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# A Multi-Week Inquiry for an Undergraduate Introductory Biology Laboratory

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*Investigating Correlations Between Environmental Variables and Leaf Stomata Density*

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*Bruce W. Grant and Itzick Vatnick*

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In the last decade, undergraduate science education has undergone a quiet revolution emphasizing opportunities for student-directed original inquiry as the curriculum.

Opportunities to conduct open-ended investigations in the laboratory portion of courses are important because they teach students how science is actually done and thereby they learn by doing it (Woodhull-McNeal 1989; Goodwin et al. 1991; Heady 1993; Ortez 1994; Sundberg and Moncada 1994).

However, several authors have commented that biology departments seem to have lagged behind in these visionary reform efforts of course curricula for a variety of reasons (Holt et al. 1969; Carter et al. 1990; Sundberg and Armstrong 1993), and our experience in teaching introductory labs leads us to concur.

Our explanation is that many of our freshmen students lack sufficient confidence, organizational skills, and content background for open-ended student-directed investigations as freshmen, and we lack the resources to supervise large numbers of independently active learners that populate our introductory courses. Our solution is to begin with an intermediate step that retains at least some of the ownership and empowerment components of open-ended inquiries as well as the efficiencies of scale of the "demonstra-

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**Table 1:** Inquiry Framework. Summary of the relations among various modes of inquiry for introductory laboratory courses in biology. Columns refer to various components of the inquiry, and the cells indicate who "owns" each component, i.e., was the component provided by the instructor or up to the student to generate? (modified from Sundberg and Moncada 1994; Ohlhorst 1995; D'Avanzo 1996)

inquiry mode	research question	study system	data collection/ statistical analysis	data interpretation	results and conclusions
open-ended inquiry	student	student	student	student	student
bounded inquiry	student/ given	student	student/given	student	student
guided inquiry	given	given	given	student	student
closed-ended demonstration	given	given	given	given	given

tion lab" format. We refer to this intermediate as a "bounded inquiry."

In bounded inquiries, students' research questions and study subjects are instructor constrained but hypotheses and study species are not a priori specified. In addition, sample acquisition, data collection, statistical analyses, and other technical details are prescribed, however, neither the students nor the instructor know what the outcome of the study will be. Finally, a critical component of this activity is that at the end, students present their research findings to their peers during an in-class research symposium using their own authentic voices. A summary table showing the relations among the various inquiry modes appears in Table 1.

We have developed a bounded inquiry for the second semester laboratory of our two-semester first-year biology course. This inquiry involves the study of the ecophysiology of terrestrial plants and is titled "Environmental Correlates with Leaf Stomata Density." During the inquiry, students investigate correlations between various environmental variables (light, temperature, carbon dioxide, and so forth) and leaf stomata density. Below, we describe the salient features of the

bounded inquiry our students conducted, and we relate some of the student results from and attitudes toward this inquiry over the past three years.

#### SYNOPSIS OF LAB ACTIVITY

##### *Lab Period #1:*

(a) We perform an introductory activity during which instructors use a combination of lecture and group questioning to teach the basic ecophysiology of leaf stomata including stomata structure, function and role in the regulation of leaf temperature, gas exchange, and photosynthesis in vascular plants (our lab handout is available on a course-specific web page linked to the Widener University biology department web site: [http://www.science.widener.edu/~grant/esa/exp2/bgiv\\_1.html](http://www.science.widener.edu/~grant/esa/exp2/bgiv_1.html)).

(b) Students work in pairs to envision a specific environmental difference that might affect stomata density, and formulate predictions about which way they would expect stomata density to vary. At this stage students are asked to generate a graph that depicts what their data would look like if their predictions were (or were not) true. The environmental difference of interest to them must be available on campus,

and before they head out they must envision exactly where to go to obtain leaves for their study. All of the above must be discussed with and approved by the instructor PRIOR to collecting data.

(c) Students bring their leaf samples back to lab and estimate their stomata densities using a clever technique of making stomata impressions using commonly available nail polish and clear tape as is described in Appendix 1 (Neill et al. 1990; Brewer 1992; see <http://www.zoo.toronto.edu/zooweb/able/volumes/vol-13/3-brewer/3-brewer.htm>). Stomata impressions are easily visible under a light microscope at 400x (see Figure 1).

##### *Lab Period #2:*

(a) Students finish counting their stomata slides, if they have not yet done so.

(b) Instructors use a combination of lecture and group questioning to teach basics of statistical analysis that students will need to analyze their data (including averages, standard deviations, statistical terminology [e.g., significance level,  $P$  value, degrees of freedom], and the use of a  $t$ -test).

(c) Instructors demonstrate the use of a simple spreadsheet and statistics package (Microsoft's EXCEL) and help students to input their data, calculate averages and standard deviations, perform a  $t$ -test using these data, and generate a clear graphic summarizing their results.

In actuality, only half of lab period #2 is devoted to the activities listed above. In the first half of lab, students perform an unrelated and more traditionally formatted lab on fruit and flower diversity. Between lab periods 2 and 3, students generate coauthored oral and written reports according to specific guidelines (included with the lab handout mentioned above).

##### *Lab Period #3:*

Each pair of students presents their data and conclusions in class in a 10-minute report to their peers dur-

ing the "Stomata Results Symposium."

#### SUMMARY OF RESULTS

During the spring semesters of 1995, 1996, and 1997 we supervised over 75 projects, all of which used green plant material available in mid-February on Widener's campus (this happened despite the fact that Widener is located in an urban landscape and in 1996 a foot of snow was on the ground).

The majority of the projects were studies of the effects of sunny versus shady environments on stomata density. About half of these compared different individuals of the same species living in different places on Widener's campus, and the other half examined leaves in the sun versus shade on the same individual plant (e.g., within a dense shrub or tree). The remainder of the projects examined other effects such as proximity to sources of carbon dioxide (the campus is adjacent to interstate 95) and the effects on grass of trampling by pedestrians.

The vast majority of the results were consistent with the student hypotheses (i.e., more stomata in the sun, fewer stomata with more CO<sub>2</sub>); however, this was not always the case. Some plants showed a great deal of variation (e.g., some holly trees) that was unexplainable by treatment. Regardless, we noticed that as the students were collecting their data they were raising additional issues and posing hypotheses about characteristics of leaves, such as color and size, that may also have been affected by the environmental variable of interest. Clearly, their minds were working in unexpected directions and attempting to construct new relationships outside of the bounds of the inquiry. Often these insights and extensions generated lively discussions among students during the question/answer portion of the Stomata Results Symposium.

Student comments on this multi-week inquiry were generally very favor-

able. Many commented that they liked designing their own experiment and presenting their results to their classmates. Negative comments most commonly reflected student frustration with using the spreadsheet/statistical package available. During the second and third years we used improved instructional materials and tutorials, and students had been exposed to the software in new labs in their fall freshman lab course, so we received fewer of these comments.

Our impressions of the effects of this activity on our teaching are also positive. Considerable effort is required to resist giving too much information to the students during the project formulation stage and to simply "let go." However, this effort was returned when we witnessed the "eureka" of understanding that students attained typically when they saw the first graph depicting the results of their data.

The transfer of ownership of the inquiry from us to the students culminated in the Stomata Symposium during which we acted only as moderators of often lively student discussion. Importantly, many students who otherwise would be silent classroom occupants were fully engaged with their peers in this format. We get a great deal of satisfaction when we witness this kind of transformation.

#### CONCLUDING COMMENTS

In closing, we wish to raise three points. First, it is important to realize that this activity requires two-and-a-half lab periods. We consider that affording students the opportunity to engage in all of the steps to a scientific

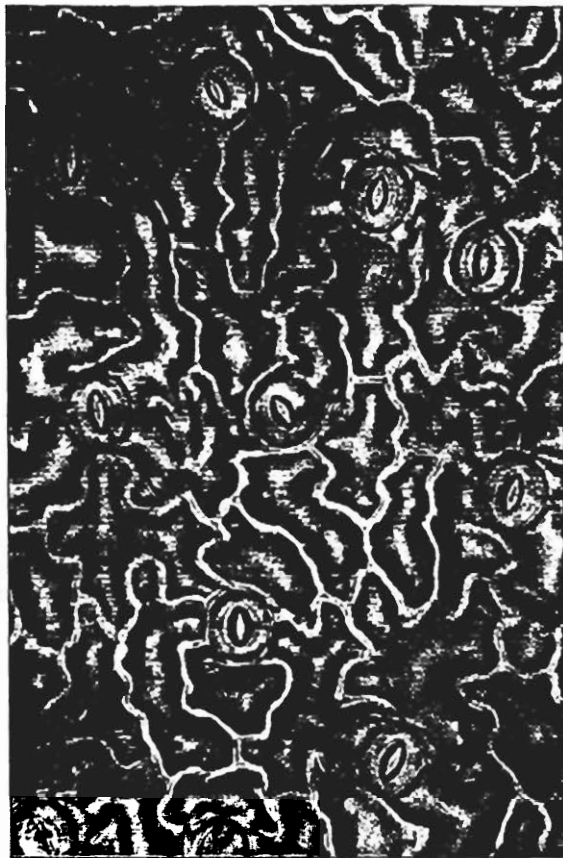


Figure 1: Stomata Impression. Photo showing what students would see in their nail polish stomata impression under a light microscope at 400x.

investigation, i.e., "teaching science by doing science," is of paramount importance to freshmen and justifies this level of lab time allocation. These students get few other opportunities to develop and wield skills in experimental design, statistical analysis, and hypothesis testing in any content area of their curriculum.

In addition, plants are very distant organisms to most freshmen, and the intensity of this project may enhance their comprehension of the evolutionary principles of plant leaf design more so than conventional lecture formats alone. We gladly devote this seemingly large portion of the laboratory time available during the semester to achieve these objectives.

Second, dozens of recent reports on laboratories based on open-ended

### Appendix 1: Detailed Procedure for Obtaining Stomata Impressions.

Obtain the leaf upon which you wish to census stomata.

On the side you wish to census stomata (typically the leaf underside) paint a rather thick swath of clear nail polish.

After the nail polish has dried (several minutes), obtain a square of VERY CLEAR tape (such as package sealing tape, but do NOT use scotch tape). Stick your tape piece to the area that contains the dried nail polish swath.

GENTLY, peel your nail polish swath from the leaf completely. You will see a cloudy impression of the leaf surface now attached to your tape piece. This is your "leaf impression." Make only one impression per leaf.

Tape your leaf impression to a VERY CLEAN slide and use scissors to cut off the excess.

Label the slide to indicate the treatment group name (e.g., leaf from sun) and other info (e.g., leaf #3) directly on the slide. Color coding slides is a very good idea.

Focus your leaf impression under at least 400x power and observe the stomata (see Figure 1).

Search around on your impression to find an area that subjectively appears to have a high density of stomata. That is, move the slide around until the field of view is away from the edge of the impression and so that there are no dirt blobs, no thumbprints, no damaged areas, and no big leaf vein impressions in view.

Count all stomata you see and record the number.

Repeat the previous two steps three times. The highest number of the three will be your datum from this impression. One datum per slide.

Repeat all steps above for at least eight different leaf impressions in each treatment group.

Students use a stage micrometer to convert their data from units of "stomata number per field of view at 400x" to units of "stomata per mm<sup>2</sup>."

Note: Students must design their own data sheets on which to record stomata counts and they are informed that they will be specifically assessed in part on how well they accomplish the tasks of data acquisition and archiving.

student investigations in *JCST* and other science education journals provide important and creative directions for our collective teaching efforts. We must all try to work with our colleagues to explore these directions and make substantive changes *along course sequences* within major programs of study and not just within single courses. Educational meta-issues such as diversity, retention, and empowerment to think scientifically depend less on a student's experiences in a single course as upon his or her tra-

jectory through the curriculum.

Third, we feel that an important goal of curricular innovation should also address the retention issue of teachers in pre-college settings and the valuation issue of teaching at the undergraduate level. As we mentioned above, we like teaching this kind of activity, and this attitude is often infectious with our students—and vice versa. At least for us, this affirmation instills a sense of renewal in our other academic pursuits.

Numerous authors have noted that

open-ended inquiries give students a sense of ownership of their learning and that this contributes to their development as life-long learners. We wish to add that teaching using these types of activities promotes the same goal in teachers as well. □

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## INCUBATION TEMPERATURE OF THE PIGEON EMBRYO (*COLUMBA LIVIA*)

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**Abstract**—This study compared the growth and development of pigeon embryos raised in the nest under normal parental care to that of eggs from the same clutch incubated in an incubator. The average daily temperatures of both environments (nest and incubator) were the same, but the hourly average temperatures and patterns of temperature fluctuations of these environments differed. Our results indicate that the small temperature fluctuations experienced by pigeon eggs in the nest under parental incubation may be the optimal thermal environment for *in ovo* growth and development. © 1998 Elsevier Science Ltd. All rights reserved

**Key Word Index:** Incubation temperature; pigeons; growth and development

### INTRODUCTION

Early definitions of incubation described it as the process by which the heat necessary for embryonic development is transferred to an egg after it has been laid (Beer, 1964). Modern definitions of incubation recognize that heat transfer is not the only factor involved in influencing the growth and development of avian embryos and therefore describe incubation as the process by which thermal environment, humidity, gaseous environment, egg turning and other physical factors are regulated to influence embryonic development (Drent, 1975; Wilson, 1991). In spite of this expanded definition there is little doubt that the incubation temperature plays a crucial role in the growth and development of avian embryos.

Over the past 50 years there has been considerable research to determine the optimal incubation temperatures of various domestic and wild bird species. Most studies of this nature were conducted by incubating eggs under artificial conditions. For example, Lundy (1969), as cited in White and Kinney, (1974) determined that the optimal incubation temperature for domestic fowl is between 37 and 38°C, and that death occurs at temperatures above 40.5 and below 35°C. The temperature range of 25–27°C was defined as the physiological zero temperature, the incubation temperature under which

no development occurs (Romanoff and Romanoff, 1972). White and Kinney (1974) concluded that the mean incubation temperature of avian eggs must be close to the optimal temperature for their development.

Unlike studies on avian eggs in artificial incubators, the incubation temperature of eggs in a natural environment varies according to parental attentiveness. Birds share incubation responsibilities in several different ways. In some species only one parent, usually the female, is the sole incubator of the eggs. In other species both sexes share the incubation responsibility. Under the dual parental care system the single incubating parent splits its days between sitting on the eggs and foraging for food. The result is intermittent incubation for the eggs, causing significant fluctuations in temperature, mirroring the periods of attentiveness and inattentiveness (White and Kinney, 1974).

Pigeons utilize bisexual incubation. One parent, the female, remains in the nest for the bulk portion of the incubation period, switching incubation responsibilities with her partner several times a day. During the entire incubation, the eggs are seldom left unexposed (Vatnick, Morrone and Davis unpublished). Therefore, one would not expect to observe a large variation in embryonic temperature throughout the entire *in ovo* development. This study was designed to determine the natural variation of the thermal environment that pigeon embryos experience throughout incubation.

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## METHODS AND MATERIALS

A flock of seven mated domestic pigeons (*Columba livia*) was purchased from a local breeder who has kept the same breeding flock for over 30 years. The pigeons were kept in a coop (2.4 m × 2.4 m × 3.25 m) housed on the roof of Kirkbride Hall (Widener University, Chester, PA). Eggs were collected from January through to September 1994. A total of 13 eggs from two-egg clutches were used in this experiment. Eggs were given identification marks within 6 hr of being laid. The day on which the egg was laid was considered day 0 chronological age. The first day of incubation was considered the day that the second egg was laid.

The first laid egg was left in the nest to be incubated by its parents. The second egg was removed from the nest on the day after it was laid and placed in an Hova-Bator Incubator (G. Q. F. Manufacturing Co., Savannah, GA) kept at approximately 36.9°C for the entire incubation period. This method was chosen in order to allow the pigeons to establish incubation, as taking the first egg would have impeded the process. The incubator was equipped with an automatic egg-turner to prevent membranes from adhering to the shell. The second egg was replaced in the nest by a water-filled dummy egg instrumented with a thermocouple connected to a CR7 Measurement and Control System data logger. A similar dummy egg was also placed in the incubator to monitor the incubation environment of the incubator eggs.

These dummy eggs were prepared using recently-laid eggs which were emptied by making a small hole in one end and injecting air into them. Eggs were rinsed, air-dried and a thermal couple was inserted and held in place with silicon adhesive and the entire egg was sealed by dipping in clear nail polish. The eggs were then filled with water to simulate the

contents of fertile eggs. In order to allow for parental manipulation similar to living eggs, the dummy eggs were not secured at the bottom of the nest. The data logger recorded the temperature of the dummy eggs every 5 min throughout incubation.

For each nest and incubator egg, an average temperature was calculated for every hour of incubation. Each hourly average corresponded to a specific hour of a 24 h day (e.g. 2400 h) for each incubation day, from day 0 to day 18. There were approximately 16–18 hourly temperature measurements for each time corresponding the same number of incubation days. These measurements were averaged to yield a 24 h pattern of incubation temperature for each individual nest. The data from all nests were then averaged to yield the overall 24 h pattern of incubation for all nests. Daily temperature averages for each nest were calculated by averaging all 288 temperature measurements for (egg temperatures were recorded every 5 min, i.e. 12/h or 288/day) a particular day. The daily averages of all nests were then averaged to yield an average daily pattern of incubation for each of the 18 days of incubation. Data taken from the incubator dummy eggs were compiled in exactly the same manner.

## RESULTS

*Average daily incubation temperature pattern*

Incubator embryos experienced a daily average incubation temperature of  $36.9 \pm 0.2^\circ\text{C}$  from days 1–18 of incubation (Fig. 1). Embryos in the nest experience lower daily incubation temperatures in their first 2 days of life. The average daily temperature at day 0 (chronological age), before incubation commenced, was  $35.3^\circ\text{C}$  for nest embryos, almost  $2^\circ$  lower ( $P < 0.5$ ) than the average day 0

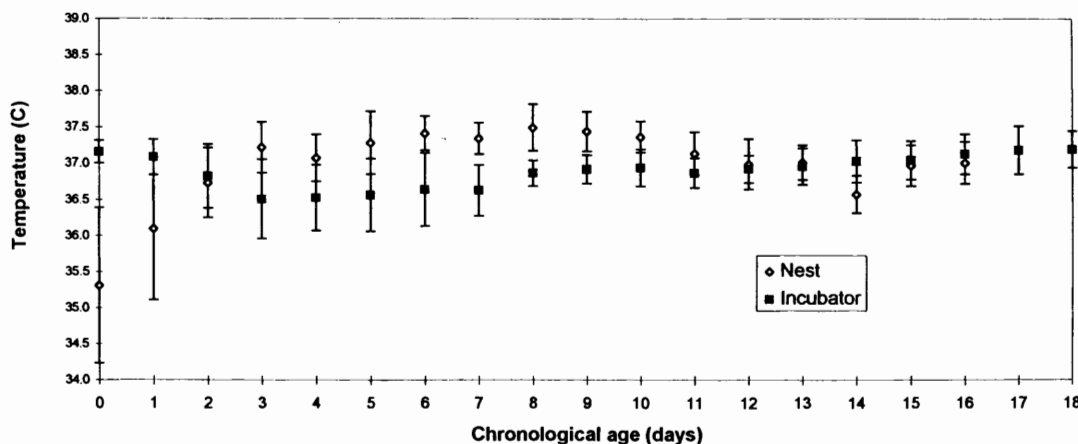


Fig. 1. Average daily incubation temperatures of pigeon embryos raised in nests and in an incubator (mean  $\pm$  standard error).

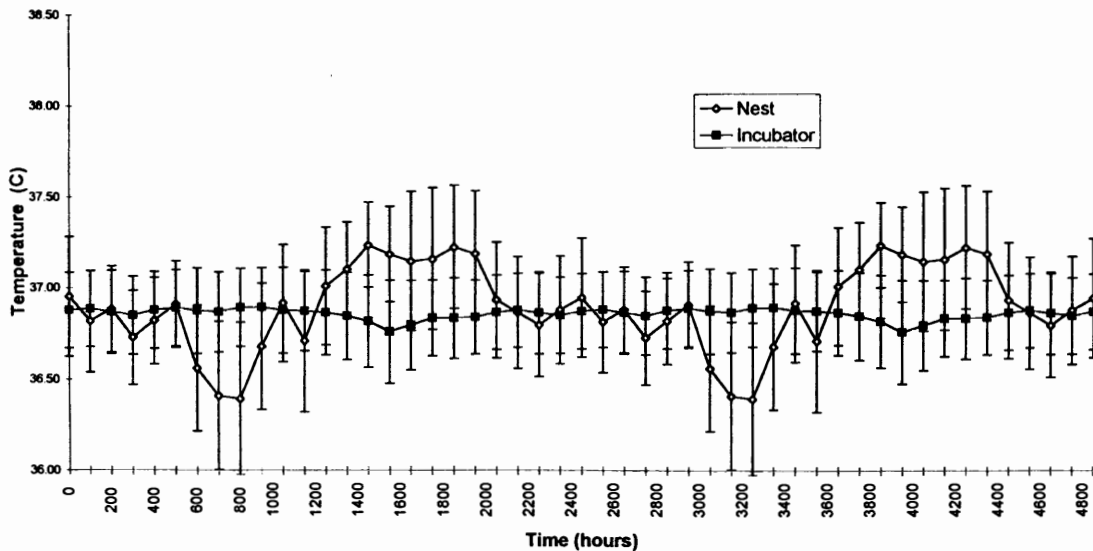


Fig. 2. Average hourly incubation temperatures of pigeon embryos raised in nests and in an incubator (mean  $\pm$  standard error).

(chronological age) temperature for incubator embryos. By day 2 (chronological age), when incubation commenced there was no difference between the average daily incubation temperatures of both treatments (approx. 36.9°C; Fig. 2). This temperature remained the same for the rest of the experiment.

#### Average hourly incubation temperature pattern

Embryos in the incubator were exposed to an average hourly temperature of  $36.9 \pm 0.2^\circ\text{C}$  that varied very little throughout incubation (about  $0.2^\circ\text{C}$ ), while the average hourly incubation temperature in the nest exhibited a diurnal pattern (Fig. 2). An average high temperature of  $37.2^\circ\text{C}$  was reached in the late afternoon between 1400 and 1800 h. An average low temperature of  $36.5^\circ\text{C}$  occurred in the morning between 0600 and 0900 h.

#### DISCUSSION

Direct measurement of internal egg temperature is difficult and can result in embryo mortality. A variety of indirect measurements have been taken by different investigators to better assess the thermal environment experienced by living embryos. Different studies measured various variables such as air, parent's body, and brood patch temperature in addition to making several temperature measurements (e.g. top, bottom and core) of non-viable eggs instrumented with thermocouples. For example, Lennerstedt (1966) looked at internal egg temperature and air temperature in his study of Capercaillie (*Tetrao urogallus* L.). Bergstrom (1989), studying Wilson's Plover (*Charadrius wilsonia*) and Killdeer

(*C. vociferus*), measured internal egg, nest bottom and shaded air temperatures. Russell (1969) and Purdue (1976) added an extra variable, brood patch temperature, in their study of white-winged doves (*Zenaida asiatica*) and Snowy Plovers (*Charadrius alexandrinus*), respectively. Measurements of air and parent body temperature were taken in addition to internal egg temperature in a study of rock pigeons (*Columba livia*) conducted by Marder and Gavrieli-Levin (1986).

While some species do seem to be incubated naturally at a constant temperature, Huggins (1941) found that "in natural incubation there is no one egg temperature, but a range of temperatures through which an egg can develop normally and the young can hatch". Many investigators have measured egg temperatures throughout the incubation period of numerous species. Caldwell and Cornwell (1975) determined the average egg temperature for Mallards (*Anas platyrhynchos*) to be  $36.3^\circ\text{C}$  for the entire 24 days of incubation. Egg temperature slowly increased over a  $7^\circ\text{C}$  range ( $T_{\text{max}} = 41.5^\circ\text{C}$ ) during incubation until hatching. They determined that "embryo temperatures of about  $38.0^\circ\text{C}$  are more constant than their data indicates" due to the difficulties of estimating embryo temperatures from probes placed in the egg air cell. The incubation temperature of Goldencrest *Regulus regulus* is held constant over the entire incubation period, independent of time of day (Haftorn, 1978).

In this study the daily average temperatures of nest eggs increased during the first 4 days of life and leveled off thereafter. The daily average egg temperature from day 5 until the end of incubation

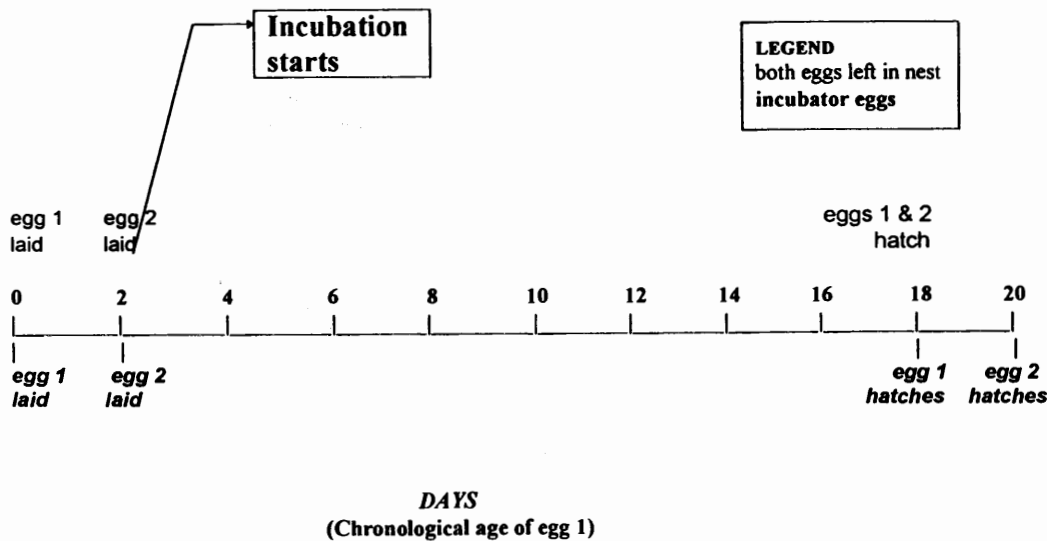


Fig. 3. Timeline of incubation. The control timeline is a result of previous observations of the incubation length of pigeons in our flock. When both eggs are left in the nest, they hatch within a few hours of each other 18 days after the first egg is laid. In this experiment, the nest eggs followed the normal incubation period, while the incubator eggs (2nd eggs) hatched 2 days later on day 20 (chronological age).

was remarkably constant (Fig. 2). Pigeons do not begin incubation of their eggs until the clutch is complete, roughly 48 h after the first egg was laid. After that time, eggs are attended by both sexes and are left unattended less than 1% of the time (Vatnick, Morrone and Davis unpublished). Therefore, one should expect that pigeon eggs experience very little fluctuation in their temperatures when incubation is well established.

A large portion of the studies of incubation temperatures of eggs of several domestic (e.g. Martin and Insko, 1935, turkeys; Lundy, 1956, domestic fowl) and wild species (e.g. Morton and Pereyra, 1985, dusky flycatchers; Lennerstedt, 1966, Capercaillie) reported mean daily temperatures. It may be that the *hourly fluctuations* in incubation temperatures throughout a given day are as important in determining growth and development of birds embryos as the average *daily temperature* which they experience. Looking at average hourly temperature, Haftorn (1983) found that the Great Tit (*Parus major*) experiences an incubation temperature range of 34.0–36.2°C ( $X = 35.4^\circ\text{C}$ ), high temperatures occurring from 0600 to 1600 h, and low temperatures from 0500 to 1800 h.

In this study the average daily temperature of nest eggs after the third day of life varied less than 0.5°C. The hourly average temperature of nest eggs in this study exhibited a discernible diurnal pattern with lows in the early morning (0600–0800 h, Fig. 2) and highs in the early afternoon (approx. 1400 h, Fig. 2). The differences between the high and low tempera-

tures were also remarkably small (about 0.7°C). In contrast, the average hourly temperature of incubator eggs was constant throughout the day and did not vary by more than 0.2°C. Perhaps the diurnal pattern of nest-egg temperature is a reflection of the daily fluctuations in the parent's body or brood patch temperature. It may be that this diurnal pattern in incubation temperature changes the rate of growth and development of pigeon embryos, contributing to the difference in the incubation length between the treatments (Fig. 3). Future studies could mimic, with the use of an incubator, the small hourly fluctuations in the incubation temperatures that pigeon eggs experience under natural incubation and examine the effect on incubation length of eggs subjected to this treatment.

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