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GENETIC AND MATERNAL ENVIRONMENTAL INFLUENCES ON EMBRYONIC PHYSIOLOGY: INTRASPECIFIC VARIABILITY IN AVIAN EMBRYONIC HEART RATES

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ABSTRACT

The heart rate of embryos of altricial pigeons (*Columba domestica*) and bank swallows (*Riparia riparia*) was measured on a daily basis to investigate interclutch versus intraclutch variability in heart rate during development. In both pigeons and swallows, the developmental patterns of heart rate change in siblings (i.e., embryos from the same clutch) are statistically much more similar than those of embryos from other clutches of the same species. These findings could be explained on the basis of (1) measurement error (which cannot account for the present observations), (2) genetic effects, in which siblings are genetically predisposed to show particular physiological patterns, or (3) maternal and/or common environment effects, where environmental influences on either the mother or the offspring cause siblings to undergo more similar patterns of physiological development. Having defined and discussed these sources of variation, we indicate how experimental protocols might be designed that could determine the relative contributions of each of these sources of variation to physiological patterns observed during development.

INTRODUCTION

Physiologists, both by tradition and design, typically express their data as mean values with some additional indicator of variance. For example, a paper might report the mean value (± standard deviation) of heart rate for a species of bird at a specific level of activity and ambient temperature. While this conveys information on the central tendency for heart performance in that population of animals, it deemphasizes any variation inherent in the data. Bennett (1987) has referred to this trend as "....the tyranny of the golden mean". He convincingly argues that physiologists, in viewing variation as an annoyance rather than a phenomenon of potential value, have failed to use the full breadth of their data in assessing physiological hypotheses. Subsequently, authors have supported and extended Bennett's assertions (see Garland and Carter, 1994 for extensive review of this and related topics), and, increasingly, physiological studies are presenting more complete data sets that analyze variability per se.

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Unfortunately, the pursuit of the golden mean still tyrannizes the field of developmental physiology. One of Bennett's (1987) assertions — that physiologists have failed to present data in a format that permits others to analyze the variability — still remains true for much of the physiological data pertaining to developmental processes. Developmentally-oriented papers in vertebrate physiology reporting on variation and its possible sources are found most frequently in the literature on reptilian reproduction. Studies in this area will typically regulate maternal and/or offspring environment (usually temperature and water availability) and then measure changes in embryo viability and physiological performance (see Vleck, 1991; Van Damme et al., 1992; Shine and Harlow, 1993, for references). In a paper that stands out because it focuses on, rather than minimizes, the impact of variability in data, Packard and Packard (1993) investigated the sources of variation in water dynamics in the eggs and embryos of the common snapping turtle. They considered egg clutch, egg container, specific environmental chamber, and water potential of the incubating medium as "treatments" and found that each of these factors influenced the rate of egg water loss to varying degrees. Some of the factors in the Packards' study are, of course, biotic, while others are abiotic. Nonetheless, this study highlights the fact that typically there are a multitude of sources of variation in physiological data.

Unfortunately, determining what proportion of apparent physiological variability in developing systems is environmentally based, and what proportion is attributable to genetics, unidentified random factors, or measurement error, is rarely straightforward. Unlike the focus on this subject in the reptilian literature, such studies rarely have been attempted in avian embryos. Perhaps this is because of the perception that there is a greater degree of variation in maternal and embryonic environments in ectothermic reptiles than in birds. Yet, the potential for a physiological influence of both maternal and embryonic environments on avian systems has been shown through studies that vary ambient oxygen through actual or simulated high altitude acclimation (see, for example, Packard et al., 1977; Carey et al., 1989; Ar, 1993; Hempleman et al., 1993).

In this paper we provide a data set on embryonic heart rate in two species of altricial birds — pigeons (*Columba domestica*) and bank swallows (*Riparia riparia*). We see in these data compelling evidence of variation among egg clutches; namely, for any given species (or subpopulation of a species) the patterns of developmental change in heart rate are much more variable among clutches than within clutches. By discussing the multiple possible causes for these findings, we hope to show that the study of intraspecific, in addition to interspecific, patterns of development in physiology will allow a deeper understanding of the processes that influence vertebrate ontogeny.

MEASURING HEART RATE IN BIRD EMBRYOS

ACQUISITION AND INCUBATION OF EGGS

Eight domestic pigeon (*Columba domestica*) eggs (four clutches of eggs with two eggs per clutch) were collected from nests in a farmyard near Amherst, Massachusetts, USA in June, 1990, and were designated as Group 1. Sibling eggs (the pair of eggs from the same clutch) were identified. The day the eggs were laid was unknown, so the age of

these eggs was determined as "days prior to external pipping". Another eight eggs (also four clutches of eggs with two eggs per clutch) were taken from a clock tower in Amherst, Massachusetts, USA in June, 1991. These were designated Group 2. Sibling eggs were identified as for Group 1. All pigeon eggs were instrumented and subsequently monitored for heart rate (see below).

The eggs of bank swallows (*Riparia riparia*) were collected from their burrow nests in cliffs along the Connecticut River (Western Massachusetts) in June 1991. Twenty-six swallow eggs were instrumented, with heart rate (f_H) being successfully measured in 20 swallow embryos during the six-day period ending with the conclusion of external pipping (EP). As was the case for the domestic pigeon eggs, the age of the bank swallow eggs was unknown, and was described as "days prior to external pipping", as for the pigeon eggs.

After measuring egg mass, eggs were placed in a still-air incubator maintained at 38 °C. Eggs were turned twice daily.

MEASUREMENTS AND ANALYSIS OF HEART RATE

All measurements of f_H were made at 38 °C inside the incubator using impedance-cardiography. This technique has been described elsewhere (Haque et al., 1994; Tazawa et al., 1994). Briefly, this technique detects changes in electric impedance of the embryo produced by cardiac contraction and blood ejection (impedance-cardiogram, ICG). On the day of collection, we placed two thin copper wires (0.1 mm diam. for bank swallows, 0.3 mm diam. for pigeons) about 2 mm into the eggs through a hole in its shell produced with the tip of a 26-gauge needle. The wires, which were 70 cm long, were held in place and the hole in the shell closed by a tiny droplet of cyanoacrylate glue. Each pair of electrode wires was labeled to identify individual eggs and was led out past the closed door of the incubator. The electrode wire pair was connected to an impedance converter (model 2992, UFI, Morro Bay, CA.) which detected the ICG. Importantly, (1) this method allowed f_H measurement without touching the eggs or opening the door of the incubator, and (2) all data reported for a given group or species were collected from a single incubator run. Thus, all eggs experienced exactly the same environmental conditions once in the incubator. Heart rate measurements were made on the same time schedule each day for each egg, except for a few eggs that were measured for f_H twice on the day before hatching. The procedure for determination of mean daily f_H from the ICG was identical to that for the pigeons and bank swallows described in detail by Tazawa et al. (1994). Mean daily f_H of individual embryos was plotted for days prior to external pipping so that the daily changes in f_H could be chronologically coincident for all the embryos.

Mean daily f_H values from groups of birds were analyzed and compared using multivariate analysis of variance (MANOVA) for repeated measurements. Daily f_H values for each group were examined for (1) variation between and within sibling groups (are f_H's of siblings more similar than nonsiblings at any given time in development?), (2) development time effects (does the time before external pipping affect f_H within a population composed of siblings and/or nonsiblings?), and (3) development time interactions (does the rate of the developmental change in f_H vary among sibling

groups?). The F statistics from a Wilks' Lambda, Pillai's Trace, and Hotelling–Lawley Trace test were determined. There were no qualitative differences between these three methods of assessment, and consequently the significance levels for the Wilks' Lambda statistic are reported. A significance level of $p < 0.05$ was adopted as a fiduciary limit. All results in the text are presented as means ± 1 standard deviation, while values in the figures are plotted as means ± 1 standard error.

PATTERNS OF HEART RATE CHANGES IN EMBRYOS

The change in mean f_H during the last 5–6 days of incubation in the embryos of domestic pigeons (Group 1) is shown in Fig. 1. f_H varied widely among the eight embryos monitored (four sibling pairs), but increased significantly during the last few days of development. Although there could be differences of 30–40 bpm between the mean f_H's of the two siblings on any given day, the pattern of change in f_H as development progressed was more similar between siblings than between nonsiblings. For example, the siblings marked by closed circles in Fig. 1 decreased their f_H one day prior to EP and then increased it during EP, while f_H in those marked by open circles was almost constant throughout the measurement period. The variation between sibling pairs in Group 1 pigeons was statistically significant (MANOVA, $p < 0.01$).

Daily changes in embryonic f_H were measured in another group of domestic pigeons (Group 2, egg mass = 17.1 ± 1.1 g) collected at a much earlier stage of development. Generally, f_H was relatively low prior to the last week of incubation, but increased significantly as EP was approached. As in Group 1, the pattern of variation in developmental patterns of f_H was significantly more similar within sibling groups than between them ($p < 0.01$). Figure 2 shows changes in three sibling pairs showing that distinctive patterns were evident for each of the three pairs.

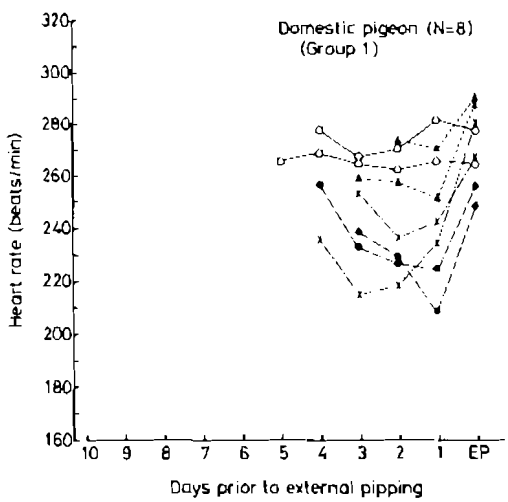

Fig. 1. Daily changes in averaged f_H of domestic pigeon embryos from Group 1. Eight embryos from the four clutches were measured (egg mass; 17.1 ± 0.9 g). Sibling pairs are represented by the same unique symbol.

Heart rate in bank swallow embryos (egg mass = 1.41 ± 0.10 g) changed significantly during the measurement period, and increased most sharply in the last day before EP. Mean daily heart rate of siblings from each of four clutches (2 or 3 eggs) of bank swallow eggs are shown in Fig. 3. The changes in f_H of swallow siblings were highly distinctive in pattern. The pattern of variation in f_H as a function of days before external pipping was significantly more similar within sibling groups than between them ($p < 0.01$). Three siblings in the top panel of Fig. 3 were measured for f_H twice on the last day of incubation. Two embryos had not pipped the shell during the first measurement. During the second measurement, all three siblings had pipped externally and showed an increased f_H. One embryo in the third panel from the top hatched before measurement during the external pipping period. It should be noted that the two siblings whose f_H is plotted in the bottom panel of Fig. 3 both required two days for external pipping to hatch, unlike embryos from any of the other clutches. Their f_H was measured twice a day on the day prior to EP and on the day on which they externally pipped (bottom panel in Fig. 3). They hatched on the same day.

EXPLAINING INTERCLUTCH VARIATION

For the purposes of this paper we will assume that each clutch of eggs is composed of full-sibs; that is, the offspring of a female mated to a single male. The present study

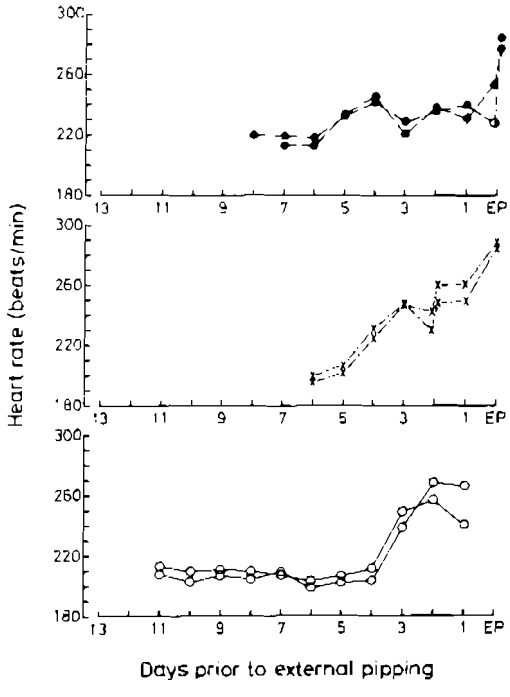

Fig. 2. Developmental patterns of f_H in three embryo sibling pairs of domestic pigeons from Group 2. Each panel represents a sibling pair. Note the distinctive f_H pattern during development characteristic of each sibling pair, resulting in much greater inter- than intraclutch variability. In the top panel, a half-closed circle indicates an embryo that did not pip the shell even though its sibling pipped during the measurement made in the morning. Then, the measurement was repeated in the afternoon by which time both siblings had pipped the shell. The two siblings in the middle panel were measured twice on the second day before external pipping. In the bottom panel, f_H in this sibling pair could not be determined during external pipping.

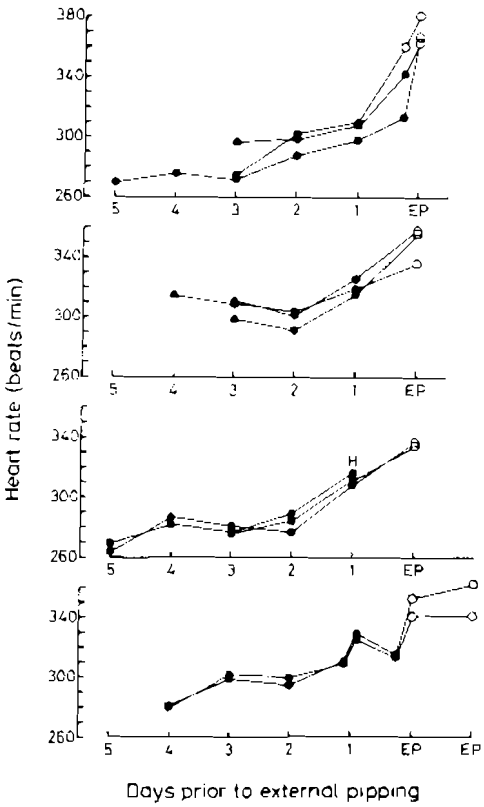

Fig. 3. Developmental patterns of f_H in four sibling groups (i.e., four clutches) of bank swallow embryos. Closed circles indicate f_H during pre-pipping stage, while open circles represent f_H during external pipping. The third panel from the top shows an embryo that hatched (H) before a measurement could be made during external pipping. In the bottom panel, both siblings required two days for external pipping.

clearly shows significant variation in f_H among clutches, or, as we assume, among sibling groups. In other words, the developmental pattern of change in f_H exhibits more variation among than within sibling groups. In addition, for bank swallows, the duration of external pipping and the time of hatching relative to external pipping vary among sibling groups (Fig. 3). Similar patterns of variation in water vapor permeability of eggshells have been noted in blackbirds and magpies, with interclutch variation greatly exceeding intraclutch variation (Sotherland et al., 1979). There are several potential explanations for these patterns: (1) measurement error, (2) genetic effects, and (3) maternal and common environment effects that influence developmental physiology. Although none of these sources of variation can be refuted as a cause of the interclutch differences we have quantified (with the exception of measurement error), we find it important to discuss the relevance of these sources of variation in the hopes of influencing how future studies of developmental physiology are conducted.

MEASUREMENT ERROR

The first source of variation among clutches that we address — measurement and experimental error — must be accounted for before any meaningful discussion of

biological causes can proceed. Although it is essential to reduce or eliminate any sources of bias in experimental protocol or measurements, the presence of variation in measurements and experimental conditions per se need not invalidate tests of interclutch variation. Even if measurements are not strictly repeatable and individuals experience slightly different laboratory conditions either before or during an experiment, these sources of variation will not contribute to variation among clutches *as long as all individuals are randomly assigned to rearing environments (incubators) and particular sequences of measurement* (see, for example, Packard and Packard, 1993). If the measurement error variation is randomly distributed with respect to sibling groups, it will contribute to variation within groups. Thus, the problem caused by measurement and experimental error is simply that it may obscure significant physiological variation among clutches.

In light of the above, we re-emphasize that in the present experiments only a single measurement technique and single protocol for egg handling were used for f_H measurement. Also, all measurements were made during the same several-day period on each animal in the same incubator. Consequently there could be no discrepancies in technique between siblings and nonsiblings within a measurement group.

GENETIC EFFECTS

If it is assumed that all of the eggs in a nest come from a single female and male pair (a reasonable assumption for many bird species), the sources of both interclutch and intraclutch variation can be discussed in the terminology of quantitative genetics. For the purposes of this discussion, we will assume that various physiological and morphological traits measurable during development have a polygenic basis and that the combination of many independent genetic and environmental influences on the expression of phenotypes results in a normal distribution of measured phenotypes within a population sample (Arnold, 1987; Falconer, 1989; Garland and Carter, 1994). In standard quantitative genetic treatments, variation among full-sib clutches can be attributed to additive genetic, nonadditive genetic, and maternal and/or common environment sources of variation (Falconer, 1989).

The different sources of variation among sibling groups are expressed as a fraction of the total phenotypic variation in a population. The importance of heritability (the proportion of total phenotypic variation that is due to variation in additive alleles) in understanding the evolution of physiology has been discussed by Arnold (1987). Basically, additive genetic variation in a population determines the resemblance between parents and offspring. Thus, a population's evolutionary response to natural selection is dependent on the magnitude of the additive genetic variation relative to the total phenotypic variation for any quantitative physiological trait (Falconer, 1989). Arnold (1987) also addressed the potential importance of genetic correlations between traits in the evolution of physiology.

The resemblance in phenotype between siblings can be due to both additive and nonadditive (dominance alleles and epistasis) genetic effects. Thus, one possibility is that the detailed patterns of f_H change during development presented in this study of altricial

birds are genetically based. A number of studies cited in Bennett (1987) have quantified heritable variation in physiological traits for vertebrate taxa. In addition, domestic chickens have pleiotropic mutations involving melanin/tyrosene metabolism (Pardue and Smythe, 1986) that can produce sibling groups with distinctive patterns of bradycardia development in the last five days of incubation (Howe et al., 1995).

Estimates of additive and nonadditive genetic variation and genetic correlations can be obtained from several different types of quantitative genetic breeding designs such as offspring-parent regression and half-sib analysis (Becker, 1984; Falconer, 1989). In a regression analysis of an offspring against mid-parent phenotype (phenotype average of both parents), many pairs of adults are randomly mated, and heritability is quantified as the slope of a linear regression of the mean physiological phenotype of offspring group against the mean phenotype of its two parents. In one of several types of half-sib analyses, each male is randomly mated to several females, resulting in progeny groups that share the same male and female parents (sibling groups) and other progeny groups that only share a male parent (half-sib families). The variance among half-sib families (estimated in a nested ANOVA) is directly proportional to the additive genetic variation in the sampled population (Falconer, 1989). Since males contribute only sperm, similarities between half-siblings reared by different mothers must occur due to the male's genetic contribution to half-siblings. Full-sib analysis can also be used to estimate genetic variation, but as we have already mentioned, the additive genetic effects are confounded with some of the nonadditive genetic and common environment effects in the variance among sibling groups. Unfortunately, to get robust estimates of genetic (and maternal) variance with any of these approaches, sample sizes substantially larger than "typical" physiological experiments are likely to be required.

In spite of this experimental complexity, Arnold (1987) and Bennett (1987) have advocated the use of quantitative genetics in physiology. We echo their recommendations since such studies are essential for a complete understanding of the evolution of physiological characters. However, given that it will be difficult for physiologists to generate large enough breeding designs to accurately quantify additive and nonadditive components of variation, we focus the rest of our discussion on several other sources of interindividual and intergroup variation that are more easily manipulated and could prove to be important in future studies of developmental physiology. These sources of variation are maternal effects on offspring physiology and phenotypic plasticity of maternal and offspring physiology.

MATERNAL AND COMMON ENVIRONMENT EFFECTS

To address the potential roles of these effects in generating interindividual variation, we must consider the expression of the physiological phenotype in parents and in offspring and distinguish what we will call "direct influences" from "indirect influences" on phenotypic expression. Direct influences occur when an individual's physiological phenotype is determined by its own genetic make-up or the environment(s) it has experienced. Indirect influences occur when some aspect of an individual's physiology is determined by its parents phenotype; specifically, the parental genotype(s) (separate from the offspring's inherited genotype) and/or parental environment(s).

Variation among sibling groups can arise from nongenetic causes such as those due to maternal and common environment. Maternal effects result from an influence of maternal genotype and/or maternal condition (related to maternal environment) on the expression of offspring physiological phenotypes. Experiments can be designed to quantify maternal inheritance (e.g., Eisen, 1967), a parameter of great interest to evolutionary biologists because of its influence on the response to natural selection (e.g., Kirkpatrick and Lande, 1989). Common environment (Falconer, 1989) can be a source of variation among families since siblings reared together share the same environmental circumstances, and environmental influences on embryo physiology can cause siblings to be more similar to each other than to nonsiblings (in other microenvironments). Although it may be difficult in practice to quantify accurately these sources of variation (maternal and common environment effects are confounded in variation among siblings), they have important implications for the study of developmental physiology. In particular, the effect of maternal condition on physiological differences among families could be used to study physiological mechanisms early in development, since maternal effects generally diminish with ontogeny.

There are several types of maternal effects that could operate in concert to influence embryo physiology. First is a maternal effect that is dependent on the maternal genotype (Arnold, 1987; Falconer, 1989). If nutritional provisions or the size or structure of the embryo and/or egg are determined by the maternal genotype, there may be an indirect genetic effect on developmental physiology of offspring. Since all siblings in a clutch may be indirectly influenced by the same maternal genotype and different maternal genotypes may exist in a population, some variation among clutches may be due to this maternal effect.

Second, maternal condition may be influenced in a predictable manner by the specific environment inhabited by each female. The susceptibility of the maternal condition (phenotype) to environmental influence is termed phenotypic plasticity, and the array of phenotypes produced in different environments for a single genotype is termed a norm of reaction (Schmaulhausen, 1949; Bradshaw, 1965). If a maternal effect on offspring developmental physiology is caused by the influence of the maternal environment on maternal condition, it could be said that there is phenotypic plasticity, or an indirect norm of reaction, for progeny physiology. For example, birds exposed to moderately high altitudes lay eggs that tend to have shells with reduced conductance for water vapor and oxygen (see Hempleman et al., 1993, for references), although the relationship between altitude and conductance is not straightforward (Ar, 1993). Since the physiology of the embryo is affected by the rate of water loss and oxygen availability, the maternal environment has indirectly influenced developmental physiology. Perhaps a more graphic example of a maternal effect in bird eggs lies in the devastation that *p, p'*–DDE, the principle metabolite of DDT, caused in wild bird populations, where maternal exposure to this pesticide directly affected the embryos, which were ill-protected by thin-shelled eggs (Kiff et al., 1979). Generally, if different females in a population are exposed to different environments, some of the variation among sibling groups could be due to this type of indirect phenotypic plasticity.

It is important to note that special environmental phenotypic variance can arise from

intraindividual circumstances that are temporary or localized in nature and are not really repeatable or predictable (Falconer, 1989). If the resulting maternal phenotype then influences the physiology of the developing embryo, this form of maternal effect would simply generate variation within and among families that would obscure other causal sources.

Maternal effects due to predictable phenotypic plasticity in the mother's condition could be particularly useful in studying developmental physiology. If the phenotypic plasticity is labile or reversible, the phenotype (or condition) of the mother can be manipulated, and the maternal effects on developmental physiology will vary among her progeny. One of several possible experimental designs for generating physiological variation among embryos in sibling groups is as follows. Mated females are exposed to two or more environments that could elicit maternal effects. The order of exposure to these environments is randomized for each female. Eggs are collected after each period of maternal exposure to an environment. Physiological measurements on these eggs are then analyzed by ANOVA with three sources of variation: among sibling groups, among maternal environments, and environmental sequence. Data from such experiments should allow maternal effects to be quantified and, moreover, could be used to determine if they are of adaptive value to the embryo. For example, hens at high altitude lay eggs with reduced gas conductance (Hempleman et al., 1993). Purportedly, this helps the embryo reduce water vapor loss at high altitude. An experimental design as outlined should allow a more complete understanding of how maternal effects are produced and what impact they have on the embryo.

In addition, the experimental design outlined above should result in regulated interindividual variability of large magnitude that could be used in the types of correlational analyses advocated by Bennett (1987) and Huey (1987). For example, if the different patterns of f_H we have observed result from maternal effects, it should be possible to generate multiple sib-ships with different heart rate patterns during development. Subsequent measurements of cardiac output, oxygen consumption, and body size at hatching (which might all be reasonably influenced by heart rate) could identify the degree to which crucial physiological events are coupled in development.

CONCLUSIONS

Comparative physiologists studying development have typically employed experiments that use changes in the external egg environment (humidity, temperature, oxygen availability) to evaluate and understand, for example, critical windows in development (see numerous papers in Deeming and Ferguson, 1991). In doing so, we have tended to focus on the central tendencies. While this continues to be a perfectly reasonable approach for elucidating general physiological processes during development, comparative physiologists in other fields of physiology have begun to embrace variation, particularly in studies on the selection of physiological traits (see Garland and Carter, 1994, for references). To date, developmental physiologists have not made the fullest possible use of physiological variation to provide additional insights into the development of physiological mechanisms.

Using a data set with intriguing interclutch patterns of variation in heart rate, we have attempted to offer several different causal explanations (all unsubstantiated as yet!). The field of quantitative genetics abounds with experimental protocols and tools that could be imported into developmental physiology to test hypotheses relating to observed causes of variation. For example, as Bennett (1987), Garland and Carter (1994), and others have suggested, correlational analyses that utilize interindividual differences can be used to study physiological mechanisms. As an extension of this approach, genetic sources of variation in developmental physiology could be manipulated to provide insights into the causes of particular physiological responses. We further recommend that physiologists working with systems where maternal or other environmental effects on egg physiology might influence their findings (probably most systems), control for this phenomenon where possible. Maternal effects in particular could also be used as an effective experimental tool. For example, it might be possible to make "designer eggs", in which manipulation of the maternal condition results in eggs with differences in shell permeability, energy stores, or rates of protein synthesis, that can be used subsequently to address specific developmental mechanisms.

In summary, it is important to recognize that variation in physiological processes appears concurrent with the onset of the process themselves. There are numerous sources for this variation — genetic, maternal environment, and common environment. Studies that incorporate variation into their experimental design should allow developmental physiologists to pose new questions pertaining to issues of development, evolution, and adaptation, and perhaps to answer long-standing questions about the mechanisms of developmental physiology.

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