 9% higher hematocrit compared with males but no difference in nestlings. Female nestlings increased mass at higher rates and attained a higher mass and tarsus length by day 21. Therefore, our results suggest that sexual differentiation in somatic traits precedes differentiation in hematological traits. Although hematopoiesis does occur in the bone marrow, throughout development it also takes place in the liver, spleen, and yolk sac (Starck and Ricklefs 1998). If erythropoiesis was primarily dependent on the size of the skeleton, we would expect to see correlational patterns of the development of hematological traits with somatic traits relating to skeleton size. Interestingly,

Table 4: Results of linear mixed effects models for ecological variables and developmental trajectory

Metric (change days 7–21)	Morphology	Hct	Hb
Year:			
df	1, 17	1, 17	1, 16
F	8.1	<.1	1.3
P	.01*	.84	.28
Lay date:			
df	1, 42	1, 43	1, 43
F	.5	<.1	2.2
P	.50	.84	.14
Brood size:			
df	1, 16	1, 17	1, 16
F	.9	.1	.9
P	.36	.73	.36
Sibling sex ratio:			
df	1, 16	1, 17	1, 16
F	2.5	1.9	.4
P	.13	.19	.54
Parent visit rate per chick:			
df	1, 16	1, 17	1, 16
F	.1	.7	<.1
P	.71	.41	.80

Note. Hct = hematocrit; Hb = hemoglobin concentration.
*Statistically significant.

21-d-old females had larger body masses but similar wing lengths compared with males. Experimental studies in common swifts (*Apus apus*) have shown that nestlings optimize body mass and wing length for wing loading ratio at fledging as a possible adaptation for maneuvering and predator escape (Wright et al. 2006), but a similar experiment in American kestrel chicks found a null result (Mitchell 2018). Previous work in our kestrel population has shown that females occupy wintering habitats with lower predation risk compared with males (Ardia and Bildstein 1997, 2001). Taken together with this study, female kestrels may prioritize fat stores over optimal wing loading during development because of their use of low predation risk habitats.

Relationships between Quality Metrics

Although the developmental trajectories and maturity values for mass, tarsus length, and wing length were correlated, there was no relationship between any of the somatic traits with trajectories or maturity of physiological traits. This is consistent with existing data in American kestrels (Dawson and Bortolotti 2000) and other systems: spotted turtledove nestlings (*Spilopelia chinensis*) reach 72%–81% of adult hemoglobin concentration and hematocrit but only 54% of adult body mass before fledging (Eklom and Lill 2006; but see Lill et al. 2013). Thus, we find no evidence of trade-offs between physiological machinery and body size. However, as suggested by Mainwaring and Hartley (2012), experimental manipulations that limit resources are needed to fully assess trade-offs. The lack of correlation that we found further emphasizes the importance of using hematological samples to assess individual quality in addition to measuring somatic traits, since hematocrit and hemoglobin concentration capture aspects of quality unique to body size with known links to fitness (Bowers et al. 2014; Minias 2015). Although correlated during development, hematocrit and hemoglobin concentration were unrelated to each other by day 21. Uncoupling between hematocrit and hemoglobin concentration during development has also been shown in white storks (*Ciconia ciconia*; Kaminski et al. 2014), Wilson's storm petrels (*Oceanites oceanicus*; Kostelecka-Myrcha and Myrcha 1989), European starlings (Kostelecka-Myrcha et al. 1972), and spotted turtledoves (Eklom and Lill 2006).

The Role of Ecological Context in Development and Nestling Maturity

Nestlings had higher hemoglobin concentration in nests with earlier lay dates (fig. 2) and in 2018. In other studies, nestling hematology, especially hemoglobin concentration, shows sensitivity to changes in ecological conditions, including lay date (Kaminski et al. 2014; Cornell and Williams 2017; Cornell et al. 2017; Kaliński et al. 2019), temperature (Kaliński et al. 2009), parasite load (Thomas et al. 2007), annual variation (Bańbura et al. 2007; Kaminski et al. 2014), and habitat (Kaliński et al. 2015; Glądalski et al. 2016, 2019). However, none of the ecological factors we studied explained the individual variation in developmental trajectory of hematocrit or hemoglobin concentration. Further studies are needed to assess other possible causes of individual variation in developmental trajectories and potential costs, such as oxidative stress. Growth rate of somatic traits was faster in 2018, which is consistent with other studies showing significant interannual variation (Hedd et al. 2002; Renton 2002; Pérez et al. 2016). Although we saw longer nestling wing lengths during 2019, previous work has shown that nestlings increase feather growth rate in response to experimental increases in corticosterone (Butler et al. 2010), maternal predation risk before egg laying (Coslovsky and Richner 2011), and ectoparasite loads (Saino et al. 1998), which may provide mechanistic explanation for the longer wings in 2019. Interestingly, the traits that relate to flight performance at fledging—hemoglobin concentration and wing length—showed the strongest, if any, relationship to ecological context in the form of lay date and year (Cornell et al. 2017). Although lay date can vary in relation to year, the interaction of these two variables was not significant in our models. Future studies should assess whether other systems show the same degree of individual variation in developmental trajectory of physiological traits shown here as well as potential ecological factors regulating development at the level of the individual.

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