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Individual Research

- Cellulose Secretion in Trichoderma reesei
Rick Waggener..... 1
- The Zero Length Spring
Scott Budzein..... 15
- Polymorphism in Lamium
Rick Sullivan..... 25

Reviews

- The Measurement of Interspecific Competition
John Pardue..... 31
- The Physiological Aspects of Stress
Steve Sharp..... 39
- The Problem of Eusociality
Tim Carter..... 52

Attitudes

- The Ethical Aspects of Abortion
Julie Mortimer..... 47

A Word With...

- Dr. Julian T. Darlington..... 58

The Southwestern Science Journal

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Preface

This first issue of the Southwestern Science Journal is the newest Southwestern publication. The purpose of this journal is to provide a forum for student and faculty research and ideas. We, the editors, feel that this publication will demonstrate to the campus and alumni the level of research found in the natural sciences. This journal will be published biannually with a winter and spring issue. We feel that this 1983 third term is appropriate for such a new publication as it is exemplary of the creativity that term three offers.

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Cellulase Secretion in Trichoderma reesei

Rick Waggener*

INTRODUCION

Cellulose is the world's most abundant renewable carbon source, but any economically feasible method of converting it into usable products such as food, fuel, or chemicals would depend upon its hydrolysis to glucose. Methods such as acid or high temperature degradation tend to break down the resulting sugars and also produce undesirable by-products from the impurities found in waste cellulose (Spano et al., 1975). But according to Montencourt and Eveleigh (1979), enzymatic hydrolysis of cellulose by the cellulases holds the most promise for the future since enzymes are highly efficient, clean, reusable, and do not generate unwanted by-products.

There are many natural sources of cellulases, but most are found amongst the microbes and the fungi. Fungal cellulase has been the most thoroughly studied, especially that of the imperfect fungus Trichoderma. This cellulase actually consists of a "complex" of three different forms of cellulase activity that act synergistically to hydrolyse cellulose (Emert et al., 1974; Nisizawa et al., 1972; Gritzali and Brown, 1979). According to Mandels and Weber (1969), Trichoderma reesei (formerly Trichoderma viride QM6a) is the best known source of all the enzymes of this complex. But recently work has also been done with hyperproducing catabolite repression-resistant mutants of T. reesei (Montencourt and Eveleigh, 1979; Ghosh et al., 1982).

Since Reese et al., (1950) first proposed a stepwise model of cellulose hydrolysis involving components he termed "CI and Cx," much work has been done to characterize the enzymes involved and their modes of action. It is now generally thought that there are three basic forms of cellulase in the Trichoderma complex: 1,4-beta-glucan cellobiohydrolase (exoglucanase), 1,4-beta-glucan glucanohydrolase (endoglucanase), beta-glucosidase (cellobiase) (Gong et al., 1979). Although there is still much disagreement about the inter-relationship of these enzymes it is generally accepted that the endoglucanase randomly hydrolyzes the cellulose chain while the exoglucanase attacks the ends yielding cellobiose, and the cellobiase hydrolyzes cellobiose to yield glucose (Gong et al., 1979; Ghosh et al., 1983).

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The production of these enzymes by Trichoderma must be induced, and many compounds have been determined successful inducers although they vary in effectiveness. These include: cellulose, lactose, sophorose, and cellulose derivatives (such as cellobiose) (Mandels et al., 1962). Sophorose is by far the most potent of the known inducers of cellulase secretion in T. reesei (Sternberg and Mandels, 1979), but the identity of the natural inducer is still not yet agreed upon.

It was my intention in this work to first establish suitable growth, harvest, and assay procedures for experiments involving Trichoderma reesei; secondly, to determine an inducer and inducer concentration most appropriate for the described conditions; and further, to establish a reproducible time course of induced cellulase secretion. The establishment of uniformity between replicate treatments in a single time course was essential before further manipulations could be attempted.

As previously mentioned, much work has been done with Trichoderma in such areas as determining cellulase complex identity and kinetics, and manipulation of enzyme yields through investigation of growth conditions and use of hypersecretory mutants. However, very little work has been done to investigate the physiological mechanisms whereby cellulase is produced and secreted in Trichoderma. It is to this that my ultimate, and as yet unattained goal addresses itself. Through the addition of various concentrations of a known metabolic inhibitor in the medium of induced T. reesei with the subsequent observation of the time course of secretion, I had hoped to learn something about the role specific target mechanisms these inhibitors play, if any, in the secretion of cellulase. However, the main body of this Directed Inquiry was, of necessity, devoted to the aforementioned establishment of a reproducible time course of secretion. This was due to inherent, but unforeseen difficulties in the form of variation between experiments, and, more importantly, variability between replicate treatments in the same time course of secretion. Through many manipulations of experimental conditions this variability was eventually overcome. This left time for only one experiment using treatments of various concentrations of cytochalasin B (an inhibitor active against microfilament-based systems), and the results of this one experiment were highly inconclusive.

This does not imply that the knowledge gained from this Directed Inquiry is not of value. An establishment of a workable methodology for producing a time course of cellulase secretion in Trichoderma reesei free from unacceptable variability will be invaluable in future work with this fungus at this institution with the facilities available. This is of special interest as I am in a position to contin-

ue with this work and investigate the physiology of secretion in Trichoderma reesei.

Materials and Methods

The procedures outlined in this section are the culmination of many previous variations, most of which are not mentioned. This was the best technique I obtained to produce and reproduce a suitable time course of secretion for lactose-induced T. reesei. A few notable previous techniques are mentioned.

A. Culture

A culture of Trichoderma reesei (ATCC 13631) was maintained on slants of potato dextrose agar (PDA). Stocks were transferred approximately every two weeks.

B. Inoculation

A spore suspension necessary for inoculation into liquid growth medium was obtained by pouring approx. 10ml of .1% Tween 80 over a 4 day PDA slant of T. reesei and agitating with a sterile glass rod. This heavy suspension was transferred aseptically into 50ml of .1% Tween 80 and a small sample of diluted suspension was aseptically removed for spore counting on a hemacytometer. The standard inoculation used was 10⁶ spores/ml liquid growth medium.

C. Growth Medium

The primary medium used in these experiments was formulated after that of Mandels and Reese (1957): .2% KH₂PO₄, .14% (NH₄)₂SO₄, .03% urea, .03% MgSO₄ · 7H₂O, .03% CaCl₂, 1ppm Fe⁺⁺, and .8ppm Zn⁺⁺ adjusted to pH² 5.3 with .1M NaOH. (This medium will be termed "MR" in subsequent references.) For normal growth (non-cellulase induced) 2g/l glucose was added as a carbon source.

D. Induction Media

Primary induction medium was MR (no glucose) with 1mM lactose. An alternate medium used simultaneously in some experiments was plain phosphate buffer and lactose: 2.31g/l KH₂PO₄ and 1mM lactose.

E. Growth and Induction

500ml of MR with glucose in a 1l Erlenmeyer flask was inoculated with 5 million spores and placed in an incubator/shaker (reciprocal) at 25 degrees C. and 100 r.p.m. (amplitude 4.5 cm). After three days mycelium was harvested by filtration, rinsed with 1l distilled water, and

resuspended in 500ml induction medium in a 1l Erlenmeyer flask. After 4-6 hours the mycelium was harvested by filtration (not washed). The medium was saved, split into two 250ml volumes and placed into replicates. Inoculation weight was 1.5-2g/l wet mycelium/100 ml medium. The two replicates were replaced in the shaker and 5ml samples were withdrawn from each replicate at 2 hr. intervals. Sampling was discontinued at 14 hrs. after induction. Each sample was assayed 3 times.

F. Notable Previous Methods

1. In an early comparison between Avicel cellulose and CF-11 cellulose for long-term induction ability, four 250ml Erlenmeyer flasks with 100ml MR were prepared. Two contained 2g/l CF-11. two contained 2g/l Avicel. These were inoculated directly with spores and incubated for six days. Each flask was sampled once and assayed once.

2. In an early comparison between low levels of lactose vs. high levels for optimum induction the previous procedure was followed using two flasks of 1mM lactose and two flasks of 5.8mM (2g/l) lactose.

3. Replicate Method

The most notable previous method followed the procedure in part E until time for induction. Three fresh 100ml volumes of MR induction medium in 250ml Erlenmeyer flasks were then inoculated with wet mycelium (1.5-2g/100ml). The flasks were sampled at 2hr. intervals also, but each sample was assayed once i.e., each flask was a replicate. (In the final method replicate assays were done on single samples from splits originating from the same medium and mycelial mass).

G. Samples

To keep mycelium out of the samples, and to aid in sampling, pipettes with specially filtered tips were used. A small strip of nylon webbing (source: commercial nylons) was wrapped several times over the tip of the pipette and affixed by a rubber band. The entire tip was submerged during sampling to prevent bubbling. Samples were withdrawn, placed in small plastic bottles, and frozen for later assay.

H. Cellulase Assay

A viscometric assay specific for the endoglucanase component was employed.

Substrate: 1.2% carboxymethyl cellulose (CMC) in 0.018M citrate-NaOH buffer, pH 5.0, make .05% in merthio-

late.

5ml CMC in size "300" Ostwald-Fenske viscometer was equilibrated at 30 degrees C. in a water bath for 15 min.

1ml sample was added, mixed, and $t=0$ flow rate was recorded. Flow rate measured again at $t=30,60,90$ min.

Enzyme units were expressed as increases in relative fluidity:

$$(1/N_{sp})/t$$

$$N_{sp} = \text{specific viscosity} = t - t_0 / t_0$$

$$t_0 = \text{flow rate for water (reference)}$$

The following method was utilized in the one experiment with cytochalasin B. As will later be discussed, this method was unacceptable, and alterations must be made in subsequent experiments.

A. Chemicals

Cytochalasin B (CB) was obtained from Sigma. A stock solution was prepared of CB in dimethyl sulfoxide (15.7mM).

B. Growth and Induction

Method identical to that above except mycelium and induction medium were split into four replicates, each of 25ml medium and .5g wet mycelium in 125ml Erlenmeyer flasks.

C. Treatment

Three flasks received CB at the following concentrations: .3ug/ml, 3ug/ml, 30ug/ml. The fourth flask received DMSO only (100microl stock: .4%).

D. Samples

2ml samples were taken at 4hr.-7hr. every 30 min. Split occurred at 4.75 hrs. and treatment at 5 hrs. Samples were also taken at 10 and 14 hrs.

RESULTS

In the early comparison between the two celluloses, Avicel and CF-11, it can be seen in TABLE 1 that Avicel-induced cellulase activity is 58% less than that of CF-11-induced activity.

In the early comparison between high and low lactose

concentrations for ability to induce cellulase secretion it can be seen in TABLE 2 that 1mM lactose-induced activity was 44% less than that of 5.8mM (2g/l) lactose-induced activity.

GRAPHS 1 and 2 show the great variation in the time courses of secretion between replicates using the earlier "replicate method" in F. of Materials and Methods. GRAPH 1 uses phosphate induction medium and GRAPH 2 uses MR induction medium. In this and previous experiments, MR induction was generally higher than levels achieved with phosphate induction (roughly twice). However, standard deviations were so great as to make this uncertain.

GRAPH 3 shows a later trial of the final technique i.e., splitting the medium as well as mycelium for dual trials and reading single samples three times for statistical accuracy. Splitting in this experiment occurred earlier than some previous trials (at 5 hrs.) and was seen to give more consistent results. Standard deviation was acceptably small and almost no variance between trials was seen up to 10 hours after induction.

TABLE 3 shows the results of cytochalasin B (CB) treatment as described in Materials and Methods. The 3ug/ml and 30ug/ml CB treatments were seen to be slightly lower in activity than both the control (100microl DMSO) and the .3ug/ml CB treatment. However it was also seen that all activities, even in the control, were much reduced from that of GRAPH 3. Indeed, this activity was five to ten times less!

Table 1

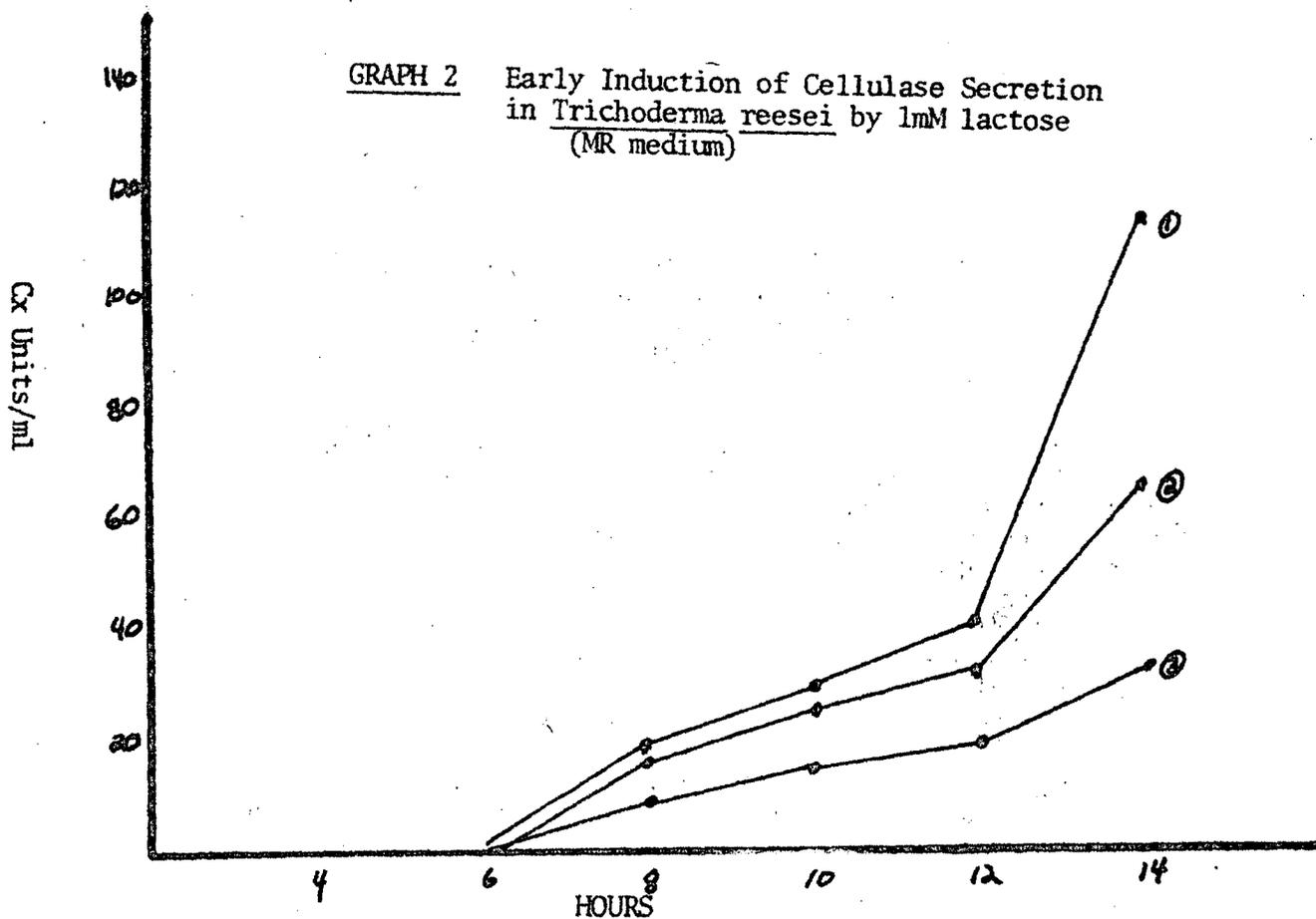
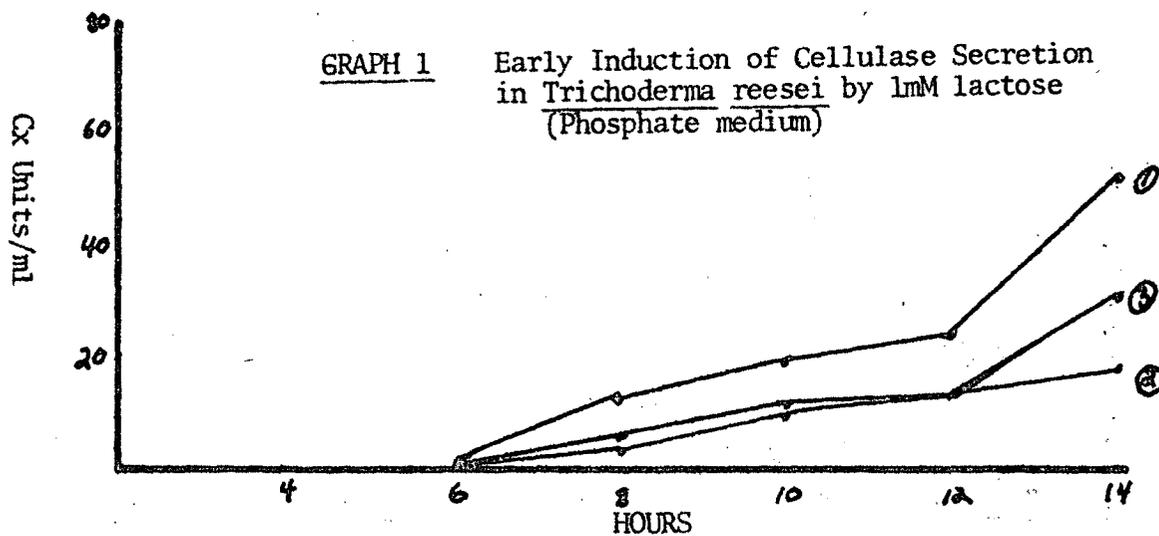
Comparison of two cellulose sources, Avicel and CF-11 as long-term inducers of cellulase secretion by Trichoderma reesei

<u>Medium</u>	<u>Cellulose</u>	<u>Incubation time</u>	<u>Cx Units/ml</u>
MR	Avicel	2 day	0.00
MR	Avicel	4 day	7.85
MR	Avicel	6 day	742.97
MR	CF-11	2 day	0.00
MR	CF-11	4 day	8.05
MR	CF-11	6 day	1760.00

Table 2

Comparison of 1mM lactose and 5.8mM lactose as inducers of cellulase secretion in Trichoderma reesei

Medium	Inducer	Conc.	Incub. time	Cx Units/ml	Average
1. MR	lactose	1mM	6 days	1204	1255
2. MR	lactose	1mM	6 days	1306	
1. MR	lactose	5.8mM	6 days	2089	2227
2. MR	lactose	5.8mM	6 days	2364	



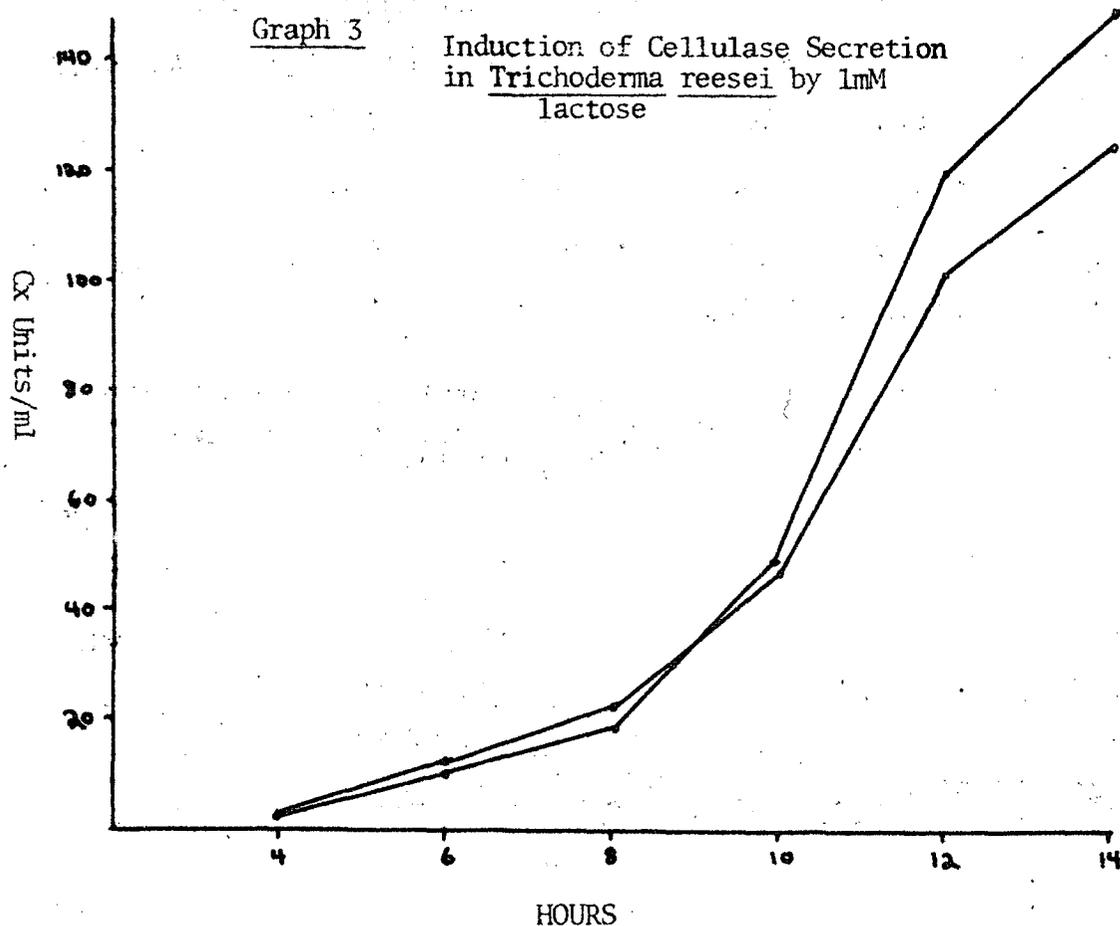


Table 3

The effect of cytochalasin B (CB)
upon secretion of cellulase
by Trichoderma reesei

Treatment	Cellulase Units/ml ^{ab}
Control (0.4% DMSO)	12.63 ± .54
0.3 g/ml CB	12.18 ± .41
3.0 g/ml CB	10.84 ± .38
30.0 g/ml CB	10.56 ± .33

^a ± standard deviation

^b 14 hrs. after induction
by 1 mM lactose

DISCUSSION

Whatman CF-11 cellulose powder was seen to be a more effective inducer of cellulase activity than Avicel microcrystalline cellulose. This seems most likely due to the crystallinity of the Avicel. According to Millet et al. (1979), crystallinity inhibits the attack of all three forms of cellulase in the *Trichoderma* system. This crystalline structure must be overcome before complete hydrolysis can occur. The exoglucanase component is generally thought to act upon this highly-ordered cellulose structure, and indeed it is sometimes referred to as "Avicelase." Wood and McCrae (1979) used isolated exoglucanase and showed that Avicel was more readily attacked than "Whatman cellulose powder." However, in my study, a balanced complex (containing endoglucanase) was assumed to be present. Thus, in the less highly-ordered cellulose powder the decrystallization would be much less important, and the other enzymes would be much more effective. Another factor could be that the surface area of the cellulose powder is greater and thus more susceptible to hydrolysis. TABLE 1 also shows the characteristic lag time of cellulase secretion as induced by cellulose. This lag may be because induction on insoluble cellulose is generally thought to involve very low constitutive levels of cellulase that produce soluble break-down products that can enter the cell and cause induction (Sternberg and Mandels, 1979). This inherent slowness in response time could be the cause of this lag.

There is a great amount of data indicating that the most potent of the cellulase inducers is sophorose, a trace contaminant in glucose (2-O-beta-glucopyranosyl-D-glucose) (Mandels et al., 1962; Sternberg and Mandels, 1979). Sternberg and Mandels (1979) report that sophorose will induce secretion at a concentration as low as 1.5ug/ml, and at 500ug/ml the lag time is from 1.5 to 2 hours. However, they also indicate that the cellulase complex induced by sophorose is deficient in one or more components.

Sophorose was not used as an inducer in my experiments because it is not sold commercially and must be synthesized through a difficult process. Its induction of a incomplete array of enzymes was also undesirable. Therefore, I chose to try lactose as an inducer. Many sources indicated its induction capabilities but also suggested low activities of cellulase and slow rates of induction. Sternberg and Mandels (1979) gave lactose-induced activity as about 1/10 that of sophorose and "at a much slower rate." Mandels et al. (1962) reported that one mg of sophorose yielded 2800 units of cellulase activity whereas one mg of lactose yielded only 12 units. However, using low levels of lactose under my specified conditions I achieved significant induction in

4-6hrs. This is a very positive indication of the utility of my methodology in that lactose is cheap and easily obtainable and, according to Gong et al.(1979), induces a complete complex of enzymes.

As seen in TABLE 2, two levels of lactose, 1mM and 5.8mM, were shown to effectively induce cellulase secretion. The lower concentration was chosen as the best for induction since lactose in the induction medium provides a carbohydrate source that can be utilized for growth by the fungus. Thus, a lower concentration of lactose that also was effective as an inducer was the more desirable treatment.

GRAPHS 1 and 2 are representative of the variability seen in many of the different techniques attempted.

One possible source of early variability was that the mycelium was originally not well washed between growth on glucose and transfer to induction in lactose. Nisizawa et al. (1972) did a representative study showing the effect of catabolite repression that glucose has on cellulase secretion in *Trichoderma*. Glucose clinging to mycelium differentially throughout the mass during filtration that is not washed off could cause parts of the mass to be more glucose-inhibited than others. Hence, variability could be caused in different replicates taken from this mass. However, later thorough washings proved ineffective in reducing the variability.

Another possible source of variability was that of inoculation size. Inoculation was usually 1.5-2g wet mycelium/100ml medium but varied within this range between experiments (replicates were always inoculated with the same mass, + or - .05g). This might account for greater inoculation mass resulting in greater enzyme output. Mandels and Wever (1969) indicate that mycelial inoculation size has little effect on enzyme yields, but they report no data for this. Sternberg and Mandels (1979) used an inoculation size of 1.5-2mg/ml. This is ten times smaller than my inoculation size and suggests testing necessary in future work. However, no such testing was done in this work so no firm conclusions could be drawn.

Another avenue explored was the differential incorporation of mycelium into samples withdrawn from induction medium. Before use of the nylon-webbed tips on sampling pipettes, mycelium would be drawn into the pipette, some receiving more mycelium than others. Then the sample would be centrifuged at 4000rpm for 1 min. to remove the mycelium. However, Berg and Pettersson (1977) suggest that cell-bound cellulases are released upon cell disruption. This centrifugation could have been too harsh, causing disruption of fungal cells and release of cellulase; but if some samples

contained more mycelium, then centrifugation would release more cellulase in these samples. This was combated through the use of the nylon-webbed pipette tips.

Thorough washing and use of filter tips still did not reduce variability to acceptable levels. This variability was finally reduced when experimental design was altered as specified as the best technique elaborated upon in Materials and Methods. This involved the splitting of inoculated induction medium, as well as the mycelium it contained, as replicates, but each was assayed three times for statistical accuracy. This method yields replicates which are much more identical. But I also feel that this method is somehow overcoming a difficulty due to glucose from the growth medium. For example, if thorough washing could not remove all glucose (perhaps small amounts are yet unutilized within the cells) then this could express itself as repressive effects for a short time in the induction medium. In the early "replicate" method, replicates were inoculated with mycelium fresh out of glucose. Replicate activities would thus be subject to a catabolite repression lag which would shortly diminish. In the final method the entire mass was inoculated into one flask of glucose-free medium for at least 4hrs. before replicates were split. This would perhaps allow time for the hypothetical repression lag to diminish. It must be noted that I include this hypothetical case only to demonstrate that the change in experimental design might not have simply resulted in more identical replicates, but that it could have overcome a large source of variability simply by nature of its different methodology.

GRAPH 3 shows the time course of secretion of a trial of the final technique. In some earlier trials of this technique an unexplained leveling occurred after splitting the dual trials. This leveling was not seen when the split occurred earlier (4.75hrs). The time course of GRAPH 3 was seen to have great future utility in experiments requiring 0-10 hours induction.

The cytochalasins have been shown to be active against microfilament-based systems, and this has also been shown true for fungal systems (Grove and Sweigard, 1980; Staples and Hoch, 1982; Thomas et al., 1974). Thus it was thought that cytochalasin B (CB) would be of value in its application to a time course of secretion in Trichoderma reesei. However, Grove and Sweigard (1980) compared the inhibition of spore germination in Gilbertella persicaria by cytoplasmic streaming and cellulase secretion in Achlya ambisexualis, and found that cytochalasin A inhibited both. Cytochalasin A (CA) would thus have been the better choice in the one experiment using cytochalasin. However, CA was temporarily unobtainable in the time available, and it was thus necessary to use the CB on hand. The concentration mentioned by

Thomas, 20ug/ml, was increased to 30ug/ml and two other replicates received 3 and .3ug/ml CB.

TABLE 3 indicates that the activities of the 30 and 3ug/ml CB replicates were lower than those of the control and .3ug/ml treatment. However, the greatly reduced overall activity (by a factor of 10) seems to indicate that an inhibitory effect other than that of CB was masking the results. This could have been due to the dimethyl sulfoxide. As well as serving as a solvent, the dimethyl sulfoxide can solubilize the membrane to allow CB to be taken into the cells. However, this increase in permeability could have allowed a component of the medium, normally desirable, to cross the membrane and alter cellular physiology. It is not that DMSO itself inhibited secretion since it was present only in .4% concentration (when many studies have used 1% DMSO as a solvent with no reported adverse effects). Another possibility could be that the new shaking geometry or other such differences arising from the down-sizing of the replicates could have caused these low activities. This down-sizing was unavoidable since only very small quantities of CB were available (due to expense).

The inconclusive results of this on treatment are disappointing, but there is yet much promise for future work in this area. After design flaws are systematically eliminated, perhaps cytochalasin effects on cellulase secretion in Trichoderma reesei can be characterized.

Acknowledgement

I would like to thank Dr. T.W. Hill for making available to me some preliminary data on Trichoderma reesei obtained during the summer of 1982.

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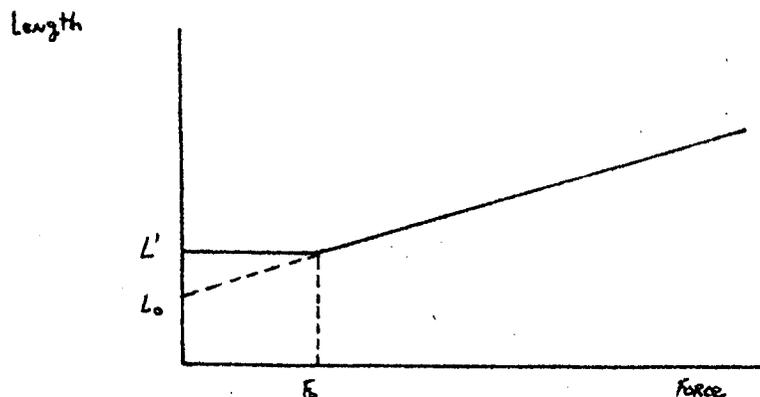
The Zero Length Spring

Scott Budzien*

In the high-technology world of modern physics, learning something new about a simple device can be refreshing. Such is the case with the La Coste zero initial length spring. First developed in 1934 by Lucien J.B. La Coste for use in a seismometer, today the zero length spring is unknown to almost all but geophysicists. However, this little known spring has some interesting properties that are worth investigating.

Though its name might suggest otherwise, the unstretched length of the zero length spring is not zero; the spring looks just like any other "run-of-the-mill" helical extension spring. However, the zero length spring differs from other springs in that its length, rather than its displacement is proportional to the load it supports. Not in any violation of Hooke's Law, this property results from the fact that the La Coste spring is a special example of an initial tension spring.

An initial tension spring is one in which the coils are pressed together by forces within the spring wire. Before the spring can elongate, these forces must be overcome. More specifically, an initial tension spring is wound in the same manner as regular helical extension springs, except that the wire is twisted as it is coiled. The twisting puts a torsional moment in the wire cross-section of each coil, which adds up over the entire spring to yield a translational tension (Andreeva, 1966). This initial tension does not affect the force constant of the spring, and once the threshold tension is reached, the spring elongates like any other spring, according to Hooke's Law.



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To illustrate the way in which an initial tension spring works, consider the length-vs-force diagram above (Bradner, 1972). The length of the spring remains at the unstretched length l' until the initial tension F_0 is surpassed, at which point the spring length changes at a rate given by the inverse of the force constant, called the compliance. Using the point-slope form, the length-force relationship can be described in the region where $F > F_0$ by

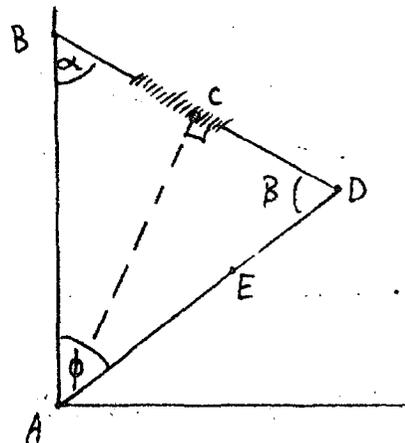
$$(1) \quad (F - F_0) = k (l - l')$$

This relationship makes sense; it is equivalent to Hooke's Law, which states that the extending force is proportional to the elongation produced by that force. In the case of the initial tension spring, the extension force is the applied tension minus the initial tension, which holds the coils together and opposes extension. In both cases the elongation is the difference between the length of the spring and its unstretched length, and the constant of proportionality is the force constant.

Using the slope-intercept form of equation 1, where the initial length is defined as the length-axis intercept, we get

$$(2) \quad F = k (l - l_0) \quad \text{where } l_0 = l' - F_0 / k$$

In one simple step, we can elicit the three most important concepts behind the zero length spring: (1) why it is called the "zero length" spring, (2) what its length-force relationship is, and (3) what specific characteristic makes it a zero length spring. Setting the initial length l_0 equal to zero gives us all of this information. First, the zero length gets its name from the fact that it has a zero initial length--perhaps it should be more accurately referred to as the "zero initial length spring." Secondly, its length, rather than its elongation, is proportional to the applied force when the applied force is greater than the initial tension. Finally, the sine qua non of the zero length spring is that its unstretched length be equal to the initial tension divided by the force constant: $l' = F_0 / k$.



The diagram above depicts the La Coste seismometer (La Coste, 1934). A weight is suspended at the end of the boom AD, which is pivoted at A. An initial tension spring BD is attached to a wall at B. The lengths AD and AB must be equal, and B must be directly above A. C and E are the centers of mass of the spring and the boom, respectively.

Assume that the center of mass of the spring is at the center of BD. Then AC is the perpendicular bisector of ϕ , and $\alpha = \beta = 90 - \frac{\phi}{2}$, since ABD is an isosceles triangle.

Given that the mass of the spring is M_1 , the mass of the boom is M_2 , and the mass suspended at D is M_3 , the total potential energy of the system is due to the gravitational potential energies of the masses plus the elastic potential energy of the spring.

$$PE = PE_{m_1} + PE_{m_2} + PE_{m_3} + PE_{\text{elastic}}$$

$$PE = M_1 g (AD \cos \phi + CD \cos \alpha) + M_2 g AE \cos \phi + M_3 g AD \cos \phi + \frac{1}{2} k (BD - l_0)^2$$

$$PE = (M_1 AD + M_2 AE + M_3 AD) g \cos \phi + M_1 g CD \cos \alpha + \frac{1}{2} k (BD - l_0)^2$$

Let $a = AB = AD$. Let $b = AE$. Then

$$PE = (M_1 + M_2 + M_3) a \cos \phi + M_1 g CD \cos \alpha + \frac{1}{2} k (BD - l_0)^2$$

$$BD = 2 CD \text{ and } CD = a \sin \frac{\phi}{2} \text{ so that } CD \cos \alpha = a \sin^2 \frac{\phi}{2}$$

Regrouping, we get

$$PE = (M_1 + M_2 \frac{b}{a} + M_3) a g \cos \phi + M_1 g a \sin^2 \frac{\phi}{2} + \frac{1}{2} k (2a \sin \frac{\phi}{2} - l_0)^2$$

At equilibrium $\frac{\Delta PE}{\Delta \phi}$ is zero.

$$0 = \frac{\Delta PE}{\Delta \phi} = -(M_1 + M_2 \frac{b}{a} + M_3) a g \sin \phi + \frac{1}{2} M_1 a g \sin \phi + k (a \sin \phi - l_0 \cos \frac{\phi}{2})$$

After collecting like terms

$$(3) \quad 0 = [k a - (\frac{1}{2} M_1 + \frac{b}{a} M_2 + M_3) g] a \sin \phi - k l_0 \cos \frac{\phi}{2}$$

Equation 3, then, is the equation that defines the equilibrium conditions of the system. If the bracketed term can be made equal to zero, i.e., $ka = g (\frac{1}{2} M_1 + \frac{b}{a} M_2 + M_3)$, and the initial length is zero, then the system will be in equilibrium independent of ϕ . This means that every position of the boom is an equilibrium position; wherever the boom is placed, it will stay. On the other hand, if these two conditions are not met, then the spring will have only one equilibrium position.

This system can be looked upon as oscillatory, but possessing an infinite period. This makes good sense. Generally, oscillating systems have one equilibrium position and, after being displaced from that position, oscillate about it with a certain period. In this case, however, all positions are equilibrium positions, so that when the boom is displaced from one position to does not return to it; its quarter-period, thus its period also, is infinite.

As an alternate way of explaining the system's infinite period, consider the following:

The concept of an oscillating system having an infinite period is easily understood in terms of the restoring moment. In this instrument... zero resultant restoring moment is attained because, under deflection, the change in the spring moment due to extension and decrease in the moment arm is exactly balanced by the change in gravity moment due to decrease of its moment arm (Sprengthener Instrument Co.).

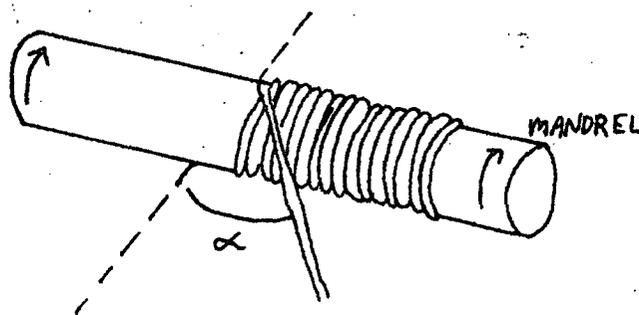
Since the frequency is proportional to $-\frac{dT}{d\phi}$, where T is the restoring torque, the frequency is zero. With the period equal to the reciprocal of the frequency, the period is infinite.

The apparatus described above is essentially the La Coste seismometer, except that the seismometer possesses a long, but finite, period. The finite period can be obtained in two ways: by deviating slightly from the zero initial length or by tilting the apparatus so that the wall is not perfectly vertical. When the apparatus is tilted, the force of gravity on the masses changed with respect to the angles of the apparatus. Tilting is the better method of the two, since it introduces less period asymmetry with respect to the position of the boom (La Coste, 1934). Also to reduce period errors, seismometers use boom positions near the horizontal. Since the initial length is such a critical parameter, small changes in the spring length, such as those due to thermal expansion, can greatly affect the device. For this reason seismometers are usually kept in constant-temperature, constant-humidity environments. In addition to that, the seismometer contains several features to eliminate temperature-induced errors.

An earthquake puts out a broad range of vibrations; the more powerful the earthquake, the more significant become the lower frequency vibrations. The La Coste-Romberg seismometer is used to detect the long-period vertical earthquake vibrations--the earth motion along the horizontal

axes requires a different type of detector. By deviating from the infinite period as described above, the seismometer can be tuned to periods from 20 to 100 seconds. The La Coste seismometer is currently used world-wide in earthquake detection centers.

As part of a student research project, I constructed a La Coste seismometer. I wound the initial tension spring, adjusted it to zero length, and built the seismometer apparatus. Unfortunately, it was not quite as simple as that, or rather, fortunately it was not as simple as that, because I learned a lot of good physics in the process.



THE PARTIAL-
OVERLAP METHOD

Initial tension springs are wound by one of two methods: the twisting method, or the partial overlap method (Andreeva, 1966). In the first method, the wire is actually twisted as it is wound onto the mandrel. In the second, the wire is wound onto the mandrel over the part of the spring already on the mandrel. In falling in line with the rest of the coils, it twists itself. This method introduces less inconsistencies to the initial tension; it is the one that La Coste recommended.

My initial tension spring was wound on a lathe, although the wire was fed by hand. We used the partial-overlap method using a small angle α . It took three tries before a relatively good spring was produced; the first two had a lot of inconsistencies in the initial tension, resulting from variations in the tension applied to the wire as it was wound. This effect is easily observable: in some sections of the spring the coils are pressed together while in other sections they are separated. This gave rise to a force constant that decreased as more tension was

applied to the spring: since the force constant is inversely proportional to the number of active coils, as each section of the spring opened up, a different number of active coils was introduced; hence a decreasing force constant.

Since the spring had straight-wire terminations with hooks on the ends, making measurements was fairly easy. Both the force constant and the initial tension can be found from one set of measurements. A pointer was attached to the lower end of the spring, and the spring was suspended vertically next to the spring. When the spring supported no mass (except its own, which produced no extension), the pointer position was measured, followed by measurements of the pointer position corresponding to several different suspended masses ranging from 450 to 850 grams. The reason for starting with 450 was to overcome the initial tension; that mass produced separation between all of the coils.

The force constant can be obtained by finding the difference of the positions corresponding to two masses. Since the force constant is defined as the elongation produced by a given force:

$$(4) \quad k = \Delta Y / \Delta Mg.$$

After computing the force constant of the spring the initial tension can be found. Recall equation 1:

$$(1) \quad (F - F_0) = k (l - l')$$

In this case $F = Mg$, and $\Delta Y' = (l - l')$ is the same as the difference in the position of the pointer with the suspended mass and the position with no mass.

$$(Mg - F_0) = k \Delta Y'$$

$$F_0 = Mg - k \Delta Y'$$

I had a problem measuring the force constant and initial tension very accurately with this method, because I used a meter stick where the smallest division was the millimeter, too large for the small ΔY produced. This problem was alleviated by later changing to a cathetometer to measure the position, accurate to 0.005 centimeters. Another problem was that the pointer had to be continually adjusted, since the bottom of the spring twisted as the spring extended. This problem was also solved by using the cathetometer. Overall, the extension method produced consistent results--consistently low estimates of k and high values of F , compared to the actual values later obtained. The reasons for this are not clear.

The other method I employed to find the force constant

and initial tension involved oscillation. For any spring the period of oscillation is given by

$$T = 2\pi \sqrt{\frac{l - l_0}{g}}$$

$$g\left(\frac{T}{2\pi}\right)^2 = l - l_0$$

From the definition of l_0

$$g\left(\frac{T}{2\pi}\right)^2 = l - l' + F_0/k$$

$$g\left(\frac{T}{2\pi}\right)^2 - l + l' = F_0/k$$

$$(5) \quad F_0 = kg\left(\frac{T}{2\pi}\right)^2 - k(l - l')$$

Let l_1 be the position of the pointer for mass M_1 and T_1 the corresponding period.

Let l_2 be the position of the pointer for mass M_2 and T_2 the corresponding period.

l' is the position of the pointer for mass of zero.

$$F_0 = kg\left(\frac{T_1}{2\pi}\right)^2 - k(l_1 - l')$$

$$F_0 = kg\left(\frac{T_2}{2\pi}\right)^2 - k(l_2 - l')$$

$$kg\left(\frac{T_1}{2\pi}\right)^2 - k(l_1 - l') = kg\left(\frac{T_2}{2\pi}\right)^2 - k(l_2 - l')$$

Dividing both sides by k and rearranging

$$(6) \quad \left(\frac{1}{2\pi}\right)^2 g [T_1^2 - T_2^2] = l_1 - l_2$$

Let $\Delta Y = l_1 - l_2$. Let $\Delta M = M_1 - M_2$. By equation 4

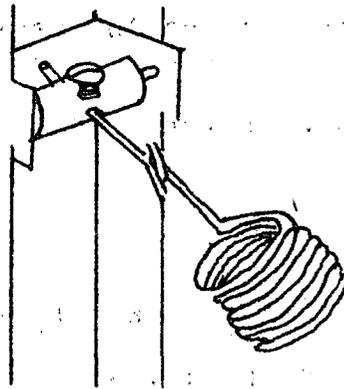
$$l_1 - l_2 = g(M_1 - M_2) / k$$

Substituting this into equation 6 and rearranging yields

$$(7) \quad k = (2\pi)^2 \frac{M_1 - M_2}{T_1^2 - T_2^2}$$

The oscillation periods and pointer positions corresponding to different masses were measured. The force constant was found according to equation 7. Then the initial tension was computed according to equation 5. This method was also consistent, though less so than the extension method. The force constant came out slightly high as did the initial tension. Once again, the reasons for the inaccuracy is unclear.

Consequently, I had to resort to using the "ball park" figures for F_0 and k in trying to tune the instrument. Tuning involves suspending the correct suspended mass to the end of the boom and adjusting the unstretched length of the spring to give an initial length of zero. The length can be adjusted by shortening the lengths of the straight wire terminations. In this case, however, the spring had a negative initial length, which meant that I needed to add wire to the spring. This was facilitated by the hooked ends. (As it is nearly impossible to explain, see the diagram below.) Using equations 2 and 3 with my estimates of k and F_0 , I attempted to determine the proper suspended mass M_3 and unstretched length l' .



ARRANGEMENT
FOR CHANGING
THE INITIAL
LENGTH

Tuning the spring turned out to be essentially a hit-and-miss process. I tried several suspended masses in the range of my theoretical ones, and tried several values for l' for each mass. The tuning process took several hours. The reason for the difficulty is that l' and M_3 both depend on k ; when k is not accurately known, neither are l' and M_3 . The situation is complicated by the fact that the apparatus "wall" must be perfectly vertical. It is almost impossible to know which of these three parameters is wrong, causing the device to have only one equilibrium position, rather than an infinite number. A further complicating factor was that my trial estimation of k was much too high. After a period of time, I finally hit upon the right combination, however.

Surprisingly, the boom was not in equilibrium for all values of ϕ when properly tuned. The boom had a range of angles (approximately $50 < \phi < 90$) over which it exhibited the infinite period property. The reason for this was a varia-

tion of the force constant as the spring extended. This did not result from a changing number of active coils, but more likely from the fact that the force constant varied over the length of the spring; you simply can't expect a "home-made" spring to be perfect.

In summary, then, the zero length spring is a special case of an initial tension spring, whose total length, rather than its elongation, is proportional to its load. The initial length depends upon the unstretched length, the initial tension, and the force constant. When placed in a certain apparatus, a zero length spring turns the system into an oscillating device with infinite period. The device, when modified slightly to yield a long, but finite, period is the La Coste-Romberg seismometer, used world-wide in earthquake detection. Possibly, in the high-technology world of modern physics, the simple zero length spring can find other useful applications.

Acknowledgements

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Polymorphism in Lamium

Rick Sullivan*

Introduction

Lamium amplexicaule is a wildflower that is common throughout most of the United States from Florida westward to California and north into southern Canada. It blooms in late Winter and continues into fall until the first frosts. It can be found in open fields, lawns, and many waste places. L. amplexicaule is commonly referred to as henbit. It is a member of the mint family, Lamiaceae, and exhibits characteristics common to its family such as square stems, bilabiate flowers, and deeply lobed nutlets. The species name "amplexicaule" means "clasping around the stem." This refers to the upper leaves which cling to and encircle the stem (Fernald, 1950).

The leaves of henbit are round to ovate and about 1.2 to 3.0 cm broad with round-toothed margins. The leaves that are directly below the flower clusters are sessile, the blades horizontal or ascending and forming a square around the stem. The lower leaves possess petioles about 1.0 to 4.0 cm long.

Henbit's stems are decumbent, although erect in flowering. They are 15 to 45 cm long and are sparsely pubescent. The stems arise from a single base and branch freely to form many stems that branch from a single tap root. Most of the stalks are arched or ascending.

The bilabiate flowers of henbit grow in groups of 6 to 10 or more in a whorl among the axils of the upper leaves. The calyx is somewhat pubescent and about 5.0 to 6.5 mm in length with long acuminate teeth. The corolla is 1.2 to 1.5 cm long with a very slender and straight tube. The pubescent upper lobe is concave and erect. It arches over two long and two short stamens. The lower lobe is notched in the middle and is quite frequently mottled. The two lateral lobes are short and curve slightly inward.

A second flower form also exists on separate plants. This form possesses a corolla that ranges from 7.0 to 10.0 mm in length. Several other floral parts are also markedly different from the first flower form. This second form is a light color blue; however, the first form is dark blue, almost purplish in color.

The main objective of this experiment was to determine

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whether or not this second flower form is a variety of Lamium amplexicaule or if it is a separate species.

Materials and Methods

The first step in performing this experiment was to obtain a large population sample of Lamium, both those that possessed light types of flowers and those with dark types. The sample was gathered from areas around the Southwestern Campus and its surrounding neighborhoods. When this had been done, the flowers were removed from their plants and three measurements were made on each flower, the length of the corolla tube, the length of the upper lip, and the width of the flower at its widest point. The mean and standard deviation for each group of measurements was tallied, and the various measurements were compared between the two types of flowers.

The next step was to examine the vegetative characters of each plant. This was done in relation to stem and leaf shape, the amount of pubescence on each plant, and the number of flowers per plant.

A third area examined was the pigments in the floral parts. The floral pigments were extracted from the flowers by soaking the floral parts of each flower type in a solution of 85% methanol that contained one drop of HCl (which allowed the pigments to dissolve in the alcohol faster by hydrolyzing the sugar molecules in the pigments). When the flowers had been allowed to soak for at least 24 hours, the pigments were spotted on Whatman 1 chromatography paper, and one dimensional paper chromatographs were run using four solvents: 15% acetic acid; butanol: acetic acid: water (4:1:1); ethyl acetate and water; and xylene: acetic acid (3:1) mixed as 1 part in a solution of 3 parts water. Two dimensional paper chromatographs were then run using the solvent systems that gave the best separation. This was performed by running one side of the paper in the BAW solution, turning the paper 90 degrees and running a second side of the same paper in 15% acetic acid. This caused the dissolved pigments to be spread out over the entire sheet of paper so that the results could be examined more exactly. The pigments in the vegetative material were separated and observed by using two dimensional chromatography in the same manner as the floral pigments.

A fourth area explored was an attempt to see if the seeds would sprout and flower so that I could attempt to cross breed the flowers. The seeds were placed in a petri dish on a piece of moist filter paper and put in a climarium. Both seeds that had been frozen to simulate winter temperatures and fresh seeds were used. This attempt to interbreed the plants was unsuccessful.

Results

Figures 1,2, and 3 illustrate the frequency of measurements for each type of flower in each of the three dimensions measured. Table 1 shows the Rf values for the floral pigments found in each flower.

Discussion

After examining the results, many similarities between the two plants were noticed as well as many very obvious differences that could not be overlooked.

One of the main morphological similarities was the vegetative material of the two plants. The leaf size, shape, and pubescence were indistinguishable between the two plants. Both plants possessed leaves that were sessile and formed a square around the uppermost parts of the stem directly below the flower clusters. The pigments in the vegetative material of the two plants were alike. When a two dimensional chromatography test on these pigments was run, all the pigments were in common. The stems of the two plants were also alike. Both plants possessed stems that were square and sparsely pubescent. The floral parts on each plant were alike. Both plants' flowers had two long and two short stamens. Both plants' had flowers that were similar in shape and form, not in size and color. Both plants could be found in the same areas, mainly around bushes and telephone poles, in large fields and open spots that are not mowed often, and in waste areas such as dumps or junkyards. Both types of plants could be found next to each other with only a few feet separating them. The seeds of each plant were similar in size, shape, and color. The pollen grains from each kind of flower were alike as far as could be seen from light microscopic observation. These similarities indicate that the plants are closely related, if not the same species.

Many obvious differences were also evident between the two flowers. The different flower sizes and colors are the main morphological difference between the two plants. The darker flower is larger than the lighter flower in all dimensions measured (Figures 1,2,3). Some overlap can be seen in all the measurements, but not until two standard deviations from the mean. Another interesting difference is seen in the floral pigments. The darker flower and the lighter flower have identical pigments except that the darker flower possesses one more pigment than the lighter flower (Table 4). This pigment difference could be due to unequal amounts of HCl added to the methanol when the pigments were extracted (resulting in only partial hydrolysis), or it could indicate the presence of an additional pigment. A third difference is the peak blooming time for each plant. The dark

Table 1. Rf Values for the Floral Pigments

Pigment #	Dark Flowers	Light Flowers
1	3, 1.2	3.2, .75
2	3.8, .25	This pigment did not appear
3	8.75, .25	7.0, .25
4	11.5, .5	10.25, .2
5	5.75, 8.5	5.5, 7.0
6	8.9, 8.0	8.9, 6.9

x, y
 x= distance in BAW
 y= distance in 15% Acetic Acid

Figure 1. The Length of the Corolla Tube

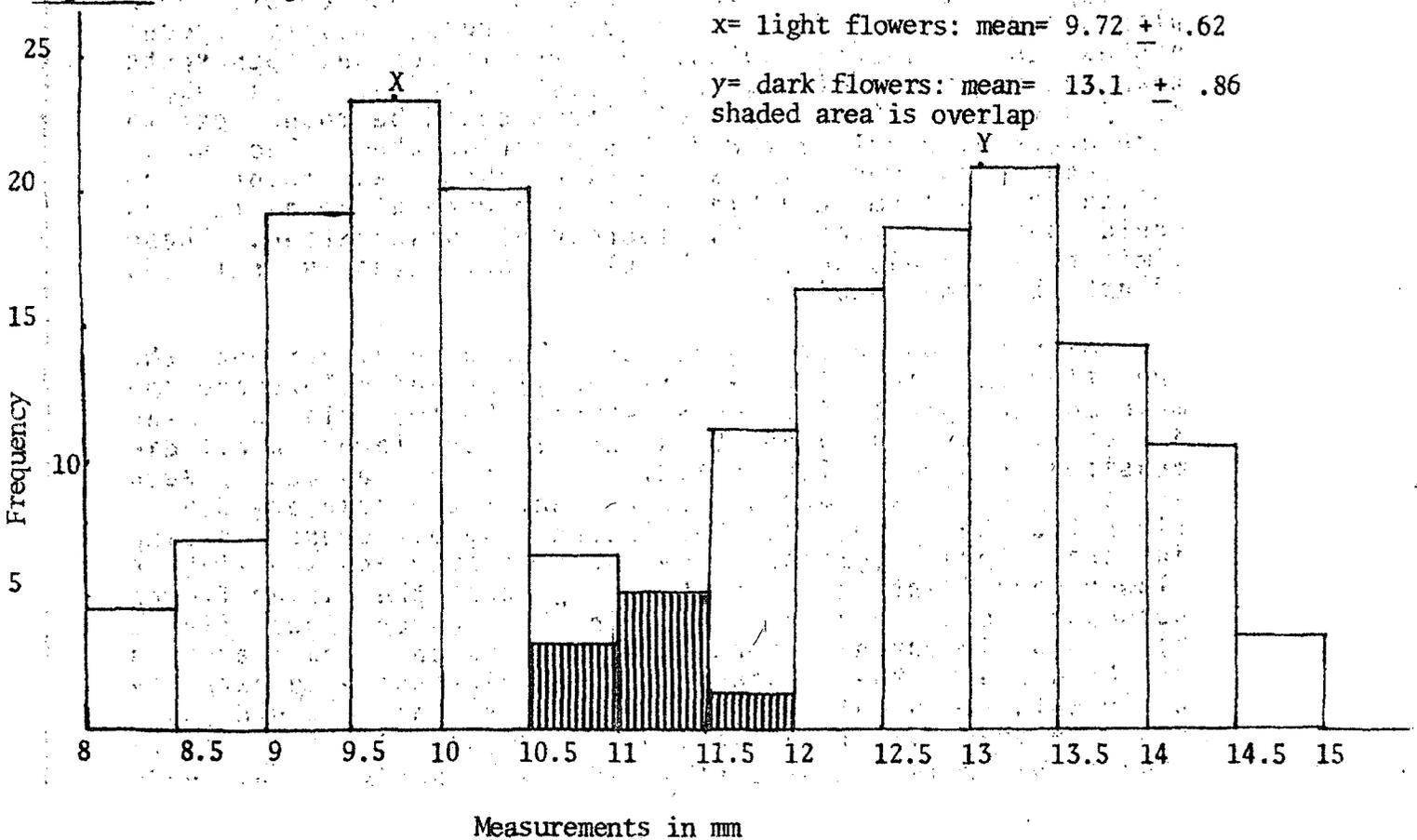


Figure 2 Floral Measurements for the Upper Lip Size

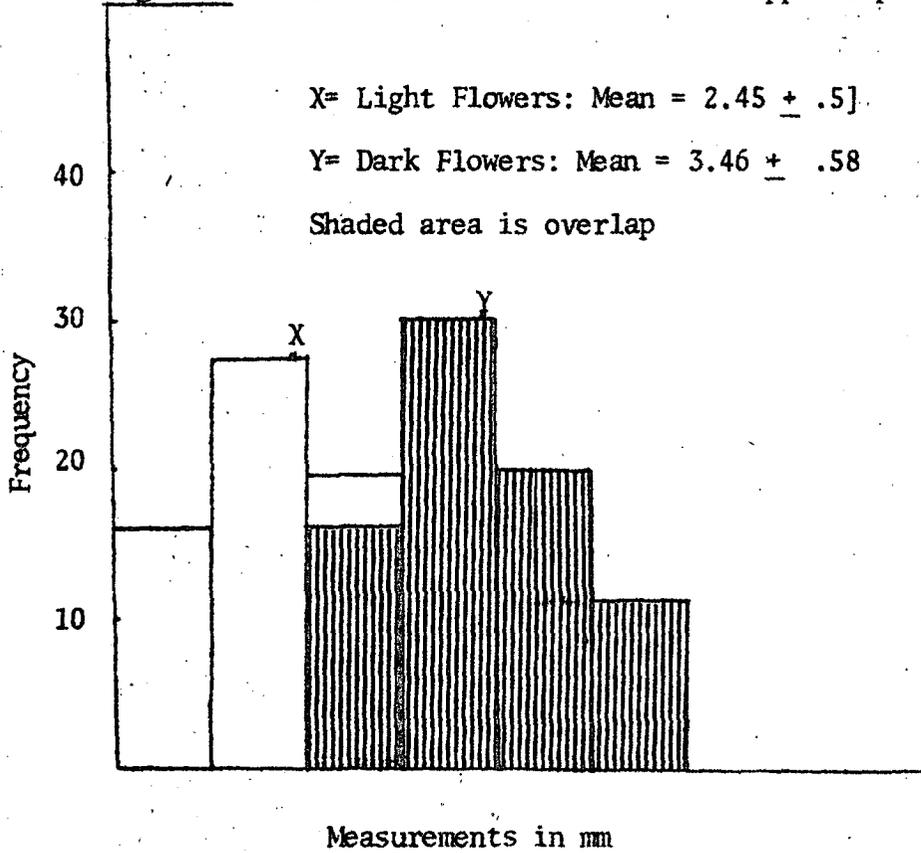
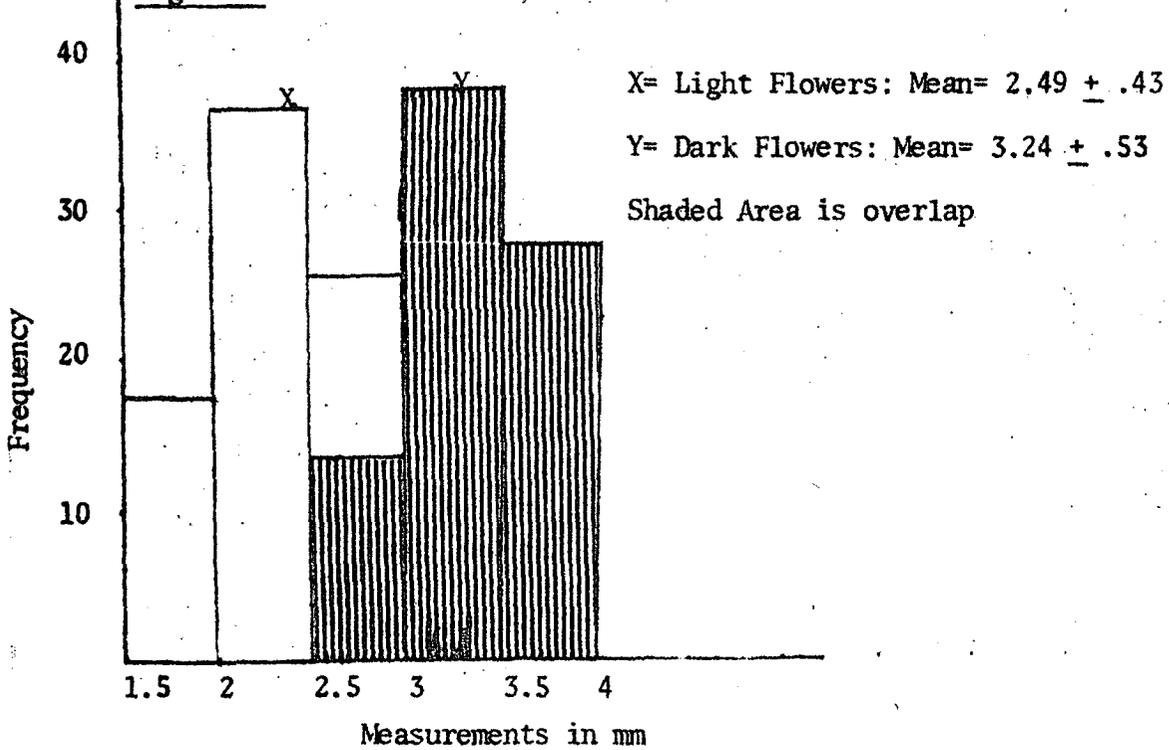


Figure 3 Floral Measurements for the Flower's Width



plants tend to have their peak blooming times on the average about two weeks prior to the peak blooming period of the lighter colored plants. This could be a form of temporal isolation between the plants to prevent reproduction with each other; however, during the entire time when one flower is in bloom, specimens of the other flower can be found nearby. By looking at the differences between the two flower types, one might very well be led to think that the flowers each belong to separate taxa, yet the similarities are too profound to ignore.

Examination of the similarities and differences causes one to ask many questions and formulate many hypotheses. The first question that I asked was whether the light flower matured into the dark flower with age. To answer this question, about a dozen plants with light colored flowers were tagged and observed to see if their flowers grew into darker ones. This did not occur. All of the tagged plants produced light flowers that gradually blossomed, shriveled, and died. None of the tagged plants produced any dark flowers. In order to see if one plant would successfully reproduce with the other, I attempted to grow seeds; however, after many attempts, the seeds would not sprout. This question can be further investigated by attempting to see if reproduction among the two types of plants occurred in nature. I was not able to observe if the flowers were self-pollinated or insect pollinated. Because I do not know whether or not the flowers have a common pollinator, I cannot make any conclusions about interbreeding between the two flower types.

Another hypothesis is that Lamium amplexicaule is polymorphic. That the two separate flower forms are two morphs of the same species. This would explain the similar vegetation, yet it does not fully account for the pronounced difference in the flower forms.

Another equally possible hypothesis has to do with another taxon of the mint family, Lamium purpureum. This plant has flowers that are very similar to the smaller light flowers. This plant grows during the same time and in the same areas as henbit. It has been theorized that hybrids between L. amplexicaule and L. purpureum exist (Fernald, 1950). If this is true, then the smaller plant could be the result of such a cross with vegetation like L. amplexicaule and flowers like L. purpureum. I did not think of this in time to test it. Had it been found earlier, I would have tested the viability of the pollen from the light flowers to see if it had the lower viability that is commonly seen in hybrids.

The error factor in this experiment cannot be overlooked. The measurements in the floral characteristics must be made with a large degree of precision. The solutions

that were made for the chromatography tests must be made accurately, and the tests run so as to minimize the chances of problems such as too large a spot on the paper or allowing the solvent to run off the paper. One error that I know I made was in measuring the flowers. I would measure flowers that had been freshly picked and some that had been frozen until time was available for measurements to be made. The flowers that were frozen tended to have smaller measurements than the others simply due to their time in the freezer. This caused the averages to be slightly lower than they should have been. This did not play a great role in comparisons because equal numbers of both flower types were frozen and measured; therefore, the measurements of all the floral parts are down slightly from the norm for both types of flowers.

In making conclusions, it is necessary that I take all things into consideration. I do not feel that I have found enough information to classify these two plants as two separate taxa; however, I feel that I have found differences that are valid and important between the two flowers. The hypothesis that Lamium is polymorphic is the one that I favor. I feel that this hypothesis accounts for both floral differences and the peak blooming season differences. I have really only opened the door into this problem. Many new ideas and factors must be considered and proven before this or any of the other hypotheses can be considered definite.

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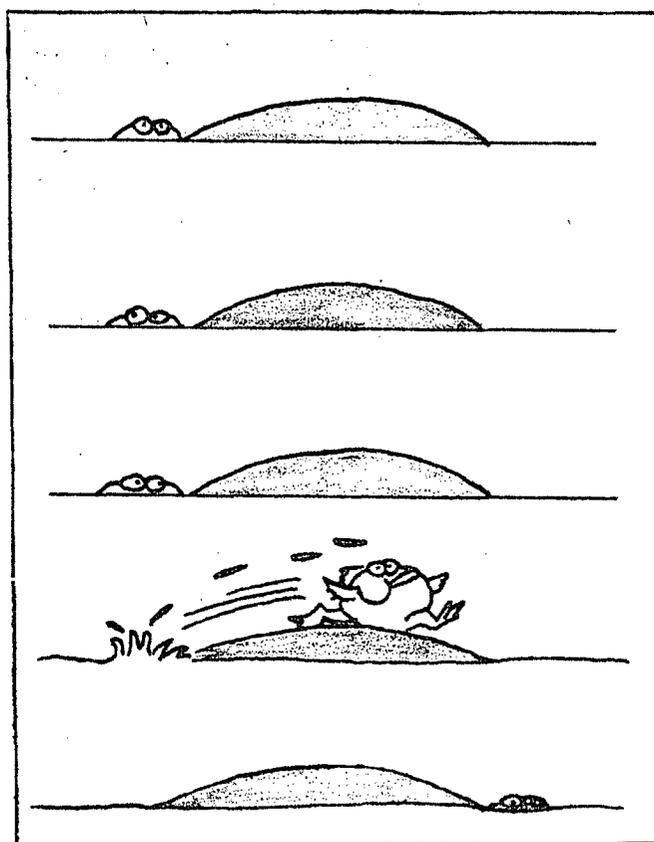
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THE FAR SIDE

by Gary Larson

Another
great
moment in
evolution.

The Measurement of Interspecific Competition

John Pardue*

Introduction

The role of interspecific competition in the structuring of natural communities has recently become the focus of an intense controversy among population biologists and ecologists (Schoener 1982). Competition, defined as the use of a resource by an organism which reduces the availability of the resource to others (Ricklefs 1976), has long been seen as the dominant ecological interaction between species (MacArthur and Levin 1967). Recently, however, the absence of conclusive quantitative data and the recognition of the importance of other biological interactions have led to the reexamination of some of the basic tenets of the competition theory. From this debate two camps have emerged. One emphasizes the relative importance of competition and other biotic factors (e.g. predation), and the other which deemphasizes these biotic factors in the structuring of natural communities.

Present competition theory is comprised of several basic principles. Thomas Schoener summarized these in his excellent review article (Schoener 1982). First, species too similar in their resource use cannot coexist for long; one will competitively exclude the other. Secondly, species can coexist because of sufficient differences in their resource use. Third, interspecific competition is a major evolutionary force, selecting for adaptations that result in species with little resource overlap. Fourth, species distributions are often determined by competitive pressures. Fifth, species may compete by interference, as well as by depletion of resources. However, interference is unlikely to evolve if resources are not sufficiently scarce.

The primary reason for the reevaluation of this theory is its inability to consistently explain and predict natural situations (Wiens 1977). Much of the early work done in this area lacked any systematic raw data of any kind and was consequently largely theoretical. The lack of raw data, however, cannot be blamed solely on the early researchers who undertook the study of competition. Competition, being an abstract concept, is very difficult to measure quantitatively. This paper will discuss recent advances in the measurement of interspecific competition, concentrating on the application of the experimental method to field work. Three examples of this work dealing with different types of organisms will be discussed.

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Mathematical Methods

Early attempts at the measurement of competition were largely modifications of the Lotka-Volterra equation. This equation had previously been used to measure rates of change of a population with a given carrying capacity. In this form of the equation the rate of population change undergoing competition is modified by a competition coefficient. This abstract, dimensionless coefficient is a relative measure of competitive ability and is used to mathematically translate the effect of individuals of one species on another's growth rate.

The idea of the competition coefficient was defined further by MacArthur and Levins who sought to quantify the idea that species cannot be too similar in their resource use and still coexist. They developed an equation which added a meaning to the competition coefficient. This equation states that the competition coefficient is equal to the sum of the fraction of this resource h used by species i , multiplied by the fraction of this resource h used by species j , divided by the square of the fraction of resources used by species i .

$$\alpha_{jh} = \frac{\sum (p_{ih} \times p_{jh})}{\sum p_{ih}^2}$$

This equation has the property by which the greater the overlap of resource use by species i and j then the larger the value for the competition coefficient (Schoener 1982).

These mathematical models have been used to make many predictions about natural communities. Unfortunately, these equations are flawed by the assumptions that have to be made in order for the calculations to work. These assumptions are that competition is the only structuring force affecting ecosystems and that resource levels are fixed. These limitations put a restriction on the validity of the predictions made by these equations and consequently a better method of "measuring" competition had to be developed.

The Controlled Field Experiment

I have already mentioned the difficulty involved in measuring such an abstract concept as interspecific competition. The competition process cannot be observed per se but its relative intensity can be determined by quantifying the effect on the population densities of the organisms being studied. The intensity of competition can be evaluated by manipulating the population densities of two species which are known to overlap in resource use. For instance, one could add members of species i to a given area and note the effect on species j . Likewise, one could remove members of

species i and see if species j responds with an increase in population density. Lotka and Volterra were correct in supposing that competition affects populations by decreasing their rate of change. However, it cannot be considered the sole factor, and the most accurate estimate of its intensity is obtained through field experiments. The usefulness of these field experiments has become so well documented that their strategy is often included as part of competition theory itself (Schoener 1982).

The controlled field experiment was popularized by J. H. Connell, a Scottish ecologist who studied the distribution of two barnacle species on the rocky intertidal zone (Connell 1961). By the use of population manipulation he determined that a special type of competition, interference competition, was occurring for space on the rocky intertidal. More important than the information he obtained about barnacles was the realization that competition could be studied in this manner. Later, Connell (1975) outlined the basic features of the field experiment. First of all, the essential aspect is that everything varies in the same way between treatment and control except for the manipulated factor, in this case the population density. Second of all, because of the inherent impossibility of finding control plots identical to experimental plots, replication is necessary. Thirdly, the effect of the experiment must be taken into account. For instance, if the enclosure or cage excludes predators then this must be evaluated along with the results.

Obviously the organisms which can be studied in this manner are limited. The organisms must be relatively non-mobile; (e.g. the use of the controlled field experiment for the study of bird competition is inappropriate). Nevertheless, the results of a very large number of field experiments has been recently published and many more are in progress. Preliminary synthesis of the ideas obtained by this work seem to confirm the relative importance of competition as an important biological interaction. However, it has also revealed that predation and environmental variability play an equally important role in some ecosystems (Schoener 1982). I will now discuss three recent attempts at the measurement of competition. These three examples emphasize different types of relationships and illustrate the problems of performing a controlled field experiment.

N.G. Hairston and Salamander Competition

Interspecific competition is assumed to almost always occur between congeneric species. This is the case because closely related species will have similar resource requirements. Therefore, these species will have a greater chance of being in competition.

In the late 1940's, while studying the local distribution and ecology of Plethodonid salamanders in Appalachia, Nelson G. Hairston, Sr. discovered a situation which seemed to fit the prevailing competition theory perfectly. Two species of terrestrial salamanders, Plethodon glutinosus and Plethodon jordani were found to have altitudinal distributions that overlapped very little on any given transect in the Black Mountains of North Carolina. On the other hand, in the Balsam (N.C.) Mountains, the two species were found to overlap by a large amount. Hairston concluded, by observation, that interspecific competition was more intense in the Black Mountains than in the Balsams. In the Black Mountains, competitive exclusion allowed only a narrow band of overlap (Hairston 1949).

About 25 years later after the situation was discovered, Hairston began to feel that his conclusions were invalid without the benefit of experimental evidence. In 1960, in fact, he had proposed in his classic work with Smith and Slobodkin that predation rather than competition was primarily responsible for community structure of organisms of intermediate trophic level (Hairston, et al. 1960). In 1974 Hairston began a series of field experiments that would measure the intensity of interspecific competition between the two species.

Simply, what Hairston intended to do was a series of removal experiments on random plots. At night he would systematically attempt to remove the total number of one of the species from an experimental plot. Later in the year he would return to census the plot to determine any response. The Plethodons in the area of narrow overlap should show a greater increase in population than those in the area of greater overlap. The intensity of the competition was reflected in the numerical response of the species released from its competitive interaction. The results of Hairston's experiments confirmed this hypothesis (Hairston 1980). The response of P. glutinosus to the removal of P. jordani was more prolonged and intense in the area of narrow overlap. These results, obtained from five years of experimentation, confirmed Hairston's original conclusion that interspecific competition was responsible for the structuring of these communities.

Rodent and Ant Competition in the Sonoran Desert

As I stated before, it is virtually assumed that interspecific competition occurs between congeneric species because of niche similarities. Recently, however, several biologists (Brown and Davidson 1977; Wright 1979) have hypothesized that it is not unlikely that very distantly related species could compete for a certain limiting resource. James Brown and Diane Davidson, in an attempt to determine

the extent of such competition, studied the relationship between seed-eating desert rodents and ants in Arizona. The study was conducted as a series of manipulation experiments attempting to determine the intensity of this possible competitive interaction (Brown and Davidson 1979).

Over a two year period they demonstrated reciprocal increases in rodent and ant densities on 0.1 ha plots from which the other taxon had been excluded. Ants were removed by poisoning the nests. The rodents were excluded by fences. The number of ant colonies increased 71% on plots where rodents were excluded. Brown and Davidson also compared seed levels in the soil and annual plant densities on the experimental and control plots. They concluded that there was strong evidence that seed was the limiting resource for both organisms.

The problems involved with this experiment were more complex than in Hairston's. The fences required to exclude rodents may have changed the normal interactions of the ants. In addition, rodent and ant censusing probably disturbed the microhabitats of the respective species. Initial differences between the experimental and control plots were not determined. On the basis of this experiment even Brown and Davidson were wary of definite conclusions. As the complexity of the field experiment increases, the accuracy and validity of the results tend to decrease.

Competition Among Darwin's Finches

Perhaps the most famous and most widely studied competitive interaction occurs on the Galapagos islands in the Pacific. Finches, of the genus Geospiza, have evolved different beak sizes and shapes in response to food selection pressures. This, in turn, affects the distribution of the various species on the archipelago. These interactions were first reported by Darwin in The Origin of Species. Later, David Lack, the British ornithologist, presented a theoretical framework for understanding the adaptive radiation of the finches in his work, Darwin's Finches. The raw data to ultimately confirm this early theoretical work is being gathered now by ecologists Peter R. Grant, Rosemary Grant, and Dolph Schulter at the University of Michigan.

The question of what originally structured this community is not really in question. There is practically universal agreement that it is the result of adaptation to different food availabilities. What is in question is whether competition is maintaining this arrangement or is it simply the result of chance.

Some ecologists, such as Peter Grant, believe that as more species arose, (there are now 14 in all), competitive

interactions became more important (Lewin 1983). There are certain regularities in the finch population (e.g. the absence of closely related coexisting species) that seem to be the result of resource competition. Gathering data to prove this competition, however, is a difficult task. Connell's manipulation experiments are useless with birds. The method used by several of the Galapagos researchers is one described by Connell (1975).

This approach consists of finding a "natural experiment". For example, if two species are distributed along an altitudinal gradient, one hypothesis is that one species is excluding the other through resource competition. This hypothesis is tested by finding the same community in the field with one of the species absent. If competition is determining the boundary, the species should extend its range of resource use in the absence of a competitor. When this method was applied in the Galapagos the emphasis was on food preferences and availabilities. If food preference alone is maintaining the community then these habits should be the same regardless of the presence of the other species. This was the basic premise behind a study done by Grant and Schuller (1982).

Grant and Schuller studied the feeding preferences of one finch, Geospiza difficilis, on the Galapagos islands of Pinta and Genovesa. G. difficilis exists on the lowland island of Genovesa alone. On Pinta island, however, G. difficilis is present in the highland region and overlaps with a lowland finch, G. fuliginosa. The diets of the G. difficilis were found to be different on the two islands. Both populations ate seeds but the highland Pinta population placed a greater reliance on insects. Because Grant and Schuller found that the lowland area of Pinta is very similar to Genovesa in terms of available food types, this contradicts the hypothesis that food preferences alone are determining community structure. Moreover, G. fuliginosa is absent from Genovesa, a situation not explainable by the absence of any food type.

Grant and Schuller obtained these results from systematic, detailed observations of behavior and abundance. They made their conclusions on the basis of very detailed autoecological data (diet information, food densities, food handling time, etc.). This approach has been seen as the logical alternative to the experimental method. However, their measurement of competition has been less than convincing. Grant and Schuller have shown that certain finch species seem to be excluded from some areas. However, they have not documented changes in population density (e.g. Hairston). In this situation it is almost impossible to do so. Their work with Darwin's finches emphasizes the ideological difficulties involved in interspecific competition measurement.

primarily that the measurement of resource overlap or exclusion does not prove that competition is affecting population densities. Until this is documented, the null hypothesis will always stand alongside the competition theory.

Conclusions

There are many inherent problems in the study of interspecific competition. I have already mentioned the danger of altering the habitat during experimental tests as well as the impossibility of finding identical control and experimental plots. Another problem is the short amount of time usually allowed for such field experiments. Evolutionary processes are very long events and are rarely detected during studies of this short length.

An even greater problem is the nature of the competition process itself. Competition is an historical process. It may have occurred in the past and not be evident now at all. When the researcher manipulates the population by reintroducing competition, the organisms involved are different from their ancestors. They have evolved as the result of the competitive process that structured the community in the first place. Whether this historical competition can be measured experimentally is a philosophical problem.

Another consideration is the idea of the variable environment. Since almost all resource levels are in a constant state of flux it has been hypothesized that competition is important only during times of ecological "crunches" when a limiting resource falls below a certain level (e.g. Dunham 1980).

The controlled field experiment, therefore, has two time constraints. First of all, competition, being a historical process, may or may not be in operation. Second of all, it is conducted at a time of given resource availability due to environmental variability. Thus, the results of the experiment are largely determined by these two factors.

Despite their shortcomings, the controlled field experiment seems to be the most useful and accurate method for measuring interspecific competition. It seems essential at present to demonstrate that competition remains an important process and worthy of study. More quantitative methods, if they are indeed possible, will undoubtedly be developed as the process becomes more understood.

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The Physiological Aspects of Stress

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Purpose

In this paper, I intend to explore the effects of stress on the body. Rather than emphasizing only one area of physiology, I want to show the effects in a more holistic frame. Two major issues I will address in this paper are the immediate physiological reactions and psychological stress. There are several reasons for this latter emphasis: 1) a great deal of experimental research has incorporated psychological stress in the form of math test for humans or immobilization for rats; 2) there is a lessened possibility of outside factors producing results (see later); and 3) it is an area I plan to research further in the future.

Justification

To understand the importance of a knowledge of the effects of stress, one must first know what stress is. Stress can be defined very simply as an organism's nonspecific response to any demand made upon it (Vigas, 1980), a type of stimulus-response reaction of the body. This definition of stress is extremely broad, so to reasonably discuss stress, we must limit either the type of stress (cold, psychological, shock, ether, etc.) or the type of response (metabolic, hormonal, immunological, etc.). However, it is clear that practically any bodily stress response (including death) can be the immediate result of a given type of stimulus. Usually the result is not so very traumatic as death, but some of the changes in homeostasis can be quite large.

Stress, and especially repeated or long-term stress, can also have long-term pathological effects. The most studied effects in recent years have been in the cardiovascular (hypertension) field and the psychological (anxiety) field. Aside from these areas, effects of stress such as ulcers, spastic colons, and even susceptibility to disease are widely studied.

Research Problems

In attempting this type of research, two major problems present themselves: the research material itself and the source of experimental stress. There are literally volumes of sources of stress research, but since stress is such a broad field, this requires a researcher to sort through a large amount of information in order to get data pertinent

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specifically to his area of study. This paper is concerned primarily with research which was performed mostly in three different decades: the 1930's, the 1950's, and the 1970's. The huge majority of research since the '50's is directed toward either long-term (pathological) effects of stress or the stress effects of specific or combined substances (drugs) injected into a subject.

The source of experimental stress is important to consider. If the source of stress is some kind of chemical (e.g. ether), it may produce some chemical reactions specific only to that particular chemical. If it is pain or injury related (e.g. operations or electric shock), there would certainly be very specific healing attempts made by the subject's body in addition to standard stress responses. To avoid these specific "side effects," I decided to concentrate on psychological stress, since it is a result of fewer external physical stimuli than are most of these other types of commonly used experimental stresses.

Findings

The total physiological reaction to stress, both acute and non-acute (either repeated or chronic), was thoroughly delineated by Hans Selye in his "General Adaptation Syndrome" (GAS) which first appeared in its totality in 1950 (Selye, 1950). As he describes the GAS, he divides stress sources and stress reactions into specific groupings, and one soon realizes that the GAS is like Plato's ideal chair: it exists in theory, but in reality it never exists in totality. This does not discredit the GAS, it merely reminds us that it is a guideline, not a hard and fast rule.

In the GAS, Selye defines stress as having three stages: "Alarm-Reaction" (AR), the "Stage of Resistance," and the "Stage of Exhaustion." (Selye, 1950). The immediate physiological response occurs in the AR; both of the other reactions present themselves later and are a kind of return to homeostasis. Since I am investigating only the immediate effects of stress, I will explore only the AR, and will do so through experimental research in four major areas: physical, metabolic, immunologic, and hormonal.

Physical

It is practically common knowledge that when under stress a subject will experience an increase in heart rate (HR) and blood pressure (BP). One further change which is not quite so well known is that the subject will also experience a rise in skin conductance level (SCP). A study performed on human subjects determined the changes in these three states simultaneously along with changes in epinephrine (E) and norepinephrine (NE). In this study, the sub-

jects, who ranged in age from 22 to 55, were seated in a sound-attenuated air conditioned room and were subjected to varied stimuli with a three minute rest period after each stimulus. The stimuli were all short-term and either emotional or cognitive, ranging from listening to music to performing mental arithmetic (subtractions of 7 from 500 to 0) to an anticipation of venipuncture. The recording equipment was in an adjoining room. Electrodes recorded the HR and transducers were affixed to measure SCL. An indwelling catheter was inserted into the antecubital vein for measurement of the hormones. The different stimuli caused varying degrees of mental distress (as determined by the unpleasantness of the situation as described by the subjects) and produced varying changes in the levels of the five determinants. The mental arithmetic produced the greatest changes in the determinants: HR rose from 71 to 110 (beats per minute), SCL rose from 50 to 70 μscm , and BP showed a significant increase. SCL showed a significant increase for all stimuli except music, whereas very significant increases for HR and BP were found only in response to mental arithmetic. NE showed an increase for all stimuli, whereas E showed significant increases for only venous catheterization and mental arithmetic (Engel, 1980). Thus we find SCL to be a determinant of whether stress is present, and HR and BP to determine the presence of severe stress.

Metabolic

Selye reports findings that acute stress will at first decrease and then raise above normal the basal metabolic rate. He attributes this to anterior-pituitary extracts [sic], particularly thyrotropin and corticotrophin (ACTH). The AR is characterized by an initial hypothermia followed by hyperthermia. Blood glucose is utilized at an increased rate, while, at the same time, hepatic glycogen is being transformed into glucose at a rapid rate (Selye, 1950). There is also an increase in pituitary glycogen. Glycogen was found to decrease by approximately 30% in the anterior pituitary and by approximately 50% in the median eminence (Jacobowitz, 1964). Gluco-corticoids in the liver are found to cause an increase in gluconeogenesis which, rather than being a transformation, increases the body's total carbohydrate content. Glycogen is also seen to be depleted in the muscles. A rise in lactic acid is seen in the muscles as a result of the fact that glucose is being metabolized (fermented) faster than oxygen can reach the site of metabolism. In fact we see that although glucose metabolism increases, arterial oxygen concentration does not (although venous oxygen concentration is greatly decreased). Lactic acid can also be found in increased concentrations in the brain and urine (Selye, 1950).

Lipids are found to be metabolized more quickly as are

carbohydrates. A sudden loss of stored fat is characteristic of the AR stage. It is often accompanied by a temporary transfer of lipids from adipose tissue into the liver. Blood lipids are found to decrease (Selye, 1950) as is serum cholesterol (Francis, 1981).

We also see a rise in plasma levels of non-protein-nitrogen as a result of increased protein catabolism, which diminishes the tissue-protein reserves (Selye, 1950). Also notable during the AR stage is a drop in serum uric acid (Francis, 1981) which accompanies a diuresis (caused by E) (Selye, 1950); a rise in serum cortisol (Francis, 1981); and the rise in elimination of corticoids in the urine (Selye, 1950).

Immunologic

The effects of stress on the immune system produce varied and sometimes opposing consequences. Most of these consequences are produced in an individual suffering repeated or chronic stress, but some rapidly occurring effects of acute stress can be seen. Agglutinins and hemolysins have been found to form after the AR brought on by ultraviolet radiation. Varying stressful stimuli can produce different food allergies or even anaphylactic shock (Selye, 1950). Some corticosteroids released during stress are immunosuppressant (Miller, 1980). There is a rise in total white blood cell (WBC) numbers, but this is found to be due to a dramatic increase in polymorphs which is accompanied by drops in numbers of lymphocytes and eosinophils (possibly caused by release of ACTH and glucocorticoids). Red blood cell numbers are also found to increase during AR (Selye, 1950). Although the number of WBCs increases, their ability to phagocytize heat-killed bacteria is lowered during stress. Interferon production is increased by stress, but it was also found that whereas low doses of E increased the interferon response, high doses of E produced a decrease in that response (Miller, 1980). Another study concerning longer-term combat stress found decreased numbers of WBCs among the attackers and no change in WBC numbers among the defenders (Gal, 1975).

Hormonal

As it is nearly impossible to discuss any physiological reaction without including hormones, I have already touched on some hormonal changes as results of stress. I would hesitate to attempt to include every hormonal reaction to every type of stress since these data would easily run into the thousands, therefore, I will cover the major stress occurring hormones and groups of hormones and their respective effects.

Epinephrine and Norepinephrine

Low levels of E and NE are normally found in the blood. Different types of stress produces differing increases in the levels of E and NE. Through both urinalysis and tail vein catheterization the responses of E and NE to stress has been tested in rats. Kvetnansky, et. al., found that forced immobilization produced a 40-fold increase in E and 6-fold increase in NE. Decapitation produced an 80-fold increase in E and an 8-fold increase in NE (Kvetnansky, 1978). After 90 minutes of immobilization there is a marked decrease in E lasting for 24 hours, while NE level does not decrease to any significant degree (Kvetnansky, 1970). They found through further investigation that initially the rate of E and NE production does not increase, but after repeated stress, the rate of production of both increases significantly (Kvetnansky, 1971). Palkovits, et. al., found that stress caused the brain NE concentration to either decrease or remain unchanged in all areas of the brain except for the median eminence, where there was a very slight increase; his conclusion from this was that these findings showed that NE was being released in the system (Palkovits, 1975f).

Both of these hormones have strong effects on the system. E increases the basal metabolic rate, raises the body temperature, produces a transitory hyperglycemia, produces a hyperlactic effect, causes diuresis and counteracts the antidiuretic effects of vasopressin (Selye, 1950), and raises the BP (Kopin, 1980). E is released from the adrenal cortex specifically, whereas NE is a product of the sympathetic nervous system in general. NE has been reported to decrease ACTH releasing factor (CRF) and thus inhibit the secretion of ACTH (Palkovits, 1975). A study of men and women exposed to identical psychological stress showed relatively unchanged NE levels for both sexes, but the E level for men nearly doubled, while it remained unchanged for women; however, in self-ratings, women expressed significantly greater distress and unpleasantness than the men. The conclusion of the researchers to this data is that the response to stress is not the same for men and women (Frankenhaeuser, 1976).

ACTH, CRF, and Corticosteroids

Two minutes after immobilization stress, CRF reaches a peak value and we find the peak of ACTH to occur 3 minutes after that (Sakakura, 1976). The increase in ACTH secretion when produced by stressful stimuli is almost completely mediated by neural pathways and CRF secretions from the hypothalamus (Ganong, 1980). This increase in plasma ACTH is found to occur with an increase in Pituitary ACTH, followed, an hour later, by a decrease in pituitary ACTH to 50-60% of original ACTH levels (Vernikos-Danellis, 1963).

Corticosteroids are found to increase significantly as a result of immobilization (Jindra, 1980). The plasma ACTH is found to be inversely proportionate to and dependent upon plasma glucocorticoids (Butte, 1972). Subsequent studies show that the turnover of ACTH is quite slow, and the stress-induced ACTH secretion represented newly synthesized, rather than stored, ACTH (Marks, 1963), (Goldman, 1963), and (Jacobowitz, 1963). ACTH secretion is increased by the presence of B-endorphin as well (Van Loon, 1980).

ACTH has been seen to raise the glycogen content of the liver, stimulate corticoid secretions, (Selye, 1950) stimulate the memory process in mice, (de Wied, 1980) and possibly to increase the blood flow around the adrenal gland, and therefore, causing an increase in adrenal secretions (Stolk, 1980).

Dopamine (DA) and Dopamine-B-hydroxylase (DBH)

Both are seen to increase as a result of stress. Plasma DBH increases approximately 25% after 5 minutes of immobilization, (Kvetnansky, 1978) and will increase further after repeated immobilization (Kvetnansky, 1971). Brain DA will increase 35-45% over normal brain DA levels (Goldstein, 1980). DA is known to inhibit the release of ACTH (Tilders, 1980). DA is also a NE precursor (Kvetnansky, 1980).

Others

Other hormones which have relationships with stress are serotonin, vasopressin, and acetylcholine. Serotonin is a regulator of neuronal function. Its most likely function in stress is to modulate the negative feedback mechanism regulating the pituitary-adrenal function (Berger, 1974). A slight rise in serotonin is seen after 30 minutes of immobilization (Kiss, 1980). Vasopressin is released during stress to control blood osmolarity, pressure, and volume (Makara, 1980). Acetylcholine stimulates corticoid production. It also causes muscle fiber contraction during states of stress (Selye, 1950).

Summary

We can see from the data examined that stress produces a reaction from every quarter of the body. These reactions also have interreactions with each other, creating a complex system of feedback mechanisms. Ultimately, the need for stress reactions is to increase the survivability or adaptability of the individual during a stress situation. These reactions are normally short-term reactions which return rather rapidly to a previously unstressed level of homeostasis. But when stress is repeated or chronic, these stress reactions can cause pathological problems in the individual.

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The Ethical Aspects of Abortion

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It is the purpose of this paper to discuss the decision-making process involved in the choice to have an abortion. I write this paper after three months of counseling abortion patients and discussing with them the decision to have an abortion. I would like to point out that by the time most of the women actually make an appointment and reach the clinic, they are firm in their decision. If not, an abortion will not be performed until they have decided for themselves that they are sure this is the choice they should make.

A woman who finds herself with an unwanted pregnancy usually finds herself in a moral dilemma, trying to decide whether to carry the pregnancy full-term or terminate it. John Lemmon in his article "Moral Dilemmas" discusses five different kinds of moral dilemmas, and while he does not tie these in with abortion, I believe several of these dilemmas represent what a woman goes through in facing an unwanted pregnancy.

The first situation is where a person feels that he or she ought to do one thing and also that he or she ought not to do that same thing. This is commonly the case with pregnant women who feel that they do not want to carry this pregnancy any further, but wonder about whether or not they should actually have an abortion. Lemmon states that this kind of choice is usually made by considering the ends of the decision. Although a woman may feel that she is responsible for being in a pregnant state, she feels strongly that she should not carry the pregnancy full-term, and decides to terminate the pregnancy.

The next situation is similar to the first, but slightly more complex. It is the case where there is some inconclusive evidence that one ought to do something, but also some inconclusive evidence that one ought not to do that same thing. In this situation, one cannot rely on one's duties or pre-assigned moral solutions, because part of the dilemma is trying to decide these duties, obligations and moral solutions. An example of someone in this situation would be a young woman who finds herself pregnant before completing school. Her boyfriend says that he will marry her and give the child a father, but he is not yet out of school and could not make a good living. The young woman is torn between giving up her school and the career she wanted, entering into a marriage she is not sure of, and keeping her child, or having an abortion and staying in school. She

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feels that she has a duty to the fetus, but also feels a duty to herself. This situation is compounded even more when the young girl has experienced birth control failure. Lemmon states that this decision is serious enough that no matter what the choice is, it imparts a changed moral outlook on the person making the decision. Lemmon does not give an easy way out of this kind of dilemma, but states that it must be thoroughly reasoned out.

The next dilemma that Lemmon mentions is the situation where a person has to make a moral decision that he or she is totally unprepared to make. This type of dilemma is rare in the case of abortion, because before most women even know they are pregnant, they have thought about alternatives they would face if they were to become pregnant. I do believe this happens occasionally in the case of a young teenager who becomes pregnant. She does not know much about birth control, pregnancy, or abortion, and often turns to someone else for help. The girl usually avoids making the decision herself and when questioned states that an abortion was her parents' or boyfriend's idea. In this case it is necessary to discuss the girl's feelings. In most cases, the girl comes to the conclusion that this is what she should do, but occasionally it comes out that she wants to keep her baby and the abortion is not performed. Lemmon states that even though resolution of this problem is painful, it leaves the agent stronger and forces them to develop a new morality.

I would like to move on from here into a discussion of the two basic types of ethical systems and see how these are used in making the decision to have an abortion.

Utilitarianism is a kind of teleological ethical system which states that an action is morally right if the person's doing it brings about good consequences. The general point here is that the goodness or badness of the consequences of an action determines whether the action is right or wrong. In this type of system the consequences of an action include everything that happens as a result of a certain action.

Utilitarianism goes a step further than the teleological system to focus on whether or not an action produces a useful result. An action is wrong if it is useful in bringing about a bad end. The basic principle to be followed is that "the right depends on the good." Some philosophers describe good as pleasure, while others describe it as happiness, and still others describe it as a unique and indefinable property of things.

The question remains as to whose pleasure or happiness matters the most. Is it the person who performs the action or others who are also affected by the action? The answer given by most utilitarians is that it is the sum total of

happiness or unhappiness that matters. If a certain action makes one person X amount of happy, but at the same time makes another person Y amount of unhappy, then if $X > Y$ the action is right, and if $Y > X$ it is wrong. No one person's happiness counts more than anyone else's. In this situation all are equal (Taylor, 1975).

I believe that without realizing that they are subscribing to an ethical theory, many pregnant women follow these guidelines while making the decision whether or not to have an abortion. One of the most common reasons given for having an abortion is that the woman is not ready to carry a child, give birth, and be a mother. I believe that the woman feels that it would cause a certain amount of unhappiness and anxiety, as well as disturb those around her. Families are mentioned quite often - an unmarried woman might state that her relatives (especially parents and siblings) would be shocked to find out she was pregnant and unmarried, and she feels that it would save lots of heartache to secretly terminate this pregnancy. Here the woman feels that whatever happiness might be felt with this pregnancy would be greatly outweighed by the unhappiness it would cause her family members. Another common reason, given mostly by younger women, is that they want to finish school before beginning a family. Here the woman feels that the happiness gained from finishing school outweighs the happiness of having a baby. In addition to this, most women who give this reason also give the reason stated above, and therefore have several different factors pointing toward the abortion decision.

The deontological system is very different from the teleological one. The deontological system maintains that it is possible for an action or rule of action to be right even though the result is not the greatest balance of good over evil. An action is right or wrong in itself and is totally separate from its consequences. One performs an action because of an obligation or duty to do so - because it is thought to be a good or right action (Frankena, 1973).

On the surface it appears difficult to justify abortion using this moral theory, but if one looks deeper I believe it is possible. An action is performed because of a duty to do so. One extremely common reason given for abortion is that the woman cannot financially manage the pregnancy, birth, and raising the child. She feels that it is her duty not to bring a child into this world when it cannot be properly cared for. Many women who have this problem already have other children and feel that by bringing another child into the family they would not be able to feed and clothe the children they already have. They feel a duty or obligation to their children not to lower their living standards even more. This obligation is felt most strongly in the

case of a single-parent family where it would be financially devastating if a woman had to take leave of absence or give up her job totally to have a child. The woman in this situation usually expresses this sense of duty or obligation to her family at the time of her abortion counseling. This is a very sad situation, but unfortunately is quite prevalent.

Another common reason for abortion given by many women is that their family is complete. Many of these women have teenagers and feel that it is their duty to their families and themselves not to interrupt their family situation.

Another reason that is given for abortion is that the pregnant woman has been undergoing some type of drug therapy known to cause birth defects. While this is not a common reason, it nevertheless does occur. This woman feels that it is her duty not to take the chance of bringing a retarded or deformed child into the world.

I believe that the teleological ethical system applies to these cases also. In financial and family stress, these pregnant women see abortion as the way out that would cause the least unhappiness.

In conclusion, I would like to state that there are many women who give reasons for abortion that are not mentioned in this paper; however, the ones discussed here are the most common. Another important note is that many women in the same circumstances as described in this paper choose to carry their pregnancy full term. Since these women are seldom seen by abortion counselors, I was unable to include this aspect in my paper.

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The Problem of Eusociality

Tim Carter*

Eusocial insects are usually characterized by a colony of non-reproducing individuals which assist a single or else a small number of reproductively active individuals. Eusocial behavior is particularly well-developed in the insect order Hymenoptera, which includes all of the ants and most of the bees and wasps. Eusociality is also evident among the termites, which are not Hymenopterans. Most ethologists define altruism as, "behavior whereby an individual increases the welfare of another individual at the expense of its own welfare (Arms and Camp, 1979). At first glance a colony of individuals which have lost the ability to reproduce and instead assist a single individual in its reproduction would seem to be extremely altruistic. This seemingly altruistic behavior presents a severe problem to evolutionary theory, which predicts that truly altruistic behavior should not evolve. Darwin himself recognized the social behavior of some insects as a major obstacle to evolutionary theory (Darwin, 1859). However, after much recent study of the social Hymenoptera, biologists have agreed that eusociality is indeed not altruistic.

Requirements of eusociality

Sociobiologist E. O. Wilson lists three qualities by which the majority of entomologists define eusociality:

- (1) Individuals of the same species cooperate in caring for the young.
- (2) There is reproductive division of labor with more or less sterile individuals working on behalf of fecund nestmates.
- (3) There is an overlap of at least two generations in life stages capable of contributing to colony labor, so that offspring assist parents during some period of their life (E. O. Wilson, 1975).

In recent years two credible hypotheses have been proposed to explain the evolution of eusociality. These are W. D. Hamilton's kin selection theory and R. D. Alexander's individual selection theory.

Haplodiploidy and the kin selection theory.

Hymenopterans possess an unique method of sex determination which is termed haplodiploidy. According to E. O. Wilson (1975), unfertilized eggs typically develop into males (hence, haploid) and fertilized eggs develop into fe-

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males (hence, diploid). The Hymenopterans together with only a few other arthropods are the only groups to show haplodiploidy (Wilson, 1975). The phenomenon of haplodiploidy was known for years, but a suitable explanation for it could not be found. Thus eusocial behavior among insects continued to be thought of as altruistic behavior.

However, W. D. Hamilton in 1964 proposed a brilliant explanation of haplodiploidy. By combining Mendelian genetics with Darwin's natural selection (Trivers and Hare, 1976), Hamilton concluded that, "if a female is fertilized by only one male all the sperm she receives is genetically identical. Thus, although the relationship of a mother to her daughters has the normal value of one-half ($1/2$), the relationship between daughters is three-fourths ($3/4$)" (Hamilton, 1964). Since daughters are more closely related to each other (by a factor of $3/4$), they would better enhance their inclusive fitness by helping their mother reproduce more daughters than by reproducing themselves. Helping to reproduce a sister to which an individual is related by three-fourths ($3/4$) is more genetically profitable than reproducing an offspring to which an individual is related by one-half ($1/2$).

Hamilton further argued that haplodiploidy predisposed the Hymenoptera to the evolution of sterile workers because females are potentially more closely related to their mother's brood than to their own offspring. Hamilton gave three requirements for worker behavior to appear:

- (1) Mothers must live long enough to encounter their progeny.
- (2) Queens must be supplied with sperm from only one individual male.
- (3) Males must be reproductively active but not participate in the work of the colony (Hamilton, 1964).

Because female workers increase their own inclusive fitness by working to increase the fitness of their sisters, Hamilton's explanation of haplodiploidy has been placed under the heading kin selection.

The concept of kin selection is most evident in truly eusocial species such as Apis mellifera, the common honeybee. In this Hymenopteran only one reproductively active queen per hive is tolerated. In the performance of her egg-laying duties, this single queen may last several years (Wilson, 1971). Thus, this queen encounters the progeny of several generations. Before the queen begins her reproductive functions, she mates with male bees. Although the queen may actually copulate with several males, most experts

believe that sperm from the first male to reach her completely fills the queen's spermatheca. Thus, sperm from all subsequent matings is not stored (Hamilton, 1964). Lastly, the males of the honeybee hive serve no function beyond providing sperm for fertilization of the queen's eggs. Indeed, in their adaptation as carriers of the male gametes, male honeybees have lost the ability to provide food for themselves and are at the mercy of the female workers for their nourishment (Wilson, 1971).

Kin selection theory: Mechanisms of evolution of eusociality.

Since the development of the kin selection theory of eusocial behavior many models have been proposed which outline the development of eusocial behavior. C. D. Michener formulated a model which included several evolutionary steps that lead up to eusocial behavior. The most primitive of these is the parasocial level where "adults belonging to the same generation assist one another in varying degrees" (Wilson, 1975). At this low level, "they may be merely communal, which means that they cooperate in constructing a nest but rear their brood separately" (Wilson, 1975). At the next level of involvement, quasisocial, "the brood are attended cooperatively, but each female still lays eggs at some time of her life" (Wilson, 1975). In the semisocial state, "cooperative brood tending is enhanced by the addition of a true worker caste; in other words, some members of the colony never attempt to reproduce" (Wilson, 1975). Finally, when semisocial colonies persist long enough for members of two or more generations to overlap and to cooperate, the list of eusocial requirements is complete and we can refer to the species as being eusocial. C. D. Michener proposed this sequence to explain the appearance of the eusocial bees.

An alternate sequence has been proposed by Wheeler to account for eusociality among ants, termites, social wasps, and a few groups of the social bees. This model begins at the subsocial state. "Here there is an increasingly close association between the mother and her offspring" (Wilson, 1975). At this most primitive level, the female provides "direct care for a time but departs before the young emerge as adults" (Wilson, 1975). Next the parental care is extended to the point where "the mother is still present when her offspring mature" (Wilson, 1975), and the offspring might next assist her in the rearing of additional brood. "It remains only for some of the group to serve as permanent workers, and the last of the three qualities of eusociality has been attained" (Wilson, 1975).

Kin selection: the Trivers and Hare test.

Hamilton's kin selection theory of eusociality received a great impetus from a work by Trivers and Hare in 1976. Trivers and Hare proposed that if Hamilton's degree of relatedness between daughters and sons were correct, then the sex ratio of a eusocial colony should be maintained at three:one (3:1) female to male. They hypothesized that this three:one (3:1) sex ratio was maintained by the worker caste because it enhanced their genetic fitness. Trivers and Hare tested their theory by measuring the ratio of dry weights of females to dry weights of males in about twenty species of ants which had worker classes. They found that the ratio did conform to three:one (3:1) female to male. As a further test, Trivers and Hare predicted that an ant species without a worker caste would have a one:one (1:1) sex ratio. They found this ratio to be true among the slavemaking ants which raid other colonies to recruit worker ants (Trivers and Hare, 1976).

As a result of the work of Trivers and Hare many writers in the field no longer regard the workers as assisting the queen in reproduction. Rather they view the workers as holding the queen hostage and manipulating the sex ratio of her progeny for their own genetic benefit (Eberthard, 1975). It must be remembered that the mother is equally related to her male and female offspring by one-half ($1/2$), thus, she should have no preference in the sex of her offspring. However, the female progeny gain a decided advantage by having the queen reproduce a majority of females. The workers can manipulate the sex of the queen's offspring by the amount of care given to the haploid eggs that she lays (Noonan, 1978).

This combination of kin selection and manipulation of the sex ratio demonstrates that eusociality is far from altruistic, because the worker caste does not lower their inclusive fitness by being sterile.

Individual selection and eusociality.

A second theory of the development of eusociality is individual selection. This theory does not deny the haplodiploid genetic thinking of Hamilton, rather it is a response to the worker manipulation theory of Trivers and Hare (Evans, 1977).

One of the assumptions of Trivers and Hare and also of Hamilton was that the queen stored sperm from a single male. H. E. Evans noted that multiple matings by queens are much more frequently observed than single matings. If the queen fertilizes her eggs with gametes from more than one male, the kin selection theory is weakened (compare to W. D.

Hamilton, 1964). As an example, if a queen has gametes from just two males and randomly uses each type to fertilize her eggs, then the average relatedness between sisters drops to one-half ($1/2$). Thus, the sisters would no longer increase their inclusive fitness by helping the queen to reproduce females (Alexander and Sherman, 1977).

A second observation also detracts from the kin selection theory. It is frequently noticed, especially among social wasps, that some individuals not related to the colony will nonetheless "join" the colony and begin serving as workers (Evans and Eberhard, 1970). The writers citing individual selection see this as evidence of a queen's complete dominance of a colony so that all newcomers, both progeny and "joiners" alike, serve her genetic interests.

This observation of queen dominance leads into the brunt of the individual selection theory. According to R. D. Alexander (1974), in the conflict of genetic interest between the queen and the progeny, the queen has won the battle. First of all the queen can control the sex of her progeny by controlling the release of sperm from the spermatheca. After the egg is laid, she can control the size of the offspring by the amount of food she makes available--this is trophogenic dominance (Evans, 1977). By this method of diet-controlled size and controlled sex, the queen produces progeny whose reproductive options have been foreclosed. If the progeny cannot reproduce themselves, then the only way for the progeny to increase their inclusive fitness is to do just those things that also raise their mother's fitness (Alexander, 1974).

An extreme example of this foreclosure of reproductive options would be "trophic eggs" which the queen lays and then feeds to workers. The queen foregoes the development of future progeny in order to nourish the existing progeny (Evans, 1977).

Another example of queen manipulation is behavioral dominance. In an often cited example, queens of Dialictus zephyrus (a sweat bee) use "nudging" and "backing" maneuvers to either move workers into guarding positions or else lure them into different parts of the nest where stimuli for nest construction or larval provisioning are present (Evans, 1977).

Another form of queen dominance employed by advanced social insects (such as honeybees) is pheromonal dominance. Here the worker individuals undergo both physiological and behavioral responses to strong pheromones (trans-9-keto-2-decanoic acid) exuded by the queen (Evans, 1977). The workers appear overwhelmed by this pheromone and in the presence of the queen they forego their reproduction and help the

queen. This effect of pheromonal dominance has been shown very dramatically in A. mellifera through studies by Jean George. In order to remove the source of the pheromone, the queen was removed from the hive. All the developing eggs were also removed to prevent the workers from merely hatching out a new queen. With no queen to direct their action, a few of the previously sterile workers were able to lay a small number of eggs (George, 1969). However, these pseudo-fertile workers did not retain their egg-laying ability, because the haploid eggs laid by the workers quickly developed into drones. When mature, the males mated with the pseudo-fertile workers. The new diploid eggs were raised as queens. The first queen to emerge was mated with a male and easily supplanted the pseudo-fertile workers as the new queen of the hive (George, 1969).

The parental manipulation theory suggests that the queen uses a variety of mechanisms to manage her progeny. Because the queen reduced the individual fitness of any one of her offspring in order to increase her own fitness, parental manipulation of progeny falls under individual selection.

Synthesis of theories.

M. J. West Eberhard provides an unique synthesis of the kin selection theory and the individual selection theory:

Although the above hypotheses are presented by their respective authors as separate and independent theories, [both] of them are perhaps best seen as... new insights, not as mutually exclusive theories. There is no reason why... kin selection... and [individual selection] cannot have contributed simultaneously or sequentially to produce the evolution of sterile castes. And it seems fruitless, in making generalizations, to argue in favor of one idea to the exclusion of the other... Rather, it seems necessary to acknowledge that all of the hypothetical factors could have operated, either alone or in some combination, especially in view of the fact that the several origins of worker sterility could each have arisen in a different combination of circumstances, making a uniform explanation impossible (Eberhard, 1975).

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Dr. Julian T. Darlington

by Lisa Timmons*

If while walking the corridors of Frazier Jelke, you perceive the aroma of tobacco among the multifarious odors haunting the building, you can probably attribute it to the pipe of Drs. Jones, Darlington, or Amy. If perchance you have just passed through a cloud of smoke outside the seminar room, you can assume there is a departmental meeting going on in there. If you are a frequent passerby, you may have noticed this haze lingering at least once a week near the door of the seminar room and if you are a curious person, you probably are wondering just what the pipe puffers are perusing.

If you ask Dr. Darlington, he will tell you they're talking biology. They're discussing the recent research projects and pet interests of the faculty, organizing, planning ahead and deciding on issues important to the whole Biology Department - where the next monies will be spent, what seminar topics will be dropped in the hat, when comps will be given, why the roses aren't blooming or who will buy the next pouch of tobacco.

Dr. Darlington will tell you many other things too. One student says with a smile, "He will give you the 'whys' before you ask." "He's very approachable. His office hours are longer than 10 to 3. You can find him most any time of the day and can talk with him about almost anything."

Even worms. Dr. Darlington's special interest is free-living flatworms and before you ask "why?" he will tell you. "They're important. Oh sure, some people question the importance of some things biologists study, like flatworms, but they fail to see how even such little animals can fit into the overall picture of life. What is really important anyway? No one can answer that question, but each little bit of information we accumulate on a primitive, lower form (of life) aids in our understanding of more sophisticated life."

The much misprized worms Dr. Darlington studies are flattened, elongated, bilaterally symmetrical and regenerative. His joy is not especially in studying their anatomy but in their classification and distribution. He has discovered four new species of flatworms, and he and fellow helminthologist Dr. Clay Chandler (of Middle Tennessee State University) have been forerunners in the documentation of the distribution of the worms west of the Mississippi River. Their quests have taken them to Arkansas, New Mexico, "Old"

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Mexico, North Carolina and other places in the South and Midwest. As a result of their works, they are ones of the few flatworm specialists in the area.

Their studies reveal a comparatively wide expansion of the habitats of a given species, considering the topical diversity of the regions it would inhabit. Some scientists try to link the distribution patterns discovered by researchers such as Dr. Darlington with the theories of continental drift. However the varieties of worms in an area changes over the years because persons are traveling and camping more and redistributing the worms. Ecologists are investigating the possibility of linking some types of worms with polluted areas, and even doctors have an interest in the worms. For example, some send specimens to Dr. Darlington to identify; however, none of the free-living species Dr. Darlington has analyzed has been a cause of disease.

When can a busy college professor find time to worm-hunt? During sabbaticals, vacations, weekends, on the biology field trip (Field Biology 403A) or whenever he is not in the classroom, in a departmental meeting or whenever he isn't serving as secretary of the faculty meetings or faculty representative for the Board of Trustees. (Dr. Darlington teaches Biology of the Invertebrates 203, Vertebrate Anatomy and Histology 208, Local Flora and Fauna 403B and Biology 100.) Students from last year's field trip testified that Dr. Darlington's exasperatingly enthusiastic worm hunts during the hikes had him looking under almost every rock and log in his path. "Most of the time, he tirelessly led the group" and undaunted by fruitless searches, continued in his mission to find a unique, previously undocumented or possibly unclassified variety of worm.

If you ask, he'll tell you how he got started in the worm business - how he was persuaded by an accidental finding of a previously unclassified variety to pursue his interest in graduate school at the University of Florida after attending Emory University. He will tell you how the lack of knowledge in his field summoned his interest and curiosity. He still affirms that "there's a lot of work to be done" in his area. Dr. Darlington can be a source of information in this and many other areas and he's willing to share it; just ask.

