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Estimation of the growth curve and heritability of the growth rate for giant panda (*Ailuropoda melanoleuca*) cubs

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ABSTRACT. Giant panda cubs have a low survival rate during the newborn and early growth stages. However, the growth and developmental parameters of giant panda cubs during the early lactation stage (from birth to 6 months) are not well known. We examined the growth and development of giant panda cubs by the Chapman growth curve model and estimated the heritability of the maximum growth rate at the early lactation stage. We found that 83 giant panda cubs reached their maximum growth rate at approximately 75-120 days after birth. The body weight of cubs at 75 days was 4285.99 g. Furthermore, we estimated that the heritability of the maximum growth rate was moderate (h^2 = 0.38). Our study describes the growth and development of giant panda cubs at the early lactation stage and provides valuable growth benchmarks. We anticipate that our results will be a starting point for more detailed research on increasing the survival rate of giant panda cubs. Feeding programs for giant panda cubs need further improvement.

Key words: Growth; Chapman model; Feeding program; Lactation; Survival rate

INTRODUCTION

The giant panda (*Ailuropoda melanoleuca*) is at high risk of extinction, which may be a result of human population expansion and habitat destruction (Liu et al., 2001; Vina et al., 2007; Zhu et al., 2013). Lineage-specific biological traits, such as difficult reproduction, a restricted diet, and a low cub survival rate during the newborn and early growth stages also contribute to the high risk of extinction (Zhu et al., 2001; Czekala et al., 2003; Liao et al., 2003; Sutherland-Smith et al., 2004; Zhang et al., 2009). The giant panda has a relatively high birth rate of twins (~45%) (Huang et al., 2002). Nonetheless, a female giant panda only feeds one cub, and the rest of the cubs require artificial feeding if the mother gives birth to twins or triplets. Accurate details regarding the growth and development of captive giant pandas in the early lactation stage (from birth to 6-months-old) will facilitate the improvement of feeding strategies to increase the survival rate of cubs.

In this study, we investigated the growth and development of 83 giant panda cubs using the Chapman model (Schepers et al., 2000; Fan et al., 2004) and accurately estimated their growth curve during the early lactation stage. We also estimated the heritability of the maximum growth rate during this stage ($h^2 = 0.38$) based on the growth curve and quantitative genetic analyses. To the best of our knowledge, this is the first report of the heritability of the growth rate of giant panda cubs. Our results will provide essential baseline information on the growth and development of giant panda cubs.

MATERIAL AND METHODS

Animals and data collection

Body weight (g) and 10 types of anthropometric indices (measured in cm) (i.e., length of the head, body, tail, forelimb and posterior limb, and the circumference of the chest, abdomen, neck, elbow, and knee) were collected from 83 giant panda cubs (40 males and 43 females). The cubs were born between 2003 and 2012, had thorough genealogical records, and included 13 prenatal stages from 0 (birth) to 120 days old. Ten days elapsed between each stage.

Analysis of growth

The Chapman growth curve model was fitted to the data to describe the growth process of giant panda cubs. The equation is as follows:

$$W_t = W_0 + a(1 - e^{-bt})^c \quad \text{(Equation 1)}$$

where W_t is the mass in g at age t (days), W_0 is the mass at birth, a is the asymptotic mass (g),

b is the mass growth rate constant (days^{-1}), and c is another growth parameter. There is an inflection point on the growth curve where individuals attain the maximum growth rate. We fitted the growth curves using a nonlinear regression procedure. The SigmaPlot (Version 12.3) curve fitter using the Levenberg-Marquardt algorithm (Mustapha and Phillips, 2000) was used to determine the coefficients (parameters) of the independent variable that gave the best fit between the equation and the data.

Forward stepwise regression was used to screen the 10 indices of body measurements that changed significantly with respect to age. The remaining indices that did not change significantly with respect to age were removed. Body measurement indices were entered or removed depending on the F value, which was calculated by SigmaPlot. The F threshold was the default value in the software. The stepwise regression model was as follows:

$$y = m + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_i x_i \qquad \text{(Equation 2)}$$

where y is the age (days), m is the constant parameter, β_i is the regression coefficient, and x_i is the index of the body measurement.

Estimation of heritability

To estimate the heritability of the maximum growth rate between birth and 120 days, we screened 23 half-sibs from the 83 giant panda cubs to form a half-sib family.

The maximum growth rate was calculated from the inflection point of the growth curve. Descriptive statistics were obtained, and data and the Shapiro-Wilk test of normality was implemented (Royston, 1992). In addition, a test for equal variance was carried out. According to quantitative genetics, narrow-sense heritability (h^2) is estimated as the ratio of the additive genetic variance (V_A) to the total phenotypic variance (V_P) (Theriault et al., 2007; Husby et al., 2011; Potti and Canal, 2011). The total phenotypic variance was calculated as follows:

$$V_P = V_S + V_W + V_\varepsilon \qquad \text{(Equation 3)}$$

where V_P is the total phenotypic variance, V_S is the paternal half-sibling variance, V_W is the maternal half-sibling variance, and V_e is the residual variance. We assumed that all sources of variation, except for the individual sire effect, were due to maternal effects. Narrow-sense heritability (h^2) was estimated as follows:

$$h^2 = 4\sigma_S^2 / (\sigma_S^2 + \sigma_W^2) \qquad \text{(Equation 4)}$$

where σ_S is the expectation of paternal half-sibling variance and σ_W is the expectation of maternal half-sibling variance. The genetic correlation coefficient of half-sibs was $r_A = 0.25$. Each of these statistics was calculated by SigmaPlot.

RESULTS

Body weight

We fitted our growth model to the body weight data for the 83 giant panda cubs representing birth to 120 days after birth. The Chapman model was successfully fitted to the body weight data ($R^2 = 0.99$) (Figure 1 and Table 1). The parameters P_{W0}, P_a, P_b, and P_c were highly significant ($P_{W0} = 0.004$, $P_a = 2.18 \times 10^{-6}$, $P_b = 1.31 \times 10^{-5}$, and $P_c = 2.27 \times 10^{-9}$). The results of the Student *t*-test between the measurements and predicted values indicated that the predicted values of this model were acceptable (Table 2). The inflection point of the growth curve suggested that giant panda cubs reached their maximum growth rate at approximately 75 days and the body weight at this time was 4285.99 g. The maximum growth rate was 74.29 g/day.

The Chapman model was also successfully fitted to the body weight data for male and female cubs ($R^2 = 0.99$ for males and females; Figure 2 and Table 1). There was significant difference in body weight between male and female cubs during the first 120 days after birth (Table 3). When the cubs reached their maximum growth rate, the ages of male and female cubs were 74.60 and 74.99 days, respectively. The body weights of male and female cubs at approximately 75 days were 4244.33 g and 4174.67 g, respectively. The maximum growth rate was 74.54 g/day for male cubs and 73.53 g/day for female cubs.

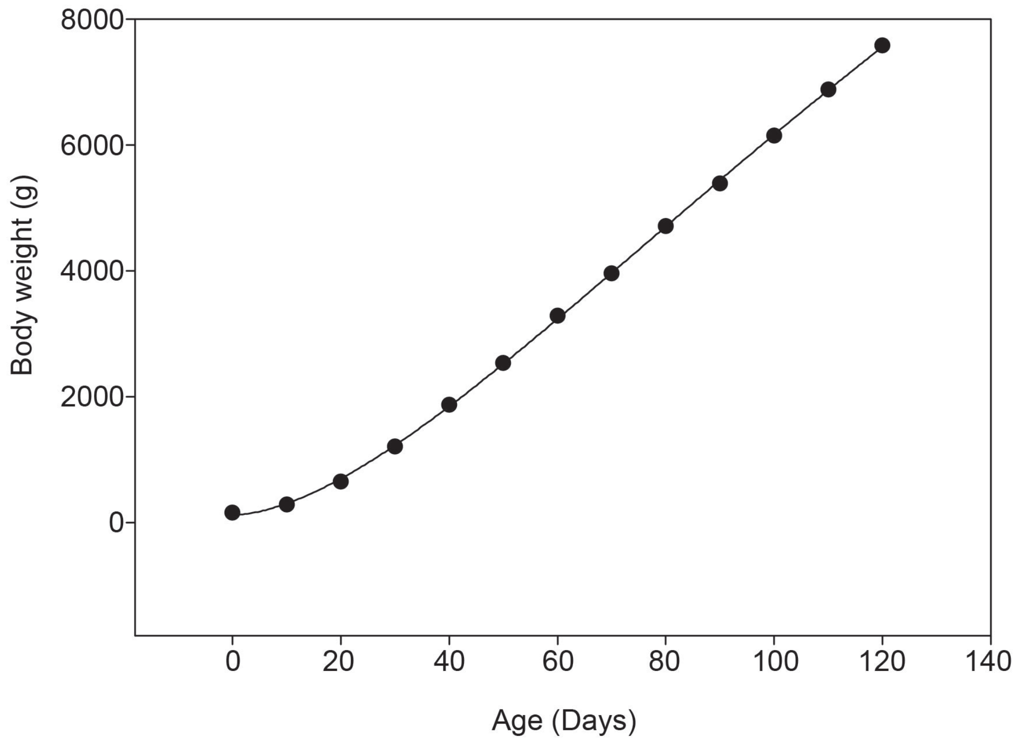

Figure 1. Growth curve of giant panda cubs from birth to 120 days. The black circles indicate raw data. The curve represents the Chapman model.

Table 1. Estimates of Chapman growth curve parameters of body weight.

	Parameters				Inflection point (days)	Maximum growth rate (g/day)
	W_0 ± SE	a ± SE	b ± SE	c ± SE		
Male	116.72 ± 37.22	18581.27 ± 2075.21	0.0076 ± 0.0011	1.76 ± 0.092	74.60	74.54
Female	119.96 ± 32.33	18011.41 ± 1772.22	0.0078 ± 0.0010	1.79 ± 0.084	74.99	73.53
All	116.18 ± 30.02	18765.70 ± 1768.25	0.0075 ± 0.0009	1.76 ± 0.075	75.58	74.29

W_0 = birth weight of individuals; a = asymptotic body weight or body measurement of the individuals; b = growth rate parameter; c = allometric growth parameter.

Table 2. Student t-test between the measurements and predicted values of body weight from 0 to 120 days old**.**

Age (days)	N	Measurements	Predicted values	t	P
0	58	151.52 ± 36.50	116.18	7.37	0.00
10	59	280.65 ± 66.74	297.53	-1.94	0.06
20	52	644.63 ± 116.90	693.07	-2.99	0.00
30	63	1205.29 ± 187.61	1222.30	-0.72	0.47
40	56	1868.90 ± 284.14	1840.82	0.74	0.46
50	58	2530.71 ± 466.42	2518.01	1.28	0.21
60	61	3282.52 ± 430.35	3231.40	0.93	0.36
70	60	3957.37 ± 522.76	3964.06	-0.10	0.92
80	59	4705.71 ± 592.49	4703.10	0.03	0.97
90	55	5384.47 ± 598.48	5438.66	-0.67	0.50
100	53	6145.85 ± 671.60	6163.25	-0.19	0.85
110	52	6877.98 ± 693.47	6871.21	0.07	0.94
120	42	7581.67 ± 734.60	7558.38	0.21	0.84

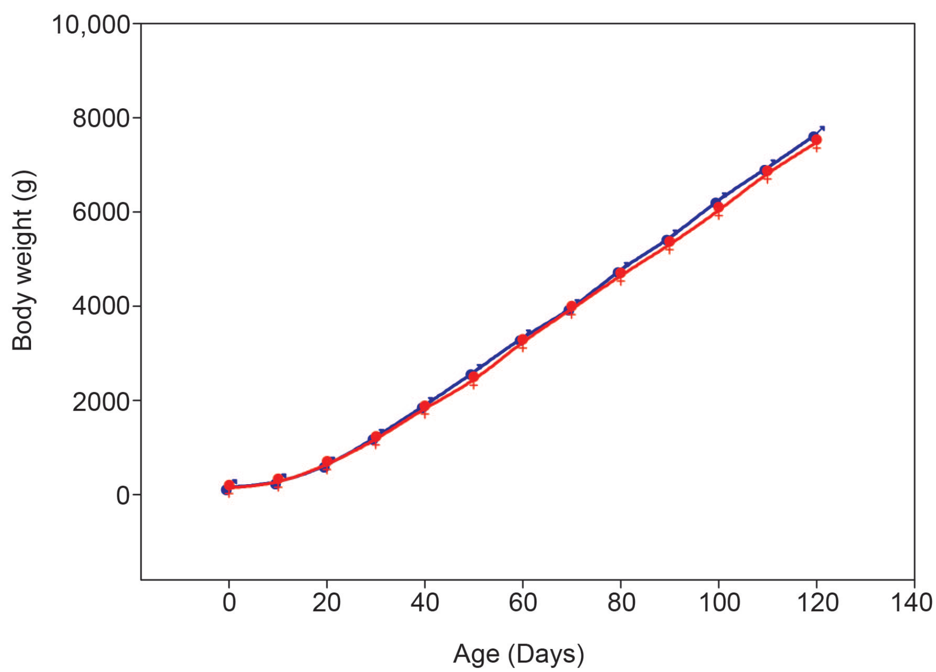

Figure 2. Growth curve of male and female giant panda cubs from birth to 120 days. The blue and red lines indicate male and female cubs, respectively.

Table 3. Result of Two Way ANOVA analysis of body weight.

Source of Variation	df	SS	MS	F	P
Age (day)	12	157397672.00	13116472.67	5096.24	0.00
Sex	1	56623.86	56623.86	22.00	0.00052
Residual	12	30885.09	2573.76		
Total	25	157485180.94	6299407.24		

SS = sum of squares of deviation from mean; MS = mean square.

Anthropometric indices

Body length was a forced variable in our model. Based on the statistical results (Table 4), we applied a total of four steps in the regression. Body length ($P = 1.57 \times 10^{-6}$), and circumference of the chest ($P = 1.10 \times 10^{-6}$) and knee ($P = 2.36 \times 10^{-4}$) were included in our model, and abdominal circumference ($P = 0.706$) was removed. Other body indices were not included in our model. The final model is shown in Table 4. Body length data as well as chest and knee circumferences were fitted by the Chapman model (Figure 3 and Table 5). Based on the growth curves, these three indices reached a maximum growth rate at approximately 20 days after birth. The maximum growth rates of body length and circumference of the chest and knee, were 0.53, 0.49, and 0.17 cm/day, respectively.

Table 4. Parameters of the stepwise regression.

Step	R^2	SE	Indices	Entered/Removed	Regression model
0		4.78	Body length (x_1)	Entered	
1	0.99	2.20	Abdomen circumference	Entered	
2	0.99	1.75	Knee circumference (x_2)	Entered	$y = -19.05 + 3.88x_1 + 3.961x_2 - 3.72x_3$
3	1.00	1.22	Chest circumference (x_3)	Entered	
4	1.00	1.16	Abdomen circumference (x_4)	Removed	

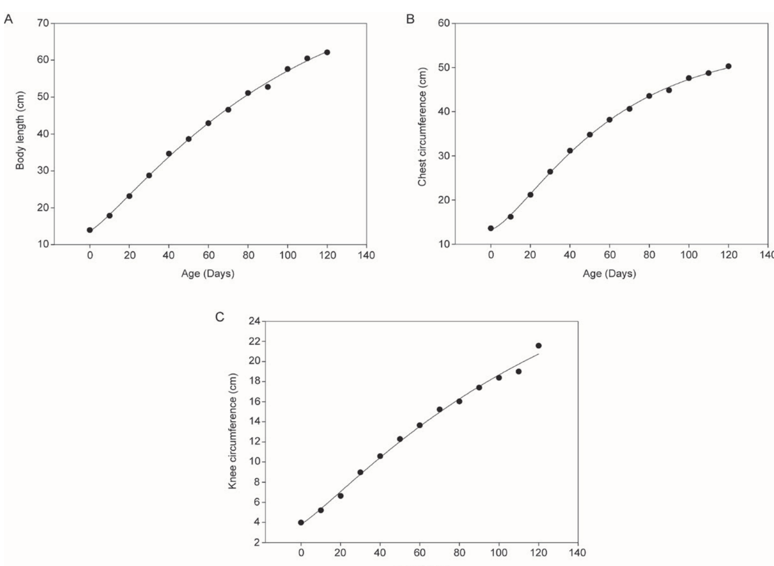

Figure 3. Body development of giant panda cubs from birth to 120 days. Panels A, B, and C show body length, chest circumference, and knee circumference, respectively.

Table 5. Estimates of Chapman growth curve parameters of body measurements.

	Parameters				Inflection point (days)	Maximum growth rate (cm/day)
	L_0 ± SE	a ± SE	b ± SE	c ± SE		
Body length	13.74 ± 0.62	70.52 ± 6.11	0.011 ± 0.002	1.24 ± 0.11	18.96	0.53
Chest circumference	13.35 ± 0.39	42.08 ± 1.27	0.020 ± 0.0016	1.50 ± 0.11	20.06	0.49
Knee circumference	3.84 ± 0.43	28.92 ± 7.98	0.0083 ± 0.0042	1.17 ± 0.22	18.83	0.17

L_0 = body measurement at birth; a, b, c are consistent with Table 1.

Estimation of the heritability of growth rate

To further estimate the heritability of the maximum growth rate, we divided 23 individuals into seven paternal half-sibling families from the total of 83 giant panda cubs. Heritability was estimated on the day when the giant panda cubs reached the inflection point of the first 120 days. There was no significant difference between the paternities (P = 0.30). The heritability of the maximum growth rate was 0.38, which was not significantly by significance testing (t = 0.43, P > 0.05).

DISCUSSION

Growth and development of giant panda cubs

We estimated the growth curve of giant panda cubs with high confidence, which describes the growth and development of cubs at the early stages (from 0 to 6 months) of the lactation period (0 days to 18 months).

The sigmoid curve has an inflection point where the individuals or species reach their maximum growth rate. This provides a valuable benchmark for breeders to adjust the diet of giant panda cubs and fully realize the growth potential of this species. In this study, the maximum growth rate of giant panda cubs (the inflection point) during the early lactation stage was 74.29 g/d. During the fastest growing period of the early lactation stage (Peng et al., 2001), artificial supplementation of breast milk is essential for improving the survival rate during weaning, and will benefit later growth and development.

Similar to the findings for other mammals (Blasco et al., 2003; Barrionuevo et al., 2004), sexual dimorphism in giant pandas does not contribute to differences in growth during early developmental stages. We did not detect a significant difference in growth between male and female giant panda cubs from 0 to 120 days after birth. Identifying the age of abandoned cubs in the wild by their weight is important, and relevant measures could be taken to guarantee their normal growth and development.

Estimation of age by body development parameters

We found that body length, as well as chest and knee circumference, showed significant changes over time. Body length and circumference of the chest reflect growth status from vertical and horizontal perspectives, respectively, and, together with body weight, can be used to confirm the age of cubs. Accurate and timely information regarding the growth and the developmental condition of cubs is valuable for breeders to adjust management plans for feeding.

Tail length does not change significantly during this time period, which suggests that tail growth does not contribute to or is not associated with survival of the giant panda (Peng et al., 2001). In the current study, the Chapman model was successfully fitted to body length and the chest and knee circumference. This suggests that the age of giant panda cubs is most accurately predicted using body measurement indices, and it also provides a potential approach to predict the age of abandoned cubs in the wild.

Estimation of heritability

In our study, the maximum growth rate of giant panda cubs was highly heritable (h^2 = 0.38), similar to Large White pigs (h^2 = 0.33 for males and h^2 = 0.36 for females) (Saintilan et al., 2012) and Angus-Brahman cattle (h^2 = 0.31) (Elzo et al., 2010). To the best of our knowledge, this is the first report of heritability estimates of the maximum growth rate during the early lactation stage of giant panda cubs.

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