Advantages of nonlinear mixed models for fitting avian growth curves

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Our understanding of avian growth rates can benefit from the use of two statistical approaches that explicitly model the sources of intraspecific variation. First, random effects can evaluate whether there are consistent differences between individuals and groups of siblings within a population, and also account for any lack of statistical independence among data points. Second, nonlinear fixed-effect functions can be extended to test specific biological hypotheses of interest, such as for differences between groups or populations. We illustrate the advantages of these methods by using nonlinear mixed models to study variation in the growth trajectories of nestling orange-crowned warblers *Oreothlypis celata*. Specifically, we quantify the sources of variation within populations, analyze the effects of asynchronous hatching, and test for a difference in the growth rates of populations in Alaska and California, which are at the northern and southern limits of the species' breeding distribution. We found that growth rates did not consistently vary between nests and individuals within populations and were not affected by asynchronous hatching, but were higher in Alaska than in California. Our extensions of traditional methods allowed us to accurately quantify this difference between populations, which is consistent with life history theory but has rarely been demonstrated in previous comparisons of intraspecific passerine populations. The methods we present can be applied to any taxonomic group and adjusted to fit any nonlinear function, and we provide code and implementation advice to facilitate the use of this analytical framework in future studies.

An organism's growth rate is a fundamental life history trait that is often closely correlated with fitness and a suite of other traits (Haywood and Perrins 1992, Roff 1992, Stearns 1992). Variation in vertebrate growth rates is thought to reflect adaptive evolution to different ecological conditions (reviewed by Case 1978, Arendt 1997, Starck and Ricklefs 1998), and studies of birds have highlighted how ecological conditions such as food availability, sibling competition, and nest predation risk underlie variation in growth rates within and between species (Ricklefs 1968, 1976, Nilsson and Svensson 1996, Royle et al. 1999, Remes and Martin 2002). Within a species, growth trajectories can be sensitive to a variety of genetic and environmental factors, potentially leading to variation at multiple hierarchical levels: between individuals, between family groups, and between populations (Ricklefs and Peters 1981, Badyaev and Martin 2000, McCarty 2001). Accurately quantifying variation at each of these levels is therefore critical for understanding the ecological and evolutionary processes that shape growth and development.

Studies of growth face several fundamental methodological challenges. First, field studies generally track the growth of related individuals through time, leading to a lack of independence between data points. This occurs at two levels: at the nest level, because siblings share genetic backgrounds and environmental conditions, and at the nestling level, because of repeated measurements of individuals. Second, the nonlinear nature of growth curves limits the types of statistical analyses that can be easily applied, so studies interested in identifying the sources of variation in growth have often used a multistep process to analyze their data. For example, passerine growth trajectories are usually analyzed by fitting a logistic function, which has three fixed parameters: the asymptotic mass, the inflection point, and the growth rate constant (Ricklefs 1967). To understand the factors affecting variation in the growth rate constant within a species, studies have divided their data and fit separate curves to different populations, sexes, nests, or treatment groups (Bancroft 1984, Gard and Bird 1992, Barrett 1996, Kaiser and Lindell 2007), or to each individual nestling (Best 1977, Ricklefs and Peters 1981, Blancher and McNicol 1988, Emms and Verbeek 1991, Badyaev et al. 2001, Searcy et al. 2004, Tilgar and Mand 2006, Tjorve and Underhill 2009). This provides a separate estimate of the growth rate constant for each group or individual, and these estimates are then used as the dependent variable in subsequent analyses that aim to identify how growth rates respond to variation in ecological conditions, parental behavior, or genetics.

The standard method of studying intraspecific variation by dividing datasets, fitting separate curves, and using parameter estimates in subsequent analyses has several drawbacks. First, it requires either the exclusion of groups or individuals with insufficient data to adequately estimate a curve, or the use of inaccurate estimates based on little data. Second, it places equal weight on each parameter estimate, although these are often generated from different amounts of data. For example, even if one individual was weighed a dozen times and another only six times, the estimated growth rate constant of each bird would be treated equally in any subsequent analyses, ignoring the larger standard error of the estimate based on fewer data. Third, it does not provide an explicit estimate of group or individual-level variation in the parameter estimates. Finally, this method does not allow for accurate tests of differences in the growth rate constant between treatment groups or populations. Instead, studies that compare estimated growth rate constants from separate curves can overstate the difference between groups, because ignoring sibling relationships or repeated measures can lead to underestimated standard errors (Brisbin et al. 1987).

Traditional statistical approaches can be extended in two ways to address the methodological problem of related data points and to more directly test biological hypotheses of interest. First, the lack of independence between siblings and between repeated measurements of the same individual can be accounted for through the inclusion of random effects, which estimate the amount of variation between groups and/or individuals (Laird and Ware 1982, Pinheiro and Bates 2000). Mixed models are therefore particularly useful for studies interested in understanding the sources of variation within populations (Bolker et al. 2009). Rather than fitting a separate curve to each individual or group, they base fixed-effect parameter estimates on all the data, accounting for the structure dictated by the random effects. Extensive research during the past two decades has led to the development of theory, approximation methods, and software for nonlinear mixed models, and these models have become widely used (Lindstrom and Bates 1990, reviewed by Davidian and Giltinan 2003). For example, nonlinear mixed models of growth are used for fisheries research, where understanding individual variation in growth trajectories provides more accurate estimates of future harvest yields (Pilling et al. 2002, Alos et al. 2010, Shelton and Mangel 2012). A second type of model extension involves altering the fixed-effect structure to directly test hypotheses of interest. As in any statistical model, nonlinear fixed-effect functions can be extended to explicitly test for differences between populations, experimental treatments, or other groups, or to determine how growth rates are affected by variation in continuous variables such as provisioning rates, climate, or parasite loads (Ritz and Streibig 2008). Here, we illustrate how the application of these methods can improve biological inference, and include implementation advice and R code (Supplementary material Appendix 1) to make these methods more accessible.

Variation in orange-crowned warbler growth trajectories

We applied nonlinear mixed models to orange-crowned warbler *Oreothlypis celata* growth trajectories to understand patterns of variation between groups of nestmates and individual nestlings, to quantify the effects of asynchronous hatching on growth trajectories, and to test for differences in the growth curves of populations breeding in Alaska and California. We used random effects to address our first goal and to produce more accurate standard errors in all our analyses. Our second and third goals were accomplished by extending the logistic equation to include fixed effects that tested for differences between asynchronously hatched young and between populations.

We assessed the amount of variation between birds in different nests and between individuals by including nest and nestling-level random effects in our models. These random effects estimated the amount of variation in each parameter at the nest and nestling levels, and because we expected variation to increase with the strength of competition (Ricklefs 1968, Rodenhouse et al. 1997), we used the random effect estimates to evaluate whether competition between siblings or between nesting pairs was stronger in Alaska or California. Birds in Alaska raise larger broods (Sofaer 2012), so if sibling competition increases with brood size, we should see more nestling-level variation in Alaska. At the nest level, greater variation in territory quality and food delivery rates could increase variation between nests. Birds in California breed at a high density on an island, respond strongly to simulated territorial intrusions, and maintain higher testosterone levels during the nestling period than birds in Alaska (Horton et al. 2010, Yoon et al. 2012), suggesting stronger competition for high quality territories in that population. Therefore, we predicted the amount of variation between nests would be higher in California. In addition to these comparisons, in each population we evaluated whether faster growing birds, or birds that reached their inflection points earlier, achieved a higher asymptotic mass.

We then tested whether birds that hatched after their nestmates (i.e. asynchronously) differed in their growth rate constant or their asymptotic mass. Birds often hatch on different days when parents initiate incubation before all eggs are laid (Clark and Wilson 1981, Ricklefs 1993), and younger nestlings often show slower growth (reviewed by Krebs 1999) and may reach a smaller size (Bryant and Tatner 1990) than their siblings. Lower nestling mass and condition can negatively affect fitness (Magrath 1991, Linden et al. 1992), but to attain the same size, younger nestlings either have to grow for a longer time period (Krebs 1999, Johnson et al. 2003) or grow at a faster rate, which is rarely observed in birds (Starck and Ricklefs 1998) and which can have negative effects on fitness (Metcalfe and Monaghan 2001, Mauck et al. 2005). We were interested in testing whether younger nestlings reached the same asymptotic mass as their older siblings and whether they differed in their growth rate constant. This test was only carried out in the population in Alaska, as asynchronous hatching was less common in California.

Finally, we tested for differences between the growth curves of warblers breeding in California versus Alaska. Life history theory shows that rapid growth is associated with faster life history strategies, and our study population in Alaska has larger clutch sizes and lower adult survival than the population in California (Horton et al. 2010, Sofaer 2012). Although several studies have found that the growth rates of passerine birds do not differ between intraspecific populations (Hussell 1972, King and

Hubbard 1981, McCarty 2001), passerine populations with different life history strategies can have heritable differences in their growth rates (Starck et al. 1995). In addition, comparisons between species have shown that birds at higher latitudes often have faster growth rates (Ricklefs 1976, McCarty 2001, Remes and Martin 2002, Schekkerman et al. 2003), further supporting the prediction that nestlings in Alaska should grow more quickly than those in California.

Methods

Data collection

We collected data on the growth of orange-crowned warblers in Fairbanks, Alaska (*O. c. celata*) from 2007 to 2008, and on Santa Catalina Island, California (*O. c. sordida*) from 2008 to 2009 (Supplementary material Appendix 2). We weighed 210 nestlings or fledglings (dependent juveniles that had left the nest) from 49 nests in Alaska, and 64 individuals from 24 nests in California; the median number of days on which we weighed each individual was 3 in both Alaska (range: 1–6 d) and California (range: 1–8 d). Birds were individually marked with non-toxic permanent marker on their toenails, and were generally weighed daily or every other day, starting on hatch day or when the nest was located. Weights were taken to the nearest 0.01 g using a My Weigh MX-50 digital scale. Some growth trajectories were incomplete due to nest failure (AK: n = 8 nests; CA: n = 13 nests). We weighed nestlings until flight feathers emerged from feather sheaths, which occurred on nestling day 6 in Alaska and day 7 in California (hatch day = day 0); we also banded birds on this day to identify individual fledglings.

We captured fledglings to accurately estimate asymptotic mass. When data collection ends before the asymptotic mass is attained, the estimated asymptotic mass may be biased low, leading to an inflated estimate of the growth rate constant (Austin et al. 2011). In Alaska we caught 26 birds on the day they left their nests, either day 9 or 10 post-hatch. This included two fledglings that fledged from nests that were not monitored. The feather development, behavior, and mobility of these birds indicated they had fledged within the past day, and they were included in the dataset as 10 d old. California birds had longer nestling periods, so we measured 10 nestlings on day 10, and 7 birds on their natural fledge day, day 13–14. We found no evidence of bias in our estimates of asymptotic mass, which were within a single standard deviation of the mean mass ($\pm$ 1 SD) of adults in each population (Alaska: 9.32 ± 0.44 g, n = 209 adults; California: 9.49 ± 0.66 g, n = 802 adults).

Analysis of variation in growth trajectories within populations

We modeled the growth curves of each study population with a logistic function, as is typical for passerine studies (Starck and Ricklefs 1998):

$$w_t = \frac{A}{1 + e^{(K(I-t))}} \tag{1}$$

where w_t = mass at time t (g), A = asymptotic mass (g), K = growth rate constant, I = the inflection point of the growth curve (days), and t = nestling age (days).

Our model estimated the amount of variation between sibling groups and individuals by including two levels of random effects, nest and nestling. The nest effects accounted for the lack of independence between nestmates due to a shared genetic background (full or half siblings), maternal effects, and a common level of parental care. The nestling effects modeled repeated measurements on individuals. We included random effects for one or more of the three parameters in the model, A, K, and I, allowing the parameter estimates to vary between each nest or nestling. For the kth measurement on the jth nestling in the ith nest the mass was:

$$w_{ijk} = \frac{A + A_i + A_{ij}}{1 + e^{((K + K_i + K_{ij})(I + I_i + I_{ij} - t_{ijk}))}} + \varepsilon_{ijk} \tag{2}$$

In all models, the random nest effects, (A_i, K_i, I_i), the random nestling effects, (A_{ij}, K_{ij}, I_{ij}), and the random errors, ε_{ijk}, were assumed to be normally distributed with a mean of zero and a variance to be estimated from the data. Within each set of three random effects for each nest or nestling, the effects were not assumed to be independent of each other. This potential dependence allowed, for example, for an earlier inflection point to be correlated with a higher asymptotic mass. Between nests and nestlings, effects were assumed to be independent of each other, as were the random errors. We report the estimated standard deviation of each random effect, which quantifies variation between groups of nestmates around the overall fixed-effect estimate or individuals' deviation from their nestmates. The fixed-effect estimates from our mixed models are applicable to a typical (i.e. median) nestling in a typical nest. These estimates are called 'subject-specific', and due to the nonlinearity of the logistic function they are not exactly estimates of the population average (Hu et al. 1998). For fixed effects we report point estimates and standard errors.

We built models that included a single random-effect level (nest or nestling) on one or more parameters as well as models that included both nest and nestling-level random effects. We first applied the same random-effect structure at the nest and nestling levels, but when an estimated correlation between parameters in a top model was near zero, we also fit a reduced model without a correlation. If a model failed to converge, if the estimated random effect standard deviation was zero, or if the absolute value of the correlation between two random effects was 0.9 or higher (often 1 or 0.99), we concluded that the model was overparameterized (Pinheiro and Bates 2000) and discarded the model from further consideration.

We used an information-theoretic approach based on Akaike's information criterion (AIC) to select a random-effect structure (Burnham and Anderson 2002). AIC values can be used for mixed models, but may be biased to favor simpler random-effect structures (Molenberghs and Verbeke 2005, Bolker et al. 2009, Greven and Kneib 2010). Therefore, when AIC values are very close, support for the more complex random-effect structure is stronger than indicated. We fit all models using maximum likelihood in the nlme package of R (Pinheiro et al. 2011, R Development Core

Team). Throughout our work, we compared mixed models to models that lack random effects to show how mixed models provided a better fit to our data and to highlight differences in the biological inferences based on each approach. Models without random effects were fit using nlme's gnls function, while mixed models were fit using the nlme function. We did not use the lme4 package (Bates et al. 2012) because it does not allow mixed models and models with only fixed effects to be directly compared, but we include example syntax for implementation in lme4 in our code (Supplementary material Appendix 1).

Although we do not discuss model diagnostics in detail, our code includes syntax to produce plots for visually checking model assumptions (Supplementary material Appendix 1). As is typical for mass data, we saw some evidence for increasing variance with increasing mass. Specifically, the ratio of the standard deviations of the residuals for birds weighing 7–9 g versus 1–3 g was 1.3 in our final model of the Alaskan population, 1.9 in the final model of the California population, and 1.4 in the final model with both populations (see below for model description). This was judged to not be severe enough to merit the use of weighted regression.

To visualize the amount of variation within a population, we calculated intervals encompassing 95% of variation at the nest and nestling levels (Fig. 1). At the nest level, this interval was calculated as ± 1.96 times the nest-level standard deviation, while the 95% interval encompassing variation between both nests and nestlings was calculated as ± 1.96 times the square root of the sum of the nest- and nestling-level variances. Although the magnitude of nestling-level variation around the asymptotic mass was nearly equivalent to nest-level variation (Results), the joint 95% interval is only slightly bigger than the nest-only interval because of the rarity with which an extreme nestling would be found within an extreme nest.

Incorporating asynchronous hatching into the analysis of growth trajectories

We extended our nonlinear fixed-effect function to test for differences in the inflection point, growth rate constant, and asymptotic mass between younger and older nestlings in Alaska, where 23 of 49 nests were known to have hatched asynchronously. We defined younger individuals (hereafter referred to as runts) as those that had not yet hatched on the first nest visit when other nestmates had hatched. The difference in the inflection point, I_r, quantified how far to the right the entire growth curve was shifted for runts, compared to their older siblings. We therefore did not have to assume that all nestlings in a nest were the same age or that runts were a full day younger; in short, the shift in inflection point captured runts' later hatching, and did not necessarily imply delayed growth after hatching. Additional parameters estimated how runts differed in their growth rate constant, K_r, and asymptotic mass, A_r; x_r was an indicator variable with a value of 1 for younger nestlings:

$$w_t = \frac{A + A_r x_r}{1 + e^{((K + K_r x_r)(I + I_r x_r - t))}} \qquad (3)$$

While this model (Eq. 3) shows only fixed effects, random effects of nest, (A_i, K_i, I_i), and nestling, (A_{ij}, K_{ij}, I_{ij}), were also included, as in model (Eq. 2). Following Zuur et al. (2009), we used model selection to select the random-effects structure with the most support, and then used hypothesis testing to evaluate the fixed effects based on our top model.

Comparison of growth trajectories between two populations

We tested for differences in the growth curves of Alaskan and Californian birds by adding a fixed site effect to each of the three parameters in the logistic function. The full fixed-effects model also included a shift in the inflection point for younger nestlings:

$$w_t = \frac{A + A_{pop} S}{1 + e^{((K + K_{pop} S)(I + I_r x_r + I_{pop} S - t))}} \qquad (4)$$

where S was an indicator variable for the Alaskan population, and A_{pop}, K_{pop}, and I_{pop} were the differences between sites. As before, while the model (Eq. 4) shows only fixed effects,
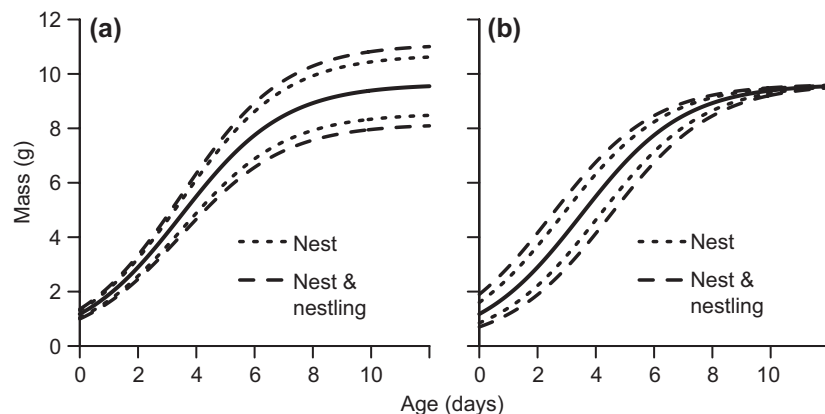

Figure 1. Variation within the Alaskan orange-crowned warbler population: (a) variation between nests and individuals in the asymptotic mass while holding the inflection point constant, and (b) variation in the inflection point given a constant asymptotic mass. This model included uncorrelated nest- and nestling-level random effects on the asymptotic mass and the inflection point. The fixed-effect structure was a three-parameter logistic function, and the solid lines show the growth trajectory of a typical nestling. Intervals encompass 95% of variation at the nest and both the nest and nestling levels (see Methods for calculation details).

we included nest- and nestling-level random effects. We included a runt effect on the inflection point, and assumed that this effect did not differ between sites, as the estimated shift in the inflection point of Alaskan runts was within one standard error of the estimate when the model was fit to Californian birds. To evaluate how the use of mixed models affected our inferences, we compared the parameter estimates and statistical significance of the differences between sites from models with and without random effects.

We calculated confidence intervals for the estimated growth trajectories in each population (Fig. 2) via the delta method, using the emdbook package in R (Bolker 2012). The intervals shown in Fig. 2 are much smaller than in Fig. 1 because these represent 95% confidence intervals on the estimated growth trajectory in each population, whereas those in Fig. 1 show 95% of the variation between nests and nestlings within the Alaskan population.

Results

Analysis of variation in growth trajectories within populations

We found unequivocal support for the inclusion of random effects in models of Alaskan birds, indicating that nests and nestlings consistently varied in the parameters that estimated their growth trajectories. The top models included random effects of nest and nestling on the asymptotic mass and the inflection point, while other models fit comparatively poorly, with ΔAIC values over 50 (Table 1). We found no support for random effects on the growth rate constant, indicating that there was little variation in the growth rate constant within this population.

Next, we determined that birds that reached the inflection point of their growth curve earlier did not show consistent differences in their asymptotic mass. The estimated
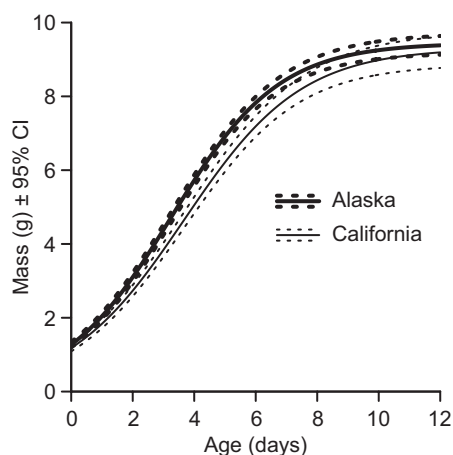

Figure 2. Comparison of estimated growth trajectories in the Alaskan and Californian populations. Alaskan birds reached their inflection point earlier and had a higher growth rate constant. The populations did not differ significantly in their asymptotic mass. Solid lines show growth trajectories based on our parameter estimates; dashed lines show the 95% confidence interval for these trajectories (see Methods for calculation details).

correlation between these two random effects at the nest level was zero ($r = 0.05$; 95% CI: -0.66, 0.71), and the confidence interval on the nestling-level correlation also included zero ($r = -0.28$; 95% CI: -0.64, 0.18). We therefore based our inference on the model with no correlations between random effect parameters, as this model had stronger or equivalent statistical support than the models with one or both correlations (Table 1). Neither the fixed-effect estimates nor the random effect standard deviations qualitatively differed between these top models.

Nests and individuals varied in their asymptotic masses and inflection points (Fig. 1). The estimated random effect standard deviations on the asymptotic mass at the nest and nestling levels were 0.55 g (95% CI: 0.39, 0.78) and 0.51 g (95% CI: 0.40, 0.64), while the estimated standard deviations on the inflection point were 0.33 d (95% CI: 0.23, 0.46) and 0.40 d (95% CI: 0.33, 0.48), respectively. Fixed-effect estimates were similar in the top mixed model and the model without random effects, but the standard error of the growth rate constant was smaller in the mixed model (0.565 ± 0.008) than in the model with only fixed effects (0.569 ± 0.016), likely because the mixed model accounted for variation at the nest and nestling levels. Fixed effect estimates from the top mixed model for the asymptotic mass and inflection point were 9.63 ± 0.13 g and 3.49 ± 0.07 d, respectively.

Results from the population in California also showed consistent intraspecific variation in the inflection point and the asymptotic mass (i.e. support for random effects on these parameters), but random effects on the growth rate constant were not supported. For models with the same random-effect structure at the nest and nestling levels, three had equivalent support (ΔAIC values < 1; Supplementary material Appendix 1, Table A1), likely because nest-level effects on the asymptotic mass and the inflection point were highly correlated, although this estimate was imprecise ($r = 0.80$; 95% CI: 0.02, 0.97). We therefore built two models with nest-level random effects on only the asymptotic mass and nestling-level random effects on both the asymptotic mass and the inflection point; one model estimated the correlation between the nestling-level random effects and the other constrained the correlation to be zero. These models avoided overfitting by eliminating highly correlated parameters. Support was strongest for the model in which nestling-level random effects were uncorrelated (Supplementary material Appendix 1, Table A1). The asymptotic mass consistently varied between nests and individuals; random effect standard deviations were 0.55 g (95% CI: 0.27, 1.13) and 0.52 g (95% CI: 0.32, 0.85), respectively. Fixed effect estimates of the growth rate constant (0.514 ± 0.014), asymptotic mass (9.52 ± 0.22 g), and inflection point (3.90 ± 0.10 d) were not qualitatively affected by the inclusion of random effects.

Incorporating asynchronous hatching into the analysis of growth trajectories

We found that runts did not differ in their growth rate constant ($K_r = 0.013 \pm 0.022$; $t_{459} = 0.62$; p = 0.54) or their asymptotic mass ($A_r = -0.42 \pm 0.28$ g; $t_{459} = -1.49$;

Table 1. Model selection results showing models with different random-effect structures for the Alaskan population. The fixed-effect structure was a three parameter logistic model. In the top models, the correlation between random effects within the nest and/or nestling level was constrained to be zero; this constraint is indicated with an asterisk.

Random effect structure						Number of random-effect parameters
Nest level	Nestling level	Δ AIC	AIC	log(L)	Total number of parameters	
A and I*	A and I*	0.00	825.53	−404.76	8	4
A and I*	A and I	0.26	825.79	−403.90	9	5
A and I	A and I	2.33	827.86	−403.93	10	6
–	A and I	55.72	881.25	−433.62	7	3
I	I	96.8	922.33	−455.17	6	2
–	I	138.87	964.40	−477.20	5	1
A	A	152.33	977.86	−482.93	6	2
–	A	202.74	1028.27	−509.13	5	1
A, I, and K	–	362.32	1187.85	−583.93	10	6
A and I	–	365.62	1191.15	−588.57	7	3
I	–	374.59	1200.12	−595.06	5	1
A	–	404.42	1229.95	−609.97	5	1
K	–	651.08	1476.61	−733.30	5	1
K	K	653.07	1478.60	−733.30	6	2
–	–	657.59	1483.12	−737.56	4	0
–	K	659.59	1485.12	−737.56	5	1

p = 0.14). However, altering the inflection point ($I_r = 0.63 \pm 0.12$ d; $t_{459} = 5.18$; p < 0.0001) to shift the growth curve to the right for younger nestlings greatly improved the fit of our model (ΔAIC = 96.6 for the top model without effects of asynchronous hatching). Including the difference in the inflection point for runts incorporated variation in hatching time into the fixed effect structure, whereas in our first model this variation was incorporated into the nestling-level random effect on the inflection point. Therefore, the estimated random effect standard deviation for the inflection point was lower in the model with the runt effects (0.14 d; 95% CI: 0.08, 0.26) than in the model without them (0.40 d; 95% CI: 0.33, 0.48). Model selection again supported nest and nestling-level random effects on the inflection point and asymptotic mass (Supplementary material Appendix 1, Table A2). The top model constrained both random effects correlations to be zero, indicating birds that reached their inflection point earlier were not expected to attain a higher asymptotic mass. Based on the top model, fixed effect estimates for the growth rate constant, asymptotic mass, and inflection point for older nestlings were 0.568 ± 0.008, 9.55 ± 0.12 g, and 3.30 ± 0.07 d, respectively.

Comparison of growth trajectories between two populations

Orange-crowned warbler nestlings in Alaska had an earlier inflection point and a higher growth rate constant than nestlings in California, but these populations did not differ in their asymptotic mass (Table 2; Fig 2). Random effects substantially improved the fit of a model testing for population differences in the growth trajectories (ΔAIC = 561.4 for the model with only fixed effects; Supplementary material Appendix 1, Table A3). Our top model included random effects of nest and nestling on the inflection point and the asymptotic mass (Supplementary material Appendix 1, Table A3). By accounting for additional sources of variation, random effects made the difference in the growth rate constant highly significant ($t_{627} = 3.26$, p = 0.001) rather than marginally significant in the model with only fixed effects ($t_{900} = 1.81$, p = 0.071; Table 2). The difference in the inflection point was significant in the mixed model ($t_{627} = -3.47$, p = 0.0006) and in the model without random effects ($t_{900} = -3.91$, p = 0.0001), while neither model supported a difference in asymptotic mass between populations ($t_{627} = 0.52$, p = 0.603 and $t_{900} = 0.43$, p = 0.663, respectively).

Table 2. Fixed-effect parameter estimates (± 1 SE) from models testing for differences between populations in growth trajectories. We compare the model without random effects to the best-supported mixed-model, which contained random effects of nest and nestling on the inflection point and asymptotic mass. Parameters with the *pop* subscript estimate the difference in the Alaskan population compared with the Californian population; I_r estimates the shift in the inflection point for younger nestlings. The mixed model provided stronger statistical support for a difference in the growth rate constant between populations.

Model	K	K_{pop}	A	A_{pop}	I	I_{pop}	I_r
No random effects	0.534 ± 0.019	0.041 ± 0.023^	9.35 ± 0.16	0.09 ± 0.20NS	3.67 ± 0.09	−0.44 ± 0.11***	0.93 ± 0.05***
Mixed model	0.525 ± 0.012	0.048 ± 0.015**	9.31 ± 0.23	0.14 ± 0.26NS	3.67 ± 0.10	−0.42 ± 0.12***	0.77 ± 0.05***

NS indicates a p-value greater than 0.10, ^ indicates a p-value between 0.10 and 0.05, **indicates significance at the 0.01 level, and ***indicates significance at the 0.001 level. The significance of K, A, and I is not noted, as it is unreasonable to expect these parameter estimates to be zero.

Discussion

Identifying the ecological and evolutionary factors that underlie variation in growth rates has been a central goal of many avian studies. By including random effects and extending the logistic function to test specific hypotheses, we were able to understand the sources of variation in growth (Fig. 1) and directly compare the growth trajectories of younger and older nestlings and of birds in two populations (Fig. 2). In each of our analyses, mixed models provided a substantially better fit to our data than models without random effects (Table 1; Supplementary material Appendix 1, Table A1–A3), and also accounted for the lack of independence between data points. Our extension of the fixed-effect function allowed us to directly test for differences between groups, whereas previous studies have used a multistep process that involved fitting separate growth curves and then using the estimated parameters in subsequent analyses. Below, we summarize our results and highlight how extensions of standard methods improved our biological understanding of our study system.

Interpretation of random effects

Within each population, we found consistent variation between individuals and nests in the asymptotic mass and the inflection point (Fig. 1), but not in the growth rate constant. We based these inferences on the amount of support for models with random effects on each parameter (Table 1; Supplementary material Appendix 1, Table A1). The lack of support for nest- or nestling-level random effects on the growth rate constant corroborates the longstanding hypothesis that the growth rate constant varies little within populations (Ricklefs 1968). We also compared the amount of variation at the nest and nestlings levels, and predicted that there could be more variation between individual nestlings in Alaska and between nests in California. Instead, we found that these two populations had a similar amount of variation in the asymptotic mass at each level; each random effect standard deviation was approximately 0.5 g in both populations. Because nestling mass can affect future fitness (Magrath 1991), and because these warblers weigh <10 g, the magnitude of this variation may be biologically meaningful. The similarity of these estimates also implies that the strength of competition between siblings may be similar in these populations, and surprisingly, suggests that competition between pairs may either not act via nestling provisioning rates or may also have a similar strength in these two populations.

We were also interested in assessing whether nestlings that reached their inflection point earlier attained a higher asymptotic mass, and to do so we used the estimated correlation between random effect parameters, which measures whether deviations around one fixed effect are related to deviations around other fixed effects. We found that variation in the inflection point was not correlated with variation in the asymptotic mass, as models in which this correlation was constrained to be zero received more support both in California and in Alaska. We saw the same pattern between populations, as birds in Alaska reached their inflection point earlier, but the two populations did not differ in asymptotic mass.

Interpretation of fixed effects

Instead of fitting separate growth curves to different groups or individuals, we extended the logistic model to explicitly test for effects of asynchronous hatching on growth trajectories, and also to test for differences between populations. We found no differences in the growth rate constant or the asymptotic mass between younger and older nestlings, indicating that asynchronous hatching did not substantially affect growth trajectories. Our results contrast with those from previous studies, which have often found slower growth rates in younger nestlings (reviewed by Krebs 1999). Although asynchronous hatching can lead to brood reduction (Lack 1968, Magrath 1990), in our study populations starvation rarely occurred for any nestlings, and runts hatched less than a day after their older siblings; these observations may explain why we observed minimal effects of hatching time on the asymptotic mass or growth rate constant. Alternatively, because we estimated multiple runt effects (i.e. one for each fixed-effect parameter) with a relatively small sample of younger birds, we may have had limited statistical power. Nevertheless, our results show that incorporating fixed differences in the inflection point substantially improved the fit of our models because an important source of variation was captured within the fixed-effect structure, rather than in the nestling-level random effects.

Our comparison between populations showed that Alaskan nestlings grew more quickly than Californian birds and reached the inflection point of their growth curve approximately half a day earlier; the two populations did not differ in their asymptotic mass (Fig. 2). Mixed models provided qualitatively stronger support for a difference in the growth rate constant compared with a model with only fixed effects (Table 2), likely because the random effects explained additional variation within populations and thereby highlighted differences between populations that previously had been masked by within-group variation. Few previous studies have found strong evidence for intraspecific variation in the growth rate of a passerine bird (Starck et al. 1995), and these demonstrations are particularly important in light of past work suggesting that passerine growth rates may be maximized and should therefore not vary between populations (Ricklefs 1969, Werschkul and Jackson 1979, King and Hubbard 1981). We found that although growth rate constants varied little within populations, they were not physiologically constrained to be equivalent between populations. These results highlight the need for studies that test whether the ecological factors that are thought to underlie variation in growth between species can also lead to adaptive variation between intraspecific populations.

Comparative studies of avian growth have focused on the growth rate constant, which can be compared between species that differ in their asymptotic mass (Starck and Ricklefs 1998), whereas few studies have considered variation in the inflection point. We show inflection points can differ between populations, and suggest that future work could evaluate whether the inflection point is under selection and shaped by ecological factors such as breeding season length and nest predation risk. Alaskan birds reached their inflection point nearly half a day earlier, and additional data from our study populations show that they also had shorter

nestling periods (mean ± 1 SD: 9.5 ± 0.8 d, n = 39 nests) than Californian birds (12.0 ± 1.1 d, n = 42 nests). However, the relative effects of faster growth rates, earlier inflection points, and differences in the developmental stage at fledging have not been integrated to understand the factors underlying variation in avian nestling period lengths.

Conclusions

We suggest that future studies of avian growth should consider the two types of model extensions we illustrate here: the inclusion of random effects and the alteration of the fixed-effect function to explicitly test hypotheses of interest. Random effects quantify variation within populations and account for the lack of independence between data points, leading to more accurate estimates of parameters and their standard errors. Not only are these features useful for intraspecific studies, but because comparative methods that control for phylogeny can now incorporate estimates of intraspecific variation (Ives et al. 2007), future interspecific studies will also benefit. More generally, mixed models are appropriate tools for ecological research because they can partition the sources of variation in a dataset and because unbalanced designs, missing data, and varied measurement intervals do not increase the complexity of implementation (Vonesh and Carter 1992). Therefore, studies should consider individually marking nestlings to allow for the inclusion of both nest- and individual-level random effects, although analyses of existing data can likely improve model fit by including at least nest-level random effects (e.g. Table 1). An increased use of models with both random effect levels would allow for comparisons of how the relative amount of variation explained at each level is affected by breeding ecology or life history strategy. For example, a greater relative importance of nest-level effects would indicate that shared genes, maternal effects, and/or a relatively even distribution of parental care overwhelm differences between nestmates. By contrast, when starvation of one or more young is common or nestmates vary considerably in mass, more variation should be captured by individual-level effects.

To understand the ecological factors that underlie intraspecific variation in growth trajectories, we suggest that studies directly incorporate tests of relevant hypotheses into the nonlinear fixed effect function. Previous methodological suggestions for studies of avian growth have largely focused on the appropriateness of different nonlinear functions (e.g. logistic, Gompertz, von Bertalanffy, and Richards functions; Starck and Ricklefs 1998, Brown et al. 2007, Tjorve and Tjorve 2010), but have not discussed how these functions may be altered to test specific hypotheses. Our study illustrates how to incorporate parameters that estimate the difference between two groups in the growth rate constant, asymptotic mass, or inflection point. Similar extensions of the fixed-effect function could be used to incorporate continuous covariate data, for example to test how variation in provisioning rate affects nestlings' growth rate constant or asymptotic mass. Because of their flexibility, nonlinear mixed models are also widely applicable beyond the study of growth curves, and we provide our R code and data

(Supplementary material Appendix 1 and 2) to encourage the wider use of these methods.

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