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DEVELOPMENT OF HEART RATE IN THE PRECOCIAL KING QUAIL *COTURNIX CHINENSIS*

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Summary

Our aim was to examine changes in heart rate (f_H) during the embryonic and posthatching periods of the smallest precocial avian species, *Coturnix chinensis*. In experiment I, repeated measurements of mean f_H were made for individual quail by ballistocardiogram (BCG) during incubation, and by both piezo-electric film and electrocardiogram (ECG) during the posthatching period (resting and thermoneutral conditions). Mean f_H of all embryos increased during the second half of incubation and the first week posthatching, but a few embryos experienced a very brief period of decreased f_H prior to internal pipping. After the first week, f_H of posthatching quail was maintained at high levels (550–650 beats min^{-1}), then decreased with age and increase in body mass. The maximal f_H of quail chicks represents a greater posthatching increase in f_H than is found in larger precocial chickens, this difference being attributable to the higher demands of thermoregulation at small body masses in the quail. In experiment II, the mean f_H of quail embryos (day 2–16) was recorded by ECG, and embryonic stage, yolk-free embryo mass (wet and dry) and water content were measured. Mean f_H was linearly related to embryo mass throughout incubation, except on the day prior to internal pipping, when the f_H of a few embryos declined below this linear relationship. Measurements of instantaneous f_H of late incubation embryos, young and adult quail all showed spontaneous fluctuations in f_H. Two main frequency components of f_H fluctuations were identified for the first time in an avian species. Low-frequency (mean 0.09 Hz, 12.6 s) and high-frequency (1.4 Hz, 0.9 s) oscillations in both young chicks and adult quail were detected and are considered to reflect baroreflex mediation of f_H and respiratory sinus arrhythmia, respectively.

Key words: non-invasive, heart rate, oscillations, growth, embryo, posthatching, quail, *Coturnix chinensis.*

Introduction

Changes in heart rate (f_H) during growth reflect the changing metabolic requirements and the state of the central nervous control of the oragnism. All avian embryos are ectothermic, at least until hatching; however, in precocial species, the development of endothermy starts with increases in metabolic intensity after internal pipping and the commencement of pulmonary respiration, which reflects the beginning of their transition to thermoregulatory control (Paganelli and Rahn 1984; Whittow and Tazawa, 1991). Other species with less mature hatchling developmental modes become endothermic later in the posthatching period. In any case, the shift from an ectothermic to an endothermic state is likely to influence the level of f_H during development.

The development of avian embryonic heart rates (f_H) is increasingly being investigated, particularly by using non-invasive techniques to obtain repeated measurements from the same individuals during incubation until hatching (Tazawa *et al.* 1991*a*,*b*, 1994; Tazawa and Whittow, 1994). In contrast, there are fewer comparative developmental studies of the cardiovascular abilities of birds during the posthatching period, and fewer still during both embryonic and posthatching periods for the same species. However, Odum (1941, 1945) described the development of f_H in house wrens (*Troglodytes aedon*) in relation to ambient temperature and gave insight into some of the factors that contribute to variation in adult and juvenile f_H in birds.

The embryonic f_H of the smallest precocial species measured by non-invasive ballistocardiography (BCG) to date is that of the highly selectively bred Japanese quail (egg mass 8–10 g, Suzuki *et al.* 1989; Tazawa *et al.* 1991*a*). In the present study, we measured embryonic and posthatching f_H of one of the smallest precocial species (egg mass 5–6 g, hatchling mass 3.5–5 g, adult mass 40–50 g), the king or Chinese painted quail (*Coturnix chinensis*, formerly *Excalfactoria*; Johnsgard, 1988).

This species is found from India to southeast China, and down into Australia, and has not been selectively bred as yet, but is being developed as a small experimental animal model in Japan (Tsudzuki, 1994). The hatchlings are capable of weak endothermic heat production at first, but are not homeothermic during the first 2 weeks of posthatching development and are close to the physiological limits for the precocial development mode (Bernstein, 1973; Pearson, 1994*a*,*b*). According to allometric predictions (Tazawa *et al.* 1991*a*), the high metabolic demands of being the smallest precocial hatchling are likely to require a higher f_H by the time the embryo hatches than measured to date. We therefore hypothesize that the f_H of the quail should also increase further after hatching, in parallel with improvements in thermogenic powers during the development of homeothermy.

Experiment I of this study examines the daily changes in mean f_H of the same individual quail during the second half of incubation and the posthatching period using non-invasive techniques under thermoneutral conditions. In experiment II, semi-invasive measurements by ECG are used to investigate the relationships between embryonic growth (wet and dry yolk-free mass), embryonic stage, water content and mean f_H. Finally, we present preliminary measurements of instantaneous f_H for late incubation embryos and quail during the posthatching period. Spontaneous variability in instantaneous f_H is examined using power spectral analysis for the first time in an avian species, and we discuss the possible physiological origins of this f_H variability.

Materials and methods

Acquisition of eggs

Quail eggs for experiment I were acquired from the University of Osaka Prefecture in October 1996, after conducting a preliminary experiment in August. These eggs were from a colony established at the university from recent imports to Japan from Taiwan. All eggs received at Muroran were identified by numbering, which indicated their parentage. Only eggs from wild-type parents were used, and all birds were in their first breeding season. Eggs were freighted by a local courier service in padded cardboard containers to Muroran Institute, measured (as described below) and then incubated immediately. Prior to shipment, the eggs from Osaka were collected each evening and held in storage at 15 °C for up to 5 days in a low-temperature incubator.

After hatching, the chicks from experiment I were raised to maturity, as described below, and paired for breeding. Eggs for experiment II were all laid by five first-generation female quail at Muroran. Eggs were collected daily and stored in a low-temperature incubator at 10–11 °C for up to 3 days before being set for incubation.

Incubation

Egg mass was measured on an AND balance (model ER-180A) to within 0.001 g immediately before eggs were placed in the incubator. Egg length, pole to pole, and maximum width across the equator were also measured to 0.05 mm using a vernier caliper. Fresh egg mass of the freighted eggs was estimated from the relationship given by Hoyt (1979) using egg dimensions. Eggs were incubated in a small still-air incubator (Zenkei table-top model 40, Japan; capacity approximately 40 chicken eggs) within a sterilised plastic tray, which permitted quick removal of the eggs from the incubator for mass or f_H measurements with a minimum of cooling of the incubator. Eggs were incubated at 38±0.5 °C and 55 % relative humidity until hatching or until the desired incubation day. Relative humidity was controlled by vents so that eggs achieved approximately 15 % mass loss during incubation according to the relationship described by Rahn and Paganelli (1990).

Posthatching rearing conditions

After hatching, chicks were removed from the incubator, hatchling mass was determined to the nearest 0.001 g, a tape leg-band was attached for identification, and the chicks were returned to a constant-temperature brooder, which was a modified glass terrarium (600 mm×300 mm×280 mm), lit and heated continuously by a 100 W infrared lamp and a commercial electric heater for hand-rearing birds (20 W). Environmental temperature, although relatively constant, was not uniform throughout the brooder, but ranged between 30 and 40 °C. The floor of the brooder was covered with wood shavings; chicks were supplied with a mixture of high-protein chicken feed, small finch seed mix, supplemented with meal worm (*Tenebrio* sp.) larvae, spinach and lettuce, and water *ad libitum*. The same brooder was also used during f_H measurements of the quail chicks. Breeding quail were housed as four pairs in a chicken rearer (Zenkei M-type, capacity 50 birds), the dimensions of each compartment being 880 mm×710 mm×820 mm, and were supplied with feed similar to that of the young quail.

Embryonic heart rate measurements

All measurements were made in a larger still-air incubator (Sakura IF-B3, Tokyo) at 38±0.2 °C. Embryonic f_H varies considerably during development, and circadian rhythms in f_H may be present, but are yet to be fully explored and were not investigated in this study. In experiment I, f_H measurements refer to a single time during the day, which was different for individual eggs, but repeated measurements during the incubation period were always made at a similar time of the day for each egg. Measurements were made on 11 eggs between 09:00 h and 17:30 h each day. The f_H of individual embryos was measured by ballistocardiography (BCG) using a single audiocartridge unit so that individual embryos were measured sequentially. Each egg was allowed to reach temperature equilibrium (45 min) before measurement and, in experiment I, eggs were measured every day during the second half of incubation until hatching.

Ballistocardiogram

Embryonic f_H was detectable non-invasively using an audiocartridge measuring system previously described in many

studies of domesticated avian embryos (Suzuki *et al.* 1989; Tazawa *et al.* 1989, 1991*a*). Inside the measurement incubator, a floating platform was hung from the ceiling, and the audiocartridge system and a small concave ceramic dish were placed on it to support the egg. This platform attenuated most of the external vibrations that contaminate f_H signals, but further attention was required to minimise machine- and human-induced vibrations in the vicinity of the experimental incubator. This was particularly important when measuring f_H in earlier embryos, which give weak signals. After the egg had been placed horizontally on the platform, the audiocartridge stylus needle was brought into contact with the egg at right angles to the egg surface. The electrical signal was amplified (Bioelectric amplifier type 4124, NEC San-ei) to a variable extent, depending on embryonic age, but generally by between 85 and 95 dB. The signal was then low- and high-pass-filtered to remove baseline wandering and high-frequency noise. Bandpass filtering frequencies varied individually between embryos, but were between 4 and 30 Hz. The final signal was digitised using a 12-bit A/D converter with an input of ±5 V every 5 ms and stored on a personal computer.

Electrocardiogram

Three copper wires (0.1 mm diameter) 30 mm long were used as electrocardiogram (ECG) leads. Each wire was bent at right angles 3–4 mm from one end and inserted into a hole made on the upper surface of the egg by carefully puncturing the eggshell and shell membranes with a 25 gauge hypodermic needle sterilised in alcohol. Epoxy glue was used to seal the hole and to fix the electrode in place with minimal reduction of the diffusive surface area of the egg. The three electrodes were inserted to form an equilateral triangle with sides 15 mm long. Prepared eggs were rewarmed in the small incubator until the epoxy hardened (1 h) before transfer to the measurement incubator. The electrical signal was similarly amplified, notch- and bandpass-filtered before being digitised and recorded on a personal computer as described above. Bandpass filter frequencies varied with both embryonic age and the quality of the signal. Embryos at 2–3 days were usually filtered between 4 and 20 Hz and later embryos between 4 and 50 Hz.

Posthatching heart rate measurements

Piezo-electric film

The f_H of hatchling (day 0) and 1-day-old quail, which were too small to be measured by the smallest ECG disc electrodes, was measured using a flexible piezo-electric polyvinylidene fluoride (PVDF) film, which is sensitive enough to detect the cardiac contractions (apex cardiograms) of hatchlings when the film is in contact with the sternum. The system used here is described in detail by Tazawa *et al.* (1993). However, quail were unrestrained during measurements in this study. A small ventilated cylindrical chamber (inclined 20–30 ° above horizontal), the diameter of which was approximately twice the width of the chick, was used to restrict the range of movements of the quail to a position standing on the film, which lined the floor of the inclined cylinder. Cotton wool was inserted between the film and the cylinder wall. The curved surfaces of the chamber also directed an active quail back towards the centre of the film. The PVDF film sensor was the same as that used by Tazawa *et al.* (1993). The output signal was amplified by 80 dB and bandpass-filtered between 5 and 24 Hz using the same system as for embryonic BCG measurements. A 1.5 s time constant was used on amplification so that both respiratory and heart rate signals were detected; this was verified on an oscilloscope display. The cylinder, which was used to measure hatchling f_H, was left continuously in the heated brooder containing the quail hatchlings so that they became accustomed to its presence. Environmental temperature at this location was thermoneutral, at approximately 35 °C. Chicks were placed individually in the chamber soon after hatching (within 2 h), and after 15 min the digitised signal was recorded as for embryonic signals (detailed below). With broodmate quail in close proximity, and clearly visible, chicks soon settled down inside the measurement cylinder.

Posthatching ECG

f_H was determined for 4-day-old and older quail using two types of non-invasive ECG electrodes dependent on quail size. Both systems were used on unrestrained quail, which were confined within an isolated area, 15 cm in diameter (mesh enclosure), of their brooder. One chick was measured at a time and was accompanied by 1–2 broodmate(s) within the enclosure. Quail were not deprived of food and water during these experiments, and all measurements were made during daylight hours. Quail were familiar with being handled by humans and soon relaxed within the mesh enclosure, often sleeping during measurement periods. The f_H of small quail between 4 and 10 days old was measured using a reusable mini Ag/AgCl skin ECG electrode system (model NT-214, Nihon Kohden, Tokyo). The lightweight discs (outer diameter 8 mm) had a 2 mm deep well on the contact surface into which the ECG electrolyte paste (Elefix paste, Nihon Kohden) was placed. The electrodes were then attached to the quail using double-sided adhesive collars specifically designed for the mini-electrodes. The f_H of larger quail, including five adults, was measured using solid-gel disposable electrodes (Vitrode A-50, Nihon Kohden), commercially available for neonate ECG/respiration monitoring. The flexible sticky gel pads (2 cm diameter) were reduced to triangles of approximately one-third of their original size. Two electrode leads were attached to the skin of the thoracic wall below the wings utilising the naked apteria, caudal to the humeral joint. A third electrode was attached to the left lateral surface of the rump, after trimming a small area of down. All leads were supported above the brooder lid to allow freedom of movement. The signal was amplified, notch- (50 Hz mains interference) and bandpass-filtered, then recorded on computer as for embryonic measurements.

Mean heart rate calculations

All the methods listed above produced a digitised f_H signal

that was recorded for 2 min periods at a sampling frequency of 200 Hz in both embryonic and posthatching periods. Measurements were made over four consecutive recordings for individual embryos during periods when the f_H signal was least disturbed by activity and external noises (approximately 10 min). The recorded data files were processed by computer using Burg's algorithm (maximum entropy method, see Usui *et al.* 1985), which divided the 2 min data file into 5 s periods and calculated the power spectrum density of each interval individually, which we defined as 'f_{H5}'. The program displayed the 5 s interval, its autocorrelation function and power spectrum distribution and an f_H value for the spectral peak with the most power on the screen. When the autoselected spectral peak was not that of the f_H signal, a secondary spectral peak was optionally selected instead. f_H was determined for undisturbed intervals only, which were always at least 50 % or more of the total 5 s intervals of the four runs (8 min in total). The mean value (± standard deviation, S.D.) of all f_{H5} values was determined and is referred to as the mean daily f_H of that embryo or chick.

Instantaneous heart rate calculations

In addition to mean f_H measurements, instantaneous f_H (f_{HI}) was determined for individual quail (20–30 min recordings) when the high-frequency QRS complex of the ECG signals could be isolated. f_{HI} was calculated from the time interval between consecutive R waves, which were recorded using a Schmidt-trigger method. Oscillations (approximately 0.004–5 Hz) in f_{HI} were examined for young and adult quail when R peaks from ECG signals were detected with a minimum of noise entering the data. Data files of f_{HI} were examined for 5–10 min segments, for subjects that were not apparently active. Time intervals were then divided into 512-point time-series segments for which the power spectrum was calculated using a Fast Fourier Transform after the data had been normalised by the least-squares method. The calculation window (rectangular) was then moved half of one time-series segment, and power spectra were recalculated for the subsequent segments. Finally, for each time interval, the cumulative average of the power spectra was calculated and examined for significant spectral peaks in the expected frequency ranges.

Embryonic growth and staging

In experiment II, the eggs were placed in a refrigerator at 8 °C immediately after f_H recordings; on the following day, the egg was opened, the embryo was removed and excess fluid was removed by blotting on tissue paper. Yolk-free wet mass was determined to the nearest 0.001 g, and the embryo was then oven-dried to constant mass at 70 °C. Staging was determined by reference to Hamburger and Hamilton (1951) for chicken embryos.

Statistical analyses

Gompertz growth functions were fitted to chick body mass (g) according to equation 1 of Ricklefs (1967) by non-linear least-squares regression analysis (SYSTAT; Wilkinson, 1990). We examined the variability in mean f_H of embryos and chicks during development using one-way analysis of variance (ANOVA) followed by pairwise multiple comparisons using the Bonferroni procedure. Values are presented as means ± S.D.

Results

Experiment 1

The mean daily f_H values of all quail embryos and that of individual embryos measured each day during the last 60 % of incubation are presented in Fig. 1A,B. An f_H signal was detectable by BCG in only a few embryos before day 9 of incubation, and these measurements were therefore omitted from the analysis. The mean f_H of all embryos prior to internal pipping (IP) varied significantly with incubation age (repeated-measures ANOVA $F_{1,6}$=6.978, P<0.001) between days 9 and

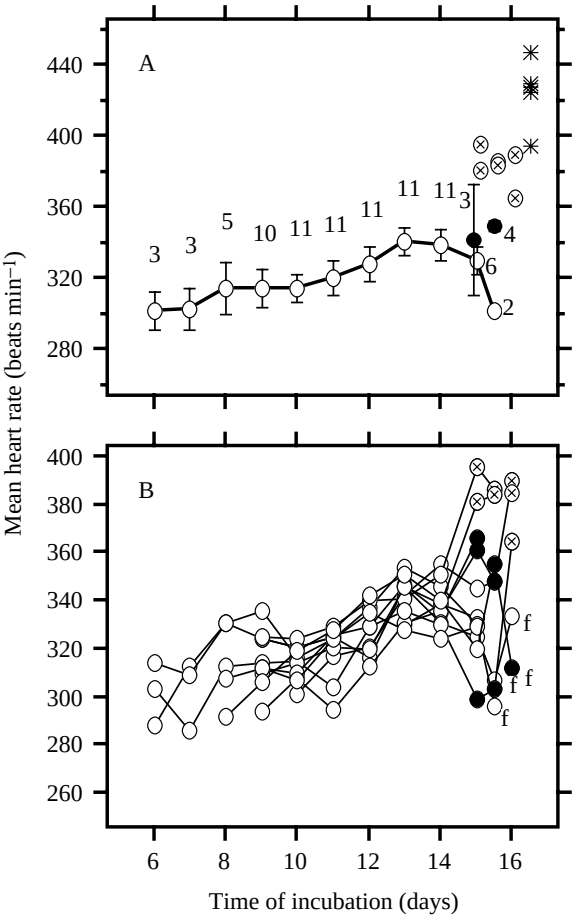

Fig. 1. Daily changes in overall mean heart rate (f_H) (A) (beats min^{-1}) and mean f_H of individual king quail (B) from mid-incubation to hatching, determined by the non-invasive BCG method. (A) Daily mean f_H (±S.D.) is presented together with the number of embryos. Open circles, pre-internal pipping embryos; filled circles, internally pipped embryos; crossed circles, externally pipped embryos; asterisks, hatchlings. (B) Symbols as in A. Four embryos which failed on the last day of incubation are indicated by an 'f'.

15. Mean f_H did not change significantly between days 14 and 15 of incubation in pre-internally pipped embryos (Fig. 1A).

On day 15, all the embryos were measured a second time, in the same order as previous measurements, in the evening (designated 15.5 days old). Six embryos were pre-IP, three embryos were IP and two embryos had already externally pipped (EP) during the day (15.0 days old). In the evening, only two embryos were still pre-IP, four embryos were IP and the same two embryos were EP, indicating that a further two embryos had internally pipped some time during that day. On day 16, five of the same embryos had already hatched prior to measurement (three were IP and two were EP at 15.5 days), and a further two hatched soon after their measurements. Two embryos (IP at day 15.5) which had been used for measurements, died without commencing hatching. Two pre-IP embryos died between days 15 and 16 (malpositioned embryos). The mean f_H of embryos that hatched was not significantly different from that for those that failed to hatch at the end of incubation (repeated-measures ANOVA $F_{1,1}$=0.146, not significant). Further, the rate of change in mean f_H during the same period was not significantly different between hatched and failed embryos (interaction term $F_{1,6}$=0.382, not significant). The period between EP and hatching was approximately 1 day for two embryos, which externally pipped early, but less than half a day in other cases. The incubation period for embryos before hatching was between 15.5 and 16.0 days.

The mean f_H of IP embryos was variable but, in contrast, all EP embryos had a high f_H of 360–390 beats min^{-1} (Fig. 1B). The mean f_H of five newly hatched quail (mean body mass 4.28±0.48 g), at 425±17 beats min^{-1}, was significantly higher than that of EP embryos (Fig. 1A) and increased further to 450±8 beats min^{-1} (N=6) on day 1. Thereafter, the f_H of the same six quail increased to a maximum between day 6 and day 10, and then varied, but showed a general trend to decrease with further increases in body mass during development (Fig. 2). A second spectral peak in the power spectral analysis of hatchling f_{H5} data, which was confirmed to be due to respiratory movements, was averaged for each f_{H5} interval and then averaged to give the daily mean f_H of each chick. Mean respiratory frequency was 98.4±20 min^{-1} (n=5 chicks) on day 0, and the calculated mean f_H to respiratory frequency ratio of the individual hatchlings was 4.5±0.8. Calculated Gompertz growth constants for individual quail were on average 0.051±0.012 day^{-1} (n=7).

Experiment II

f_H of pre-IP embryos increased from 180 beats min^{-1} at day 2 to 300 beats min^{-1} on day 6, then increased more slowly to a maximum mean f_H at day 12 (Fig. 3A). Changes in mean f_H of pre-IP embryos during incubation were significantly different (ANOVA $F_{1,52}$=33.198, P<0.001). Significant pairwise comparisons of mean f_H by the Bonferroni procedure indicated that f_H values on days 2, 3 and 4 were significantly lower than on all subsequent days and that the following comparisons were also significant: day 5 < days 10–13; day 6 < days 11 and 12; and day 7 < day 12. Mean f_H was positively correlated with both yolk-free embryo mass (Fig. 4A) and embryonic developmental stage (Fig. 3B), but was highly variable between embryos during most of the incubation period. A Gompertz function could not be fitted to yolk-free embryo mass on incubation age by non-linear regression analysis. However, the logarithm of the yolk-free embryo mass (wet and dry mass) increased in a significant linear manner with incubation time up until hatching (Fig. 4B), with less variability in embryo mass than in embryonic f_H. Similarly, the relationship between decreasing embryonic water content and increasing incubation age was less variable between embryos than the relationship between embryonic water content and mean f_H during incubation (Fig. 5). Mean embryonic f_H is not statistically comparable between experiments I and II because of differences in methods; however, despite similarities in early incubation and IP–EP mean f_H, maximum mean f_H was higher (360 beats min^{-1} *versus* 340 beats min^{-1}), and achieved

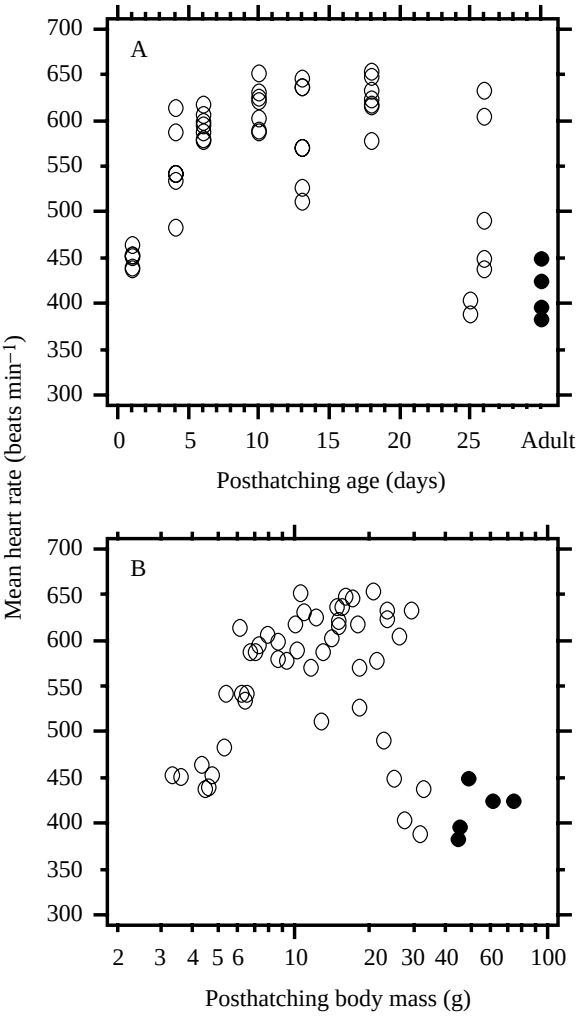

Fig. 2. Changes in the mean f_H (beats min^{-1}) of individual quail chicks (open circles, n=7 quail) in relation to posthatching age (days) (A) and chick body mass (g) (B) for the same quail that hatched in experiment 1 (Fig. 1) and for five adult quail (filled circles).

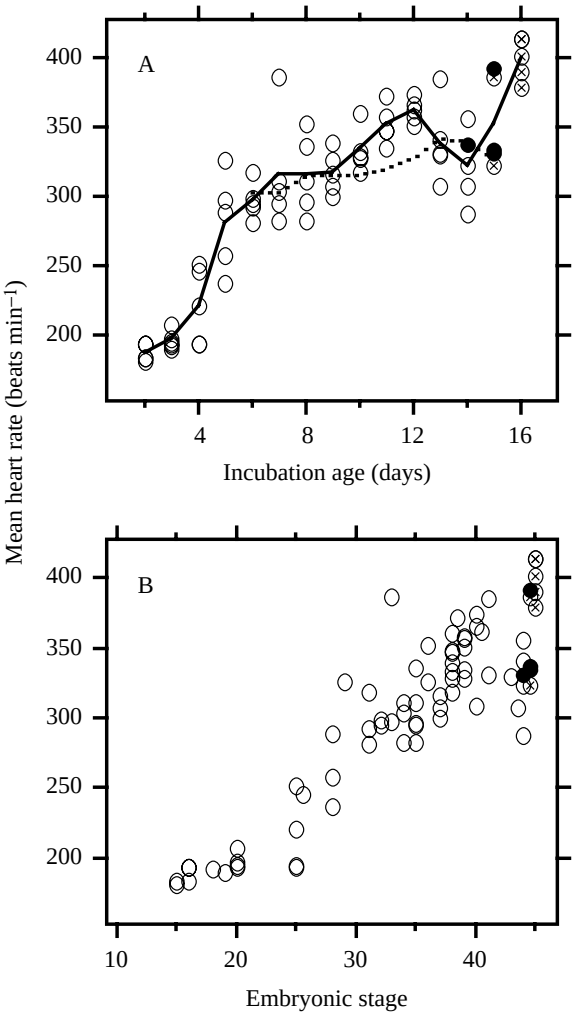

Fig. 3. f_H of individual quail embryos from day 2 to day 16 of incubation (five per day, N=75), determined by the ECG method (experiment II) in relation to day of incubation (A) and embryonic stage (B). Each symbol represents a single embryo (symbols as in Fig. 1). The solid line is the mean f_H for each day and is compared with the mean f_H of pre-internally pipped embryos from experiment I (dashed line).

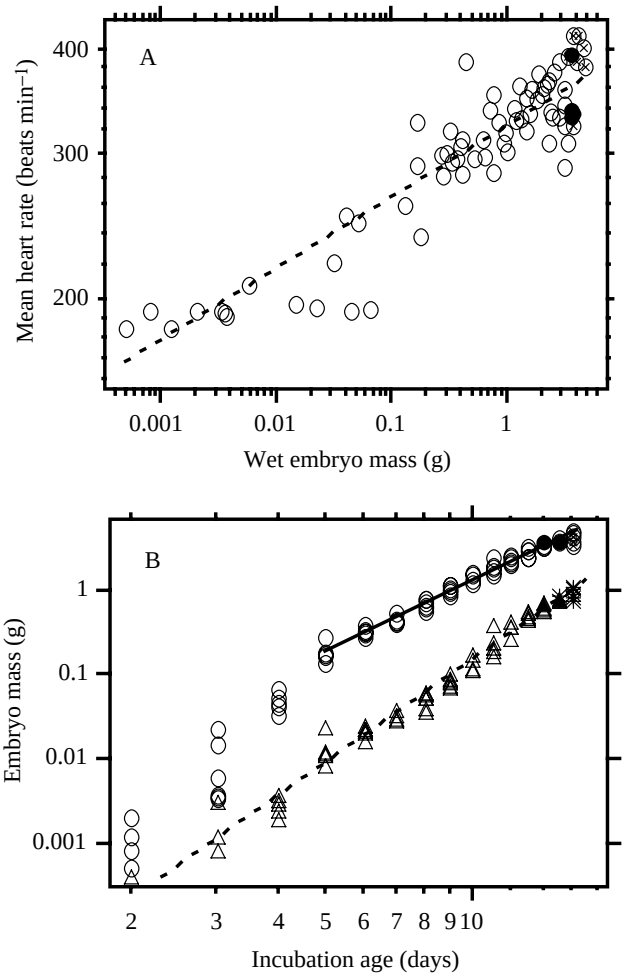

Fig. 4. (A) The relationship between embryonic mean f_H (beats min^{-1}) and wet embryo mass (g). Symbols as in Fig. 1 (dashed line: logf_H=2.508+0.086logm_w, where m_w is wet mass; r^2=0.858, s_b=0.004, $F_{1,73}$=451.75, P<0.001). (B) The relationship between yolk-free embryo mass (wet and dry; g) and incubation age (days) for the same quail embryos. Wet mass: open circles, pre-internal pipping; filled circles, internal pipping; crossed circles, external pipping. Solid line; age >4 days, logm_w=–2.667+2.765log$a$, where m_w is wet mass and a is age; r^2=0.982, s_b=0.049, $F_{1,61}$=3237.41, P<0.001. Dry mass: open triangles, pre-internal pipping; filled triangles, internal pipping; asterisks, external pipping. Dashed line; logm_d=–4.873+4.038log$a$, where m_d is dry mass and a is age; r^2=0.955, s_b=0.104, $F_{1,73}$=1509.38, P<0.001.

earlier (day 12 *versus* day 13–14), in experiment II (Fig. 3A; solid and dashed lines) than in experiment I.

Heart rate variability

Recordings of f_{HI} for quail embryos over 1 h periods also indicated that f_H was generally stable during most of the incubation period (up to day 13) (Fig. 6). f_H irregularities such as bradycardia (decreases of 20–30 beats min^{-1}) were intermittent at day 12–13, when mean f_H was maximal during incubation. Baseline f_H became increasingly unstable, with frequent bradycardia and tachycardia events, at day 14–15. The f_H of embryos on the day before hatching (day 15; Fig. 6) showed oscillations of up to 80 beats min^{-1} over short periods of 5 min. After hatching, f_H variability over short periods was greatest in young quail (first week) with the highest mean f_H and decreased with age in resting quail (Fig. 7). The amplitude of f_H variability changes was 100–200 beats min^{-1} in quail 6–7 days old and decreased to approximately 50–100 beats min^{-1} in adult quail. f_H variability was examined by Fast Fourier Transformation (FFT) to determine the period of the low-frequency oscillations in f_{HI} seen in Fig. 7. Fig. 8 shows an example of spontaneous variability in f_H for a 7 min sample of f_{HI} for a young female quail and the calculated spectral frequencies for oscillations in f_H over that sample. Low-frequency oscillations had a mean frequency of 0.088±0.029 Hz (period 12.6±3.6 s; n=8 quail, N=24 samples)

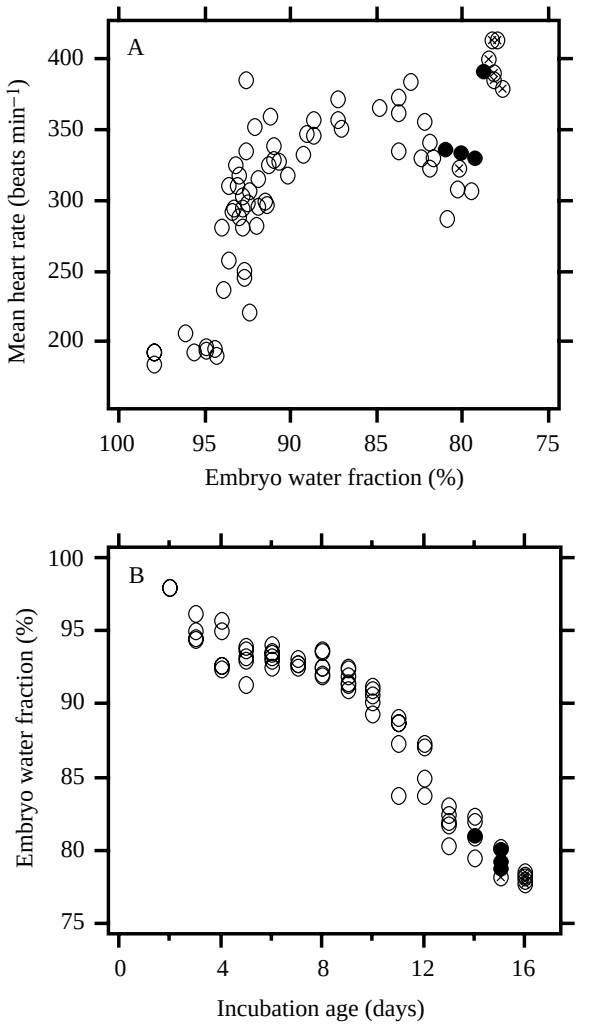

Fig. 5. Relationships between (A) embryonic f_H (beats min^{-1}) and embryonic water fraction (%), and (B) embryonic water fraction (%) and incubation age (days) for quail embryos in experiment II. Symbols as in Fig. 3.

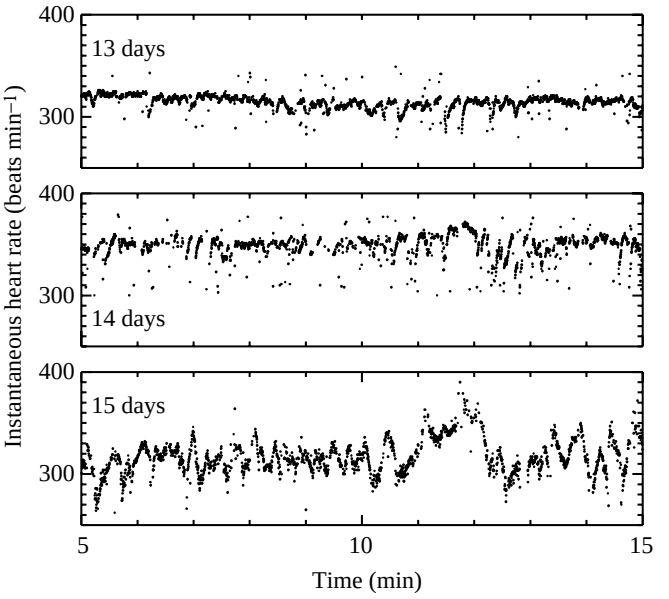

Fig. 6. Examples of typical late-incubation variability in instantaneous f_H (beats min^{-1}) of quail embryos (experiment II) for 10 min intervals. Baseline f_H was generally stable until days 12–13, but occasionally disturbed by spontaneous f_H irregularities (upper panel). Prior to internal pipping, quail baseline f_H became increasingly variable and more frequently disturbed by large fluctuations (day 14 and day 15 embryos).

over 5–10 min samples for young and adult quail combined. There was a trend for f_H oscillation periods to decrease as mean f_H increased (Fig. 9) and, as a result, the average low-frequency oscillation of young quail, 0.097 Hz (11.5 s; n=4, N=16) was shorter in duration than the mean adult low-frequency oscillation frequency of 0.089 Hz (14.9 s; n=5, N=8). However, the small number of quail measured prevents analysis of this trend. The oscillation periods of two examples from two female quail were of much lower frequencies than those of other quail (asterisks in Fig. 9) and were considered to be very low-frequency oscillations. In a few cases, it was possible to detect a high-frequency oscillation, which had a mean value of 1.36±0.58 Hz (0.86±0.32 s; n=8, N=16).

Discussion

Using several non-invasive measuring systems, we have been able to describe the developmental pattern of f_H in both embryonic and posthatching phases of growth for individuals of one of the smallest known precocial avian species, the king quail *Coturnix chinensis*. The first aim of this study was to establish a mean pattern of f_H development for this quail species, for comparison with that of much larger precocial species. To achieve this aim, short-duration f_H measurements were conducted on several quail raised simultaneously under the same conditions during incubation and posthatching. Despite the brief sampling time, mean daily heart rates of embryonic quail show similar patterns of development between individuals (Fig. 1B).

Effects of incubation delays

Embryonic mortality of the eggs transported for experiment I was higher than reported by Tsudzuki (1994) for quail reared at Osaka Prefecture University. However, there were no significant differences in mean f_H (days 9–15) between the four embryos that failed on the last day of incubation (malpositioned) and the seven embryos that hatched (ANOVA $F_{1,1}$=0.146, not significant). Both increased mortality and malpositioning and malformities are known to occur in chicken embryos after periods of storage prior to incubation (Haque *et al.* 1996). Mean maximal f_H of embryos in experiment II was higher and was achieved earlier than in experiment I (360 beats min^{-1} on day 12 for experiment II and 340 beats min^{-1} on day 13–14 for experiment I). Delays before eggs were set for incubation in experiment I because of the long transport distance and unknown conditions during

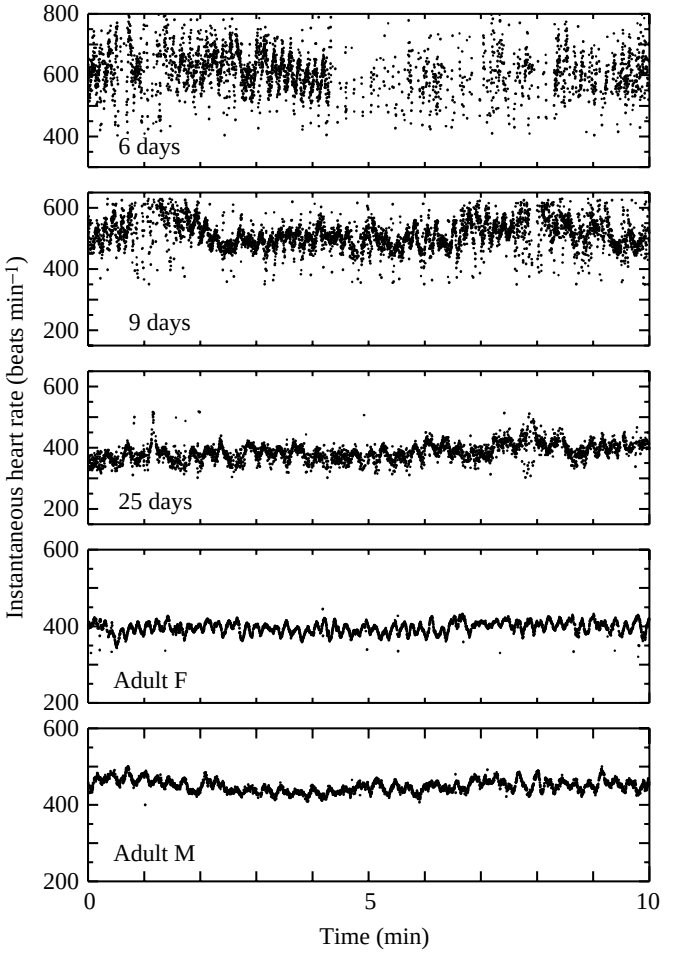

Fig. 7. Examples of spontaneous variability in instantaneous f_H (f_{HI}; beats min^{-1}) of quail during posthatching development and in two adult quail. Young quail had a higher f_H on average and exhibited larger changes in f_H over short periods (s) than did older and adult quail. Mean f_{HI} (± S.D.) from top to bottom: 515±43, 582±48, 391±24, 396±15 and 449±16 beats min^{-1}, respectively. The scarcity of points between 5 and 8 min in the upper panel reflects variable signal strength and ECG peaks that fell below the threshold trigger and were, therefore, not recorded, as was often the case for the smallest quail. F, female; M, male.

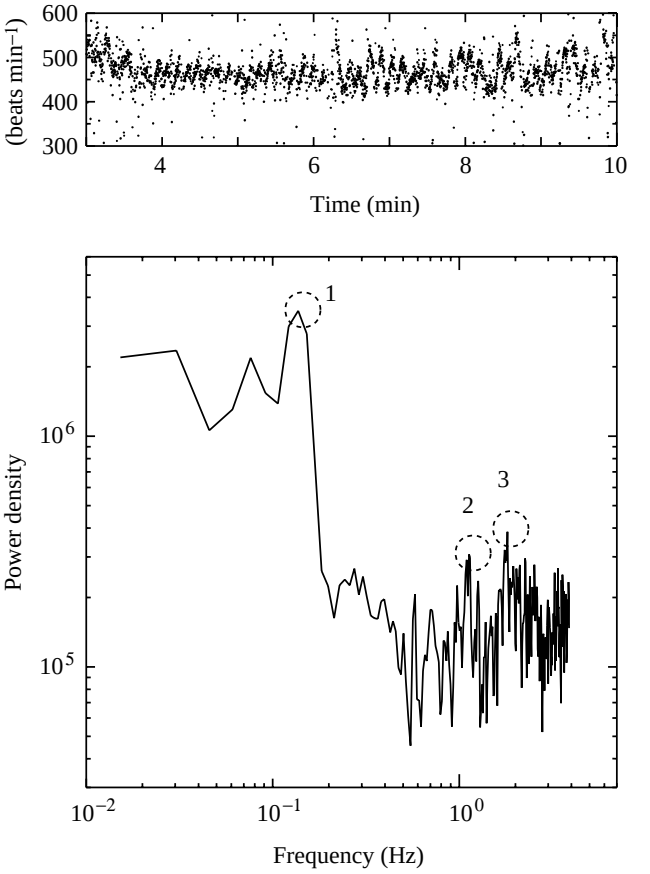

Fig. 8. A 7 min interval of instantaneous f_H (f_{HI}; beats min^{-1}) for a 17-day-old quail (upper panel, mean f_{HI}=468±38 beats min^{-1}) and the power spectrum distribution of heart rate variability for that interval (lower panel). Spectral peaks 1 and 3 indicate the low- (0.14 Hz, 7.3 s period) and high- (1.81 Hz, 0.55 s) frequency components of the f_H variability, respectively, the origin of which is discussed in the text. Peak 2 indicates a third component (1.13 Hz, 0.89 s), the origin of which is unclear.

handling, including ambient temperature, may have increased embryonic mortality in the present study as eggs laid by the adult quail subjects reared in the present study (at Muroran) have a high hatchability (J. T. Pearson, personal observation). However, more importantly, such incubation delays also have significant effects on the growth and development of embryos that hatch normally.

Changes in heart rate of quail throughout development

The average daily f_H of all quail embryos increases slowly from 300 to 310 beats min^{-1}, at which point a cardiogenic signal is first detectable non-invasively at day 6–7 (40 % of incubation) to day 10 (Fig. 1A), but the developmental pattern of individual embryos during this period shows considerable variability (days 6–9, Fig. 1B). It has yet to be determined whether this variability reflects significant differences between embryos or periodic short-term changes in f_H over an incubation day. However, it is noteworthy that, in experiment II, the same pattern of intra-embryonic f_H variability is recognisable on days 5–8 (Fig. 3A). Such variation may reflect intra-embryonic differences in the timing of maturation events or growth rates. Nevertheless, between days 10 and 13, there is less variation and all embryos consistently increased f_H to approximately 340 beats min^{-1}.

The mean f_H of newly hatched quail is significantly higher than that of EP embryos, which in turn were 40–50 beats min^{-1} above pre-IP mean f_H levels. This contrasts with the decrease in mean f_H of chickens from a maximum at EP of 310±20 beats min^{-1} to a mean hatchling value of 280±20 beats min^{-1} (Tazawa *et al.* 1992). The mean f_H of quail continues to increase throughout the first week after hatching, reaching a maximum during the second week with a doubling

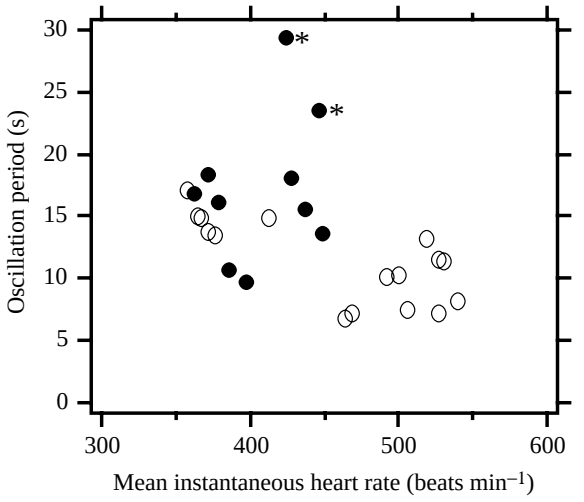

Fig. 9. The relationship between low-frequency components of heart rate variability oscillation period (s) and mean instantaneous f_H (f_{HI}; beats min^{-1}) for short measurement periods (5–10 min intervals) as determined by power spectral analysis. Open circles, young quail (n=4 quail, N=16 samples); filled circles, adult quail (n=5, N=10). Asterisks indicate two oscillation peaks that were attributed to very low-frequency components of f_H variability.

of their body mass (Fig. 2). However, the poorly insulated quail chicks have high thermoregulatory costs (Bernstein, 1973; Pearson, 1994*a*,*b*), and mean f_H is maintained at high levels (500–600 beats min^{-1}) until they achieve at least half their adult body mass (40–50 g; Tsudzuki, 1994). Mean f_H peaked at 3 days after hatching (342±39 beats min^{-1}) in the chicken, much earlier than in the small king quail, and then decreased with further development (Tazawa *et al.* 1992). Chickens achieve homeothermy within the first week of hatching after maximal f_H has increased by only 22 % above that of hatchlings. Therefore, the phenomenal 200 beats min^{-1} increase that occurs by the second week after hatching in king quail undoubtedly represents the higher energetic burden of achieving homeothermy at small body masses.

Cardiac contractions of the youngest quail (day 0–1) were detected by piezo-electric film, since the ECG electrodes were too large for hatchlings. The filtered signal from the film was generally contaminated by respiratory movements (amplifier time constant of 1.5 s), and so respiratory frequency was determined from the same power spectral analysis of 5 s intervals. Mean respiratory frequency was 98 min^{-1} for hatchling quail (n=5), 50 % higher than the value reported by Calder (1968) for adult king quail. Despite their small mass, quail hatchlings are able to maintain a high respiratory rate proportional to their body mass, and the average f_H to respiratory rate ratio of 4.5 for individual chicks was therefore similar to the average ratio for adult birds (passerine and non-passerine) found by Calder (1968).

Allometric relationships between heart rate and egg mass

The mean f_H of king quail during incubation is higher than that of the larger Japanese quail and the chicken, but changes in f_H with incubation time are similar (Tazawa *et al.* 1991*a*). However, most king quail embryos do not show significant decreases in f_H during the last stage prior to IP, unlike the chicken and the more striking examples of declining f_H in the duck and goose (Tazawa *et al.* 1991*a*), and so king quail embryonic f_H generally remains high until IP. Tazawa *et al.* (1991*a*) have noted a significant allometric relationship between pre-IP embryonic f_H and egg mass of larger precocial species. Embryonic metabolic rate at the pre-IP stage is a function of egg mass, which is attributed to an eggshell-conductance limitation on oxygen transport (Paganelli and Rahn, 1984). Therefore, f_H at the pre-IP stage is directly related to embryonic metabolic rate. The mean f_H of king quail of 341±8 beats min^{-1} (n=11) at pre-IP is not significantly different from the allometric prediction of Tazawa *et al.* (1991*a*) despite the smaller size of the egg (Fig. 1).

Mean heart rate in relation to embryonic growth

We conclude from experiment II that the changes in embryonic f_H of king quail are closely related to the changes in embryonic growth rate throughout most, but not the entire, incubation period. The relationship between quail embryonic f_H and incubation age for 6-day-old and older embryos was similar in general to that found in experiment I, even though the methods used for f_H measurements (BCG *versus* ECG, and repeated *versus* non-repeated sampling) were different (Fig. 3A). Embryonic f_H increased more in the first 6–7 days of incubation than during the remaining period of pre-IP incubation (Fig. 3B). Between IP and hatching, f_H once again increased significantly. The large increase in mean embryonic f_H from 170 to 300 beats min^{-1} up to day 6 was correlated with a small change in embryonic water fraction (Fig. 5A), since the rate of accumulation of solids in embryonic tissues is equal to the rate of decrease in tissue water content (Fig. 4B). From day 6, a slow rate of average increase in f_H, from 300 to 400 beats min^{-1}, was associated with a decrease in embryonic water fraction by the time of EP and hatching (Fig. 5). However, there is a noticeable sudden decrease in mean f_H for embryos with water fractions of 80–83 %, which deviates from the negative correlation between mean f_H and water content during the second half of incubation. Since quail embryos increased in yolk-free body mass exponentially throughout the incubation period, as did the embryonic f_H relationship with embryo wet mass (Fig. 4), we suggest that the decline in f_H of some late king quail embryos is not related to embryonic growth rates, which remained high, or to the rates of change in embryonic wet and dry mass, which remained unchanged after day 6. In the case of larger precocial species, the declining mean f_H of embryos over a period of up to several days prior to IP reflects a possible oxygen-conductance-limited stage in metabolism (Tazawa *et al.* 1991*a*; Whittow and Tazawa, 1991). As the rate of oxygen diffusion through the eggshell, shell and chorioallantoic membranes is fixed during incubation, oxygen consumption of the late embryo becomes limited (plateau phase of metabolism) and therefore f_H is

decreased independently of embryonic growth. However, we found that mean f_H continued to increase until as late as day 13–14 (81–88 % of incubation) and that only a few king quail embryos significantly decreased their f_H before pre-IP (days 14–15), and only for a period of approximately 1 day. Although f_H no longer increased according to the linear trend shown earlier in incubation in many embryos (Fig. 3B), some embryos, which externally pipped on day 15, probably never decreased their f_H during late incubation (Fig. 1). There is some evidence to suggest that f_H immediately prior to IP is very variable over even short periods (10–20 min), as reflected in Fig. 6. The day 15 (pre-IP) embryo illustrated clearly demonstrates oscillations in f_{HI} that cover a range from 290 to 380 beats min^{-1}. The variability in mean f_H of embryos on days 14–15 of incubation (Fig. 3A) reflects to some extent the short-term duration of measurements (mean f_H over 10 min), but it also suggests that the embryonic king quail f_H is not permanently suppressed or functionally limited by the oxygen-conductance of the eggshell during the final stages of incubation prior to IP, as suggested for larger precocial species. Possibly, intermittent increases in vagal activity in the embryonic quail may decrease the f_H baseline during the final stages of incubation (J. T. Pearson, unpublished observations).

Heart rate variability

The highest mean f_H of young quail was recorded from the end of the first week after hatching (Fig. 2). These high resting f_H levels are correlated with the higher metabolic demands of thermoregulation at small body masses during this period of transition to homeothermy (Bernstein, 1973; Pearson, 1994*a*,*b*). It is obvious from Fig. 7 that, while the mean f_H changes little, spontaneous oscillations in f_H or beat-to-beat intervals are often large. Heart rate variability, that is spontaneous fluctuations in the baseline f_H, is also greatest at the end of the second week after hatching. Variability decreases both before and after this point in development in the embryonic and posthatching phases. Short-term oscillations in f_H were often found to have detectable frequencies by power spectral analysis (Fig. 8). Spontaneous variability in f_H is a well-studied phenomenon in mammals (Sayers, 1973; Akselrod *et al.* 1985; Cerutti *et al.* 1994) and some fishes (for references, see Altimiras *et al.* 1996), but not in birds. These authors generally consider there to be three main physiological contributors to this f_H variability in vertebrates. A high-frequency component is associated with vagal mediation and the mechanical influence of respiration on the heart so that oscillations are usually centred at the respiration frequency (Sayers, 1973). Low-frequency (0.1–0.15 Hz) and very low-frequency (0.04–0.08 Hz) components are also recognisable and are considered to be due to the blood pressure control loop and to thermoregulatory fluctuations in vasomotor tone, respectively (Sayers, 1973). The precise frequency ranges of each component appear to vary between mammals (Sayers, 1973; Akselrod *et al.* 1985; Cerutti *et al.* 1994), and in this study we also note further differences in the low-frequency component. The low-frequency component was, on average, 0.088 Hz (12.6 s) in both adult and young quail combined (Fig. 8) over the range of f_H from 350 to 550 beats min^{-1}. This is a little lower than the 0.1–0.15 Hz range reported for humans and dogs (Sayers, 1973; Akselrod *et al.* 1985) and much lower than the 0.27–0.74 Hz of the rat (Cerutti *et al.* 1994). However, the low-frequency oscillations of young quail, which maintained a higher mean f_H, were on average 0.097 Hz, which is closer to values found for dogs and humans. The decrease in the frequency of the low-frequency component of f_H variability that takes place in king quail during posthatching development needs further investigation. The high-frequency component varied between 0.7 and 2.5 Hz (0.9 and 1.5 s) and was found in fewer of the recordings, with generally low spectral power, but was similar to values reported for the respiratory frequency of resting quail (Calder, 1968). Multiple unidentified components in power spectra of f_H variability were sometimes found in samples between the low-frequency and high-frequency components. The physiological origins of these components are unknown, but may be related to the respiratory rhythm, as distinct from the respiratory rate (see Sayers, 1973), and also warrant close examination in future studies.

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