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Cover image: On display in the British Museum of Natural History, this remarkable fossilized plesiosaur was found in southern England in the early 1830's by Mary Anning of Lyme Regis. The photograph is courtesy of Ethel Stanley

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Deadlines for Submissions

February 1, 2001 for the March 2001 Issue

April 1, 2001 for the May 2001 Issue

Spectrophotometric Assay of Gene Expression: *Serratia marcescens* Pigmentation

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Abstract: Studies of the physiology of unicellular organisms often involve assays for gene expression in conjunction with changes in growth or environmental conditions. We have exploited the property of pigment production by the nonpathogenic bacterium *Serratia marcescens* to design a simple assay for gene expression that is easily and inexpensively adaptable to the teaching biology laboratory. Our assay uses a spectrophotometer to simultaneously measure bacterial growth and expression of the red, cell-associated pigment prodigiosin. This work defines a unit value for prodigiosin expression and characterizes pigmentation through variations in cell density, growth temperature and growth medium. Spontaneous pigmentation mutants of *S. marcescens*, which can be easily isolated due to variations in colony color, may be quantitatively assayed for prodigiosin expression throughout the population growth cycle.

Keywords: *Serratia marcescens*, prodigiosin, gene expression, absorption spectroscopy

INTRODUCTION

Assays of bacterial gene expression make attractive teaching tools for several reasons. First, bacteria modulate their gene expression quickly in response to environmental cues such as cell density, growth temperature and growth medium. Bacterial messenger RNA molecules are typically degraded with half-lives measured in minutes versus the hours of stability for eukaryotic transcripts. Second, many bacteria express pigments under certain conditions. Because most pigments absorb light at some defined wavelength, pigment expression may be easily monitored spectrophotometrically. Third and perhaps most importantly, bacteria are easy to propagate in the teaching laboratory.

The *Escherichia coli* lactose operon has become a paradigm of the environmental modulation of gene expression (Brock, 1990). The *lac* operon encodes the enzyme beta-galactosidase, and this gene product can be easily assayed due to the commercial availability of a chromogenic substrate for enzyme activity. The colorless compound *o*-nitrophenyl-beta-D-galactopyranoside is hydrolyzed by beta-galactosidase enzyme to liberate *o*-nitrophenol. This latter compound is yellow in color, and changes in its concentration over time are followed spectrophotometrically at 410 nm (Miller, 1972). While this assay is relatively simple, it does require chemicals and cell permeabilization with chloroform. For classroom use we have designed a simpler gene

expression assay in which growth rate and pigment expression can be measured simultaneously for the nonpathogenic and easily cultivated soil bacterium *Serratia marcescens*.

S. marcescens produces a cell-associated pigment called prodigiosin (Gerber, 1975). Interestingly, prodigiosin is expressed as a secondary metabolite in the general method of gene expression called quorum sensing (Fuqua et al., 1996; Williams et al., 1992). Growth in liquid culture at low cell density allows low-level expression of a membrane-permeable positive regulator of prodigiosin expression. The intracellular concentration of the regulator remains low at low cell density due to its diffusion across the cell membrane after synthesis. However, as cell density increases in a closed system, the intracellular concentration of regulator increases to a threshold needed for activation of prodigiosin expression. Thus, high levels of prodigiosin are expressed in liquid culture only at high cell density. A similar phenomenon operates with colonies grown from single cells on agar plates (P. L. Haddix, unpublished observation). Very small and widely spaced colonies are initially non-pigmented; coloration first develops near a colony diameter of about 1 millimeter.

In this work we determine the wavelength at which cell-associated prodigiosin absorbs light and use this to define a unit of prodigiosin expression on a per-cell basis. Further, we characterize prodigiosin expression kinetics as functions of cell density,

temperature and growth medium. Our system is easily adaptable to teaching biology labs in which the environmental regulation of gene expression is studied.

MATERIALS AND METHODS

S. marcescens Nima, American Type Culture Collection (ATCC) #29632, was used for most prodigiosin expression experiments. A spontaneous Nima mutant, isolated by its resistance to 25 µg per ml ampicillin and designated Mutant 5, was employed for prodigiosin unit definition studies. Mutant 5 will be formally characterized elsewhere, but it was used here as a non-pigmented reference strain which totally lacked prodigiosin under all conditions tested. *Pseudomonas aeruginosa* Boston 41501 (ATCC # 27853) was also used as a non-pigmented reference bacterium. A spontaneous pigmentation mutant of *S. marcescens* Nima, designated *S. marcescens* Red, was isolated as a darker than normal small colony from a mixed growth experiment with *P. aeruginosa*. Bacterial stocks were maintained in phosphate buffer + 40% glycerol at -20°C (Miller, 1992).

Bacteria from frozen stocks were initially cultured at 30°C on nutrient agar or blood agar base slants (Becton Dickinson and Co., Cockeysville, MD). Dehydrated nutrient broth (Becton Dickinson) and M9 minimal salts (Sigma Chemical Co., St. Louis, MO) were reconstituted according to the manufacturers' instructions. Maltose monohydrate was added to nutrient broth at 0.5% (w/v). Complete M9 + 0.5% (w/v) glycerol medium was made per liter as follows (Miller, 1972). 10 g dehydrated M9 salt mixture was dissolved in 800 ml distilled water, and 10 ml 50% glycerol was added. The volume was made to 1 L with distilled water, and the medium was sterilized in an autoclave. A mixture of 20 ml 10 mM CaCl₂ and 2 ml 1 M MgSO₄ was sterilized separately. After cooling to re-dissolve the precipitate, 11 ml of the CaCl₂ / MgSO₄ mixture was added to the M9 salts with glycerol mixture to complete the medium. Media, glassware and unwanted bacterial cultures were sterilized in a Tuttnauer / Brinkmann 2540E autoclave on liquid (slow exhaust) cycle for 20 minutes at 121 C.

Cultures designated "pre-growth" were inoculated from 1-2 day 30°C agar slants to below visible turbidity in 25 ml liquid medium in 250 ml Erlenmeyer flasks. Cultures designated "growth" were inoculated into similar flasks to the desired optical density at 620 nm (OD₆₂₀) using a calculated volume of dense pre-growth as described previously (Haddix et al., 2000). All flasks were aerated at the indicated temperature ± 0.5 C in a Lab-Line™ model 3582 shaking water bath.

All absorbance measurements were made with a Beckman Model DU640B ultraviolet-visible spectrophotometer. Experiments requiring cell washing by centrifugation employed a Beckman Avanti-30 centrifuge and F0650 rotor. Cells were

pelleted at 7,000 rpm (4602 x g) for 10 minutes at room temperature.

Values given as averages are reported with the number of component measurements *n* and sample standard deviations *s*. Bacterial doubling times were calculated as follows using either PSI Plot 3™ (Poly Software International, Inc.) or Axum 6.0™ (MathSoft, Inc.) statistical software. The logarithm of optical density at 620 nm was plotted against time in hours. The software performed least squares linear regression analysis using a minimum of four growth curve time points which best reflected exponential growth during early log phase. Correlation coefficients for each regression line exceeded 0.95. Slopes of the regression lines were divided into the logarithm of two to calculate generation times in hours; these values were multiplied by 60.0 to convert them to minutes.

We quickly discovered that several physiological parameters are not independent of initial cell density and/or the volume of conditioned pre-growth medium added to fresh medium for growth experiments. These parameters include population doubling time, onset of prodigiosin expression and prodigiosin yield per cell. For this reason our reported growth data were taken from cultures standardized to begin at a calculated initial optical density of 0.2.

PRODIGIOSIN UNIT DEFINITION

We first determined the peak wavelength at which pigmented *S. marcescens* cells absorb visible light. Figure 1 shows absorption spectra for *S. marcescens* Nima, non-pigmented Nima mutant 5, and *Pseudomonas aeruginosa* grown to stationary phase in nutrient broth plus maltose. Pigmented *S. marcescens* cells in their conditioned medium show a two-humped absorbance peak with a maximum at 499 nm (Fig. 1). Since this peak is not present in non-pigmented Nima mutant 5 and *P. aeruginosa* stationary phase cultures, peak absorbance in this region is due to prodigiosin expression.

We also tested the absorbance properties of prodigiosin-containing conditioned medium in which pigmented cells had been removed by centrifugation (data not shown). Because prodigiosin is only minimally soluble in water, the pigment found in conditioned medium is most likely associated with cell membrane material. The absorption spectrum of this material showed a selective enrichment of the left hump of the prodigiosin absorbance peak. Purified prodigiosin has an absorbance maximum of 535-540 nm in acidified alcohol (Williams et al., 1971; Heinemann et al., 1970), and this wavelength corresponds to the right hump of the prodigiosin peak (Fig. 1). 499 nm, the peak value of the left hump, was chosen as the wavelength for the measurement of prodigiosin in actively growing cultures.

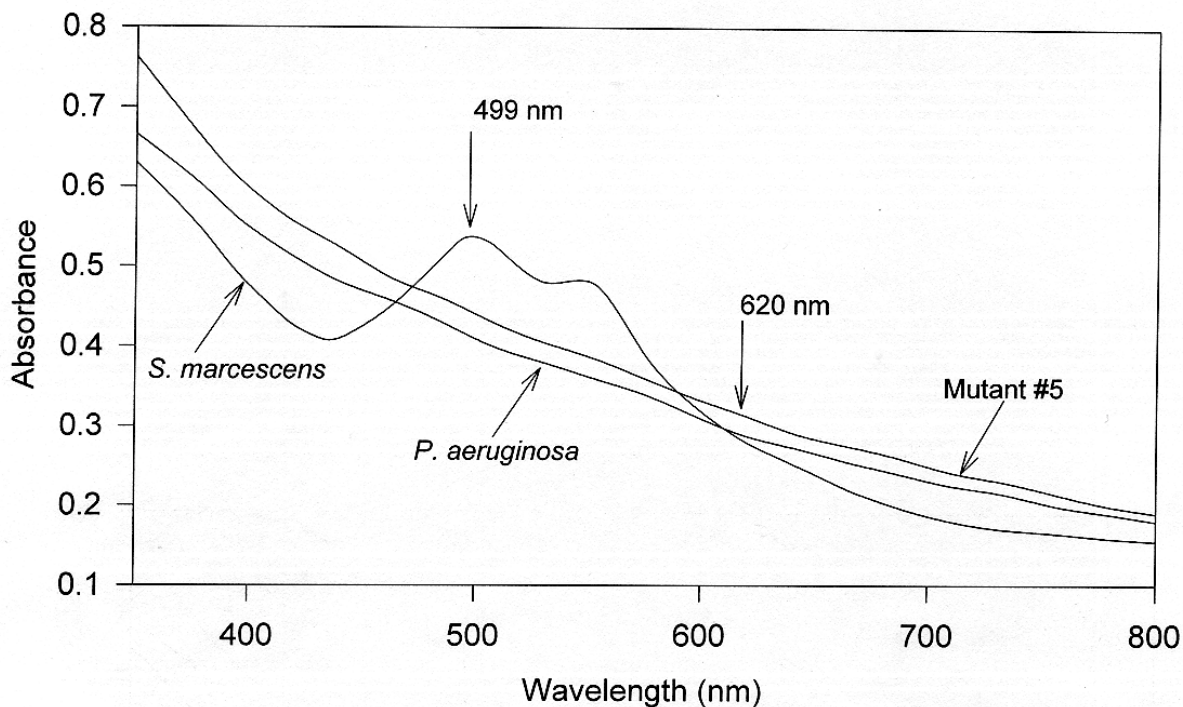


Figure 1. Absorbance spectra of bacterial cultures. The figure is a composite of visible spectra from *S. marcescens* Nima, *S. marcescens* ampicillin-resistant Mutant 5, and *P. aeruginosa* grown under conditions that induce prodigiosin expression. Cells from day-old slant cultures were grown to saturation in nutrient broth + 0.5% maltose. They were diluted in this same medium to optical densities within the linear range for both prodigiosin and whole cell quantification. Each sample was scanned at a speed of 20 nm per second.

Figure 1 also indicates that optical density values at 620 nm (OD_{620}) can be used to measure cell density without interference due to prodigiosin absorbance. *Escherichia coli*, another non-pigmented bacterium, produced an optical density profile similar to those for non-pigmented *S. marcescens* and *P. aeruginosa* (data not shown). Further work has identified the range of linear relationship between absorbance at 499 nm (A_{499}) and pigmented cell concentration. This range was found to be approximately 0.0250 to 1.6000 absorbance. Similarly, OD_{620} varies linearly with non-pigmented or pigmented cell concentration in the approximate range of 0.0570 to 0.9850. Accurate quantification of dense cultures therefore requires dilution into the 0.0570-0.9850 OD_{620} linear range.

The above information has allowed us to define a unit of prodigiosin expression based solely upon absorbance data from broth culture samples. First, we observed that the quotient A_{499} / OD_{620} is constant for each growth phase of non-pigmented *S. marcescens* Mutant 5 cells grown with aeration in nutrient broth plus maltose. Early log phase cells have an average ratio of 1.3267 ($n = 12$; $s = 0.03728$), late log phase cells show an average ratio of 1.3955 ($n =$

11; $s = .01537$), and stationary phase cells have an average ratio of 1.4274 ($n = 18$; $s = 0.01948$). These three averages were themselves averaged to calculate the A_{499} / OD_{620} quotient across all phases of non-pigmented cell growth: 1.3831 ($n = 3$; $s = 0.05134$). The quantity (1.3831X OD_{620}) therefore represents the contribution of cells alone, excluding prodigiosin, to the measured absorbance value at 499 nm. If the optical density due to cells alone is subtracted from the total absorbance at 499 nm, we have an expression representing the absorbance due to prodigiosin alone:

$$A_{499} - (1.3831 \times OD_{620})$$

(expression 1)

Finally, the absorbance due to prodigiosin may be divided by the optical density at 620 nm to express prodigiosin units on a per-cell basis. The factor 1000 is included to avoid working with numbers smaller than one:

$$\frac{\{A_{499} - (1.3831 \times OD_{620})\}}{OD_{620}} \times 1000$$

(equation 1)

We have found that this unit definition gives negative numbers for the early logarithmic portions of growth curves. This was expected due to the use of an average value for the A_{499} / OD_{620} quotient in expression 1. Figure 2 shows that the occurrence of negative “prodigiosin” units in non-pigmented cells correlates with growth through early log phase. Changes in prodigiosin units that are unrelated to prodigiosin itself have not exceeded +50 units in our hands (Fig. 2). If one desires, equation 1 may be modified to assign all values less than 50 units a value of zero units when the cells are grown in nutrient broth

with maltose. We have not made this correction, however, because we consider this limitation an important issue for student discussion. All graphs in this work therefore reflect the prodigiosin unit calculation described in equation 1. Prodigiosin unit values recorded in conjunction with measurements of population size over time accurately convey trends of increasing or decreasing prodigiosin expression as confirmed by visual inspection. A slightly more laborious prodigiosin assay that uses pigment extracted from broth culture samples and a centrifugation step has been described (Heinemann et al., 1970).

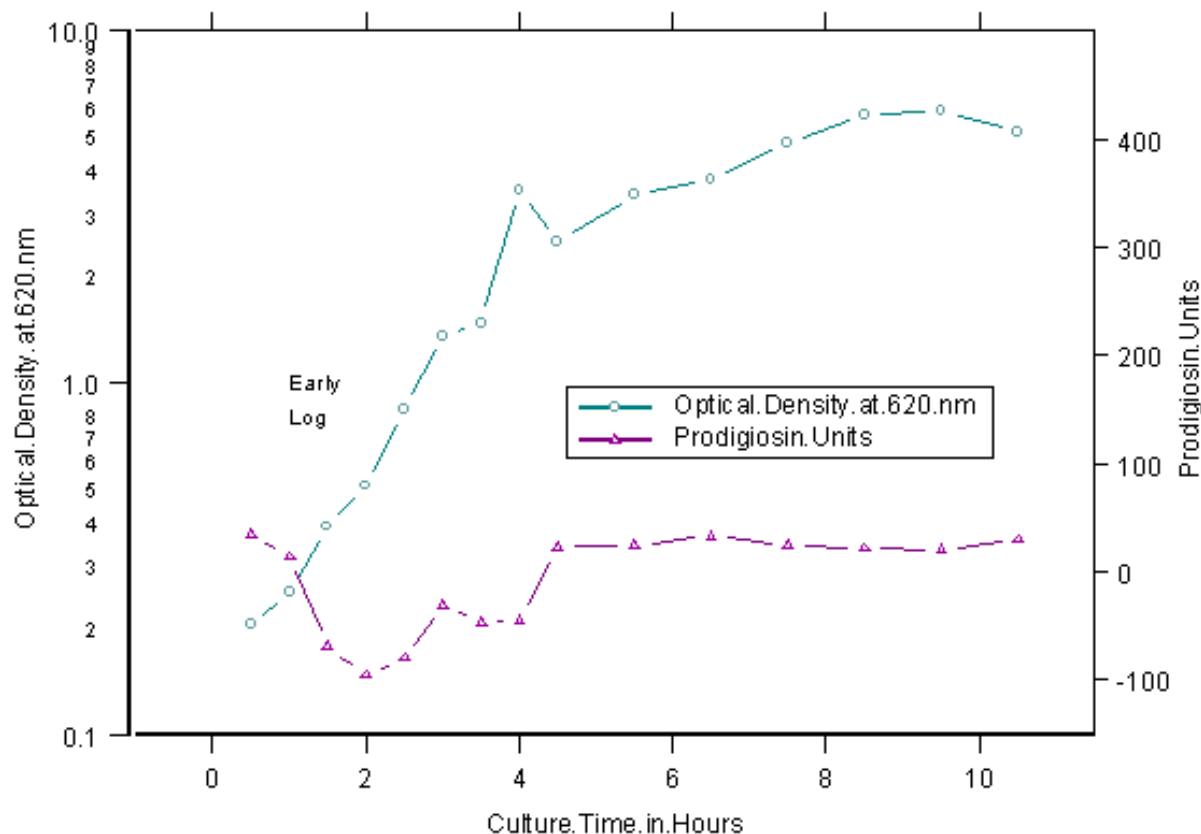


Figure 2. Prodigiosin unit background in nutrient broth + maltose. *S. marcescens* non-pigmented mutant 5 was pre-grown and grown in nutrient broth with maltose at 30 C. Growth shown in the figure began at a calculated $OD_{620} = 0.2$, and samples were taken periodically for simultaneous measurement of growth and cellular background as prodigiosin units. The average early log phase doubling time was 54.6 minutes ($n = 3$; $s = 3.85$).

CELL DENSITY-DEPENDENT PRODIGIOSIN EXPRESSION

Figure 3 shows both logarithmic growth and prodigiosin expression over time by *S. marcescens* Nima in nutrient broth plus maltose at 30 C. These conditions represent our standard inductive conditions for prodigiosin expression by *S. marcescens*. Prodigiosin levels declined initially due to low cell density and growth through early logarithmic phase.

However, prodigiosin expression began to increase as the cells entered late logarithmic growth. The observed density-dependent regulation of prodigiosin expression in Fig. 3 validates our unit calculation as a reasonable estimate of cellular pigmentation. The medium nutrient broth plus 0.5% glucose allowed only limited prodigiosin expression and growth to approximately half the cell density achieved in maltose-containing medium (data not shown).

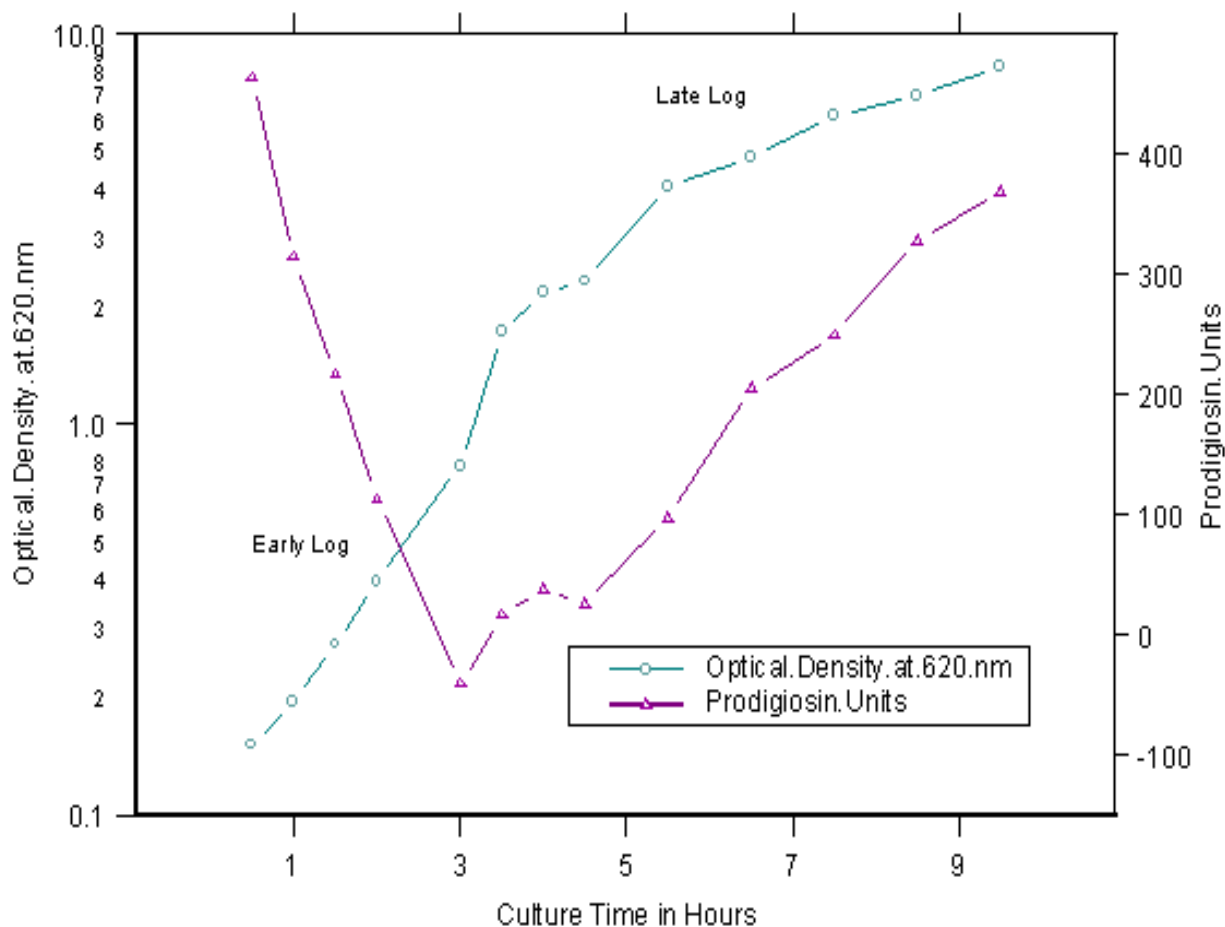


Figure 3. Cell density-dependent prodigiosin expression. *S. marcescens* Nima was pre-grown and grown in nutrient broth + 0.5% maltose at 30 C. Growth shown in the figure began at a calculated $OD_{620} = 0.2$. Samples were taken periodically for simultaneous measurement of growth and prodigiosin expression. The average early log phase doubling time was 54.0 minutes ($n = 4$; $s = 3.87$).

THE EFFECT OF TEMPERATURE ON PRODIGIOSIN EXPRESSION

An early study of prodigiosin expression showed that the pigment is not made by non-shaken cultures when the bacteria are incubated at 38 C (Williams et al. 1971). Figure 4 shows prodigiosin expression at 37°C following a pre-growth under the inductive conditions of 19 C in nutrient broth plus maltose. As expected, prodigiosin levels declined initially due to growth at low cell density. As late log phase was reached, however, prodigiosin synthesis recovered slightly but quickly decreased to background levels. Interestingly, the doubling time for *S. marcescens* Nima at 37 C is not significantly different than its value at 30 C (Fig. 4).

The fact that population growth kinetics are unaltered by a temperature increase to 37 C (Fig. 4) suggests that the higher temperature evokes a physiological rather than a genetic change to repress prodigiosin expression. Further support for the physiological role of temperature in pigment expression is shown in Figure 5. Here, cells were pre-grown at 37 C and then shifted to the inductive temperature of 30 C. As expected, pigment expression recovered at the lower temperature as the cells reached late logarithmic phase (Fig. 5). Earlier studies with non-shaken cells incubated in rich medium showed that the ability to recover prodigiosin expression decreases considerably after about 48 hours of incubation at the higher temperature (Williams et al., 1971).

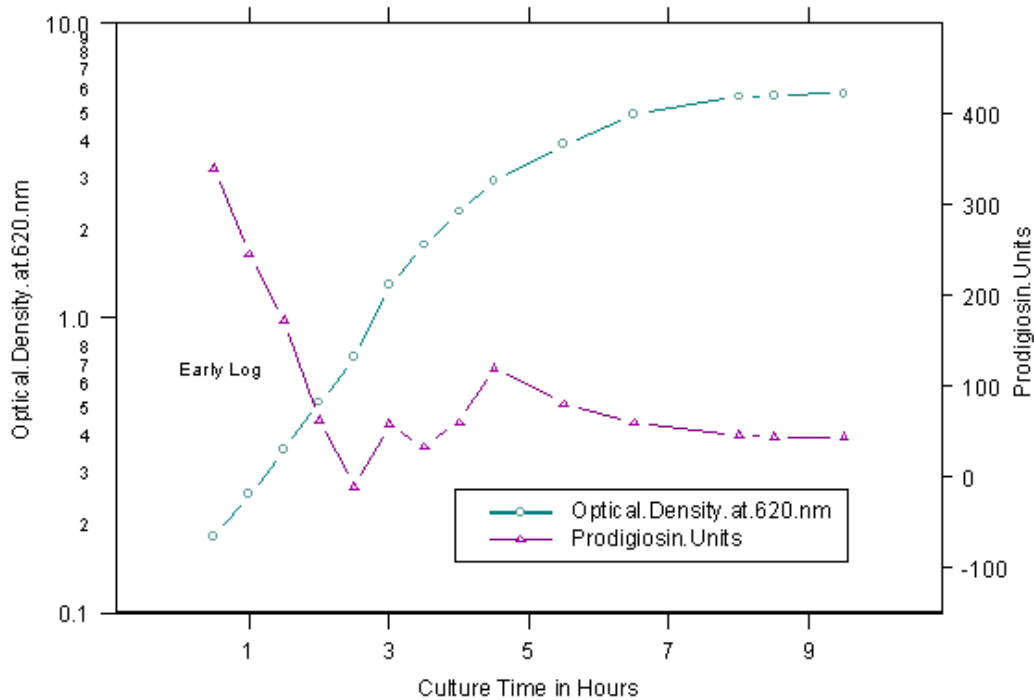


Figure 4. Prodigiosin repression at 37 degrees. *S. marcescens* Nima was pre-grown in nutrient broth + 0.5% maltose at 19 C. The pre-growth was diluted in the same medium to a calculated $OD_{620} = 0.2$ for growth at 37 C as shown in the figure. Samples were taken periodically for simultaneous measurement of growth and prodigiosin expression. The average early log phase doubling time was 56.5 minutes ($n = 2$; $s = 3.96$).

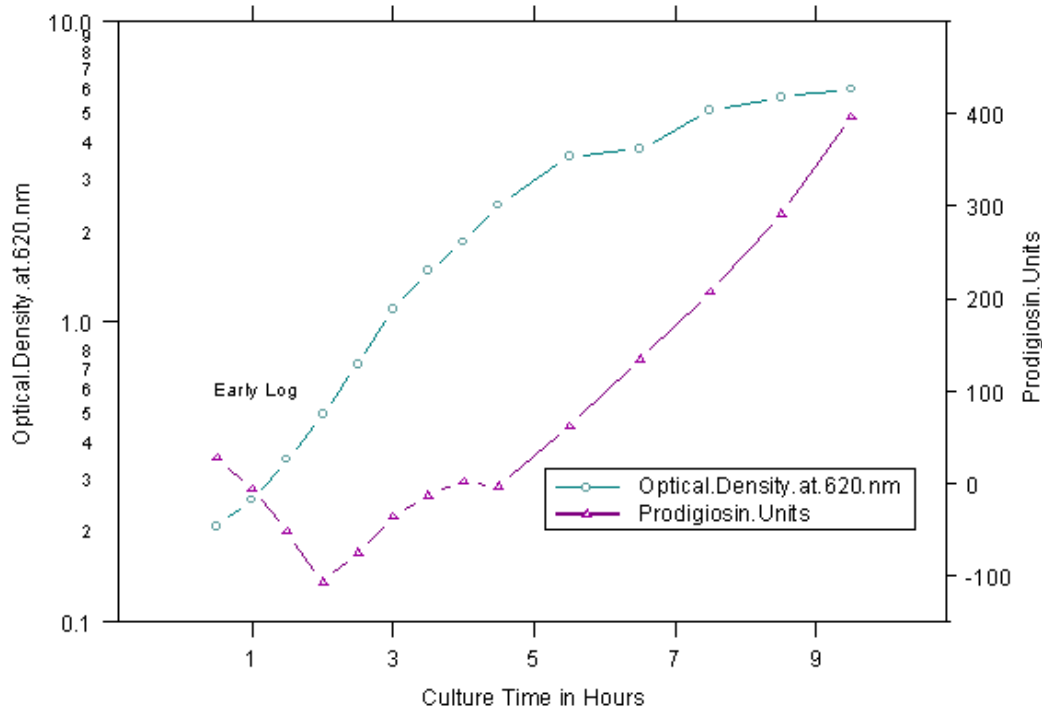


Figure 5. Recovery of prodigiosin expression by temperature downshift. *S. marcescens* Nima was pre-grown in nutrient broth + 0.5% maltose at 37°C. The pre-growth was diluted in the same medium to a calculated $OD_{620} = 0.2$ for growth at 30 C as shown in the figure. Samples were taken periodically for simultaneous measurement of growth and prodigiosin expression. The average early log phase doubling time was 56.9 minutes ($n = 2$; $s = 0.990$).

GROWTH MEDIUM VARIATION AND PRODIGIOSIN EXPRESSION

We next tested M9 minimal medium plus glycerol, a nutritionally poor medium, for prodigiosin expression. Figure 6 shows that prodigiosin unit measurement peaked near 100 units after growth to late log phase. This level is indistinguishable from the cellular background of about 100 units detected with non-pigmented *S. marcescens* mutant 5 grown in this medium (data not shown). Dense, stationary phase cultures of *S. marcescens* did not appear pigmented in

this transparent medium when viewed with the naked eye. However, concentration of such cultures by centrifugation yielded slightly rose-colored cell pellets. Spectrophotometric analysis of concentrated, stationary phase cultures routinely showed between 100 and 150 prodigiosin units (data not shown). Further work is necessary to determine whether the nature of the carbon source, its concentration in the medium or both affect final cell yield and prodigiosin expression.

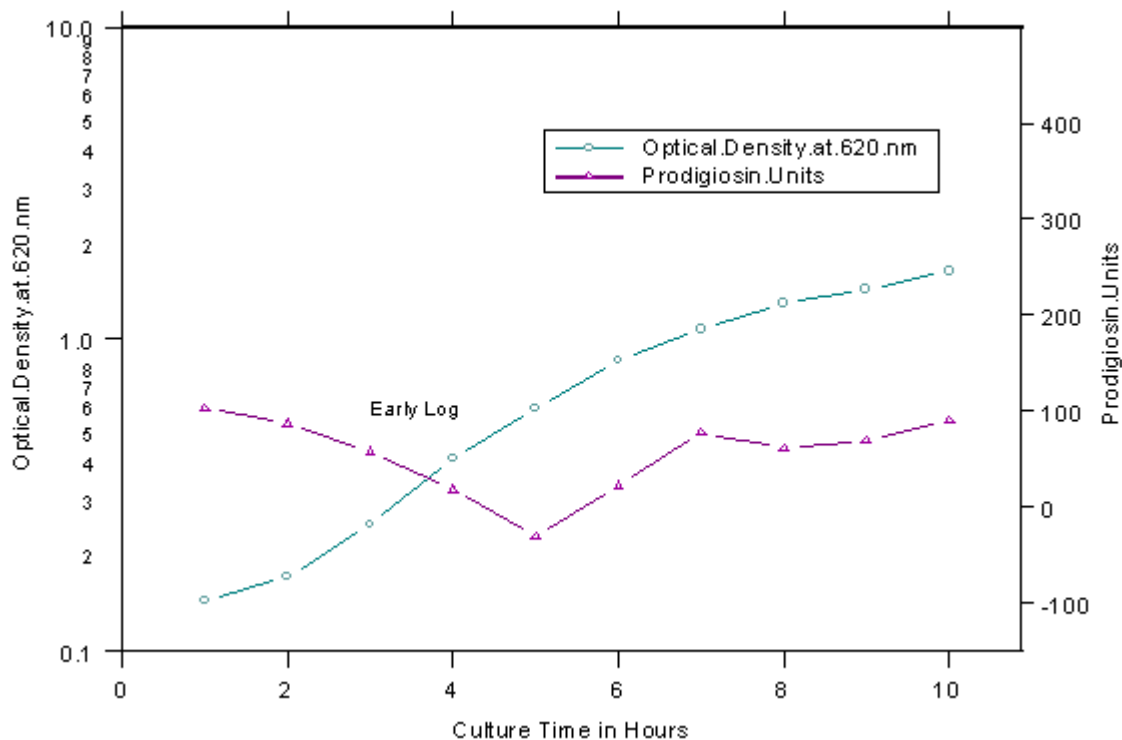


Figure 6. Low-level prodigiosin expression. *S. marcescens* Nima was pre-grown and grown in M9 + 0.5% glycerol at 30 C. Growth shown in the figure began at a calculated $OD_{620} = 0.2$. Samples were taken periodically for simultaneous measurement of growth and prodigiosin expression. The average early log phase doubling time was 105 minutes ($n = 2$; $s = 2.83$).

We have seen that prodigiosin is strongly expressed by dense populations growing in a nutritionally rich medium. Further, pigment is weakly expressed when *S. marcescens* is grown in a nutritionally poor medium which supports growth to lower densities. Therefore, we expected that a switch from rich to poor medium would repress prodigiosin expression and a switch from poor to rich medium would induce pigmentation. Both of these phenomena should be subject to normal density-dependent regulation. These ideas were tested in Figures 7 and 8, respectively. Figure 7 shows that cells pre-grown in nutrient broth plus maltose, a rich medium, failed to show density-dependent restoration of strong pigmentation when switched to the poor medium M9

plus glycerol. Interestingly, prodigiosin expression leveled off near 130 units, just above the background of ca. 100 units seen in this medium (cf. Fig. 6). The data obtained from a reverse experiment is shown in Figure 8. Cells pre-grown in M9 plus glycerol and switched to nutrient broth plus maltose show density-dependent restoration of high-level prodigiosin expression (Fig. 8). The data shown in Figures 7 and 8 were from experiments performed with cells washed in the final growth medium prior to dilution for growth into the new medium. Other work has indicated that unwashed cells in heterologous conditioned medium can be added up to at least 2% of the final volume and still produce results similar to those from washed cells (data not shown).

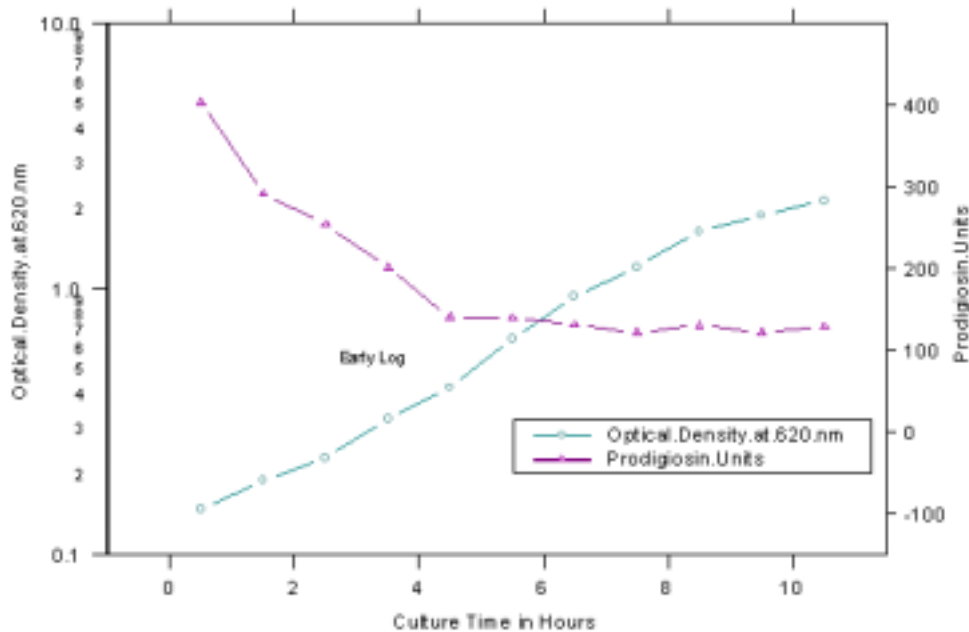


Figure 7. Prodigiosin repression by medium change. *S. marcescens* Nima was pre-grown in nutrient broth + 0.5% maltose at 30 C. The cells were washed once in an equal volume of M9 + 0.5% glycerol at room temperature, and they were diluted to a calculated $OD_{620} = 0.2$ in M9 plus glycerol. Growth and prodigiosin expression at 30 C were monitored periodically as shown in the figure. The average early log phase doubling time was 122 minutes ($n = 2$; $s = 1.41$).

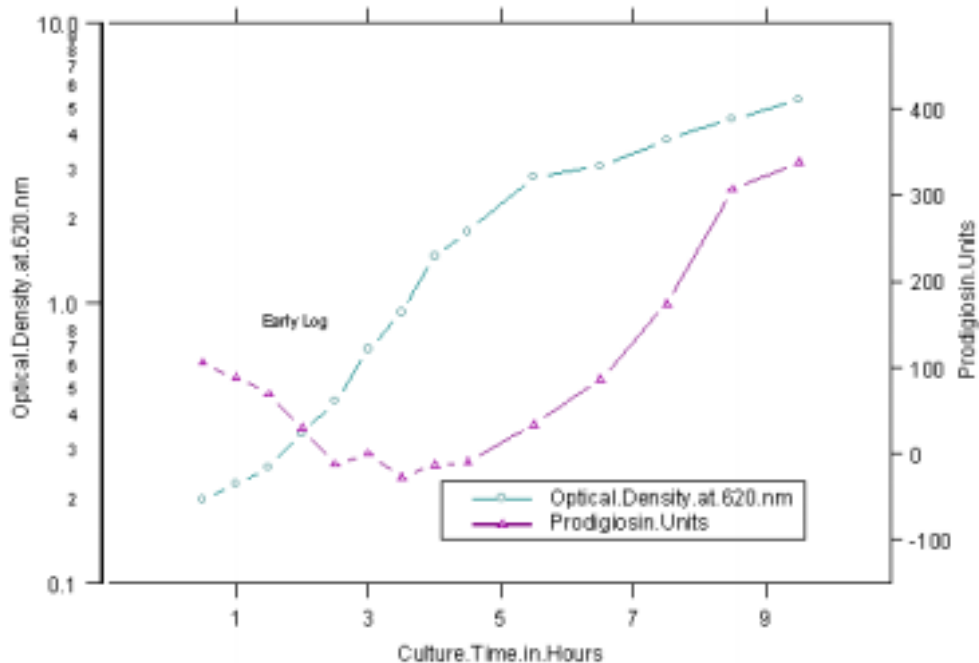


Figure 8. Prodigiosin induction by medium change. *S. marcescens* Nima was pre-grown in M9 + 0.5% glycerol at 30 C. The cells were washed once in an equal volume of nutrient broth + 0.5% maltose at room temperature, and they were diluted to a calculated $OD_{620} = 0.2$ in nutrient broth plus maltose. Growth and prodigiosin expression at 30 C were monitored periodically as shown in the figure. The average early log phase doubling time was 58.1 minutes ($n = 2$; $s = 6.08$).

CHARACTERIZATION OF A PIGMENTATION MUTANT

The results obtained with strain Nima indicate that our prodigiosin expression assay may be extended to the characterization of pigmentation by Nima mutants. Figure 9 shows growth and prodigiosin expression for *S. marcescens* Red, a Nima variant which was isolated as a redder than normal colony. Mutant prodigiosin expression kinetics are similar to strain Nima except for the apparent higher minimum

prodigiosin expression during early log phase (cf. Fig. 3). This finding suggests that strain Red over expresses prodigiosin. This mutant retains normal cell density-dependent regulation of pigmentation (Fig. 9). Therefore, its genetic lesion(s) probably affect steady-state prodigiosin levels. Further work is needed to determine if the observed up-regulation is due to increased synthesis, decreased degradation, or some combination of both.

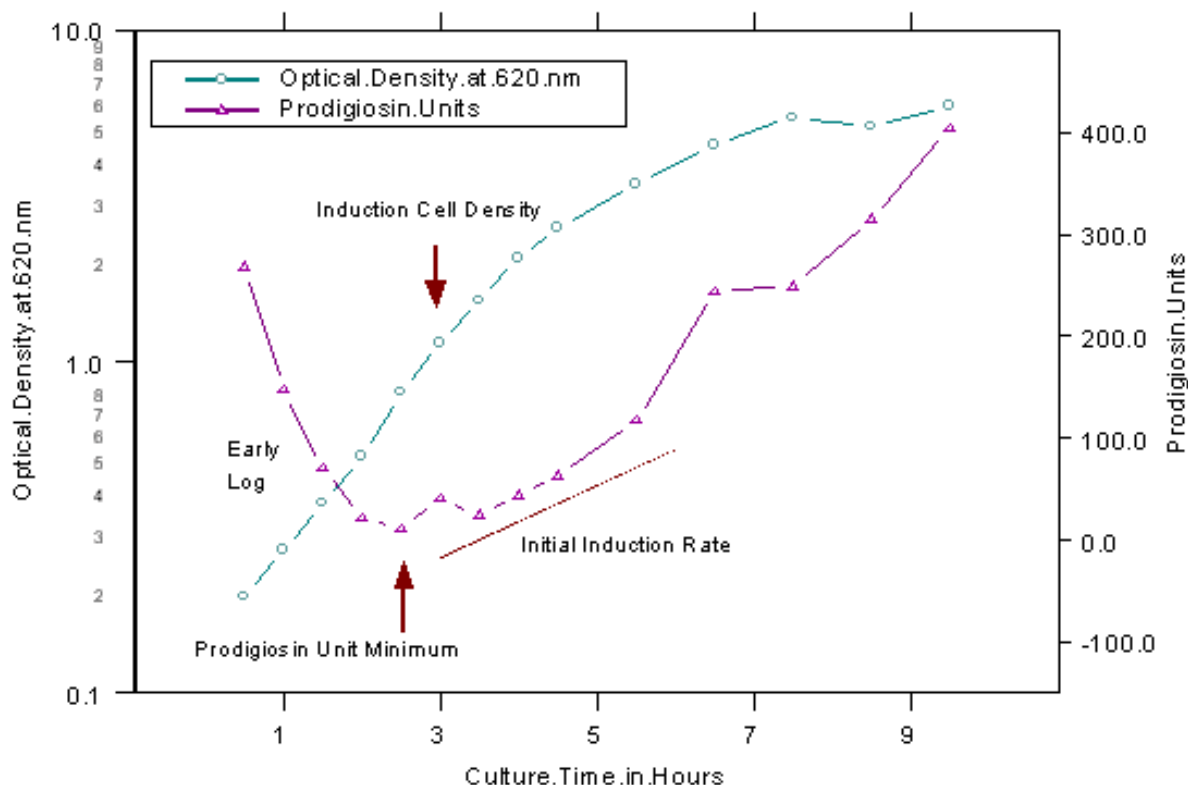


Figure 9. Characterization of a pigmentation mutant. *S. marcescens* Red was pre-grown in nutrient broth + 0.5% maltose at 30 C. The pre-growth was diluted in the same medium to a calculated $OD_{620} = 0.2$ for growth at 30 C as shown in the figure. Samples were taken periodically for simultaneous measurement of growth and prodigiosin expression. The average early log phase doubling time was 59.5 minutes ($n = 2$; $s = 0.141$). Prodigiosin kinetic parameters are indicated with arrows and an unbroken line

Because pigmentation is gradually induced in *S. marcescens*, the assignment of an absolute number describing a mutant's prodigiosin level should not be considered reliable by itself. Further, we have observed that spontaneous Nima mutants often have multiple alterations related to the pigmentation phenotype. These include doubling time, culture density at the onset of prodigiosin induction, final cell yield when grown to stationary phase, and prodigiosin yield per cell at stationary phase. We define below some useful kinetic parameters for direct comparisons of Nima and its mutants. Care must be taken to assay these parameters in the same experiment with cells

grown under standard inductive conditions, including identical initial optical densities.

We define one useful kinetic parameter as the *prodigiosin unit minimum*. This value is the lowest prodigiosin unit measurement during early log phase growth. High mutant values relative to strain Nima suggest higher than normal prodigiosin expression. A second parameter is *induction cell density*. This value is determined from the late log phase time point corresponding to the first appearance of prodigiosin units greater than the +50 unit background in nutrient broth with maltose (Fig. 2). The OD_{620} value at that time point is multiplied by the conversion factor $1 OD_{620} = 5.80 \times 10^8$ cells per ml to give the induction

cell density. Third, the *initial induction rate* is defined as the slope of the regressed line of a plot of prodigiosin units vs. culture time in hours. Time points are chosen beginning with the induction cell density point and ending three hours later. These parameters are highlighted in Fig. 9.

A more complete analysis of strain Red's expression kinetics relative to wild-type strain Nima is presented in Table 1. Strain Nima expressed prodigiosin at a greater rate once induction began. On the other hand, strain Red expressed more prodigiosin during early log phase than the parent strain. A

different indicator of prodigiosin levels, single assay of stationary phase prodigiosin from culture pre-growths, showed higher levels in the mutant. Taken together, these results show that strain Red over expresses prodigiosin. Strain Red has practical value as an isolate whose prodigiosin unit values do not dip below zero if the culture's initial optical density is at least 0.2. This avoids the shortcomings of Equation 1 that arise due to growth through early log phase. We will send *S. marcescens* Red to interested individuals upon request for use in classroom laboratory exercises.

	Nima	Red
Prodigiosin unit minimum, units	-97.5	-54.7
Induction cell density, cells/ml	1.76×10^9	1.55×10^9
Initial induction rate, units/hr	75.2	44.0
Stationary phase prodigiosin, units	436	577

Table 1: Kinetic analysis of a pigmentation mutant. Bacteria were pre-grown and grown at 30 C in nutrient broth plus maltose at an initial OD_{620} of 0.05. The medium was made as a single batch for this experiment, and the cultures were grown simultaneously in the same shaking water bath.

CONCLUSIONS

The regulation of gene expression is central to cellular homeostasis. Simple assays of gene expression are therefore valuable teaching tools for the influence of environmental cues on overall cellular metabolism and phenotypic properties. Bacteria make ideal model organisms for such assays, since they modulate gene expression relatively quickly in response to environmental changes. We describe here a simple gene expression assay that utilizes the light-absorbing properties of prodigiosin, the bright-red, cell-associated pigment of the soil bacterium *Serratia marcescens*.

Our assay accurately describes the known pattern of cell density-dependent fluctuation in prodigiosin expression. Further, our work has identified both temperature and nutritional variables that influence prodigiosin expression. Our unit definition allows growth and prodigiosin to be assayed simultaneously with a simple spectrophotometer. Because pigmentation is easily detectable with the

naked eye, *S. marcescens* growth curves can function as qualitative teaching tools for gene expression studies in labs devoid of instrumentation. Earlier work (Haddix et al. 2000) has shown that *S. marcescens* is an excellent organism for classroom studies of antibiotic resistance in bacteria. This present work extends the utility of *S. marcescens* as a classroom model organism by developing a simple assay of gene expression. This assay may also be adapted for phenotypic analysis of easily detectable spontaneous pigmentation mutants.

ACKNOWLEDGMENTS: Laboratory instrumentation was purchased with generous financial assistance from the National Science Foundation (grant number 9650766) with matching funds from Harris-Stowe State College. We are grateful to the National Center for Biotechnology Information and Washington University (St. Louis) for bibliographic and library resources. We also thank Allen C. Haddix and Michael Young for critical reading of this manuscript.

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Call for Nominations

Honorary Life Award

The **ACUBE Honorary Life Award** is presented to ACUBE members who have made significant contributions and/or service to ACUBE and the advancement of the society's mission. The award is presented at the annual fall meeting of the society.

If you wish to nominate a member of ACUBE for this award, send a Letter of Nomination citing the accomplishments/contributions of the nominee and a *Curriculum Vita* of the nominee to the chair of the Honorary Life Award committee:

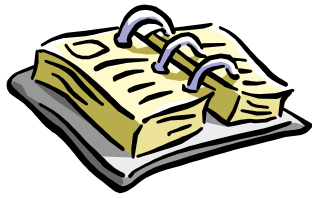
Dr. William J. Brett, Department of Life Sciences, Indiana State University
Terre Haute, IN 47809, Voice -- (812) 237-2392, FAX (812) 237-4480
E-mail -- lsbrett@scifac.indstate.edu

Call for Nominations

President-Elect, Secretary & Steering Committee Members

ACUBE members are requested to nominate individuals for the office of President-Elect, Secretary and two at large positions on the ACUBE Steering Committee.

If you wish to nominate a member of ACUBE for a position, send a Letter of Nomination to the chair of the Nominations Committee: Dr. Lynn Gillie, Dept. of Biology, Elmira College, One Park Place, Elmira NY 14901, Voice -- (607) 735-1859, E-mail -- lgillie@elmira.edu



Travel and Housing Preview

45th Annual ACUBE Fall Meeting

University of Nebraska at Kearney

October 11-13, 2001

Travel by Air

- Kearney has four arrivals and departures daily via United Express. All flights connect with Denver International Airport, the hub for United Airlines.
- Lincoln is just under 2 hours to the east of Kearney and is served by TWA, Northwest, United, and US Air Airlines. Omaha is just under 3 hours to the east with non-stop service several times a day from St. Louis (TWA), Chicago (United – O’Hare and Southwest – Midway), Minneapolis (Northwest), Milwaukee (Midwest Express), and Indianapolis (United).
- Eppley Express, a shuttle service, has three daily departures and arrivals from both the Omaha and Lincoln airports to Kearney. The fare is \$34 one way from Lincoln and \$42 from Omaha. The drop point in Kearney is the Country Inn Suites.

Travel by Car

- Kearney is 5 hours by car from Kansas City, 5 hours from Denver, 7 hours from Minneapolis, and 10 hours from Chicago.

Lodging

Kearney has 20 motels; several of which are on the Second Avenue corridor just off Interstate 80 and about one mile south and one mile east of the University of Nebraska at Kearney campus. Among those motels with tentative arrangements expressly for ACUBE meeting participants are:

Holiday Inn

110 2nd Ave.
 P. O. Box 1925
 Kearney, NE 68847
 308-237-5971 or 800-248-4460

Hampton Inn

118 3rd Ave.
 Kearney, NE 68847
 308-234-3400 or 800-426-7866

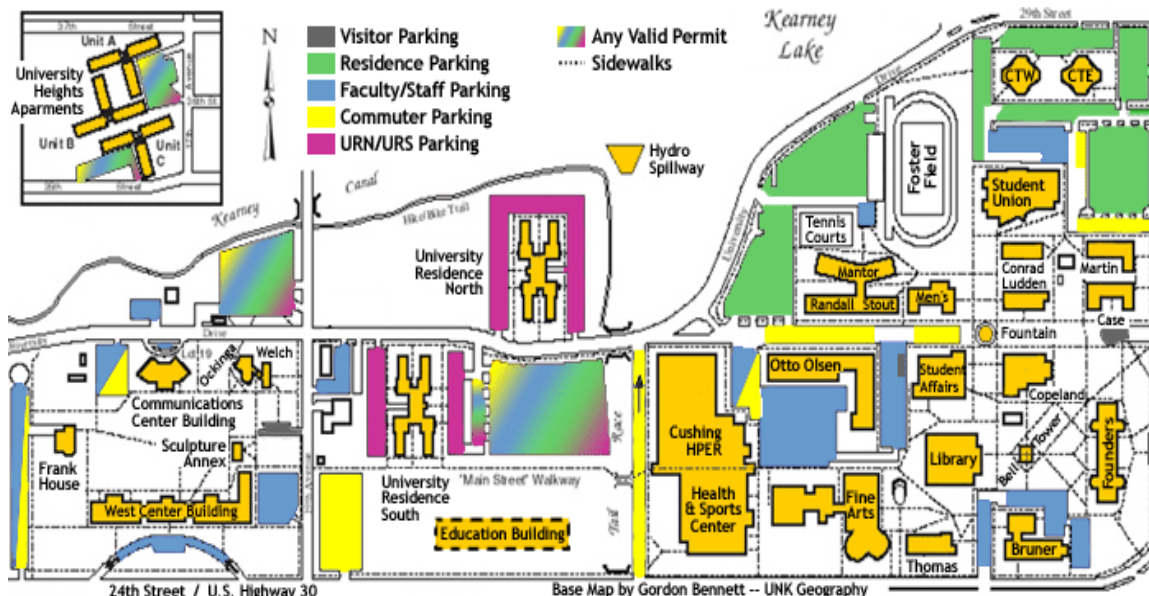
Wingate Inn

108 3rd Ave.
 Kearney, NE 68847
 308-237-4400 or 800-800-8000

Country Inn & Suites *

105 Talmadge St.
 Kearney, NE 68847
 308-236-7500 or 800-456-4000

* Location of the Eppley Express shuttle drop.



Using Dinosaur Models to Teach Deductive Reasoning Skills in Vertebrate Biology Lab

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Abstract: Continued explosion of scientific knowledge and increased emphasis on application calls for a change in teaching methods in the science classroom. The ability to apply knowledge in a problem-solving manner to real-life situations is more important to a biology student than possessing an exhaustive accumulation of facts. Described is a teaching approach that employs three-dimensional whole animal models to teach the student how to use deductive reasoning and critical analysis to discover functional correlates of structure. Using dinosaur models, which the student finds fascinating, encourages active participation. These exercises hone communication and leadership skills, and teach students to work in a collaborative manner.

Keywords: Deductive reasoning, problem-solving, vertebrate biology, dinosaurs

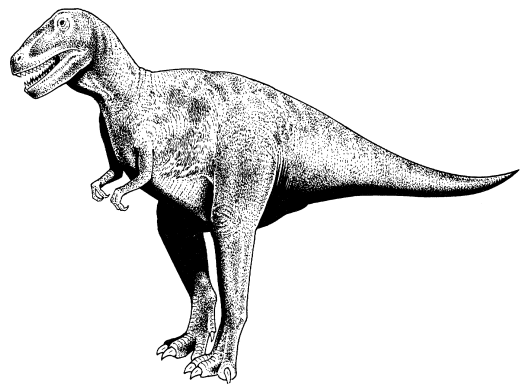
Current Concerns in Science Education

Despite the availability of information resulting from recent scientific research advances, basic college student scientific literacy in the United States is still low (NSF, 1996. P. iii). The problem does not simply lie in a deficiency of scientific knowledge, but, more importantly, in a lack of skills to use the knowledge in a problem-solving manner. To be able to apply knowledge to real-life situations, students must possess good deductive reasoning and critical thinking skills, good verbal and written communication skills, as well as the ability to work in a collaborative manner. Like scientific facts, these skills are learned. Educators are recognizing the importance of limiting the amount of factual information taught, and instead are introducing into the science classroom learning experiences that hone communication skills through collaborative learning. The focus is on inquiry and problem solving rather than on memorization (Weiss, 1992-1993). Described here is a teaching approach that hones all of these skills by incorporating deductive reasoning exercises into the labs of an undergraduate Comparative Vertebrate Biology course.

Using Problem Based-Learning in Vertebrate Biology Laboratories to Address Current Concerns in Science Education

Major challenge in teaching a comparative vertebrate biology course is that the breath of issues addressed ranges from phylogenetic history,

development and growth phenomena, to anatomic, physiologic and behavioral characteristics of organisms studied. Understanding the connection between such diverse topics is complicated by the fact that majority of junior students still use memorization rather than thinking as a primary study tool. The result is knowledge of a series of seemingly unrelated facts that will soon be forgotten. This problem can be circumvented by systematically incorporating into vertebrate biology course problem-based questions which require the student to critically analyze factual information presented in class, apply newly acquired knowledge to concrete situations, and seek the connection between the phenomena studied. Such exercises also teach the student how to work with the peers in a collaborative manner, and help hone verbal communication skills.



Because comparative vertebrate biology course has a significant laboratory component, it lends itself ideally to learning through problem-based, hands-on exploration. I have thus added deductive reasoning exercises to each laboratory session in which the students traditionally study skeletal material or dissect preserved specimen. These exercises consist of a set of problem-based questions that are addressed by analyzing animal models of extinct reptiles, representing whole body reconstructions of their external appearance. To find solutions to problems, the student must first understand the concepts discussed in the classroom and next be able to apply them to laboratory specimen. Since different individuals can use the same factual information in different manner, the problems often lend themselves to multiple interpretations. Thus, for example, a group of students may view a massive tail primarily as a propping device to maintain a bipedal posture during feeding from trees; other students may regard the same type of tail as a balance device for locomotion. The aim of exercises is to propose solutions that can be supported by scientific facts, rather than to seek the absolute truths.

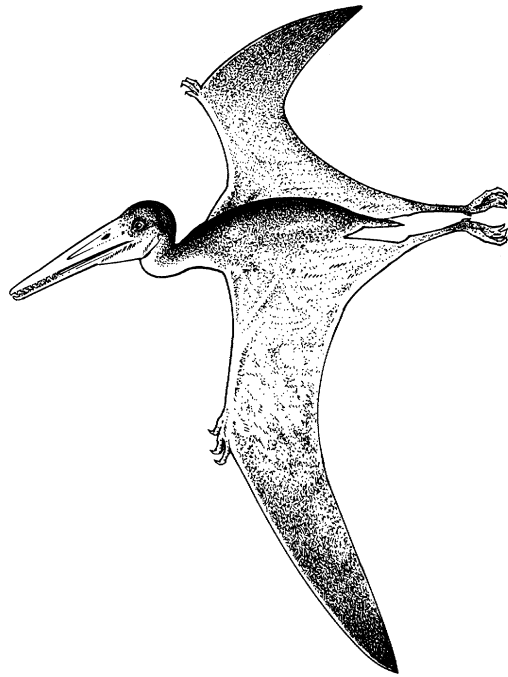
Questions are designed so as to be also pertinent to lab assignment for a particular day and related material discussed in the classroom. They frequently refer the students to pictures of the skeleton of the extinct reptile under consideration and the articulated skeletons of various vertebrates, conveniently displayed on workbenches. To arrive at valid justifications, the students must also refer to specimen studied or dissected in the particular lab. In analyzing the questions, students often consult their lecture notes and the textbook. To expedite the process, they are sometimes referred to specific textbook pages where they can find useful information.

Using Whole Body Dinosaur Reconstructions as a Model for Problem-Based Exercises

Given the importance of the spatial relationships of the anatomical structures, and the ease with which students relate to hands-on exploration of three-dimensional objects, it is beneficial to select for problem-based exercises a three-dimensional model that can be physically explored by the student. Most importantly, the model should represent a subject matter which the student finds fascinating and stimulates a curiosity for further exploration.

I discovered by accident that a study of whole body reconstructions of dinosaurs fulfills the above requirements, while permitting exploration of a wide range of biological issues. A poster of dinosaurs and flying reptiles, displayed in the lab at the beginning of the semester, generated a surprising amount of interest among the students. They repeatedly stopped by the poster to ponder over the questions accompanying the pictures, e.g., "Which head features of this extinct flying reptile suggest that this was not a bird?" I thus

promptly incorporated whole animal dinosaur models from my personal collection, charts of their skeleton, photographs of fossilized material from professional and popular journals, and a set of relevant questions into the ongoing skeletal labs. The level of spontaneous student interaction increased. They began to explore with interest the correlates between the external anatomy of dinosaur models and their skeletal features, and to draw similar structure-function correlates for modern vertebrates. Dinosaur models thus became a standard feature in vertebrate labs, from those focusing on skeletal anatomy and muscles, to those involving dissection of various organ systems.



Structure of Problem-Based Labs

A typical vertebrate biology lab is structured as described below. All labs except those dealing with embryologic development include questions based on Dinosaur models.

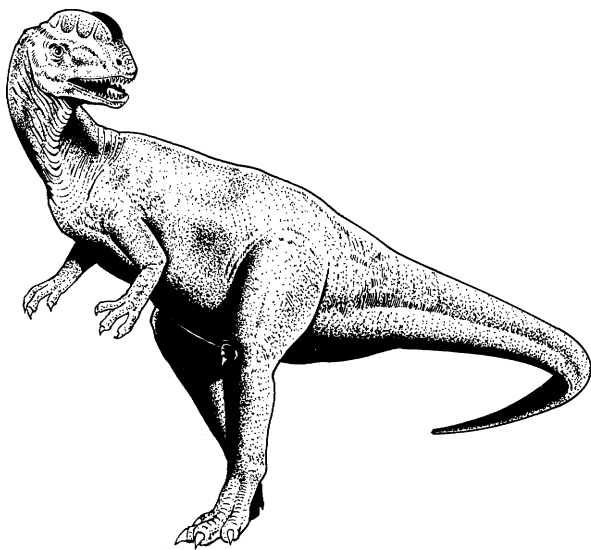
1. Work on lab assignment. During the first half of each three-hour lab period, students work in pairs on completion of their laboratory assignment. This may involve analysis of the overall vertebrate body plan, comparative study of skeletal elements discussed in class, or dissection of a set of muscles in shark, mud puppy and mink.

2. Study Dinosaur models and address problem-solving questions. About an hour and a half into the lab period, the students are instructed to form four-member teams, take a Dinosaur model from the demonstration table to a work area, and jointly seek solutions to the problem-based questions found with the model. As the teams analyze different animal models on display, each team member is expected to take a turn in acting as a group leader in coordinating

the discussion and taking notes on the team's solutions to problems. Examining four to five animal models in each lab allows ample time for discussion in a class composed of 20 students.

3. Present to the class team responses to questions. During the last 30-40 minutes of the lab period, as team members come to a consensus on the responses to questions, each team is responsible for explaining to the class the conclusions and rationale for their analysis of one animal model of their choice. The only stipulation is that we discuss all models on display. Since the questions lend themselves to multiple interpretations, to be valid, the conclusions must be justified on the basis of scientific reasoning and concrete factual information.

All team members are expected to participate in the presentation of the group's findings, based on division of labor planned by the team. As class discussion unfolds, the instructor may write down on the board the key words and phrases used by the students to help them recognize similarities and/or differences in the interpretation by different lab teams. The primary role of the instructor is to keep the discussion focused, help refine ideas by posing additional questions, and help summarize the outcome of class discussion.



Examples of Deductive Reasoning Exercises

A sample of problem-based questions follows. The questions are designed to reinforce the focus of each laboratory assignment and complement theoretical issues discussed in class.

1. Can the external anatomy of the head tell us, which sensory organs were of importance for the survival of the following extinct reptiles: *Pterosaurus*, *T. rex* and *Stegosaurus*?

a. Which characteristics of the head suggest that *Pterosaurus* had a better visual acuity than *Stegosaurus*? Based on the external anatomy of

the head, what would you conclude about *T. rex* in that regard? What features of the head generally suggest a relatively good sense of smell? How do these three extinct reptiles compare in that regard?

[The expectation is that the student will draw conclusions by comparing the overall size of the head to the relative size of different head regions housing special sensory organs (e.g., the size of the eyes or the snout). The role of the sensory organs for animal survival in different habitats (arboreal vs. terrestrial, etc.) has been discussed in class.]

b. Based on your conclusions regarding dependence of the three extinct reptiles on their visual sense, propose how differences in visual acuity affect an animal's way of life. Justify your answer.

*[Students may decide to consider how an animal's visual sense correlates with its ability to identify food source and other targets of interest. They may propose that a reasonably good visual acuity in a *T. rex*, thus capability to recognize prey at a distance, would serve a useful purpose only if a strong musculo-skeletal system co-evolved to support running to catch the prey.]*

c. Which features of the head skeleton of the three extinct reptiles support your conclusions regarding their dependence on the visual sense for survival? How do these compare to distinguishing characteristics of the head skeleton of a pigeon? (Reader: consult **Fig. 1**.)

*[Students will likely recognize a relatively large orbit size in *Pterosaurus* and the pigeon, but not in *Stegosaurus* and, to their surprise, *T. rex*. As they debate a relatively small orbit size but well developed nasal bones in *T. rex*, they begin to question whether this reptile may have also used the sense of smell for hunting. They may further decide to compare the anatomy of the occipital region and notice that, unlike the pigeon, these two extinct reptiles are missing the occipital prominence. Based on class discussion of the functional significance of major external features of the skull in relation to development of the occipital or olfactory regions of the brain, the students may propose the evolutionary changes in development of the brain of modern birds as compared to extinct reptiles.]*

Timetable: Questions **a.** and **b.** can be addressed early in the course as vertebrate body plan and the principal characteristics of various vertebrate classes are discussed. The same animal models should be revisited later in the course as vertebrate head skeleton is studied, to address question **c.**

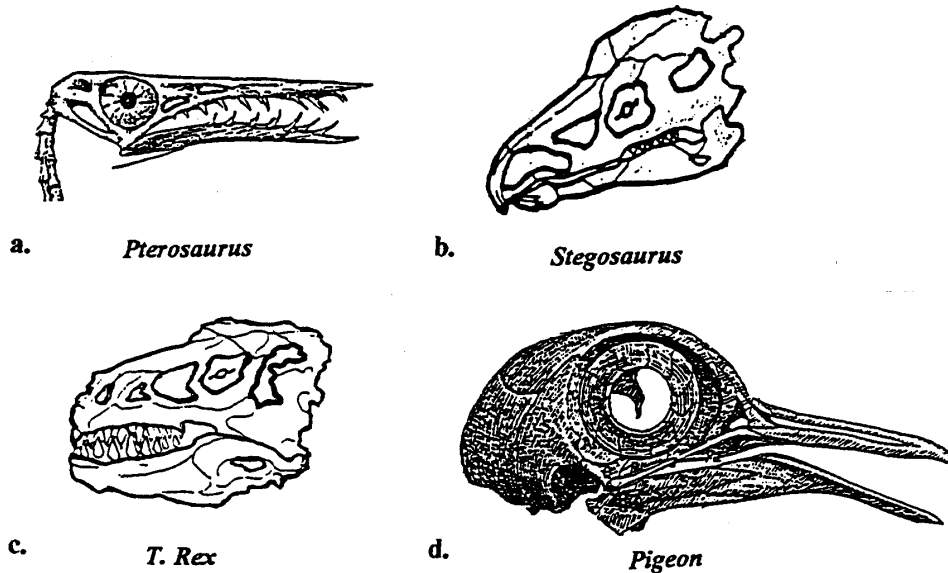


Figure 1 Comparison of the head skeleton of three extinct reptiles and the pigeon. **a. Pterosaur:** Large orbit size relative to the skull is the anatomical correlate of large eyes and a keen sense of vision. Absence of the occipital prominence suggests poorly developed occipital cortex and simple processing of visual information. **b. Stegosaurus:** Small orbit (o) in comparison to overall size of the skull is indicative of a relatively poor vision. **c. T. rex:** Small orbit (o) of this massive skull suggests that this animal may not have had a very acute visual sense. Prominent nasal bones signal a relatively large area housing the olfactory epithelium. **d. Pigeon:** Unusually large orbits correlate with keen visual sense, while slender nasal bones and reduced nasal compartment correlate well with poor olfactory sense.

2. Did flying reptiles evolve the anatomical prerequisites for true flight? Compare Pterosaur to modern birds and flying mammals.

a. What anatomical features of a take off mechanism can you find in the Pterosaur animal model? What suggests that this reptile could not have used its hind limbs for take off? Compare its skeleton to that of the pigeon and propose how differences in skeletal anatomy correlate with differences in take off mechanisms.

[The student is reminded to compare details of the animal model's external anatomy (wing claws in this case) to the skeleton in the same limb region. Early in the course, before limb skeleton is discussed, the student is already able to recognize that finger bones appear rudimentary in the pigeon. The student also recognizes that the relative size of the hind limbs, and presumably limb bones, in two animals differs.]

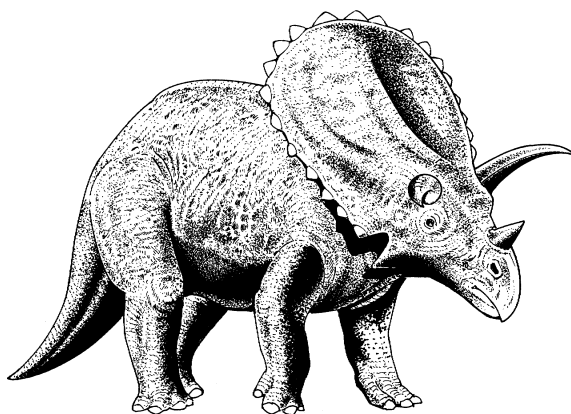
b. Which features evident on the Pterosaur model suggest that this animal was better adapted to soaring than to true flight? Justify your answer.

[The student may note the presence of a beak with teeth. In line with weight reduction principles discussed in class, the justification would imply recognizing that the jaws with teeth are heavier than a toothless beak. Wing specimens are provided to prompt the student to use the weight argument when comparing a skin membrane to a true wing. Students never fail to wonder about the light weight of the large wing specimen.]

c. Study the Pterosaur model and the picture of its skeleton. Also study the preserved bat specimen and the skeleton of the pigeon. How does the attachment of the wing membrane to the limb skeleton compare to the attachment of the flight feathers? Which of the two shows more extensive attachment to the skeletal elements and why? Can one justify the observed differences on the basis of differences in physical characteristics of skin membranes vs. true wings?

[The expectation is that the students will recall from class, that skin is heavy and prone to tearing. A student may use this argument to propose that a skin membrane requires more extensive anchoring than a true wing.]

Timetable: Questions **a.** and **b.** can be addressed early in the course but should be also revisited later on, once the skeleton or organ systems (e.g., the integument) have been discussed. Knowledge of skeleton will permit more detailed analysis of anatomy of the limbs, thus allowing the student to draw more precise structural correlates with function. Question **c.** should be addressed after the limb skeleton has been discussed to allow the student to make more accurate observations. At that time the student will be prepared to bring into the discussion more subtle points, e.g., the possibility of the presence of pneumatic bones in *Pterosaurus*.



3. Can one deduce from the external body anatomy how quickly dinosaurs could run? Compare *Triceratops* and *Stegosaurus* to alligator.

a. Which body features evident on animal models suggest that *Triceratops* was a quicker runner than *Stegosaurus*, despite the fact that both animals were very heavy? Consider the same body features in the alligator, and propose how its speed of locomotion compares to the presumed running speed in the two extinct reptiles. Justify your answer. Propose what makes the rhinoceros a good runner despite its heavy weight and relatively short legs?

[The expectation is that close examination of the relative length of limbs would help the student recognize that Triceratops stands higher off the ground and could have had a longer stride than the Stegosaurus. In making a comparison to the alligator, the student may recall class discussion on the relationship of limb rotation to speed of locomotion, and may thus propose that differences in limb positioning relative to major body axis correlate with differences in speed of locomotion.]

b. Propose a mechanism for reduction of the overall weight of the skeleton in *Triceratops* and *Stegosaurus*, given their large body mass. Are similar mechanisms used in living Ungulates?

[The student may recall from prior discussion that long bones of the limbs in terrestrial

animals contribute significantly to the overall weight of the animal. The expectation is that the student will also remember that long bones retain their strength despite the overall reduction in bone mass due to their hollow shaft filled with bone marrow.]

Timetable: These questions are best addressed once limb skeleton has been studied. They can be also revisited at the time of limb muscle dissection, and finally as the circulatory system is studied. At that time the student will be able to make the connection between the evolution of the four-chamber heart, increased efficiency of muscle oxygenation, and more aggressive use of the musculo-skeletal system.

4. What can head skeleton specializations of extinct reptiles tell us about their behavior and social life?

a. Compare position, shape and size of *Triceratops* horns to similar structures in living Ungulates. Propose multiple functions for *Triceratops* horns based on what you know about their function in Ungulates. What function is suggested by the following observation: head fossils of male *Triceratops* show puncture holes that match the size of two large *Triceratops* horns.

[Based on class discussion of integumentary and bony specializations of the head in Ungulates, the student may use parallel reasoning to propose various functions for Triceratops horns. Additional questions by the instructor may help the student relate skull puncture holes to fights for mate selection.]

b. Could *Triceratops* have used its massive frill as a protection device? Consider its plane of orientation and its position relative to body regions that need extra protection. Since a major function of large bony surfaces is to provide the site for muscle attachment, could this argument be used for interpreting the functions of the frill? Consult the picture of *Triceratops* skeleton and justify your reasoning.

[The student may view the frill as a protection device, but would encounter difficulty in proposing justifiable behavioral tactics that would permit the use of the frill as a shield for vulnerable parts of the body, the head and the chest. Regarding the second question, the student will likely attempt “to hook” some of the nearby muscles, e.g., jaw and perhaps neck muscles, to the frill. The justification would imply looking for the evidence of skeletal specializations on the frill (e.g., bony ridges for muscle attachment) in support of this argument. To help the students arrive at stronger scientific arguments on the basis of parallel thinking, the teacher may pose additional questions, e.g., “What could be the functional significance of

the pronounced occipital ridge in the primate skull in our collection?” The students may propose that the strong neck muscles attach to bony ridge.]

c. What could have been the function of the prominent bony ridges along the dorsal edge of the orbits of *T. rex* and other similar carnivorous dinosaurs in our collection? Justify your answer.

[In proposing a role of bony ridges for muscle attachment, the student may encounter difficulty in defining the muscles that would attach to such a peculiar site. This question represents a particularly good exercise in critical thinking since, proposing that bony ridges act to reinforce the skull would require recognizing that reinforcement at the supraorbital site can be justified only if the animal used its head as a weapon.]

Timetable: Questions a., b., and c. can be addressed early in the course when vertebrate orders are discussed from a holistic prospective. At that time the student already understands the principal anatomical correlates of function (e.g., using horns as a weapon, or to create illusion of a larger body size) and recognizes their relationship to animal behavior and social life (e.g., using horns to ward off enemies, or attract mate). Questions a. and b. should be revisited later when the student dissects the muscles and become familiar with the muscle attachments to skeleton. At that time the student will be able to understand the relationship between the jaw muscles and head skeleton and provide more scientifically sound arguments in support of the proposed function of head specializations. The same questions can be addressed as the importance of skin derivatives for survival is being discussed.

Some other issues that can be particularly well addressed on dinosaur models

What are weight reduction mechanisms in flying reptiles and terrestrial dinosaurs of large size?; Were dinosaurs built for strength, speed or both?; What are the advantages and disadvantages of large body size?; What is the functional significance of various body decorations?; What is the reason that Sauropods with long necks also evolve long tails?; How could a small-headed *Stegosaurus* have consumed enough food to support its weight?; What does the size of the body cavities tell us about the internal organs?; What were some of the multiple functions of tails in dinosaurs?

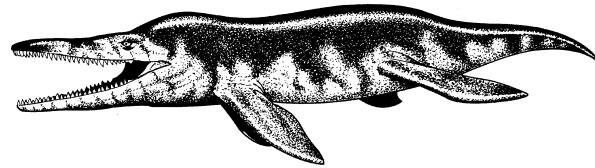
Laboratory Resources

To allow students to make accurate observations, it is essential that the models come from a reliable source and have anatomically correct proportions. Our best models, expensive but made of high quality durable plastics, are scaled-down replicas of the

Smithsonian dinosaur model collection, purchased in gift shops specializing in science museum items. Other models, also accurately built but lighter and more prone to damage, come from a cheaper source: Safari Limited, P.O.Box 630685, Miami, FL 33163.

Changes in Course Structure

To create ample time for analysis of problem-based questions, laboratory component of the course has undergone substantial modifications. Some of the traditional lab exercises had to be eliminated. We no longer dissect abdominal and hind limb muscles. Musculature of both appendages is discussed in class, but dissection done on front limb only. Some of the laboratory material studied in the past had to be reduced. In the study of palatine complex of the skull we now identify only a few major bones and emphasize the overall position of the palatine shelves in relation to their function, instead of identifying all skeletal components of the two palates. Tedious dissection of fine muscles in *Necturus* has been reduced to larger, more readily accessible muscles. When dissecting major blood vessels of the limbs in the mink (e.g., subclavian or iliac arteries) we dissect their origin and identify their general targets but no longer trace their branches. Given the importance of deductive reasoning in acquisition of knowledge, the above course modifications in course content proved to be a worthwhile sacrifice.

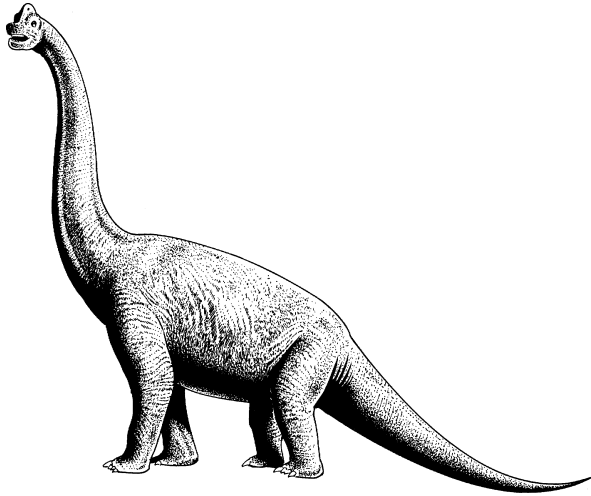


Assessment of Learning

Student responses regarding the effect of problem-based exercises on their learning have been consistently positive. They include statements: “I think dinosaur exercises made the mechanics of locomotion easier to understand.”; “Dinosaur questions really helped me understand why do you always emphasize all that conceptual stuff in lectures; “Being challenged to think made the lab interesting.”; “I like the idea of having to take turns as a group leader. That helped me personally, since one person in my group always tried to take control.”; “Having to go back and forth between the skeleton and the model really helped me see the connection.”; “I always found anatomy boring. When we worked from models we had to think about the live animal. That made it [anatomy] really fun.”; “I wish we had more thinking exercises like these in other courses.”; “I think my lab grade improved because dino questions helped me understand how musculo-skeletal system works”.

I have taught this course to a class of 35 to 40 students for eight years. Problem-based exercises were

added to the course for the past three years. The average percentage of students/year earning grade B during these three years was 42%, as compared to the 29% average for the five years before inclusion of exercises into the course. The average percentage of students earning an A grade did not appreciably change in the course of eight years. However, the average percentage of students earning grades C, D, or failing the course was 45% for the three years after the introduction of the exercises into the course, as compared to 60% average for the five years before.



Discussion

The challenge in teaching a comparative vertebrate biology course in an age in which the students most readily identify with terms “molecular biology” or “genetic engineering”, is that many students take the course only because it is required. Introducing dinosaur models into the lab early on stimulates students’ curiosity about the course. Positive student responses to introduction of dinosaur reconstructions into the lab suggest that this is an efficient model for teaching a variety of useful skills. The unmatched diversity in dinosaur adaptations and the large variety of the available models make dinosaurs particularly suitable for addressing a diverse array of biological questions, ranging from structure-function correlates to behavioral and social organization issues.

Thinking is the instrumental part of learning and the prerequisite for understanding abstract concepts. However, the majority of biology undergraduates relate more easily to concrete than to abstract. Questions based on models that can be physically explored by the student introduce thinking exercises into vertebrate labs in an unobtrusive manner. Since these exercises do not require coming up with the “correct” answers, the students do not find them intimidating and learn to enjoy them. They are quick to learn that “dinosaur questions” reinforce the concepts discussed in class and observations made in the lab, and thus increase the

chances of improved performance on both written and practical exams. Improved student performance on exams after the introduction of interactive exercises based on dinosaur models, suggests that this approach to learning is particularly beneficial to the student who has difficulty with the course content and is more likely to earn a lower grade. Student’s responses to introduction of problem-based exercises suggest that they are helping them discover the importance of mastering thinking skills. This confirms the observation of Weiss (1992-1993) that one of the best ways to convince students that thinking skills are indeed necessary is to show them that thinking and learning are connected.

Incorporating deductive reasoning exercises based on whole body models has multiple benefits for the student. Questions that accompany the models call for justification on the basis of scientific arguments, and thus encourage reasoning and interpretation. Such exercises enhance students’ ability to handle abstract ideas. As in the problem-based learning model described by Barrows and Tamblyn (1980), a beneficial lesson for the student in exercises involving thinking is that scientific judgment cannot be based on personal opinions but must be justified on the basis of scientific observations. Revisiting the same dinosaur models later in the course, as students’ knowledge increases, helps the students recognize the weakness of prior arguments (e.g., “*Pterosaur* could probably see well because it had large eyes”). Students also discover that new knowledge empowers them to make more accurate scientific interpretations (e.g., “*Pterosaur* must have had keen vision because its large orbits, relative to the size of skull, could accommodate a large retinal surface needed for increased visual acuity”). Sundberg and Moncada (1994) reported that undergraduate students gain similar insights when engaging in problem-solving, investigative laboratory exercises.

Using dinosaur models to address problem-solving questions helps maintain a holistic view of vertebrates as organisms adapted to specific habitats. This holistic perspective is easily lost as the students dissect organ systems and address more technical issues. By revisiting whole animal models, they begin to view each system not as an end in itself, but as a part of an individual experiencing development and growth, behavioral responses to stimuli, and interaction with other living things in its habitat. If this link is to be recognized by the student, the instructor may have to stimulate class discussion by providing additional information that excites students’ curiosity for further analysis. For instance, one can re-examine Saurischian models as one dissects major blood vessels. If one mentions, as the students dissects large neck blood vessels, that the giraffe pumps sufficient blood to its brain with the help of multiple hearts in its neck, they begin to wonder whether the same may have been true

for the long neck Saurischians. This may in turn lead to a discussion of whether these animals grazed only from tall trees, given that giraffe sometimes grazes on the ground. Further brainstorming may lead to questions about what food was available in the habitat of these long-necked beasts and whether the competition for the same food resources may have played a role in the evolution of the long neck.

The key to learning is finding the connection between the known and unknown. If the student is to maximally benefit from exercises involving discovery of new relationships through deductive reasoning, it is important to formulate the questions so that the student can draw from prior experience and knowledge, as in the case study approach in teaching biology reviewed by Waterman (1998). This may require considerable insight on the part of the teacher, since the questions must direct the student to approaches to problem they may not readily consider. For instance, as the students ponder over differences in the use of hind limbs by various dinosaurs, it is helpful to suggest to the students that they compare the relative length of thigh and shank bones on pictures of a dinosaur skeleton to that of different vertebrates from our skeleton collection. Linking the known to unknown expedites the learning process by providing the students with an opportunity to arrive at the conclusions on the basis of what they already know.

In described approach to learning, students hone their leadership and communication skills, by taking turns as group discussion leaders and by participating in presenting team interpretations to the class. Welty (1989) observed that effective use of discussion as a learning tool requires allowing the students a fair amount of authority. Likewise, I found that students show greater willingness to engage in conversation when a good deal of the control of discussion is in their hands. As different lab teams begin to exchange ideas, discussion often moves away from its initial focus. It is important to recognize that students benefit from such side explorations. If one attempts prematurely to bring the discussion back into focus, spontaneous exchange among the students often stops.

An important benefit of addressing problem-based questions as a team is learning how to work in a collaborative manner with other group members. Since I have instituted dinosaur-based exercises, I have noticed an increase in spontaneous peer interaction and coaching. To guard against a student taking over the discussion and control of the group, it is important to rotate members responsible for coordinating group discussion.

For maximum benefit to the student, lab classes should be kept small, 20 students or less. Higher mean SAT scores for high school students that attend smaller classes suggest that the quality of learning improves as class size decreases (Ghosh, 1999). This most likely reflects the fact that smaller class size allows for more discussion and contact between the individuals.

Dinosaur lab exercises have a positive impact on teacher, as well. They help develop a bond between the student and the teacher by encouraging the exchange of ideas. After listening to student interpretations, the teacher often begins to see things from a different perspective. It is wise to share these insights with the students. Discovering that the teacher can also learn from their views fosters student's self-esteem, desire to learn, and willingness to share ideas. This in turn helps the teacher achieve the ultimate goal of education, e.g., teaching the students how to think.

To help college students develop critical thinking skills, we must increase our efforts to incorporate into college science courses a learning format, which teaches the student how to use scientific knowledge to critically analyze issues and arrive at justifiable solutions to problems. Described approach to teaching helps the student learn how to use deductive reasoning and parallel thinking to interpret the biological phenomena. Students also learn how to justify their interpretations by applying the acquired knowledge of scientific facts. Implicit in the format of described exercises is learning how to play an active role in decision making and how to work with peers in a collaborative manner. All of these skills will prove invaluable in the world the student faces upon graduation.

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Sequencing cDNAs: An Introduction to DNA Sequence Analysis in the Undergraduate Molecular Genetics Course

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Abstract: DNA sequence analysis is a cornerstone of modern molecular genetics, yet it is difficult to provide students in the teaching lab with an experience that is meaningful and cost effective. At Millikin University in the upper division Molecular Genetics course, a series of lab exercises that culminates with a cDNA sequence that students can use for homology searches and open reading frame analysis has been implemented. The exercises begin with students picking a single colony from a *Drosophila melanogaster* embryo cDNA library. They purify the plasmid then analyze the insert through restriction digests and gel electrophoresis. In this semester's example, three cDNAs were then randomly picked from the group and sent out for sequencing. The students had an ownership and interest in the sequence data because they represent an unknown gene and were selected by them.

Key Words: *Drosophila melanogaster*; cDNA; DNA sequencing; molecular biology; undergraduate teaching

Introduction

Molecular biology has become a fundamental tool in virtually all aspects of biology. From developmental genetics to taxonomy and environmental biology, the analysis of DNA sequence is a part of the array of techniques necessary for carrying out experiments that answer important questions. These days, a college course in molecular genetics has to bridge the gap between all of the historical and biological information and the huge amount of technical knowledge needed to work in the lab. Many of these techniques are relatively easy to carry out in the teaching lab and follow fairly simple "recipe" formats. Things like DNA isolations, gel electrophoresis and restriction digests have become exercises that are carried out in some high schools. A difficulty in the teaching lab setting occurs when more complicated ideas and techniques are incorporated. In this age of genome sequencing one of the most important concepts is becoming DNA sequence analysis. This requires the fusion of a wide range of techniques including all of the basic techniques mentioned above as well as computer analyses and homology searches. The importance of this area of

molecular biology is apparent in the current literature and in the job market. As a way of exploring this critical concept, I developed a sequence of lab activities that allows students to isolate cDNA clones, characterize the inserts and have them sequenced by a remote facility. The resulting DNA sequence was recovered through the Internet and was analyzed for open reading frames and used to carry out homology searches. The resulting data provided a wide range of discussion opportunities. The students felt an ownership in the process and the kinds of difficulties, surprises and excitement that comes from doing research. The cost of DNA sequencing purified plasmids is well within the range of a lab budget.

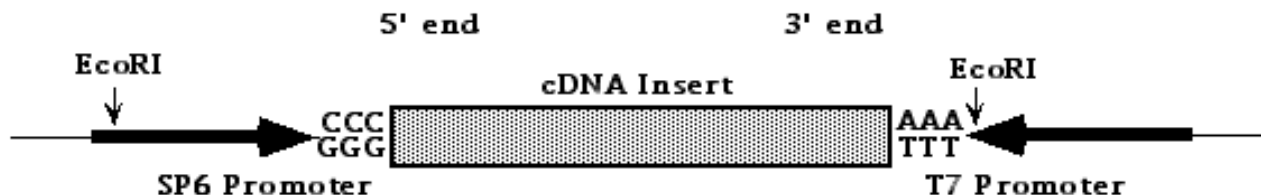
Materials and Methods

cDNA Library

For this laboratory exercise, I chose a *Drosophila* cDNA library made with mRNA from 6-18 hr developing embryos (Brown and Kafatos, 1988). This library offers three advantages. First, the library is constructed in a plasmid that makes the DNA much simpler to isolate compared to a bacteriophage. Second, the plasmid is constructed in such a way that

the cDNA is directionally cloned and oriented in a specific way (Fig. 1). Third, the completion of the *Drosophila* genome sequence made the sequence analysis more detailed. Aliquots of an amplified stock

of the library are available from the author; however, this stock should not be used for research purposes, as it has been amplified from an original stock and does not contain a representative set of cDNAs.



pNB40 cDNA Vector Cloning Site

Figure 1. The cDNA cloning site of the pNB40 vector is shown. The insertion site is flanked by SP6 and T7 viral promoters. These allow for the use of standard DNA sequencing primers. The cDNA library was constructed in an orientation specific manner. The 5' end of the cDNA was cloned by using G-C tailing and the 3' end was cloned by using the poly-A tail. The *EcoRI* restriction sites were used to excise the insert for size analysis. The *EcoRI* restriction site flanking the SP6 promoter is approximately 300 bases from the beginning of the G-C tail.

Plasmid Isolation

Each student in the lab picked a bacteria colony from a prepared plate and streaked it out to recover a single isolated colony. The bacteria medium was standard Luria broth supplemented with 100mg/ml ampicillin (Sambrook, et al., 1989). Bacteria containing plasmid were grown in liquid culture overnight at 37 C overnight. The students isolated plasmid DNA using the alkaline lysis or boiling miniprep method according to standard protocols (Sambrook, et al., 1989). Isolated plasmid DNA was digested with *EcoRI* restriction enzyme. This liberated the cDNA insert along with a small fragment from the plasmid (Fig. 1). The cDNA insert was sized using 1% agarose gels run in 1x TBE (Sambrook, et al., 1989). We chose to prepare three samples for DNA sequence analysis. These plasmids were purified using the ClearCut miniprep plasmid DNA isolation kit (Stratagene Inc., La Jolla, CA). This insured that the DNA would be of sufficient purity to allow for a good sequencing reaction and was recommended by the sequencing facility (Iowa State University Core Molecular Biology Facility). The DNA was quantified by gel electrophoresis and UV spectrophotometry.

DNA Sequencing and Analysis

Our cDNAs were sequenced by the Iowa State University Core Molecular Biology Facility. They use a PCR based cycle sequencing protocol that works effectively on double stranded DNA to easily provide

up to 500 bases of quality sequence. By using the SP6 DNA sequencing primer we were able to unambiguously obtain sequence from the 5' end of the cDNA clone. The sequence facility uses the industry standard PCR cycle dideoxy-sequence protocols and the Applied Biosystems™ fluorescent DNA sequencing system. They post the data to their computer and we recovered them through the Internet as a data file that is read with ABI's Edit View™ program that is supplied free by the sequencing center. The sequencing center also supplies a straight text data file.

The cDNA sequences were analyzed, by the students for open reading frames, and used in homology searches. We used a set of sequence analysis tools on the web called "The Molecular Tool Kit" (<http://arbl.cvms.colostate.edu/molkit/index.html>) to determine open reading frames and to translate the cDNA into a putative protein sequence. The National Center for Biotechnology's BLAST™ web site (<http://www.NCBI.nlm.nih.gov/BLAST>) was used to perform homology searches (Altschul et al., 1997).

Results and Discussion

Molecular Genetics at Millikin University is an upper division course that is taken by juniors and seniors who have already completed the Genetics course and the Molecular and Cell Biology course. The lab portion of the course covers a variety of techniques including basic nucleic acid manipulations and purifications. The lab experience described here

was carried out over a four-week period. Initially all twelve of the students in the course picked colonies from the cDNA library, isolated plasmid DNA and performed restriction digests. All of the digests were analyzed by gel electrophoresis. After this point, three clones were arbitrarily picked from the class for sequencing. The inserts ranged in size from 800 bases to 1.7 kilobases. The plasmid DNA from the three clones was purified using a spin column kit in order to prepare a DNA template that is optimal for double stranded sequencing. This step is recommended by the sequencing facility.

The reason for this experiment was to provide an unknown cDNA sequence that could be analyzed for open reading frames and used as a query sequence in a BLAST™ homology search. This demonstrated a variety of sequence analysis tools and served as an excellent starting point for discussions on molecular biology.

We recovered and sequenced three different DNA sequences. All of the templates provided quality data and gave at least 300 bases of sequence from the 5' end of the clone. Homology searches were very clear. One clone was a partial cDNA from the squid gene. A second clone was from the ribosomal protein L8 (rpL8) gene, while the third clone most likely represented an artifact of the library construction as the

homology indicated that the sequence was the pGEM plasmid.

This lab exercise developed a number of excellent discussion points. The students could readily see the effects of sequence errors on open reading frame analysis. This was particularly apparent with the squid and rpL8 gene, since the complete sequences are in the GenBank™ database and any errors were seen through the homology search. The discovery of pGEM plasmid contamination in the library brought up a number of questions and possibilities for how this artifact could have been created. Most likely, there was plasmid contamination in a reagent during the cDNA synthesis or vector preparation.

In the next few years, the analysis of DNA sequence is going to become one of the most important jobs in molecular biology. This lab experience effectively introduces the students to techniques and skills required for sequence analysis. The students were able to prepare lab reports requiring interpretation of significant amounts of data. The complex, multi-step procedures provided an excellent series of exercises that brought DNA sequence analysis and its associated problems into the classroom.

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ACUBE 45th Annual Meeting

University of Nebraska at Kearney

Kearney, NE

October 11-13, 2001



Biology in the Light of Evolution

News and Views

ACUBE Presidential Address: Evolution is Good Science

Buzz Hoagland

Indiana State University
14 October 2000

I recently had the privilege to represent ACUBE at an invited-only conference to discuss the status and future of teaching evolution. The National Conference on the Teaching of Evolution¹ was funded by the National Science Foundation and hosted by the University of California Museum of Paleontology 5-8 October 2000. This conference was unique in that it brought together, for the first time, representatives from greater than 50 professional associations and societies who have a vested interest in the teaching of evolution. This four-day event was a real eye-opener. After spending many hours in intense discussions with geologists, physical anthropologists, K-12 educators, members of the media, and other biologists, I realized how narrow my views on evolution and evolution education have been. My mantra, Dobzhansky's "Nothing in biology makes sense except in the light of evolution," needs revision.

Partly because evolution is intellectually challenging, and partly because I believed that many undergraduates are not interested in evolution, I am guilty of giving evolution short shrift in many of my courses. Have I ever asked students enrolled in my courses if they are interested in learning about evolution? No. Did I know that when Time Magazine places a cladogram of human evolution on their cover, sales increase significantly? No. Will I change my courses? Yes. I am a science educator, and evolution is good science.

There are numerous reasons why students should learn about evolution. First and foremost is the fact that evolution is good science. And, because of its inherent richness, a curriculum designed with evolution as its unifying theme is a mile wide and a mile deep. The very nature of evolution invites students to participate in the broader aspects of scientific inquiry. They learn to formulate questions, generate hypotheses, test hypotheses, ... in short, undergraduates who study evolution participate fully in the process of science.

The study of evolution can also assist undergraduates as they begin to develop a worldview. Through an understanding of the processes and patterns of evolution, students can more knowledgeably address questions of their own origin, origins of other

organisms, origins of life itself, and origins of planets, solar systems, and the universe.

Many undergraduate students express sincere concerns about overpopulation, the loss of biodiversity, the use of pesticides, and emerging infectious diseases. Each of these issues is more fully understood through an understanding of evolution. Evolution is good science.

So, how do we as biology educators facilitate improved student understanding of evolution? First, we need to know what our students know and do not know about evolution. How do we obtain this information? Ask them. It may also be helpful to know that the majority of U.S. citizens (55-75%, depending upon which survey one reads) do not accept evolution. This may be a result of our failure to teach evolution or our failure to teach evolution in ways that students can truly understand its enormous significance. Second, we must ensure that evolution is the unifying theme in biology courses, and that students learn and understand its central position in science. Third, we must be proactive in our effort to improve understanding of evolution science. For example, WGBH (the PBS station that produces NOVA) is developing an eight-hour, seven-installment program entitled Evolution is Happening that will air during the fall 2001. Preceding the broadcast will be a \$1-2 million advertising campaign. We need to begin discussing strategies about how we can take advantage of this program and media blitz to improve evolution education in our home institutions and communities. This is a once-in-a-lifetime opportunity to propel evolution onto center stage. Fourth, we need to embrace those educational practices that are founded in sound pedagogical research. Inquiry-based learning appears in national and state K-12 science curriculum standards and frameworks and should play a central role in undergraduate evolution education. Having made this pronouncement, I am well aware of the fact that this will only occur when the appropriate resources and necessary support are made available. The National Academy of Science has produced numerous resources for improving science and evolution education and they are available from the National Academy Press (NAP) website. NAP titles that I have

found particularly useful include: *How People Learn*², *Inquiry and the National Science Education Standards: A Guide for Teaching and Learning*³, and *Teaching About Evolution and the Nature of Science*⁴. The American Society of Naturalists has published a useful document entitled *Evolution, Science, and Society*⁵, which is available from their website as a PDF document. *Good Science, Bad Science: Teaching Evolution in the States*⁶, also known as the Lerner Report, was published in September 2000 and is available online as well. This report evaluates state K-12 science standards with respect to their treatment of evolution.

There are also numerous online resources available and in the development stage. One member of the Society for the Study of Evolution has received significant NSF funding to develop an online journal of Evolution Education and include a wide variety of educational resources as well. This website is currently in the development phase and should be available in 2001. The NSF has also funded a project by the AAAS to develop a national digital database that will be available in 2001-2002. WGBH is building an

evolution education web site to accompany the Evolution is Happening series. The Executive Committee and the editors of *Bioscene* are committed to keeping the membership informed of the availability of these types of resources as they become available.

In conclusion, I am excited about the future of biology education, especially evolution education. I am pleased that we have chosen evolution education as our theme for our 45th Annual Meeting at the University of Nebraska, Kearney. And I am also pleased that ACUBE has adopted the Evolution is Good Science resolution and that we will proudly display this statement on our website.

Notes:

- 1 - <http://www.ucmp.berkeley.edu/ncte/>
- 2 - <http://www.nap.edu/catalog/9853.html>
- 3 - <http://www.nap.edu/catalog/9596.html>
- 4 - <http://www.nap.edu/catalog/5787.html>
- 5 - <http://www.amnat.org/meagher.html>
- 6 - <http://www.edexcellence.net/>

In Memoriam

John Bliese

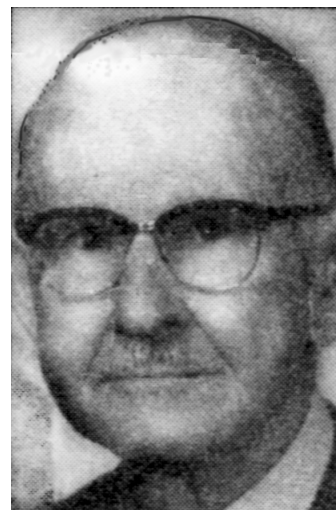
1977 Honorary Life Member

John C. W. Bliese, 87, of 107 E. 27th St. died Monday, Nov. 27, 2000, at St. John's Center in Kearney, NE. He was born March 10, 1913 in Waterloo, Iowa to August and Caroline (Kolm) Bliese. He graduated from East Waterloo High School in 1931 and received his bachelor's degree in biology from Iowa State Teachers College in 1935. He received a masters degree from Columbia University in 1936, and his doctorate in economic zoology from Iowa State College in 1953. He also receive additional schooling at the University of Southern California, the University of Michigan, State University of Iowa, Colorado State College and the University of Colorado.

He taught biology and physical sciences at Cedar Falls High School and at Teachers College High School, both in Cedar Falls, Iowa. He was an instructor of biological sciences at Cornell College in Mount Vernon, Iowa, and Iowa State College in Ames. He moved to Kearney, NE, in 1953 where he was a full-time biology professor at Kearney State College until retiring in 1983. He served as head of the biology department from 1962 to 1966. He also lectured about birds and led tours on crane viewing.

John was a member of the Nebraska State Education Association, National Education Association-Retired, National Retired Teachers Association, American Scientific Affiliation, International Crane Foundation, Whooping Crane Conservation Association, North American Crane Working Group, National Audubon Society, Nature Conservancy, Nebraska Statewide Arboretum, and the Nebraska Chapter of the Wildlife Society, He was also a member and past-president of the Big Bend Audubon Society, Nebraska Academy of Sciences and the Nebraska Ornithologist Union.

He received the Honorary Life Membership Award from ACUBE in 1977, the Johnny Appleseed Award in 1982 the Howard Juhl Conservation Award in 1982, the Kearney State College Distinguished Service Award in 1985, Admiral in the Great Navy of the State of Nebraska in 1987, the National Audubon Society Visionary Award in 1992, and the Crane Conservationist of the Year Award in 1994.



ACUBE Steering Committee

First Fall Meeting

Thursday October 12, 2000

Location: Room 205 Science Hall, Indiana State University

Time: 9:35 p.m.

Present: Charlie Bicak, Bill Brett, Austin Brooks, Tom Davis, Mary Haskins, Buzz Hoagland, Cynthia Horst, Pres Martin, Tim Mulkey, David Prentice, Nancy Sanders, Ethel Stanley, Robert Wallace, Margaret Waterman.

I. Called to order by President Hoagland

II. Announcements

Buzz Hoagland attended a meeting sponsored by NSF on the theme of Teaching of Evolution, and given the widespread misconceptions, teaching of this topic must improve. For our action, those at the conference were asked to pass resolutions supporting the teaching of evolution. Buzz is submitting a resolution to the resolutions chairman, Dick Wilson.

III. Approval of Agenda

Motion to approve agenda and send to Bioscene m/s/a

IV. Approval of minutes from 5-6 February Winter Business Meeting

Motion to approve minutes (to reconfirm email vote) m/s/a

V. Report of Officers of Standing Committees

A. Executive Secretary. Presley Martin.

Total assets as of October 12, 2000 were \$14,642. Carlock Memorial Fund has \$5590.

Total income for the year was \$6431.

Expenses for the year were \$13,171

There were 478 members on the membership list, of these, 114 paid dues through 1998, 141 paid through 1999 and 223 paid through 2000.

Additional details of the executive secretary's report are in the report submitted by Pres Martin.

Executive secretary's report approved: m/s/a

B. Membership. Tom Davis

Membership committee made no special call for membership this year, as planned, except for developing the new member letter. The mailing by McGraw Hill is scheduled for next year.

It was noted by Buzz that no one at the AIBS meeting had heard of ACUBE, but the name went on a bookmark that was distributed, and networking helped to get the name out. Others commented that we need to be presenting in ACUBE's name at meetings and maybe have literature available for distribution.

New Members letter went out, welcoming them to ACUBE. Will try to get new folks together at this meeting and plan for it at next meeting.

C. Nominations. Nancy Sanders

Nominees for steering committee are Ben Dolbear, James Rooney, and Lynn Gillie. One more is needed.

Nominees for president are Terry Derting, Marc Roy and Malcolm Levin.

Additional nominees will be solicited by a call from the floor at the first business meeting. CVs are posted by the exhibits. Voting will be before the banquet.

D. Honorary Life Membership and Carlock Awards Bill Brett.

Awards to be presented at the banquet. Honorary life membership is to be presented to Neil Baird. Other names for future honorees were suggested.

Name of winner of Carlock award: Andrew Bishop, University of Nebraska, Kearney

E. Bioscene Editors. Ethel Stanley and Tim Mulkey.

Since the move of printing from Beloit, the cost per issue of Bioscene has gone from \$1600 to \$1400.

Other changes include:

- color covers,
- direct bulk mailing of 550 issues (up from 400), and
- added ADC codes (required by the Postal Service).
- improved quality of proofing
- coordinated cover art with articles

Delays in the publishing schedule: Official business scheduled to be published is frequently late.

Once received, it takes 6 weeks to get set up, printed and mailed. It's up to us!!!

There were enough submissions to produce an extra issue this year.

At present have enough articles on hand for three more issues.

More contributions from our members are needed. Many of the articles come from outside ACUBE membership.

Can we collect areas of expertise on registration forms, as a way to help editorial board identify potential reviewers? Pres Martin thought it would be possible.

Four people are completing their time on the editorial board. Suggestions for additional board members were solicited.

F. 2001 Program Planning Committee – Mary Haskins and Charlie Bicak.

The theme for the meeting will be in keeping with Buzz Hoagland's experience at the AIBS meeting. Official theme is "Biology in the Light of Evolution." The theme will be announced and abstracts solicited at the banquet.

Transportation to Kearney was discussed, as was the scheduling of the meeting.

G. Resolution Committee: Dick Wilson (not present)

Tabled

VI. Old Business

A. Dues increase – to be \$30.

B. ACUBE is slow to cash checks for membership. Need to be deposited faster.

C. Web page: Tabled

D. Constitution update: Tabled

E. Future meetings

2002 -- Columbia College of Chicago

2003 -- Truman, however, the building is not on schedule.

2004 -- ? check with Terry Derting.

VI. New Business.

A. The call for a new chair of the editorial board was withdrawn, since Bill Brett agreed to take on this role again.

Adjourn 10:58 p.m. p.m.

First Business Meeting Friday October 13, 2000

Place: Hulman, Dede 1

Time: 12:30

Called to order by President Buzz Hoagland

I. Announcements:

NIH Bridge program linking 2 and 4-year colleges is looking for proposals. See Buzz or contact Irene Eckstrand at the Society for the Study of Evolution.

(Mary Haskins, Charlie Bicak) 2001 meeting in Kearney NE. Theme is: *Biology in the Light of Evolution*; October 11-13, 2001 (Thursday – Saturday)

Membership directories are available

II. Award nominations - call for nominees -- Bill Brett

III. Call for resolutions from the floor (no response)

IV. Nominations – Nancy Sanders

Announced nominees for Member At Large and President.

Abour Cherif was nominated from the floor for Member At Large.

No further nominations.

Motion to close nominations: m/s/a

V. Motion to raise dues from \$25 per year to \$ 30 per year, effective immediately m/s/a

VI. Call for Bioscene manuscripts from the membership

Announcement of a table at breakfast tomorrow to discuss manuscript ideas.

Editorial Board meeting announcement

VII. Don't forget to get your ACUBE memento: a canvas bag and new ACUBE mug!!

VIII. Program: Dr. Jane Stutsman, National Science Foundation. "NSF in a Changing World"

Adjourn. 1:45

ACUBE Second Business Meeting October 13, 2000

Location: Hulman, Dede 1

Time: 7:30 p.m.

I. Candidate forum. Each candidate for office made a two minute presentation.

II. Balloting

III. Carlock Award presentation by Ed Kos to Andrew Bishop of the University of Nebraska, Kearney

IV. Honorary Life Award presented by Harold Wilkinson to Neil Beard

V. **Evening Program** – Dr. Brian Dengenhardt “A Holistic Approach to Students – Mind, Body and Spirit”

Adjourn 8:45 p.m.

ACUBE Third Business Meeting Saturday October 14, 2000

Location: Hulman, Dede 1

Time: 11:00

I. Announcements

II. Presidential Address Buzz Hoagland “Evolution teaching issues”

III. Incoming Presidential Address: Tom Davis. “Did they get it? What students know”

IV. Business

A. Resolutions.

The Resolution from the National Conference on the Teaching of Evolution was passed by the membership.

Thanks to program speakers and presenters

Thanks to Indiana State University

Thanks to Program Chair

All resolutions passed by the membership

B. Annual Meeting 2001

Charlie Bicak and Mary Haskins discussed theme and abstracts

C. Bioscene editors report – Ethel Stanley and Tim Mulkey

Calls for papers from meeting

Cost per issue has decreased

Thanks to Dick Wilson for organizing the retyping of the proceedings and the Midwest Bioscenes – a huge project, well done

All Bioscene articles will be PDF format, easy to read and download

Archive is complete to where a CD can be made

We are getting an ISSN so to make library cataloging of Bioscene possible.

New editorial board members:

Bill Brett, Chairman

Margaret Waterman

Neil Baird

Conrad Toepfer

D. Executive Secretary report (see above in Steering Committee I meeting)

E. Thoughts on this meeting: Send comments to Tom Davis. About 100 people attended.

Adjourn 12:20 p.m.

ACUBE Steering Committee Meeting

Saturday October 14, 2000

Place: Room 205, Science Hall, Indiana State University

Time: 12:30

Present: Charlie Bicak, Bill Brett, Austin Brooks, Tom Davis, Ben Dolbeare, Lynn Gillie, Mary Haskins, Buzz Hoagland, Cynthia Horst, Ed Kos, Malcolm Levin, Tim Mulkey, David Prentice, Ethel Stanley, Robert Wallace, Pres Martin, Margaret Waterman

I. Call to order, Tom Davis

Annual Meeting -- Reflections on 2000

Having elections on Friday worked out exceptionally well.

Issue of low attendance on Saturday was discussed – try scheduling field trip or something more exciting.

II. Annual Meeting 2001

Keep same general format

Discussion of concerns about travel

Eliminate Thursday field trip, schedule an extra field trip for Saturday afternoon.

Major speaker, on evolution, but not the evolution/creation controversy. Maybe someone on the evolution of AIDS, emerging diseases, etc.

Perhaps have a roundtable of controversial ideas in evolution

III. Date for winter meeting: Sat 2/3

IV. Executive Secretary

Concerns about lack of payment of dues; dues will be \$30 for 2001.

Will shift dues notices to January/February

Will add dues line to the meeting registration form. These dues will cover the upcoming year. (So, for the October 2001 meeting, dues submitted will be for 2002 January- December.

114 members have not paid 1999 or 2000 dues. Time to drop from list??

Why are some regulars no longer showing up?

V. Constitutional update: Tabled for Winter Meeting.

VI. ACUBE stationery was discussed. Will keep the electronic format, but eliminate steering committee names. Only Pres Martin's name as executive secretary.

VII. Bioscene: Needs information from other steering committee members, per schedule distributed last year.

VIII. Electronic minutes to be voted on by mid November, published Dec. 1

IX. Lynn Gillie: Will be new nominations chair

X. Web Page committee: Karen Klyczek is chair. No Report

XI. List serve: Table to winter meeting.

XII. Historian – no report

Adjourned 1:35 p.m.

Respectfully submitted

February 3, 2001

Margaret Waterman

ACUBE Secretary

Call For Resolutions

The Steering Committee of ACUBE requests that the membership submit resolutions for consideration at the 2000 Annual meeting to the Chair of the Resolutions Committee. Submit proposed resolutions to:

Dr. Richard Wilson, Dept. of Biology, Rockhurst University, 1100 Rockhurst Rd
Kansas City, MO 64110, Phone (846) 501-4048, wilson@vax1.rockhurst.edu

Call for Applications John Carlock Award

This Award was established to encourage biologists in the early stages of their professional careers to become involved with and excited by the profession of biology teaching. To this end, the Award provides partial support for graduate students in the field of Biology to attend the Fall Meeting of ACUBE.

Guidelines: The applicant must be actively pursuing graduate work in Biology. He/she must have the support of an active member of ACUBE. The Award will help defray the cost of attending the Fall meeting of ACUBE. The recipient of the Award will receive a certificate or plaque that will be presented at the annual banquet; and the Executive Secretary will provide the recipient with letters that might be useful in furthering her/his career in teaching.

Application: Applications, in the form of a letter, can be submitted anytime during the year. The application letter should include a statement indicating how attendance at the ACUBE meeting will further her/his professional growth and be accompanied by a letter of recommendation from a member of ACUBE. Send application information to:

Dr. William J. Brett, Department of Life Sciences, Indiana State University, Terre Haute, IN 47809;
Voice -- (812) 237-2392 FAX (812) 237-4480; E-mail -- lsbrett@scifac.indstate.edu

If you wish to contribute to the **John Carlock Award** fund, please send check to: Dr. Pres Martin, Executive Secretary, ACUBE, Department of Biology, Hamline University, 1536 Hewitt Ave., St. Paul, MN 55104.

ACUBE Gratefully acknowledges the support of the following sponsors and exhibitors of the 44th Annual meeting:

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Manuscript Guidelines for *Bioscene: Journal of College Science Teaching*

A publication of the Association of College and University Biology Educators

Manuscripts submitted to the Bioscene should primarily focus on the teaching of undergraduate biology or the activities of the ACUBE organization. Short articles (500-1000 words) such as introducing educational resources provided by another organization, reviews of new evolution software, suggestions for improving sampling methods in a field activity, and other topics are welcome as well as longer articles (1000-5000 words) providing more in depth description, analyses, and conclusions for topics such as introducing case-based learning in large lectures, integrating history and philosophy of science perspectives into courses or initiating student problem solving in bioinformatics.

Please submit all manuscripts to editor(s):

Ethel Stanley
Department of Biology
Beloit College
700 College St.
Beloit, WI 53511
stanleye@beloit.edu
FAX: (608)363-2052

Timothy Mulkey
Department of Life Sciences
Indiana State University
Terre Haute, IN 47809
mulkey@biology.indstate.edu
FAX: (812) 237-2418

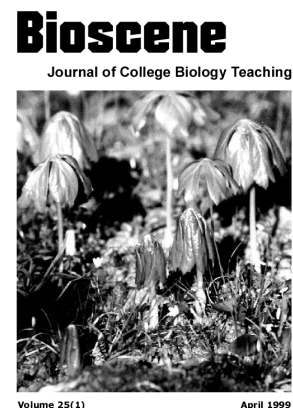
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Please refer to issues of the Bioscene from 1998 or later for examples of these items. You can access these issues at: <http://acube.org/bioscene.html>

Graphics are desirable! Lengthy sections of text unaccompanied by tables, graphs or images may be modified during layout of the issue by adding ACUBE announcements or other graphics. While tables and graphs may be included in the manuscript file, images should be submitted as individual electronic files. If you are unable to provide an image in an electronic format such as TIFF for Macintosh or BMP for Windows, please include a clear, sharp paper copy for our use. At this time, graphics will be printed as grayscale images with a minimum resolution of 300 dpi and a maximum resolution of 1200 dpi. Cover art relating to an article is actively solicited from manuscript contributors.

Upon receipt of your manuscript, an email or fax will be sent to the author(s). The editor will forward your manuscript to the chair of the editorial board. Within the next two weeks or so, your manuscript will be sent to two reviewers. You should receive comments when changes are recommended from the reviewers prior to publication of the article. Manuscript format is usually retained as accepted; however, limits of publishing the issue may affect the length of an article. Graphics may be added by the editors when lengthy sections of text are unaccompanied by tables, graphs or images. Previously published work should be identified as such and will be reviewed on a case-by-case basis. Your article will appear in the Bioscene and then on the ACUBE website: <http://www.acube.org> shortly after the issue date.



Call for Presentations

Association of College and University Biology Educators (ACUBE)

45th Annual Meeting

University of Nebraska at Kearney

Thursday October 11- Saturday October 13, 2001

Biology in the Light of Evolution

Theodosius Dobzhansky stated, "nothing makes sense except in the light of evolution".

Evolution has, once again, claimed national attention because some states have either removed and/or downplayed evolution from the curriculum objectives in the K-12 system. Although the scientific community understands the distinction between science and religion, the public may not. The upcoming PBS television broadcast on evolution will continue to promote public discussion and controversy. How can we address our students' need, and the public's need, to understand this distinction?

Presentations, posters and workshops addressing other topics are welcome, but here are some examples of possible presentations:

Issues in teaching evolution to non-majors/majors (creationism vs. evolution;
Simulation software used in lecture and/or labs; Investigative labs; Evolution of ideas and/or theories in scientific disciplines; Evolution of Scientific methodologies;
Evolution of processes, human practices and/or cultures; Analogies used in teaching evolution

Many of you have addressed these issues in creative ways. Please consider sharing your ideas and techniques at the ACUBE 45th Annual Meeting in Kearny, NE in 2001.

Please email your abstract **AND** mail or FAX a hard copy of the abstract with the completed form **BEFORE July 1, 2001** to:

Mary Haskins, Biology Department, 1100 Rockhurst Road,
Rockhurst University, Kansas City, MO 64110
Phone (816)501-4006 FAX: (816)501-4802 email: mary.Haskins@rockhurst.edu

Proposed Title: _____			
Presentation type:	Poster	45 minute paper	90 minute workshop
Name of presenter : _____			
Work address of presenter : _____			
Equipment/facilities needed:	_____ 35 mm slide projector	_____ Overhead projector	
	_____ Macintosh projection system	_____ Macintosh computer lab	
	_____ PC projection system	_____ PC computer lab	
	_____ Other: (explain)		
Phone No. presenter: _____ email _____			
Please include names and contact information for additional presenters and a 200 word abstract:			

ACUBE

Association of College and University Biology Educators

NAME: _____ DATE: _____

TITLE: _____

DEPARTMENT: _____

INSTITUTION: _____

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MAJOR INTERESTS

- 1. Biology
- 2. Botany
- 3. Zoology
- 4. Microbiology
- 5. Pre-professional
- 6. Teacher Education
- 7. Other _____

SUB DISCIPLINES: (Mark as many as apply)

- A. Ecology
- B. Evolution
- C. Physiology
- D. Anatomy
- E. History
- F. Philosophy
- G. Systematics
- H. Molecular
- I. Developmental
- J. Cellular
- K. Genetics
- L. Ethology
- M. Neuroscience
- N. Other _____

RESOURCE AREAS (Areas of teaching and training): _____

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How did you find out about ACUBE? _____

Have you been a member before: _____ If so, when? _____

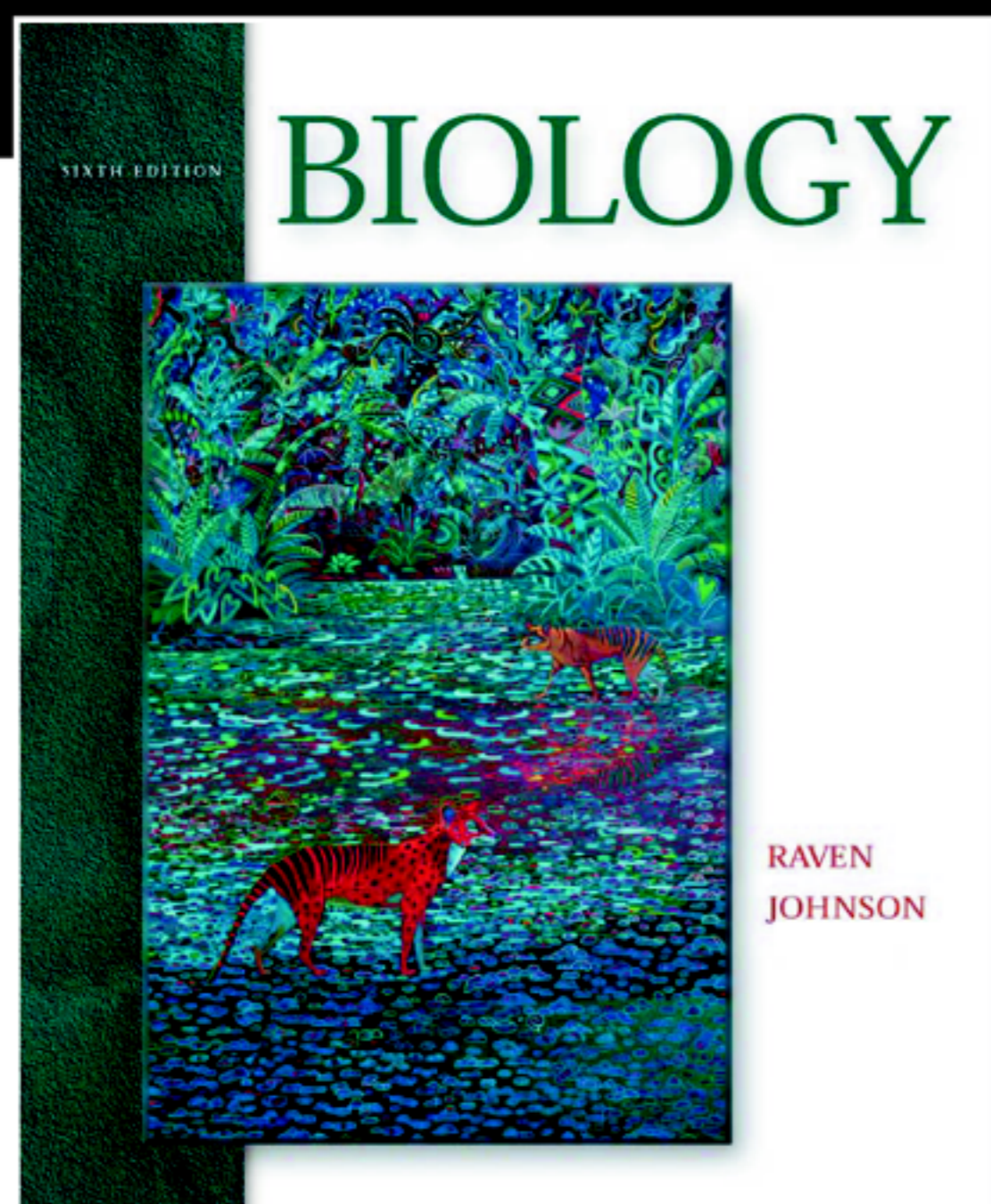
DUES (Jan-Dec 2001) Regular Membership \$30 Student Membership \$15 Retired Membership \$5

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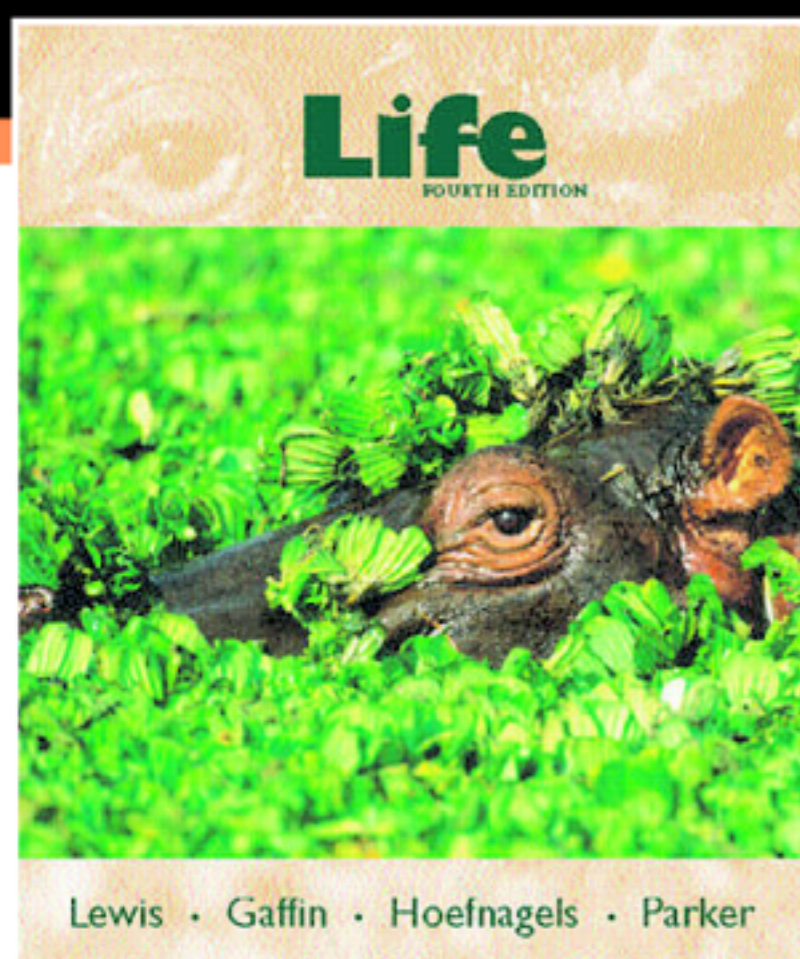
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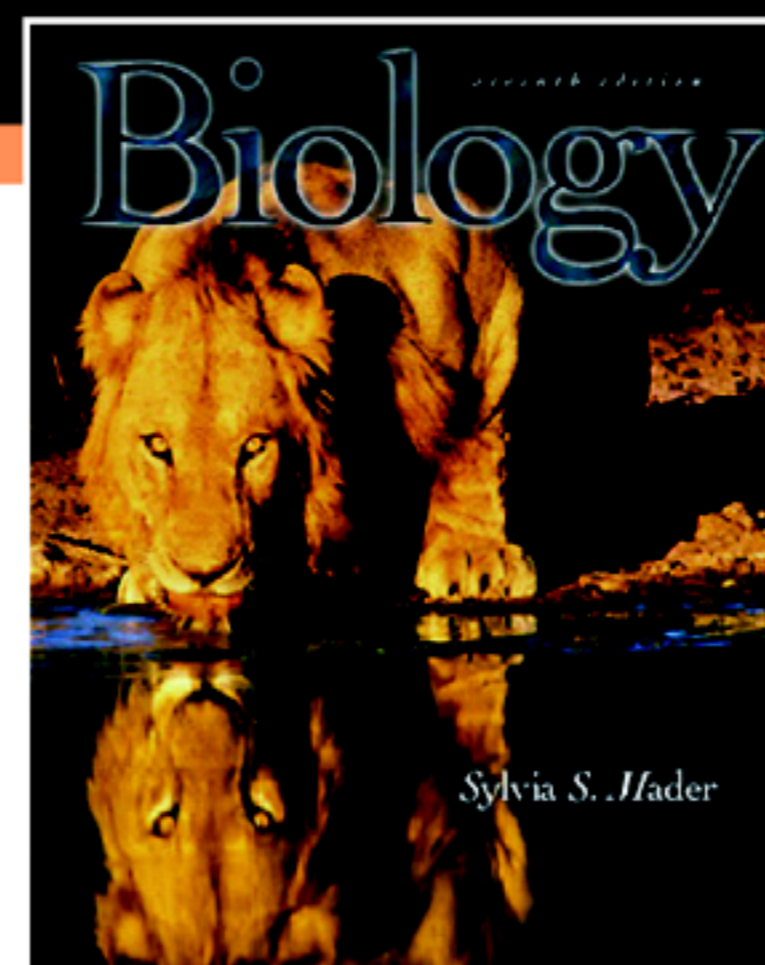


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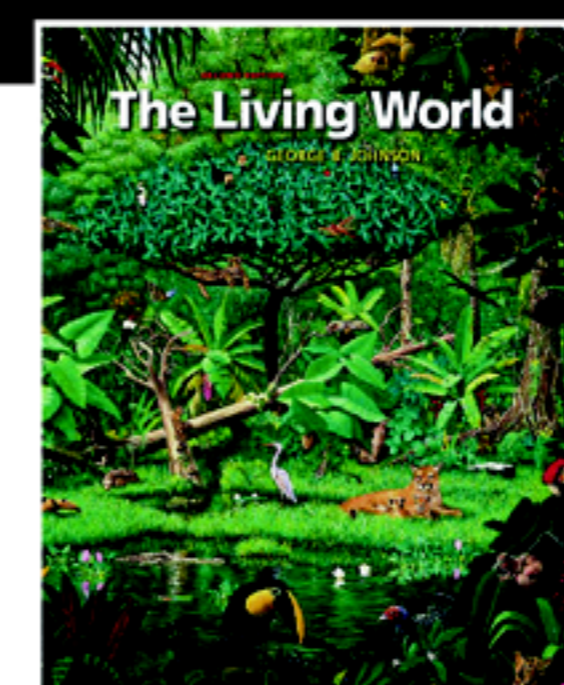


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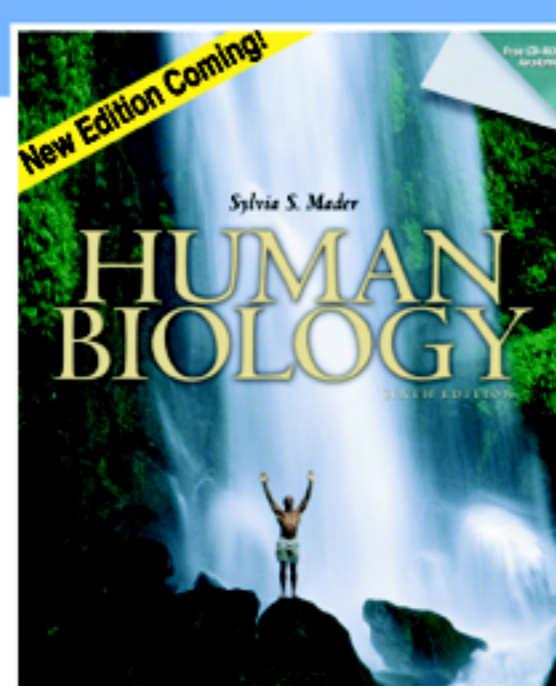


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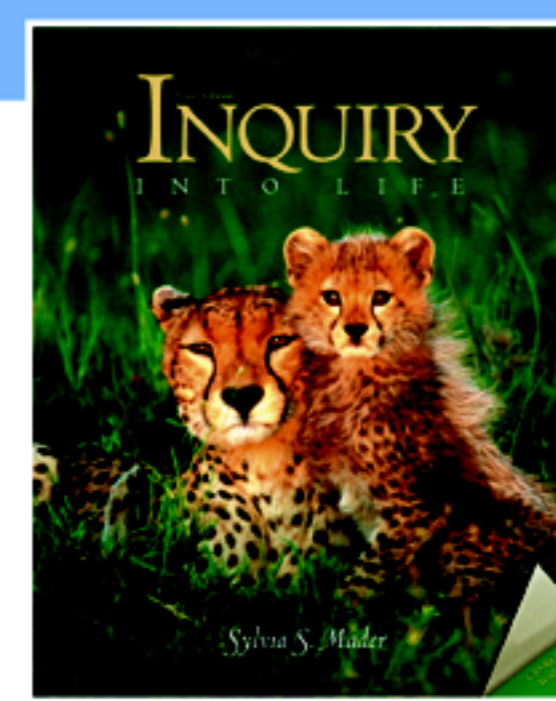


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