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Decoupling Morphological Development From Growth in Periodically Cooled Zebra Finch Embryos

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ABSTRACT Temperature affects growth and development, and morphometry can provide a quantitative description of how temperature changes affect the resulting phenotype. We performed a morphometric analysis on zebra finch (*Taeniopygia guttata*) embryos that were either exposed to periodic cooling to 20 or 30°C throughout incubation over a background temperature of 37.5°C, or were incubated at a constant temperature of 37.5°C. Using a principle components analysis, we found that the relationship between the multivariate size (first principle component) and dry embryo mass depended upon the thermal treatment to which the developing embryos were exposed. Periodic cooling resulted in a smaller embryo mass, but had no effect on the multivariate size of the embryo. This suggests that the growth of phenotypic traits such as the length of long bones and the skull are less affected by temperature than is growth of other soft tissues such as muscle and organs that contribute to body mass. J. Morphol. 269:875–883, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: Zebra finch; *Taeniopygia guttata*; morphology; embryo; temperature effects; incubation

For many oviparous organisms, developing embryos experience temperature variation that occurs seasonally, daily, or more frequently because of adult behavior patterns (Flatt et al., 2001; Hipfner et al., 2001; Shine, 2004). Birds provide parental care to their offspring in the form of heat-provisioning to the eggs (Deeming, 2002), and for many species, eggs are left exposed periodically when the incubating adult leaves the nest during obligatory foraging bouts throughout the day (Zerba and Morton, 1983; Morton and Pereyra, 1985; Weathers and Sullivan, 1989; Hainsworth et al., 1998; Conway and Martin, 2000). This results in variable egg temperatures and a reduction in the overall mean egg temperature during incubation. High temperatures accelerate development and shorten incubation periods (Deeming and Ferguson, 1991), and this is widely seen as beneficial for bird eggs, which are commonly at high risk of predation (Bosque and Bosque, 1995; Martin, 1995). Maintenance of high temperature is also seen as beneficial because low temperature results in abnormal embryonic growth, deformities, and increased embryonic mortality in domestic poultry (Lundy, 1969; Drent, 1975); yet, the effects of temperature variation on development of passerines is largely unknown (Reid et al., 2002).

In nonavian reptiles, the effects of temperature on embryonic phenotype are striking, particularly in species with long developmental periods relative to birds. For instance, in the American alligator (*Alligator mississippiensis*), embryonic growth (increases in embryo mass) and development (differentiation of tissue) show different temperature sensitivities—a 3°C drop in temperature negatively affects both development rate and growth rate, but effects on development rate are stronger. Therefore cooler temperatures result in longer development times where embryos are less developed for a given body size (Deeming and Ferguson, 1989). Incubation at low temperatures has been shown to alter other aspects of reptilian growth and development, including neonatal behavior, running speed, morphology, and residual yolk reserves (Rhen and Lang, 1999; Booth et al., 2000; Shine, 2004).

Birds exhibit rapid embryonic growth rates and require higher incubation temperatures (Deeming and Ferguson, 1991); yet, it is unclear how changes in incubation temperature affect avian embryonic development. Early experimental work on thermal effects on chicken (*Gallus gallus*) development showed that embryos incubated at constant temperatures ranging from ~20 to 30°C resulted in "disproportionate development" with increased rate of deformities (Edwards, 1902; Lundy, 1969). These classic studies identified developmental consequences during the earliest stages of chicken development under conditions of artificial incubation where eggs were held at static temperatures. If these results are generalizable to dynamic thermal states that are more characteristic of natural incubation, phenotypes may be commonly influenced by incuba-

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tion temperature during development, thus creating variation among individuals on which natural selection can act (Badyaev and Martin, 2000; Kaplan and Phillips, 2006). If changes in temperature differentially affect the progression of growth and development, consequences such as altered timing of development relative to embryo mass or size may be apparent. This is important because the effects of altered phenotypes that occur during development may carry over as fitness and survival consequences later in life (Gebhardt-Henrich and Richner, 1998; Bateson et al., 2004; Gluckman et al., 2005; Kaplan and Phillips, 2006).

Across many avian species that range from having altricial to precocial modes of development, the developmental timing of tissue and organ differentiation is quite conservative (Starck, 1998). Where variation does occur it is in the degree of tissue maturity and rates of cellular proliferation of certain organ systems (Starck, 1998). Developmental variation and covariation among traits changes through ontogeny (Zelditch and Carmichael, 1989), resulting in different patterns of allometry (trait size versus overall size) throughout development. For example, nestling house finches (*Carpodacus mexicanus*) show heterochrony (changes in the developmental timetable) between body and bill growth with body size increasing early and bill growth increasing late in development (Badyaev and Martin, 2000). If temperature variation influences the maturity of traits relative to one another, this would be reflected by differences in the allometry of these traits at a given body size.

Previously, we examined the consequences of periodic cooling to the energetic physiology of zebra finch embryos (Olson et al., 2006) and found periodic cooling reduced growth rate and growth efficiency (conversion of yolk solids to tissue). Both of these potentially affect hatchling size and morphology and may have long-term consequences later in life. In this article, we analyze linear measurements and developmental maturity of these same zebra finch (*Taeniopygia guttata* Vieillot) embryos under different thermal regimes to test how temperature variation affects their morphometric phenotype.

MATERIALS AND METHODS

Study Organism

Zebra finches are small passeriform birds that lay eggs that weigh between 0.8 and 1.2 g. This species has an incubation period of about 14 days (Zann and Rossetto, 1991), after which their altricial chicks hatch, featherless, and unable to thermoregulate. Adults generally form breeding pairs and both parents participate in incubation. This results in a relatively consistent egg temperature over the length of the incubation period. However, eggs in the wild are occasionally left exposed (Zann and Rossetto, 1991), and in the lab eggs are able to withstand exposure to cool temperatures for several hours and are able to hatch after incubation at variable temperatures (C.R. Olson, unpublished).

Artificial Incubation and Morphometric Measurements

We supplied nest boxes to 12 female zebra finches in a captive population and collected fresh eggs within 2 h after they were laid. Detailed methods for how eggs were maintained in incubators are described in Olson et al. (2006). In brief, we collected at least three eggs from each breeding female and assigned them to one of three incubation treatments: hourly periodic cooling to (a) 20° or (b) 30° 15 times a day, then returning to 37.5°C, or (c) constant incubation at 37.5°C through out the entire incubation period. These treatments exposed eggs over the length of the experiment to mean temperatures measured at 37.4°C ± 0.04 SD, 37.0°C ± 1.5, and 35.4°C ± 4.3 for control eggs and those cooled to 30 and 20°C, respectively. These eggs were incubated for 12 days before embryos were measured. We chose to examine the period prior to hatching to minimize potential loss associated with high mortality during the hatching process; however, there was no difference in mortality among the temperature treatments in eggs incubated to 12 days ($P > 0.5$). We also incubated a group of eggs for 8–13 days at a constant temperature (37.5°C) to generate a baseline growth curve for this species to which we can compare the eggs that were periodically cooled. Humidity was controlled by varying the surface area of water in the incubator. Egg mass loss averaged ~11%, projected over a typical 14-day incubation period, which is within normal limits (Rahn and Ar, 1974; Drent, 1975). Mass loss did not vary by temperature treatment ($P = 0.12$).

To examine the effects of temperature variation on embryo morphology, development was terminated by placing eggs in a freezer, and a short time later they were thawed and dissected into components of eggshell, albumin and extra-embryonic tissue, remaining yolk, and embryo tissue. Embryos were refrozen at −80°C for later measurement. We thawed the embryos and photographed each from dorsal and left lateral perspectives (Fig. 1). We used a Nikon DXM 1200 digital camera mounted on a Nikon SMZ 1500 stereomicroscope. A mm scale was included in each digital photograph.

We used tpsDig software (Rohlf, 2004) to make linear measurements. Nine linear measurements were collected in triplicate from each specimen and the mean values of each measurement were used for the analysis. Traits measured are shown in Figure 1, and include (a) the tarsometatarsus (tarsus), (b) tibiotarsus (thigh), (c) distal length of wing that includes the carpometacarsus and the phalanges (wingtip), (d) the length of the wing which includes the ulna and radius (wingarm), (e) the diameter of the eye (eye diam), (f) upper culmen distance (culmen), (g) the gape from the tip of the maxillary to the corner of the mouth (gape), (h) the head width from the top, and (i) the length of the body from the pygostyle (tail) to the insertion of the neck into the body (body length). At this stage of development, bones are incompletely ossified and tissues are malleable, so that the shape of the embryo encased within the eggshell is probably different than those we measured stretched flat on a horizontal surface. Embryos younger than 9 days did not clearly exhibit all traits and were very fragile, so we did not include them in the dataset. There were 12 embryos in the 20°C treatment, 9 embryos in the 30°C treatment, and 31 embryos that developed at constant 37.5°C. Thirteen of the 31 constant temperature embryos developed for 12 days, whereas the remainder were measured at incubation ages of 9–13 days.

The developmental normal stage of each embryo was assigned using combined staging criteria based on Hamburger and Hamilton (1951) for chickens, and Yamasaki and Tonosaki (1988) for the society finch (*Lonchura striata*). Traits used as staging criteria (Table 1) were easily visible in the digital photographs from which the linear measurements were taken, and authors one and two scored them without knowledge of the embryos' treatments or age. Although the relationship between staging criteria and incubation time is nonlinear for chickens and society finches in the earliest stages of incubation (Yamasaki and Tonosaki, 1988; Ricklefs and Starck, 1998), in the range of

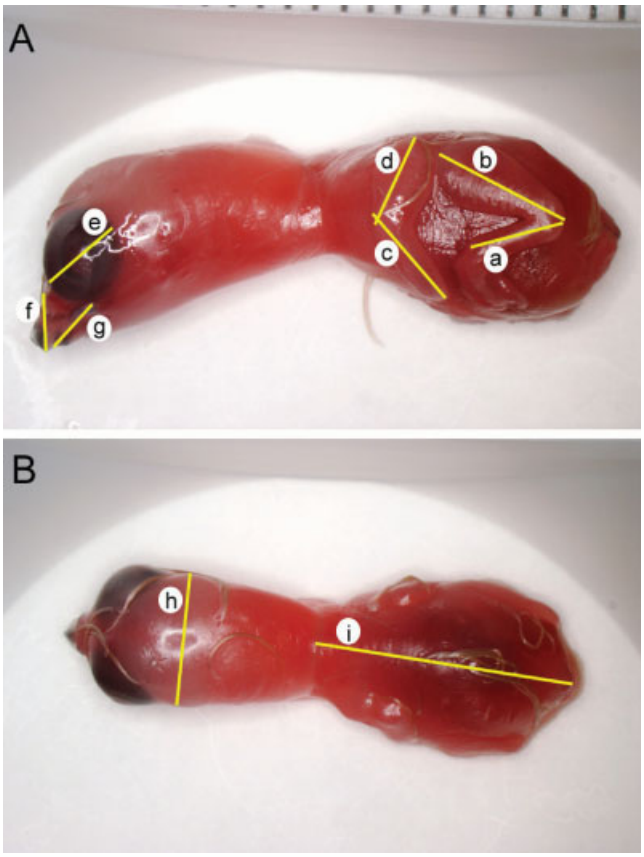


Fig. 1. Sample specimen of a single zebra finch (*Taeniopygia guttata*) embryo from (**A**) the side and (**B**) the top with yellow bars denoting the linear measurements. Linear traits are described in the text.

stages 36–46, where individuals from this study were sampled, the relationship is linear. Visual inspection of embryos revealed variation in the development of the *Musculus complexus* hatching muscles located dorsally on the neck and inserting in the rear of the skull. These muscles are typically hypertrophied in avian embryos to enable the chick to break the shell at hatch (Brooks and Garrett, 1970). We also scored the development of this muscle independently of normal stage, from 1 (absent) to 4 (well developed), while blind to treatment group and age.

Data Analysis

The primary design of this experiment was to let embryos grow under conditions of periodic cooling and compare them with embryos that grew at constant temperature for the same development time, thermal dose (degree · days) and body mass (Olson et al., 2006). This allows the effects of development time and temperature to be separated among the cooling treatments. To understand the effect of thermal treatment on ontogenetic growth we first analyzed the growth trajectory over 9–13 days of development at constant temperature. The effects of the three temperature treatments on embryo morphology at 12 days of age were then compared to the baseline growth trajectory.

Linear body measurements were $\log_{10}$ transformed before analysis. To partition variation due to embryo size from that of shape, we executed principle components analyses (PCA) on the transformed linear measurements based on the covariance matrix (Ricklefs and Miles, 1994; Klingenberg, 1996). Directions in growth trajectories among cooling treatments were compared by running a separate PCA for each temperature treatment, then calculating the angles between the PC 1 vectors. The angle θ between two PC 1 vectors, **a** and **b**, was computed as $\theta = \arccos(\mathbf{a}'\mathbf{b})$ (Klingenberg, 1996). Differences in θ between the three groups were tested against a Monte Carlo randomization of PC 1 coefficients (999 iterations) where specimens were randomly reshuffled among groups (Collyer and Adams, 2007). We then ran a PCA of all embryos together to obtain multivariate size from the PC 1 scores, and examined how multivariate size changed with dry embryo mass and normal developmental stage. Lastly, the remaining shape variations in PC 2–9 were analyzed in a MANOVA to test the effects of embryo dry mass and treatment on ontogenetic variation in shape.

RESULTS

Multivariate Size

Among eggs incubated at constant temperature from 9 to 13 days, the first principle component represented 72.1% of the variation in the dataset, whereas the second and third contained an additional 9.6% and 5.7% of the variation, respectively (Table 2). Multivariate isometry, defined by $1/\sqrt{p}$ (Klingenberg, 1996; Weston, 2003), where p is the number of traits examined, is 0.33 for the analysis of 9 traits. Linear measurements of limbs and the body exhibited positive allometry (>0.33) and traits associated with the head showed negative allometry (<0.33), although a bootstrap analysis showed few traits by themselves differed statistically from isometry (Fig. 2A). The hind legs of these embryos showed the greatest positive allometry, while the di-

TABLE 1. Normal staging criteria used to describe developmental maturity for late-term zebra finch embryos (Taeniopygia guttata) based on Hamburger and Hamilton (1951) and Yamasaki and Tonosaki (1988)

Stage 35	Beak: lower beak begins to elongate, but does not reach length of upper beak; no cornification evident
Stage 36	Beak: Lower and upper beak equal in length; egg tooth begins to be cornified
Stage 37	Tail: Extends straight out from body
Stage 38	Tail: Tail curves ventrally
Stage 39	Beak: egg tooth cornified at base; cornification not evident on rest of bill
Stage 40	Beak: upper beak begins to be cornified at the top; lower bill not cornified
Stage 41	Beak: upper cornified in the anterior 1/3, continuous to egg tooth; top of lower bill begins to be cornified Feet: claws are pink
Stage 42	Beak: Cornification spreads proximally to anterior 50%; lower bill 50% cornified Feet: claws cornified and white
Stage 43	Beak: Cornified to the base in upper and lower bill; Beginnings of gape visible
Stage 44	Beak: Cornification complete; gape characterized by a prominent zone of pigmentation Skin: thin
Stage 45	Beak: gape pronounced with distinct downward bend Skin: wrinkled and thickened
Stage 46	Hatch

TABLE 2. Scaling relationships for 9 linear morphometric measurements of zebra finch (Taeniopygia guttata) embryos incubated at constant temperature over a range of 9–13 days

Eigenvalue	0.0207	0.0028	0.0016	0.0012	0.0009	0.0007	0.0004	0.0003	0.0002
% variation	72.09	9.59	5.69	4.24	3.05	2.33	1.45	0.91	0.65
Eigenvectors	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Tarsus	0.40	−0.33	−0.29	0.24	0.38	−0.09	−0.48	−0.42	0.17
Thigh	0.43	−0.28	−0.10	−0.58	0.04	−0.35	−0.05	0.46	−0.20
Wingtip	0.36	0.34	−0.33	−0.15	−0.45	−0.27	0.33	−0.46	0.13
Wingarm	0.34	−0.19	−0.22	0.44	−0.42	0.32	0.05	0.49	0.28
Eye dia.	0.11	0.18	−0.24	0.18	0.66	−0.05	0.61	0.19	0.12
Culmen	0.32	−0.02	0.69	0.41	−0.04	−0.47	0.12	−0.01	−0.05
Gape	0.33	0.77	0.08	0.00	0.14	0.18	−0.43	0.20	−0.09
Head width	0.30	−0.14	0.01	0.09	0.00	0.45	0.22	−0.22	−0.76
Body length	0.29	−0.11	0.45	−0.41	0.11	0.49	0.17	−0.18	0.47

ameter of the eye increased in size the slowest relative to overall body size.

Multivariate size (PC1) increased with development time ($P < 0.0001$), thermal dose ($P < 0.0001$), and dry embryo mass (Table 3; $P < 0.0001$) for embryos held at constant temperature. Because at constant temperature thermal dose is strictly dependent on the amount of time that eggs develop, we used a model that examined multivariate growth at constant temperature in terms of dry embryo mass and development time, but without the thermal dose term. This model explained 86% of the variation in PC 1 ($F_{3,27} = 60.13$, $P < 0.0001$), and there was a significant time by mass interaction ($F_{1,27} = 4.85$, $P = 0.036$) indicating that the effect of mass on PC 1 changes with development time. Dry mass was the primary determinant of PC 1 ($F_{1,27} = 44.7$, $P < 0.0001$), while incubation time alone had no significant effect ($F_{1,27} = 1.69$, $P = 0.20$) in the model.

All treatment groups appeared to share a common allometric growth trajectory ($p_{\text{interaction}} = 0.235$) with limbs tending to show positive allometry and head measurements showing negative allometry (Fig. 2B). However, considerable variation between temperature treatment groups in the PC 1 coefficients also indicated that this dataset of nonrigid, soft embryonic structures was inherently noisy and/or that sample sizes were too small to detect temperature effects. The angles of multivariate growth trajectory (PC 1 coefficients) of embryos that developed for 12 days in the three temperature treatment groups varied from $\theta = 24^\circ$ to 31°, but these angles did not differ from random in pairwise comparisons among the three treatments (Table 4).

PC 1 scores (multivariate size) of individual embryos were compared among the cooling treatments and the controls at the same thermal dose, incubation time, and dry embryo mass. We employed Wilcoxon ranked-sign tests to compare the PC 1 scores of cooled embryos to the expected values obtained from a linear regression of constant temperature PC 1 scores against the explanatory variable (Table 4) and Bonferroni-corrected for 6 sequential comparisons. Periodic cooling did not affect the relationship between multivariate size and incubation time ($P > 0.5$) or multivariate size and

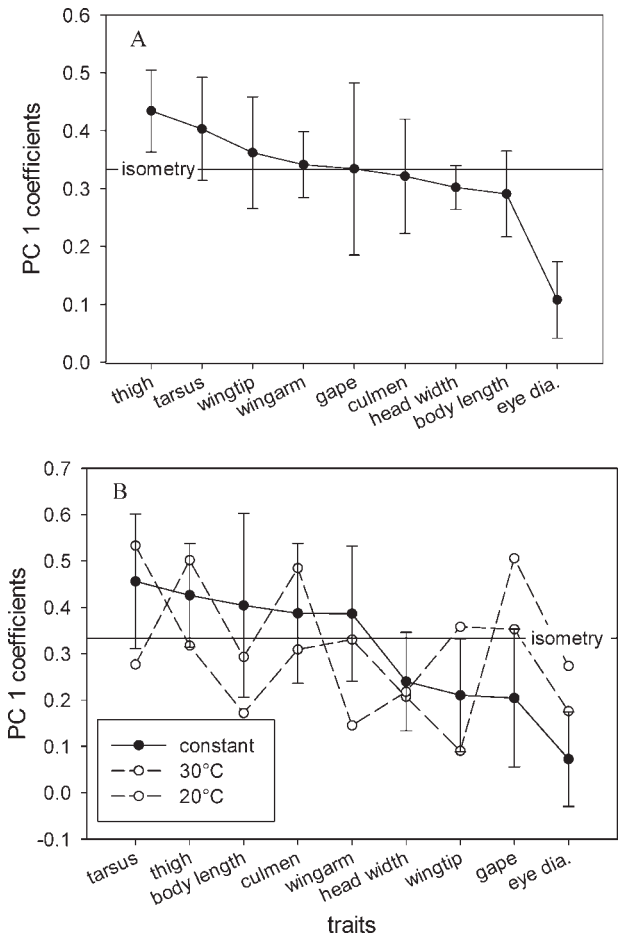


Fig. 2. Comparisons of first principle component coefficients for zebra finch (*Taeniopygia guttata*) embryos grown (**A**) at constant temperature over the ontogenetic sequence of 9–13 days, and (**B**) under different regimes of periodic cooling for 12 days. Plots show allometric relationships of different traits relative to an isometric growth coefficient of 0.33. Error bars are shown for embryos incubated under constant conditions and represent 95% confidence intervals calculated from bootstrap simulations resampled with replacement 999 times. Note the reordering of traits between the two analyses.

TABLE 3. Linear regressions of PC 1 against incubation time, thermal dose (TD), and dry embryo mass for zebra finch (Taeniopygia guttata) embryos

				Wilcoxon			
				30°C treatment		20°C treatment	
		R^2	P	$\|z\|$	P	$\|z\|$	P
Time	$0.088x - 1.017$	0.63	<0.0001	0.5	>0.5	−21.0	>0.5
TD	$0.0024x - 1.015$	0.63	<0.0001	−0.5	>0.5	−5.0	>0.5
Mass	$7.71x - 0.32$	0.84	<0.0001	−1.5	>0.5	39.0	<0.0006

Wilcoxon ranked sign tests of PC1 scores obtained from cooling treatments of 20°C and 30°C treatments were compared to the estimated values based on the linear relationships at constant temperature.

thermal dose ($P > 0.5$). There was a significant treatment effect on the relationship between multivariate size and embryo dry mass between the 20°C and constant temperature embryos (Fig. 3A; $P < 0.0006$), but not between the 30°C and the constant temperature embryos ($P = 0.66$). Analysis of covariance also revealed that PC 1 scores of embryos cooled to 20°C differed from those of constant-temperature eggs ($F_{1,40} = 6.58$, $P = 0.014$). Those eggs that were periodically cooled to 20°C had longer linear body measurements for their body masses compared to the controls (Fig. 3A).

The hypertrophy of neck muscles (*Musculus complexus*) increased with embryo age (linear regression, $P < 0.02$) and embryo mass ($P < 0.05$). We therefore calculated the residual of the neck score based on embryo age and mass and tested differences between embryos in the different cooling treatments. Embryos cooled to 20°C had less-developed neck muscles compared to the constant-temperature embryos (Welch's t (for unequal variances) = 5.40, $P = 0.016$), indicating that cooling negatively affects the growth of muscle tissue.

Normal Development Stages

By 12 days of incubation embryos had grown to a range of normal stages (38–45) that varied significantly with treatment (ANOVA $F_{2,30} = 8.9$, $P < 0.001$). Post hoc examination showed embryos cooled to 20°C were at a lower stage compared to the constant and 30°C treatments (pairwise student's t, $P = 0.0005$ and 0.003, respectively). Embryos in the 30°C treatment were at the same developmental stage as embryos that grew at constant temperature ($P = 0.86$). For all embryos, including those that developed for 9–13 days at constant temperature, normal stages were positively correlated with development time ($R = 0.67$, $P < 0.0001$).

Normal stages were positively correlated with dry embryo mass (Fig. 3B; $R = 0.75$, $P < 0.0001$) and multivariate size (Fig. 3C; PC1; $R = 0.80$, $P < 0.0001$) for all eggs that developed from 9–13 days. Residuals of normal stage accounting for dry embryo mass did not differ between treatments (ANOVA, $F_{2,48} = 0.63$, $P = 0.54$); cooled embryos did not suffer a setback at least in terms of the traits used to stage the embryos. However, residuals of stage accounting for PC1 did differ between treatments ($F_{2,48} = 3.54$, $P < 0.04$), with embryos from the 20°C treatment at a lower developmental stage than embryos from either the constant or 30°C treatment. That is, embryos periodically cooled to 20°C had a larger multivariate size for a given normal stage than embryos from the control treatment (Fig. 3B; ANCOVA $F_{1,40} = 6.06$, $P < 0.02$).

Multivariate Shape

In the dataset including only embryos which developed at constant temperature for ~9–13 days, there was no difference in body shape (PC 2–9) with incubation time and dry embryo mass (Wilks' $\lambda_{16,42} = 0.52$, $P = 0.47$). By day 9 of incubation the body plan of zebra finch embryos was well-defined in terms of shape and did not subsequently change with ontogenetic growth. A bivariate plot of PC 2 (11.2% of the variation) and PC 3 (9.2% of the variation) showed no separation among the treatment groups (see Fig. 4). We used a model that incorporated the effects of embryo dry mass and the three temperature treatments to test shape variation among embryos at day 12 of incubation. The whole model showed shape variation was present in this dataset (MANOVA, Wilks' $\lambda_{24,70.2} = 0.23$, $P = 0.02$), while treatment (Wilks' $\lambda_{16,48} = 0.42$, $P = 0.09$) and dry mass ($F_{8,24} = 0.64$, $P = 0.10$) alone were marginally nonsignificant. There were no significant interactions between treatment and dry embryo mass. Pairwise examination in principle component scores showed a treatment effect in PC7 ($F_{2,31} =$

TABLE 4. Pair-wise comparisons of growth trajectories (PC 1 coefficients) for zebra finch (Taeniopygia guttata) embryos that experienced thermal regimes of periodic cooling to 20°C, 30°C, and constant 37.5°C for 12 days of incubation

Angle between vectors θ	20°C	30°C
30°C	30.91 $P = 0.235$	
Constant	24.13 $P = 0.383$	26.16 $P = 0.395$

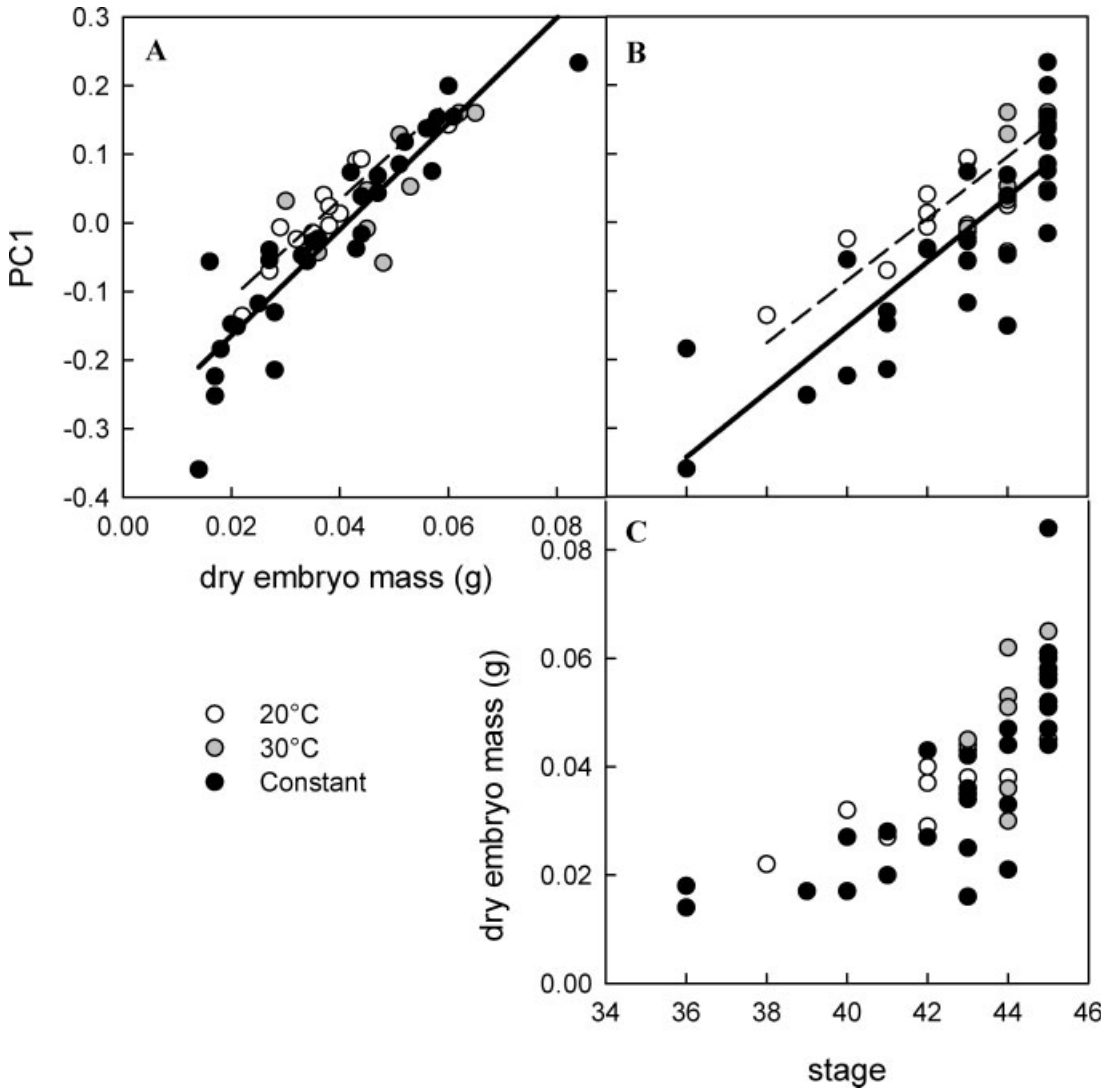


Fig. 3. Scatterplot matrix of developmental traits for zebra finch (*Taeniopygia guttata*) embryos incubated from 9–13 days, including (**A**) dry embryo mass and principle component 1, (**B**) normal stage and principle component 1, and (**C**) dry embryo mass and normal stage. Black symbols are embryos incubated at a constant 37.5°C, gray symbols are embryos cooled to 30°C, and open symbols are embryos periodically cooled to 20°C. Least square regressions are shown for the constant temperature (solid line) and 20°C embryos (dashed line) when these two treatments differ.

5.90, $P = 0.0068$), composed primarily of differences in eye diameter, and PC 9 ($F_{2,31} = 3.85$, $P = 0.0321$), composed primarily of head width.

DISCUSSION

Our results with periodically cooled zebra finch embryos are similar to studies on reptile embryos that grow at different incubation temperatures: linear traits grow faster relative to body mass at lower incubation temperatures (Deeming and Ferguson, 1989; Booth et al., 2000; Flatt et al., 2001). The greater multivariate body sizes (PC 1 scores) for a given body mass and normal stage in the 20°C cooling treatment can be achieved by enhancing the growth of linear traits compared to embryos that developed at a constant temperature, or reducing the growth of certain traits that were not reflected by the linear length of the skeleton. Temperature treatments did not affect the trajectory of multivariate growth, as seen by the similarity of PC 1 vectors between temperature treatments. Rather, the longer body sizes for a given mass in the periodically cooled embryos may be the result of reapportioning nutrients to maintain growth along the longitudinal axis, possibly at a cost to the growth of muscles and other organs that have an effect on body mass and developmental stage. The effect on embryo shape by incubation temperature was subtle, as differences between the temperature treatments were not seen in the shape axes that carried the most remaining variation (PC 2 and 3).

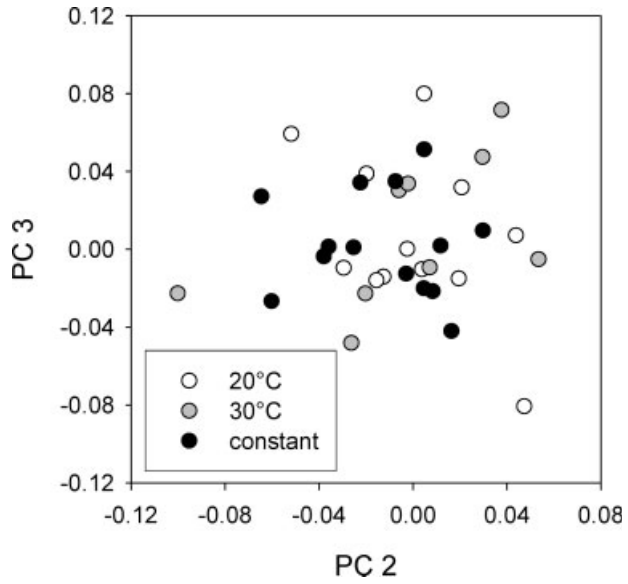


Fig. 4. Bivariate plot of shape variables, PC 2 and PC 3, among zebra finch (*Taeniopygia guttata*) embryos grown for 12 days of age under conditions of periodic cooling to 20, 30, and at constant 37.5°C.

However, it is important to point out that significant variation among smaller principle components that describe shape differences may be significant to survival and fitness in the wild (Ricklefs and Miles, 1994).

Periodically cooled embryos showed reduced prominence of the muscle complex on the back of the neck that is necessary for hatching to occur. These results are similar to cold exposure in chicken and quail embryos, which slows the growth rate of muscle tissues (Hohtola and Visser, 1998). In growing organisms, muscle growth may occur through either hyperplasia, the increase in muscle fiber number, or hypertrophy, the increase in muscle fiber size. In turkey (*Meleagris gallopavo*) poults, decreases in mean incubation temperature by as little as 2°C reduces the number of muscle fibers in breast muscle (Maltby et al., 2004). It is thought that adult organisms can only increase muscle size through hypertrophy, so that compensation for reduced muscle growth in embryos might be restricted to hypertrophy of muscle cells in adults because at some point in development the number of muscle fibers becomes fixed. It is not clear how a small *Musculus complexus* would affect hatchability of the finches in this study because they were not allowed to hatch, but zebra finch eggs exposed to hourly periodic cooling as low as 20°C can hatch (C. R. Olson, unpublished). It is likely that reduced muscle development in the neck is indicative of muscle deposition in other regions of the body, and future work should examine the effects of temperature variation on the structure and composition of muscle fibers.

Cold exposure may affect embryonic development in a similar way to that of food restriction in placental mammals (Hales and Barker, 1992; Petry et al., 2001). Mammals, when faced with placental insufficiency where delivery of nutrients is restricted by a small placenta or restricted flow in the uterine arteries (Sadiq et al., 1999; Petry et al., 2001), favor growth of organ systems that are essential for short-term survival over those that are less-essential. In chickens, embryos exposed to cold have reduced uptake of yolk solids (Feast et al., 1998), and over time this may result in a low-level, but chronic, nutritional stress of the growing embryo. Periodic cooling may disrupt the absorption and transport of nutrients from the yolk into the growing tissues, and these effects may accumulate over the incubation period by altering growth. In ectotherms that experience cooling, barriers to nutrient transport would exist at multiple levels due to the temperature effects at different levels of organization, including reduced diffusion rates across biological membranes and decreased membrane permeability at lower temperatures (Hochachka and Somero, 2002).

Given that reptiles and birds both show a similar response to low temperatures it is not unreasonable to query the adaptive nature of this relationship. For one, a developing embryo facing a thermal challenge may appear to alter its growth phenotype to enhance its short term survival. However, the different relationships between multivariate size and mass between the treatments may be due to a nonadaptive canalization of linear growth over the developmental time period (Arendt, 1997; Boersma and Wit, 1997), resulting in an apparent trade-off between growth of different structures in the body that corresponds to an event where the short-term survival of the embryo is enhanced. This would result in hatchlings that achieve a sufficient size within a prescribed incubation period, and with a delay in the development of more plastic organ systems.

Alternatively, we suggest that high predator-induced mortality at the nest during the incubation and nestling stages may have adaptively favored linear growth and tissue maturation over accumulation of tissue mass when temperatures are unfavorable. Nest predation is thought to have a strong selective influence on the evolution of the rapid growth rates typically seen in birds (Bosque and Bosque, 1995; Angelletta and Sears, 2003), possibly resulting in the most rapid growth rates among the vertebrates (Case, 1978). In the event of severe periodic cooling, which would be expected to reduce growth rates and lengthen growth periods, this strategy would minimize the time spent in the nest as eggs and allow the chicks to leave the nest in a timely way. Sibling competition may also have selected for large multivariate size and rapid maturation (Ricklefs, 1993; Lloyd and Martin, 2003) if

this allows more chicks to succeed in the competitive environment of the nest. Because life-history variants such as avian incubation periods should be under selection (Remeŝ and Martin, 2002), future studies that address the effects of periodic cooling on the morphology and function of specific systems such as muscle, neuro-sensory and skeletal systems, would be informative.

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LITERATURE CITED

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