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### Contents

**Research Articles** 

129 Ethnicity Influences the Distribution of Cytochrome P450
3A4 Gene Polymorphism—David T. Arrington, Jr., Brenda
D. Mangilog, Sebron Harrison, Xinchun Zhou, and Olga
McDaniel

- 138 Effect of an Acid Hydrolyzate on Southern Pine Softwood on the Growth and Fermentation Ability of Yeast *Saccharomyces cerevisiae*—Yi Zhang, Huey-Min Hwang, Maria F.T. Begonia, Ken Lee, and Kui Zeng
- 144 The Historical Distribution of Prairies in the Jackson Prairie Belt and in Western Mississippi—John A. Barone

#### Departments

- 149 President's Column—Sarah Lea McGuire
- 150 Junior Academy of Sciences—Aimée Lee
- 152 Photographs of the Annual Meeting
- 154 Divisional Reports
- **158 2005 Annual Meeting Abstracts** (Supplement)

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### Ethnicity Influences the Distribution of Cytochrome P450 3a4 Gene Polymorphism

#### David T. Arrington, Jr.<sup>1,2</sup>, Brenda D. Mangilog<sup>2</sup>, Lee Y. Tee<sup>2</sup>, Sebron Harrison<sup>2</sup>, Xinchun Zhou<sup>2</sup>, and D. Olga McDaniel<sup>2,3,4</sup>

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CYP3A4, a member of CYP3A family, exhibits an interindividual variation in the level of gene expression and enzyme production. It is hypothesized that such variations in the level of gene expression are caused by polymorphisms within the regulatory element of the gene. The variant most studied is at position -290 and involves an A to G transition. We have studied DNA samples from 206 African-American and 108 Caucasian patients and controls. DNA was tested in a PCR assay using sequence specific nucleotide primers and Taq polymerase. The amplification product was analyzed by 2% agarose gel electrophoresis. Genotypes were detected based on the presence or absence of the amplification product. CYP3A4 G genotype was present with a higher frequency in African-American individuals as compared with Caucasians (83% VS 3%, p < 0.0001, Relative Risk = 3.9). The homozygous AA allele was predominantly present in Caucasians (97%) but only 17% in African-Americans (p < 0.0001, Relative Risk = 2.5). In contrast, the homozygous GG allele was only detected in African-American group (14.6%). Consequently, the frequency distribution of heterozygous AG alleles were higher in African-Americans (68.4%) as compared with Caucasians (3%) (p < 0.0001; Relative Risk = 2.56). The frequency distribution of the AG heterozygous genotype was significantly lower in African-American CTx patients (30.4%) as compared with African-American RTx patients (68%) or African-American unrelated controls (82%) (p < 0.001, Relative Risk = 0.3; p < 0.0001, Relative Risk = 0.2 respectively). Interestingly, 52% of African-American patients, who had undergone cardiac transplantation (CTx) carried the GG allele as compared with 14% of the patients with renal transplantation (RTx) (p < 0.0002, Relative Risk = 4.3), and 3% of unrelated controls (p < 0.0001, Relative Risk = 6.0). Suggesting that possession of the CYP 3A4-G variant might influence the underlying clinical characteristics that have exposed these patients to an end stage heart or kidney failure. In summary, CYP3A4 genotype demonstrated remarkable interindividual variations between African-American and Caucasian populations, and a gene dose effect was present in African-American patients. Such polymorphism in CYP3A4 gene might play a role in disease susceptibility independent of the clinical course associated with variation in drug metabolic rates and immunosuppressant clearance in transplantation.

Cytochrome P-450 (CYP-450) is a heme-containing enzyme and the isoform CYP3A, accounts for about 60% of the total CYP-450 family. The members of CYP 3A are mainly found in the liver, intestine and peripheral blood cells (Shimada et al., 1994; Cholerton et al., 1992). The CYP 3A plays an important role in bioactivation of environmental carcinogens, the metabolism of a variety of drugs and anticancer agents (Shimada et al., 1994; Shinderman et al., 2003; Koch et al., 2002). Four different CYP 3A isoforms: CYP 3A4, CYP 3A5, CYP 3A7, and CYP 3A43 have been identified (Gellner et al., 2001), which are located in tandem on chromosome 7. CYP 3A4 is the major enzyme, which metabolizes immunosuppressive drugs, such as Cyclosporine, Rifampin, and Tacrolimus (Neylan, 1998; Chenhsu

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et al., 2000; Pichard et al., 1992). In addition, many antibiotics and calcium channel blockers are metabolized by CYP3A4 (Watkins, 1992; Bertz and Granneman, 1997). The catabolic activity of the CYP 3A4 may vary up to 90-fold in different individuals (Kuehl, 2001). Because of individual's variability in drug uptake and clearance, this might have a great impact on the outcome of allograft survival after transplantation.

CYP 3A4 exhibits an interindividual variation in the level of gene expression (Wandel et al., 2000; Amirimani et al., 2003). It is hypothesized that such variations in the level of expression are caused by polymorphism within the regulatory region of the gene (Amirimani et al., 2003). The majority of variations are single nucleotide polymorphisms (SNPs) and might affect the level of enzyme production. However, the functional significance of such polymorphism is unknown (Amirimani et al., 2003; Rebbeck et al., 1998; Galant et al., 2001).

A transition of  $(A \rightarrow G)$  at position -290 from transcription site of CYP 3A4 gene, caused by a point mutation in the 5'-flanking region of the gene, has been reported (Rebbeck et al., 1998; Sata et al., 2000). It is speculated that the mutated genotype (CYP 3A4-G) might have a clinically significant role in pharmacogenetics of drug metabolisms because of its effect on the level of CYP 3A4 expression and activity (Amirimani et al., 2003; Ball et al., 1990). There have been reports demonstrating an association between CYP3A4 genotypes and disease conditions, including tumors of higher clinical stages and grades in prostate cancer (Rebbeck et al., 1998; Paris et al., 1999; Kittles et al., 2002), secondary leukemia (Felix et al., 1998), and breast carcinoma (Galant et al., 2001). However, some studies disagree with the association of this variant and the risk of breast cancer (Sprudle et al., 2002). Such discrepancies suggest that there might be population differences in the frequency distribution of the genotypes causing the clinical phenotypes. Indeed, the frequency of CYP 3A4-G variant varies substantially in different populations. It has been reported at a low frequency in Caucasians (2-9%), high in African-Americans (53%) and absent in Chinese (0%) (Wandel et al., 2000; Ball et al., 1990; Walker et al., 1998). In addition, a CYP 3A4-G variant has been found more frequently in Caucasians who had low levels of CYP 3A4 protein production as compared with Caucasians with higher levels of CYP 3A4 protein (Lamba et al., 2002). This indicates the significance of the CYP 3A4-G in regulation of the

gene transcript, and furthermore, it might suggest variation in the level of expression in the population. Given the importance of functional activity of the CYP 3A4 in drug metabolism and clearance, the association with various disease states, particularly, differences in various population, assessment of the genotype variation in the population might have important consequences for patients such as allograft recipients who are subjected to multiple drug therapy. Our initial study demonstrated that African-Americans in Mississippi, predominantly carry the CYP3A4-G allele. Since the majority of transplant patients at the study center were African-Americans, this study was designed to investigate the frequency distribution of CYP 3A4 genotype variations in African-American individuals as compared with Caucasian patients and controls. The knowledge of the frequency distribution in the population will have a major impact in establishing the clinical relevance of CYP3A4 genotyping in disease susceptibility and for optimization of drug dose and therapy.

#### MATERIALS AND METHODS

**Blood samples.** Two hundred six African-American and 108 Caucasian individuals were studied. Of these, 65% were patients, who either had undergone cardiac or renal transplantation. One hundred eight unrelated controls from the same geographic area were studied. Informed consent was obtained as part of a protocol approved by the University of Mississippi Medical Center Review Board for obtaining blood samples from the participants.

*Genomic DNA.* DNA was isolated from whole blood using a modification of Blin and Staford method (Blin and Stafford, 1976), followed by phenol extraction and precipitation with 3 M sodium acetate and ethanol. DNA was stored in TE Buffer (10 mM Tris/2 mM EDTA; pH 8.0) at 4 °C until analysis of CYP 3A4 genotypes by polymerase chain reaction (PCR) method.

**Polymorphisms.** CYP3A4 genotypes were determined by amplification of a 186-bp CYP 3A4-G or a 187-bp -A DNA fragment. The primers were specific either for G or A polymorphism. A third set of primers were used to amplify a 440-bp generic fragment flanking 3' from the polymorphic site as a positive control, to confirm the integrity of the amplification pattern. The primer sequence and the location relevant to the CYP 3A4 gene are given in Table 1.

PCR. One hundred ng genomic DNA was ampli-

fied at initial cycle of 94 °C for 2 minutes followed by 25 cycles of 94 °C for 30 sec and 72 °C for 60 sec and an additional cycle of 72 °C for 2 minutes. The amplified DNA fragments were visualized by 2% agarose gel based on the presence or absence of the target DNA fragment (Figure 1).

*Statistical analysis.* The Frequency of the genotypes was compared using contingency 2 x 2

table of Fisher's exact test. A *p* values less than 0.05 was considered statistically significant. An INSTAT3 program was used to analyze the relation of CYP 3A4 genotypes based on a relative risk (RR) value obtained between patients and controls. The RR was calculated to categorize the study subjects into two groups of positive or negative for the possession of the CYP 3A4 genotypes.



**Figure 1. A representative illustration of the CYP 3A4 genotype patterns in an agarose gel electrophoresis.** The top panel demonstrates a 187-base pair A allele; the bottom panel demonstrates a 186-base pair G allele. Alleles were detected based on presence or absence of the bands. 1-13 represents different individuals. B: a negative control (water was substituted for DNA. M: a standard molecular marker.

#### RESULTS

The study group comprised 206 transplant patients of which 90 had undergone cardiac transplantation (CTx) and 116 had undergone renal transplantation (RTx). In addition, a total of 108 controls, 67 African-American and 41 Caucasians were included in this study. The control subjects had no history of disease and were from the same geographic region as the patients.

The frequency distribution of the CYP 3A4 -A and -G genotypes in African-American and Caucasian groups is given in Table 2. The frequency was analyzed based on gender and ethnicity. The CYP 3A4-G variant was present in 3% of Caucasian individuals, whom were all male, but among African-American groups, it was present in both male and female (79.4% and 87% respectively) and the difference was statistically significant (Table 2). The CYP 3A4-A genotype was present in a higher frequency in Caucasians as compared with African-American (p < 0.0001, Relative Risk = 0.6) individuals. Overall, 90% of the study population carried the CYP 3A4-A genotype. In contrast, the CYP 3A4-G genotype was detected in only 3 out of 108 (2.8%) Caucasians as compared with 171 (83%) of African-Americans (p < 0.0001, Relative Risk = 3.9).

The CYP 3A4, AA homozygous allele was found in 35 (17%) African-American individuals as compared with 105 (97%) Caucasians (p < 0.0001; Relative Risk = 2.5). Whereas the homozygous GG allele was detected in 30 (14.6%) African-American group, but it was absent in Caucasians.

The frequency distribution of the CYP 3A4 was analyzed in patients and controls. We observed significant differences in CYP 3A4 genotype distribution among the two groups of patients with CTx or RTx. The CYP 3A4-A genotype was detected only in 11 (48%) of the African-American patients with CTx as compared with 100 (86%) of the African-American patients with RTx (p < 0.0002, Relative Risk = 0.3), and 65 (95%) of controls (p <0.0002; Relative Risk = 0.2), shown in Table 3. On the other hand, the CYP 3A4-A genotype was present in 100% of both the Caucasian patients and controls. The frequency distribution of homozygous AA allele was 100% in Caucasian patients with CTx, and 93% of the Caucasian controls, but it was only 17.4% in African-American patients with CTx, 18% with RTx and 15% in African-American controls. As shown in Table 3, the data demonstrated a significant frequency difference across the ethnic population.

There was no CYP 3A4-G genotype, found in Caucasian CTx patients. However, 12 (52%) of the African-American patients with CTx, carried a homozygous GG allele as compared with 16 (14%) of the patients with RTx, and 2 (3%) of the controls (p < 0.0002, Relative Risk = 4.3; p < 0.0001, Relative Risk = 6.0 respectively). The frequency distribution of the AG heterozygous allele was lower in African-American CTx patients (30.4%) as compared with African-American RTx patients (68%) or African-American controls (82%), (p < 0.002, Relative Risk = 0.3; p < 0.0001, Relative Risk = 0.2respectively). Only, 3 out of 41 (7.3%) Caucasian controls were heterozygous for the AG allele. A comparison of gender difference between African-American RTx and CTx and the presence of CYP 3A4 variants are shown in Figure 2. The frequency distribution of the CYP 3A4 homozygous G and A were inversely present in female and male patients with CTx or RTx. The GG alleles were a 1.5-fold increased in males with CTx as compared with females with CTx, but, it was a 1.5-fold decreased in male patients with RTx as compared with females. In contrast, the homozygous AA genotypes were 1.2fold decreased in male patients with CTx as compared with females, but it was 2-fold increased in males with RTx. The differences were statistically significant where a comparison was made between the same gender and disease. As shown in Figure 2, the CYP 3A-GG was present in 40% of female with CTx as compared with 17.3% with RTx (p < 0.0005; Relative Risk = 1.7). Similarly, it was found in 61.5% of males with CTx as compared with 11% of males with RTx (p < 0.0002; Relative Risk = 6.6). While these variations must have some associations with clinical characteristics of disease, the small number of subjects and disease variations in these patients has limited the stratification of the genotypes and the underlying clinical characteristics in this study.

Genotype	5'-3' sequence	Location	Fragment size (bp)
(A) forward	ATG-AGG-ACA-GCC-ATA-GAG-ACA-AGG-GCA-A	-789, - 810	187
reverse	GAA-TCA-CAC-ACA-CAC-CAC-TCA-CTG-ACC-TC	-971, -946	
(G) forward	TGA-GGA-CAG-CCA-TAG-AGA-CAA-GGG-TAG	-790, -813	186
(C) forward	AAC-AGG-CGT-GGA-AAC-CCA-AT	-535, -554	440

Table 1. Sequence-Specific CYP 3A4 Amplification Primers.

The sequence for reverse amplification primer was same for both alleles and the control. A: forward primer for amplification of allele A; G: forward primer for amplification of allele G and C: forward primer for amplification of positive control.

	AFAM*				CAU*	
Genotype	Male n = 107 + (%)	Female n = 99 + (%)	Total n = 206 + (%)	Male n = 79 + (%)	Female n = 29 + (%)	Total n = 108 + (%)
А	92 (86)	84 (85)	176 (85.4) <sup>a</sup>	79 (100)	29 (100)	108 (100) <sup>a</sup>
G	85 (79.4)	86 (87)	171 (83) <sup>b</sup>	3 (3.8)	0 (0)	3 (3) <sup>b</sup>
AA	22 (20.6)	13 (13)	35 (17) <sup>c</sup>	76 (96)	29 (100)	105 (97) <sup>c</sup>
AG	70 (65.4)	71 (72)	141 (68.4) <sup>d</sup>	3 (4)	0 (0)	$3(3)^{d}$
GG	15 (14)	15 (15.2)	30 (14.6) <sup>e</sup>	0 (0)	0 (0)	0 (0) <sup>e</sup>

Table 2. Frequency distribution of CYP 3A4 genotypes in African-Americansand Caucasians.

\*AFAM stands for African-American and CAU stands for Caucasian

a: p < 0.0001, Relative Risk = 0.6

b: p < 0.0001, Relative Risk = 3.9

c: p < 0.0001, Relative Risk = 2.5

d: p < 0.0001, Relative Risk = 2.56

e: p < 0.0001, Relative Risk = NA

Table 3. CYP 3A4 genotype distribution in patients and controls in African-American and Caucasian populations.

		AFAM*	k			CAU*	
Genotype	CTx n = 23 + (%)	RTx n = 116 + (%)	Controln = 67+ (5)	PValue	CTx n = 67 + (%)	Control n = 41 + (%)	PValue
А	11 (48) <sup>a</sup>	100 (86)	65 (95)	а	67 (100)	41 (100)	NS
G	19 (83)	95 (82)	57(85)	NS	0 (0)	3 (7.3)	NS
AA	4 (17.4)	21 (18)	10 (15)	NS	67 (100)	38 (93)	NS
AG	7 (30.4) <sup>b</sup>	79 (68)	55 (82)°	b, c	0 (0)	3 (7.3)	NS
GG	12 (52) <sup>d,e</sup>	$16(14)^{\rm f}$	2 (3)	d, e, f	0 (0)	0 (0)	NS

\*AFAM stands for African-American and CAU stands for Caucasian

a: AFAM CTx was compared with AFAM controls, p < 0.0002; Relative Risk = 0.2

b: AFAM CTx was compared with AFAM RTx, p < 0.002; Relative Risk = 0.3

c: AFAM CTx was compared with AFAM controls, p < 0.0001; Relative Risk = 0.2

d: AFAM CTx was compared with AFAM RTx, p < 0.0002; Relative Risk = 4.3

e: AFAM CTx was compared with AFAM controls, p < 0.0001; Relative Risk = 6.0

f: AFAM RTx was compared with AFAM controls, p < 0.02; Relative Risk = 1.46



**Figure 2. A comparison of the frequency distribution of the CYP 3A4 genotypes in African-American RTx and CTx patients.** The associations were stratified on the basis of gender. The frequency distributions of the homozygous alleles were inversely present in female and male patients.

#### DISCUSSION

CYP3A4 genotype demonstrated remarkable interindividual variation between the African-American and Caucasian population as assessed by direct analysis of blood nucleated cells using single nucleotide polymorphism (SNP) detection. CYP 3A4 plays a major biological role in the metabolism of numerous compounds, including, steroid hormones, glucocorticoids, and carcinogens (Shinderman et al., 2003; Pichard et al., 1992; Zhao et al., 2002; Kobavashi et al., 2000). In addition, many antibiotics and immunosuppressive agents such as cyclosporine, and tacrolimus are metabolized by CYP 3A4 (Shimada et al., 1994; Neylan, 1998; Chenhsu et al., 2000; Hesselink et al., 2003). The study of CYP 3A4 genotype variation has at least a two-fold importance. First, the ethnic distribution of the CYP 3A4-G variant, 83% in African-American and 3% in Caucasian, may confer genetic susceptibility to diseases. Second, the CYP3A4 genotype variation might account for the differences in drug clearance between different individuals, which might affect the outcome of therapeutic management and intervention. In addition, the difference observed between African-American CTx and RTx patients in this study might have an association with clinical background of the individuals who experienced an end stage heart or kidney failure and whom were predisposed to the cardiac or kidney transplantation. Thus, this study was designed to analyze the frequency distribution of the genotypes in the population, and to investigate whether clinically different populations of patients, such as cardiac and kidney transplant recipients, might genetically have a common background for CYP 3A4 genotypes.

To our surprise, the frequency distribution of CYP3A4-G variant was 1.5-fold higher in the African-American population in our study than previously reported by others (Paris et al., 1999; Walker et al., 1998; Tayeb et al., 2000). This inconsistency between the observations might be due to a higher percentage of population admixtures present in other studies, given that the CYP 3A4-G variant is less common in Caucasians and a recent report (Zeigler-Johnson et al., 2002) have shown a high frequency distribution of the CYP 3A4-G variant among the African population. As shown in Table 3, number of subjects with the CYP 3A4-G genotype was almost rare in the Caucasian group as compared with African-American [3(3%) vs 171(83%), p < p0.0001, Relative Risk = 3.9]. Previous studies have shown an array of frequencies in different ethnic populations. The G variant of CYP 3A4 was nonexistent in Chinese and Asians, 6.5% in Caucasians, 8.9% in Saudis, 11% in Latinos, and 79.2% in Africans (Walker et al., 1998; Tayeb et al., 2000; Zeigler-Johnson et al., 2002). Including this study, the frequencies significantly vary (53%–83%) between African-Americans from different geographic regions (Rebbeck et al., 1998; Paris et al., 1999; Kittles et al., 2002; Walker et al., 1998). The variations in frequency distribution of the genotypes become significantly important where the prevalence of the disease varies considerably between populations. Such associations for CYP 3A4-G variant have been observed between African-American and Caucasian men with prostate cancer (Paris et al., 1999; Walker et al., 1998).

The CYP 3A4-A was a dominant genotype in Caucasian patients with CTx (100%) but not in African-American patients with CTx (48%), p <0.0002, Relative Risk = 0.6 (Table 3). The CYP 3A4-A genotype difference between CTx patients was independent of the ethnicity, since the CYP3A4-A genotype was present with high frequency in African-American, both RTx (86%) and control (97%) groups. While the variable frequency of the CYP 3A4 homozygous GG in African-American CTx vs RTx patients requires further investigation, in general, this data have demonstrated a significant variation in the frequency distribution of the CYP 3A4 genotypes between African-American and Caucasian population, and in particular, between the African-Americans from other regions of the United States. The substantial variation in frequency distribution of homozygous CYP 3A4-G genotype in African-American patient population demonstrated here was intriguing with regards to the difference between CTx and RTx patients (Figure 2). In terms of clinical characteristics, they both suffer from a multiple complex diseases including vasculopathy, hypertension and autoimmunity, which these diseases are associated with a complex interaction between environmental and genetic factors. Thus, a proportional variation in disease association with CYP 3A4 genotypes as well as proportional differences in prevalence of the disease and gender effect might contribute to the outcome as seen in CTx and RTx patients in this study.

Two important issues relevant to the frequency distribution of homozygous GG allele between African-American CTx and RTx patients might be raised: (1) Clinical characteristics that exposed these patients to transplantation, (2) Prevalence of cancer such as prostate, breast and secondary leukemia among these patients. At present the underlying disease characteristics for these patients are under investigation. We know that cardiovascular disease (CVD) was in large, a major cause of the heart failure in these patients. Thus, considering the complexity of the nature of CVD, a small population size such as this has offered a limited sufficient interpretation. Our results, however, suggests that variation in frequency distribution of CYP 3A4 might have a great impact in stratification of clinical manifestation of disease between CTx and RTx. The relationship between functional expression of the CYP 3A4 genotype and the clinical response to a drug clearance may considerably be depended to the status of a disease. Such observations are complicated by several factors, including involvement of CYP 3A4 in gastrointestinal and hepatic activity of Cyclosporine metabolism and other drugs (Lindholm et al., 1988; Lown et al., 1994; Schuetz et al., 2000; Combalbert et al., 1989), resulting in a variable absorption in a variety of clinical settings. Studies to observe precise quantification levels of CYP 3A4 gene expression and protein production are in progress. Such studies require a sufficient follow-up and patient management and at present it is not the focus of this study.

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Journal of the Mississippi Academy of Sciences

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# Effect of an Acid Hydrolyzate of Southern Pine Softwood on the Growth and Fermentation Ability of Yeast *Saccharomyces cerevisiae*

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An alternative energy source could come from microbial fermentation of the sugars present in acid hydrolyzates derived from agricultural biomass. The fermenting microbes must be tolerant of acidic conditions and able to ferment sugars present in the hydrolyzates. Saccharomyces cerevisiae has been shown as an ideal microorganism that can ferment hexose sugars present in lignocellulosederived hydrolyzates. The objective of this study was to assess the effect of the acid hydrolyzate of a southern pine softwood sawdust on the viability and fermentation capability of S. cerevisiae. Acid hydrolyzate of the sawdust (pH -0.63) was diluted with fermentation media at 100, 300, 400, and 800 fold. Four-day old cultures of S. cerevisiae (ATCC #765) were then added. During the 96-hour incubation (30 °C), turbidity of the medium, colony count and ethanol production were monitored. Viable number of cells and ethanol production in 300X- and 400X- diluted groups were significantly higher than other groups in the first 24 hr and reached the maximum after 24 hr and 48 hr of incubation, respectively. In a separate experiment pH of the fermentation medium was adjusted with H<sub>2</sub>SO<sub>4</sub> (SA) to simulate those of the 300X and 400X diluted acid hydrolyzate (AH) groups. The results indicated that the colony counts of both AH300 and SA300 were significantly higher than the other groups after 48 hours, with AH300 being the highest among all groups. However, this pattern was not paralleled by ethanol production activity. Thus ethanol fermentability of S. cerevisiae exists in the extreme environment such as diluted acid hydrolyzate.

Keywords: acid hydrolyzate, ethanol, fermentation, Saccharomyces cerevisiae

Fossil fuels have been the major resource to meet the demanding need for energy. With the constant increase of energy consumption and the constant use of nonrenewable fuel chemicals, there is a need to find a more economical, environmentally friendly and renewable energy source (Sun and Cheng, 2002). Research has proven that ethanol meets these qualifications. The fact that ethanol is a byproduct of microbial fermentation makes it a renewable energy source. As an oxygenated fuel, ethanol is environmentally friendly. The major challenge is how to produce ethanol economically. Ethanol production costs have been the limiting factor in the ability of gasohol and ethanol blended fuels to compete with current gasoline cost. Processing methods and feedstock are the major factors driving ethanol cost and of the two, feedstock contributes over 50% of the total cost. Production costs can significantly be reduced with use of low cost feedstock (Mani et al., 2002; Zerbe, 1992).

Lignocellulosic biomass, such as sawdust and wood chips, is of low commercial value but can provide inexpensive sources for ethanol production. Lignocellulosic biomass is usually composed of three primary constituents including cellulose, hemicellulose and lignin (Reshamwala et al., 1995; Cheung and Anderson, 1997; Boopathy, 1998; Dewes and Hünsche, 1998). Although wood residues such as sawdust and woodchips are readily available. the sugars suitable for fermentation processes and alcohol yield are only found in cellulose and hemicellulose constituents. Cellulose contains hexose sugars (mainly glucose) and hemicellulose contains pentose sugars (mainly xylose). In order to obtain the sugars needed for fermentation pre-treatment of lignocellulosic biomass is necessary.

The most widely used pre-treatment method is acid hydrolysis. Concentrated acid hydrolysis, one of the acid hydrolysis methods, involves use of concentrated acid (sulfuric acid,  $H_2SO_4$  or hydrochloric acid,

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HCl) in the presence of high temperature (190  $^{\circ}C$  – 215 °C) to split cellulose and hemicellulose into simple sugars (Springfield and Hester, 1999). The concentration of sugars tends to be higher if the lignocellulosic biomass is treated with extreme acidity and high temperature; however, the products of acid hydrolysis (acid hydrolyzate) become verv acidic and may contain a variety of inhibitory compounds such as furfural, 5-hydroxymethylfurfural (5-HMF), acetate and other phenolic compounds (Luo et al. 2002; Palmqvist and Hahn-Hägerdal 2000). In order to maximize recycling of the acid present in the hydrolyzate, it is desirable to use acid-tolerant microorganisms to conduct ethanol fermentation process. Saccharomyces cerevisiae has been shown as the yeast that can best ferment hexose sugars present in lignocellulose-derived hydrolyzates due to its ethanol-producing capacity and high inhibitor tolerance (Hahn-Hägerdal et al., 1991; Olsson and Hahn-Hägerdal, 1993). Even though S. cerevisiae grows optimally around pH 5 (ATCC 2001 Product Information Sheet for ATCC #765), one study showed that the yeast is able to grow at a pH as low as 2.5 (Taherzadeh et al., 1997b). The objective of this study was to assess the effect of the acid hydrolyzate of a southern pine softwood sawdust on the viability and potential fermentation capability of S. cerevisiae. The ultimate goal is to develop a specific acid hydrolysis procedure to minimize the amount of inhibitory hydrolyzate constituent for S. cerevisiae and to maximize ethanol fermentation within the most tolerable acidity level. Consequently, the cost for recycling the sulfuric acid and the associated impact on environmental health would be reduced.

#### MATERIALS AND METHODS

A 4 day old culture of *Saccharomyces cerevisiae* (ATCC#765) was used in the experiment. The 4 day old culture was prepared by rehydrating the freezedried culture in 5 mL sterile distilled water overnight and then transferred to potato dextrose broth (24 g/L, pH 4.2) for enrichment (ATCC 2001 Product Information Sheet for ATCC #765). According to chemical analysis with the method developed by National Renewable Energy Laboratory (NREL) at Golden, Colorado (Ruiz and Ehrman, 1996), pre-treatment of southern pine softwood sawdust by acid hydrolysis (H<sub>2</sub>SO<sub>4</sub>) yielded an acid hydrolyzate (pH -0.63) that contained the following sugars and other organic compounds: 26.65 mg/mL glucose, 6.7 mg/mL mannose, 4.48 mg/mL xylose, 2.78 mg/mL galactose, 1.4 mg/mL arabinose, and 1.64 mg/mL acetic acid. Xylitol, lactic acid, glycerol, ethanol, HMF and furfurals were not detected. In Pyrex flasks containing 90 mL fermentation media (Atlas, 1995) (1% trypticase, 0.1% beef extract, 0.5% dextrose and 0.5% NaCl, pH 6.4), 10 mL of S. cerevisiae (in PDB, cell density 15x10<sup>5</sup>/mL) was added. A different amount of acid hydrolyzate was added to the flasks to reach a final concentration of 100, 300, 400, and 800 fold dilution of the acid hydrolyzate. The flask containing only fermentation media and the cells served as the control. Fermentation flasks were incubated at 30 °C and sampled at time points 0, 24, 48, 72, and 96 hours. The following assays were conducted: (1) turbidity of the growth medium (Benson, 1994); (2) colony count on potato dextrose agar plate (Benson, 1994); and (3) ethanol production. Briefly, turbidity (Klett unit) was measured with the photoelectric colorimeter at spectral range 520-580 nm (Tube Model 800-3, Scienceware, Bel-Art Products, Pequannock, NJ). Concurrently pH and colony count (CFU/mL) on potato dextrose agar were recorded. The samples were then filtered through a 0.2 µm HT Tufftyn<sup>®</sup> membrane filter (Acrodisc syringe filter, Pall Corp., Ann Arbor, MI) and alcohol production was measured by gas chromatograph (Shimadzu GC-14A, Shimadzu Corp., Kyoto, Japan) with a flame ionization detector (FID). Samples were separated on a 30 m x 0.53 mm fused-silica capillary column with 1.0 µm thickness film. The oven temperature was programmed as follows: 40 °C (0.3 min), 40-85 °C (20 °C/min), and 85-125 °C (40 °C/min). Injector temperature was 230 °C. Injection volume was 0.1 mL.

In a separate experiment the pH values of the 300- and 400-fold diluted acid hydrolyzate were simulated by adding 0.6 and 1 mL of 2N H<sub>2</sub>SO<sub>4</sub> to the Pyrex flasks containing the mixture of 90 mL fermentation media and 10 mL *S. cerevisiae*, respectively. Various sugars present in the diluted acid hydrolyzate were added at the corresponding concentrations. The same assays were conducted following the aforementioned procedures. Difference in the experimental data between the treatment groups or between a treatment group and its relevant control group is determined with either SAS or Student's ttest (p < 0.05).



Figure 1. pH (a), Klett unit (b), cfu (c), and ethanol production (d) of *Sacchar-omyces cerevisiae* culture (pH 4.06) mixed with diluted acid hydrolyzate at the ratio of 1:10. All samples were run in triplicate. Variations were <10% among the replicate.

#### **RESULTS AND DISCUSSION**

One of the requisites for efficient growth and ethanol production is the tolerance of the fermenting microbe (*S. cerevisiae*) to the wood hydrolyzate. However, our previous experiments showed that the undiluted wood hydrolyzate (pH -0.63) inhibited the growth and the fermentation ability of *S. cerevisiae* in the media containing undiluted hydrolyzate (data not shown). Due to the toxicity of the undiluted acid hydrolyzate, the hydrolyzate was diluted with fermentation medium up to 800 fold. After 1, 2, 3, and 4 days of incubation, the mean pH, growth in

terms of Klett unit and number of colony forming units per mL of medium, and percent ethanol production for each treatment (or dilution) and for the control were determined. As evidenced by the pH data of the control group (yeast in fermentation medium without acid hydrolyzate), fermentation process decreased pH from the initial value of 5.8 to 4.6 and stabilized it around 4.6 within the 4-day incubation period. Consistent pH values were also observed for the other groups, with the 100X group maintaining the lowest pH number (Figure 1a). Concurrent measurements of viability, biomass and ethanol production levels are indicated in units of



Figure 2. pH (a), cfu (b) and ethanol production (c) of samples. Saccharomyces cerevisiae (pH 4.06) culture was added to 300 and 400 fold diluted AH (AH300 and AH400) or fermentation media with pH adjusted by  $H_2SO_4$  to its corresponding diluted AH (SA300 and SA400). All samples were run in triplicates. Variations were < 10% among the replicate.

Klett turbidity unit (Figure 1b), cfu  $\times 10^{5}$ /mL (Figure 1c) and percent ethanol production (Figure 1d), respectively. All numbers of the 100X group remained low throughout the incubation period, indicating that cellular growth and fermentation activity of the yeast culture were inhibited by the 100 X-diluted acid hydrolyzate (pH 1.8 – 2.0). At extracellular pH values below the pKa value of acetic acid (4.75), the undissociated acid can diffuse through the cellular membrane and dissociate intracellularly. ATP consumption rate will increase and hence the growth rate will decrease when the proton

pump process is activated to maintain a constant intracellular pH (Taherzadeh et al., 1997a).

Viable number of cells (expressed as colony forming units x  $10^5/mL$  of medium) and ethanol production of the test yeast in 300X- and 400Xdiluted groups were significantly higher than other groups in the first 24 hr and reached the maximum after 24 hr and 48 hr of incubation, respectively (Figure 1c & 1d). Figure 1b indicates that Klett units of the 300X and 400X-diluted groups are the highest among all groups; however, the turbidity units of all groups continued to increase during the incubation period with the exception of 100X group. Although clinically Klett colorimetry has been extensively used to monitor the growth curve of microorganisms, the turbidity scale does not necessarily correlate with active or living biomass of the test microorganisms, except during the exponential growth phase. The experiment was repeated several times and the yeast populations consistently grew and fermented better in the 300-400X groups. Yeasts exhibit a considerable tolerance to extremes of pH. They are capable of maintaining active fermentation in glucose solution between a pH range of 2.4 to 7.4 (http://www.home.earthlink.net, August, 2004). For optimum production of ethanol, routine practice dictates that the pH of the fermenting medium is maintained within the range of 4.0 to 6.0. The explanation to the test yeast's ability to grow and ferment in 300X, 400X- and 800X-diluted groups (pH 3.0 - 4.2; Figure 1a & 1d) is that the intracellular pH of the yeast cells remains quite constant at about pH 5.8, regardless of the wide pH variations in the surrounding environment. The enzymes involved in fermentation thus operate in an optimum pH environment within the yeast cells. Addition of acetate to a growth medium during anaerobic continuous and batch cultivations was shown to lower the biomass yield while enhancing the ethanol yield by S. cerevisiae (Verduyn et al., 1990; Taherzadeh et al., 1997a). The increase in ethanol yield was speculated to occur due to the uncoupling effect of acetic acid on ATP reductions, provided that byproduct formation does not increase during the sugar to ethanol conversion process (Taherzadeh et al. 1997b). Our results partially contradict those findings. In the case of 300X and 400X-diluted groups in our batch study, both biomass and ethanol production rates were significantly enhanced in 24 hours (SAS; p < 0.05). The net effect of acetate on biomass yield or ethanol formation rate may vary, depending on the growth conditions or whether the specific growth rate is affected (Taherzadeh et al., 1997a). Further studies are needed to confirm the effect of acetic acid on biomass yield and ethanol production.

In a separate experiment the pH of the fermentation medium was adjusted with  $H_2SO_4$  (SA) to simulate those of the 300X- and 400X-diluted acid hydrolyzate (AH) groups (Figure 2a). Colony count and ethanol production of the SA and AH groups were compared (Figures 2b and 2c). Colony counts of both AH300 and SA300 were significantly higher than the other groups at 48 hours (Figure 2b), with AH300 being the highest among all groups (p < 0.05; Student's t-test). However, this pattern was not paralleled by the ethanol production (Figure 2c) in which AH300 and SA300 were indistinguishable from other treatments in the first 48 hr. Except for acetic acid, all sugars in the diluted acid hydrolyzate were added to the 300X and 400X SA groups. Since various sugars and acetic acid were the only prevalent organic species detected in the acid hydrolyzate, the significant increase in the number of viable cells of the 300X groups could not be attributed to the presence of acetic acid. Apparently more study is needed to understand the complicated growth energetics of S. cerevisiae in the presence of acid hydrolyzate. Results of this study indicate that the ethanol fermentation capability of the yeast exists in the extreme environment such as diluted acid hydrolyzate. However, low sugar concentrations in the diluted acid hydrolyzate could not support the test yeast's fermentation activity. Other pre-treatment methods, such as overliming and ion exchange, were reported to result in higher ethanol productivity (Palmqvist and Hahn-Hägerdal 2000). Comparison studies on the effects of the aforementioned pretreatment methods on sugar concentrations and ethanol production will be conducted in the near future.

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### The Historical Distribution of Prairies in the Jackson Prairie Belt and in Western Mississippi

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Blackland prairie was once found in two regions of Mississippi, the Black Belt and the Jackson Prairie Belt. The latter is located on the Jackson Formation and stretches from central Yazoo County across the center of the state to the border of Alabama. The extent and location of prairies in this region and elsewhere in the state (excluding the Black Belt) was determined using plat maps from General Land Office surveys conducted in the 1830's. The plat maps show that prairies were most common in Madison and Rankin Counties, and that a large number of prairies were found on alluvial soils in the Delta. In total, 19,555 hectares of prairie were marked on the plat maps. Comparison with other historical records and the locations of extant prairie remnants suggest that many more prairies existed than were marked on the plat maps. Nonetheless, the locations of these prairies should be helpful in conservation efforts and in understanding the historical vegetation of the state.

Grasslands once formed a small but important part of the landscape of the southeastern United States (DeSelm and Murdock,1993). One type of grassland, blackland prairies, occurred on calcareous substrates that typically had dry soil conditions that limited tree growth and promoted fires (Peacock and Schauwecker, 2003). In Mississippi, these blackland prairies occurred in two general regions or "belts": the Black Belt (or Northeast Prairie Belt) and the lesser-known Jackson Prairie Belt (Peacock and Schauwecker, 2003).

The Jackson Prairie Belt stretches from the Loess Hills in Yazoo County toward the southeast, ending a short distance into Washington County, Alabama (Moran, 1995; Elsen and Wieland, 2003). Prairies in the Belt were found on slopes and uplands and were surrounded by either pine or hardwood forests (Moran et al., 1997).

Little is known about the original prairies in the Jackson Prairie Belt. Moran et al. (1997) review historical descriptions of the vegetation in the Belt, citing several sources that mention prairies in the region. Additionally, a survey of the Belt located more than 54 remnant prairies, ranging from less than 1 to more than 65 hectares for a total of 324 hectares (Wieland and Gordon, 1991 in Moran et al., 1997). But it is not clear how much prairie existed in the Belt prior to the pervasive land alterations of the 19<sup>th</sup> and 20<sup>th</sup> centuries.

Early land surveys provide one source of infor-

mation about the distribution of prairies in the Jackson Prairie Belt. The use of such data to characterize historical landscapes is increasingly important as landscape managers try to conserve or restore disappearing habitats (Noss, 1985, Landres et al., 1999, Laughlin and Uhl, 2003). While the technique has limitations (Noss, 1985, and see below), such surveys provide a starting point for developing maps of historical vegetation.

This study used data from historical land surveys to create a map showing the location and extent of prairies in the Jackson Prairie Belt. These surveys were also examined to look for areas of prairie outside of the Jackson Prairie Belt and Black Belt, to see how common prairies were in other parts of Mississippi.

#### MATERIALS AND METHODS

The Jackson Prairie Belt region is underlain by the Yazoo Clay Formation of the Jackson Group and is composed of Eocene-aged sediments (Moran et al., 1997). Prairie soils are clayey and calcareous with organic matter contents of 4 to 6% (Moran et al., 1997). At the western edge of the Belt, the formation is often covered by loessal soils (Elsen and Wieland, 2003). The Belt covers approximately 566,572 hectares (Elsen and Wieland, 2003).

Data for the prairie map were taken from land surveys conducted by the General Land Office

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(GLO). The GLO employed a rectangular mapping system that established townships in a grid system. As part of this system, the surveyors produced plat maps for each township that frequently show significant features of the landscape, such as rivers, swamps and prairies. These plat maps, along with notes made by the surveyors, have been used in numerous studies to describe the historical vegetation of the United States (e.g., Delcourt, 1976; Schafale and Harcombe, 1983; Nelson, 1997; Bragg, 2003). The surveys are the most comprehensive source of information available on the historic landscape of much of the United States. In Mississippi, the GLO surveys were conducted primarily during the 1830's, prior to the widespread conversion of land to agriculture in most of the state.

To create a map of the prairies in the Jackson Prairie Belt and other parts of the state, all of the plat maps for the state of Mississippi were examined, either as microfilm (at the Department of Archives and History, Jackson, Mississippi) or as paper copies of the originals (at the Office of the Secretary of State, Jackson, Mississippi). Areas of prairie on these plat maps are shown as irregular shapes la-

Table 1. Areas Prairie Belt and GLO plat maps	of prairie in the Jae d adjacent regions l s.	ckson based on
County	Area (hectares)	Prairies
Attala	12.8	1
Carroll	1274.9	19
Clarke	519.7	11
Coahoma	180.4	3
Grenada	114.9	6
Hinds	577.1	18
Holmes	3474.3	39
Jasper	143.6	9
Kemper	14.8	1
Leflore	1305.5	12
Madison	4538.4	64
Rankin	2540.6	23
Scott	1498.4	12
Simpson	39.9	1
Smith	27.5	2
Warren	52.5	1
Wayne	84.7	3
Yazoo	3154.7	40
Total	19555.0	265

April 2005 Vol 50, No. 2

beled with the word "prairie." Often these shapes were sur- rounded by small drawings of trees, to emphasize that the prairies were bordered by forest. Copies of all plat maps showing prairie were scanned to make JPEG files, and then georeferenced using the GIS software program ArcMap (ESRI, Inc., Redlands, CA). The areas of prairie were compiled by county to produce a larger map, showing areas of prairie when the GLO surveys were conducted. Excluded from this map are the prairies in the Black Belt region of Mississippi.

Data on the soil formations of the state were taken from the Mississippi Automated Resource Information System (http://www.maris.state.ms.us), which in turn were derived from the Office of Geology of the Mississippi Department of Environmental Quality (Moore, 1969).

#### **RESULTS AND DISCUSSION**

Based on the plat maps of the GLO surveys, 19,555 hectares of prairie existed in the Jackson Prairie Belt, neighboring areas, and western Mississippi at the time of the surveys (Tables 1 & 2). The greatest concentrations of prairie were located near the western end of the Jackson Prairie Belt, in Madison and Rankin Counties, but sizeable areas of prairie were found outside the Belt, especially in Yazoo and Holmes Counties (Figure 1). In total, 18 counties had at least small areas of prairie in this portion of the state. Areas of prairie were most

Table 2. Area of prairies based on GLO plat maps and grouped by soil formation.	
Soil Formation	Hectares
Alluvium	7268.7
Catahoula	74.7
Cockfield	1531.3
Cook Mountain	22.6
Forest Hill/Red Bluff	342.5
Jackson Group	9998.7
Kosciusko	115.7
Tallahatta/Neshoba	48.9
Vicksburg	50.9
Wilcox	26.6
Winona	72.3
Total	19553.0

common on soils of the Jackson Group and the neighboring Cockfield soil formation (Table 2). However, over 7000 hectares of prairie were located on alluvial soils, along a line from southern Yazoo County to the northeast, suggesting the presence of an "alluvial" prairie region in Mississippi.

The use of surveys and other historical descriptions to characterize historical landscapes has limitations (Noss, 1985). Two types of error are possible in this case: the surveyors may have failed to draw in prairies that were present or drawn in prairies that were not there. The problem of ignoring actual prairies was apparently common, because in several instances prairies are shown crossing the boundaries to adjacent plat maps, where they were not drawn in. How many areas of prairie were simply not shown on the maps is difficult to evaluate, though evidence discussed below suggests it was common. In addition, smaller prairies were more likely to be missed than large ones simply because the surveyors were less likely to encounter them.

The other problem, of drawing in prairies that were, in reality, not there, appears unlikely under most circumstances. The surveyors could, of course, confuse other types of habitats with prairies, but in this instance the only likely error would be the confusion of different types of grasslands. Another possible mistake would be if the surveyors mistook old fields for prairies. This appears unlikely as the surveyors often marked fields on the plat maps, and on some maps prairies and fields occur together.

An additional consideration is that the plat maps represent the extent and distribution of prairies as they were at a particular time. Presumably, as fire (both natural and anthropogenic), drought, temperatures and other factors changed through time, the area of the prairies responded. The map thus represents a snapshot of a dynamic system.

Confirming the accuracy of the GLO plat maps is difficult because few independent descriptions of the Jackson Belt prairies exist. Two early accounts do, however, provide locations for particular prairies, allowing for a partial assessment of the map in Figure 1. Harper (1857), a state geologist of Mississippi, describes the Jackson Prairie formation and notes that "the largest of these prairies are on the line of Wayne and Clarke counties, near Miltonville, in Wayne county, in T[ownship] 10, R[ange] 6 W[est], containing here from five thousand to six thousand acres of fine land." This prairie was marked on the plat map for this township (and is located on Figure 1), though it was much smaller than described by Harper (1857). On the other hand, Harper (1857) also mentions the presence of prairie in Township 3 Range 10 in Jasper County. The plat map for this township does not have any prairie marked on it (though prairie is shown in Township 3 Range 12). Finally, Harper (1857) provides a geological map of Mississippi that indicates 15 areas of prairie in the Jackson Belt. Only seven of these appear to correspond to areas on the plat maps.

Hilgard (1860) also provides both a general description of the Jackson Prairie Belt (see Moran et al., 1997) and the locations of three specific prairies in Rankin County. All three of these prairies are shown on the GLO plat maps for the corresponding townships. Hilgard (1860) also mentions the presence of "small spots of black prairie" in west central Jasper County, but these are not marked on the plat maps.

Another means of assessing the accuracy of the map is to compare extant prairie remnants with historical locations. For example, J.G. Hill (pers. comm.) located a prairie remnant in section 17 of Township 5 north, Range 9 east in Scott County. This prairie is present on the plat map for this township. Moran (1995) published the locations (by township, range and section) of four prairie remnants in Jackson Prairie Belt, in Scott, Smith and Newton Counties. Of these only one corresponds clearly to a prairie on the GLO maps. Thus, both historical and contemporary data suggest that the plat maps show only some of the prairies that were originally present in the region, but it is difficult to estimate how many prairies are missing on the plat maps.

The large area of prairies on alluvial soils outside of the Jackson Prairie Belt was an unexpected result. Most of these areas lie close to the Loess Hills at the eastern edge of the Delta. These prairies are not mentioned by either Harper (1857) or Hilgard (1860), but they do appear in one historical source. Hodgson (1823), when describing a trip to the town of Elliot in Grenada County in the early 1820's, writes "We had a delightful ride along our Indian Path, through a forest of fine oaks; which, within ten or twelve miles of the Yuloo Busha, was occasionally interspersed with small natural prairies, and assumed the appearance of an English park." No other historical descriptions of these prairies have been located. However, in eastern Arkansas an extensive region of prairie, called the "Grand Prairie" occurred on loessal deposits in the alluvial plain of the Mississippi River (Irving et al., 1980). The alluvial prairies seen in Figure 1 may have been similar to the Grand Prairie



Figure 1. Map showing the location and extent of prairies in the Jackson Prairie Belt and the surrounding region of Mississippi during the 1830's, based on General Land Office surveys. The black areas represent the locations of prairies. The shades of gray represent different soil formations as indicated.

in origin. The nature of these alluvial prairies in Mississippi deserves further attention.

#### CONCLUSION

The GLO surveys suggest that over 19,000 hectares of prairies were present in central and western Mississippi in the 1830's, with about half of

this area in the Jackson Prairie Belt. This figure is almost certainly an underestimate since many areas of prairie documented by other sources do not show up in the GLO surveys. Despite these inaccuracies, the map made from the surveys should prove a valuable tool in locating and conserving areas of prairie and in understanding what the natural vegetation of the state was like prior to extensive agriculture.

#### ACKNOWLEDGMENTS

I would like to thank the staff of the Department of Archives and History in Jackson, Mississippi for their assistance, J. G. Hill for unpublished information and help, two anonymous reviewers for their comments and Columbus State University for its support.

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#### **President's Column**

It was an honor and a privilege to preside over the 69<sup>th</sup> annual meeting of the Mississippi Academy of Sciences, which was held at the Oxford Conference Center in Oxford, Mississippi, February 17-18, 2005. This meeting celebrated the 75<sup>th</sup> anniversary of the Academy, and I am pleased to report that it was a tremendous success. The highlight of the meeting was the annual Dodgen Lecture, presented by Dr. Bruce Alberts, President of the National Academies of Science, followed by a combined poster session and reception. Continuing the tradition begun at the 2004 meeting, the 69<sup>th</sup> annual meeting included a number of interesting symposia which covered a variety of topics, including earthquakes, astronomy, drug delivery, supercomputing, advances in health science, and the biotechnology industry. Several educational workshops were also conducted, continuing the Academy's long-standing tradition of supporting science education in Mississippi.

The success of this meeting is due to the hard work and dedication of many people, who have my most sincere gratitude: John Boyle was responsible for bringing Dr. Bruce Alberts as our Dodgen Lecturer, as well as serving as abstracts coordinator and maintaining the web page; Charles Swann was responsible for arrangements with the Oxford Conference Center; Roy Duhe developed and planned the Biotechnology Industry Symposium; the format of the scientific program was developed by a team of persons that included Ham Benghuzzi, Roy Duhe, Maria Begonia, Rob Rockhold, Larry McDaniel, and Cynthia Huff; and the program itself was assembled by Ken Curry, Ann Curry, and Ann Marie Kinnell. I would also like to thank each of the division chairs for their work in designing divisional programs, symposia, and educational workshops which continue to enhance the quality of the annual meeting. I would also like to thank the chairs of standing committees for their valuable contributions: Ken Butler (Publicity; Awards and Resolutions), Michelle Tucci (Membership), Elgenaid Hamadain (Exhibits), and Lance Yarbrough (Poster Chair). In addition, the on-site assistance of Ryan Day, Shruti Chandna, Brandon Fontenelle, Madeleine Leake, Michael Yablick, and Michael Jackson was invaluable. Without the efforts of these persons, the meeting would not have been successful. Finally, I would like to thank our major sponsors for the meeting, The University of Mississippi and Millsaps College, whose sponsorship enabled us to conduct a first-class meeting in a quality location. I look forward to continued success of the Academy and its annual meeting next year!-Sarah Lea McGuire



From left: President elect Larry McDaniels, President Sarah Lea McGuire, and Dodgen lecturer Bruce Alberts.



## Mississippi Junior Academy of Science

http://www.mjas.org/

### **2005 MJAS Competition Report**

The annual MJAS competition was held on February 11, 2005, at the Hinds Community College – Raymond Campus. Dr. Robert A. Thomas, a biologist from Loyola University-New Orleans, gave the keynote address. He presented a very informative talk entitled "Communicating Science: The oft forgotten, but essential element of being a scientist." Attendance increased this year and the quality of presentations was outstanding. I plan on attending the MSTA meeting in October as well as regional science fairs to encourage MJAS participation next year. If you have any connections at the high school level and/or have ideas to help increase our numbers, please contact me at aimee.lee@usm.edu. Thanks to all of the academy members who volunteered their time by judging the presentations.

Attendance report:

# of presenters	14
# of observers	30
# of parents	9
# of teachers	5
# of judges	9

Total # in attendance 77

Schools represented:

Jim Hill High School (Jackson) Murrah High School (Jackson) Oak Grove High School (Hattiesburg) Presbyterian Christian High School (Hattiesburg) St. Andrew's Episcopal High School (Jackson)

### **2005 MJAS Competition Overall Winners**

First Place Winner (Clyde Sheely Award) – Jonathan Priester, Murrah High School Second Place Winners - Antoinette Dawson, Murrah High School Special Recognition – Raven Worthy, Murrah High School

### **2005 MJAS Competition Divisional Winners**

#### **Class I Division Environmental and Behavioral Sciences**

Sandesh Shettar, St. Andrew's Episcopal School & Heather Smith, St. Andrew's Episcopal School Special Recognition

Mary Anne Messer, Presbyterian Christian High School

#### Class II Division Behavioral Sciences, Sociology and Psychology

Samanthia Thompson, Jim Hill High School & Raven Worthy, Murrah High School

#### Class II Division Medicine & Health

Jonathan Priester, Murrah High School & Antoinette Dawson, Murrah High School

### 2005-2006 MJAS Executive Board

President:	Heather Smith alizrdluvr@yahoo.com St. Andrew's Episcopal School
Vice-President:	Sandesh Shettar shettars@gosaints.org St. Andrew's Episcopal School
Secretary:	Mary Anne Messer May10889@yahoo.com Presbyterian Christian Episcopal School
Board Members:	Antoinette Dawson Antdawson88@yahoo.com Murrah High School
	Jonathan Priester Jpriester06@Linuxmail.org Murrah High School

### **Photographs from the 69<sup>th</sup> Annual Meeting of the Missississippi Academy of Sciences** February 2005—Oxford, Mississippi

Posters are a popular way to present research.







Charles Swann, MAS Executive Officer, discusses earthquake hazzards in Mississippi.

Sarah Lea McGuire, MAS President (front row, third from left), and students promote science at Millsaps College.





MAS members enjoy discussing various academic efforts around the state highlighted by displays. (top and bottom photographs)



Robert Fritzius, MAS Division Chair for Physics and Engineering, working with students.



#### **Divisional Reports**

#### **Agriculture and Plant Sciences**

The 2005 meeting was a great success. There was a record number of oral as well as poster presentations. In all there were 25 presentations, 13 oral and 12 poster. All the students who made oral or poster presentations were awarded cash prizes totaling \$625. Money collected for these awards was contributed by Dr. S.L. Sethi \$250.00; Dr. Dinesh Goel, \$100.00; Dr. Sampat Shivangi, \$100.00; Mr. Bodhraj, \$100.00 and Farmers' Co-op, \$100.00.

The award winners are listed below:

Oral competition:	
1 <sup>st</sup> place - \$100.00	Rachel Stout, MSU
2 <sup>nd</sup> place - \$75.00	Chonthida Kaewplong, MSU
3 <sup>rd</sup> place - \$50.00	Maritza Abril, USM
3 <sup>rd</sup> place - \$50.00	Peter Ampin, MSU

Honorary mention: John McGillivray (MSU), Edward Heard (MSU), Melanie Patterson (MVSU), and Preeti Kumar (UM).



Winners of the oral competition (from left): Preeti Kumar, University of Mississippi; Chonthida Kaewplang, Mississippi State University; John McGillivray, Mississippi State University; Dr. O.P. Vadhwa, Chair, Agriculture and Plant Science Division, Alcorn State University; Edward Heard, Mississippi State University; Melanie Patterson, Mississippi Valley State University; Dr. W.L. Kingery, Vice-Chair, Agriculture and Plant Science Division, MSU

Robert Kroger (USM)
Ashley Andrews (MSU)
Gloria Miller (JSU)

Honorary mention: B.C. Owens (MSU), G. Somsamorn (MSU), and Youkai Lu (MSU).



Winners of the poster competition (from left): Gloria Miller, Jackson State University; Youkai Lu, Mississippi State University; Dr. O.P. Vadhwa, Chair, Agriculture and Plant Science Division, Alcorn State University

Please note that the checks for all these awards are yet to be mailed.

There were three judges that participated in the evaluation of oral and poster presentations. These judges were: Dr. Frank Matta, Professor of Horticulture, Mississippi State University, Dr.William L. Kingery, MSU and Mr. Earl Gordon of Stoneville, USDA. Dr. William L. Kingery (Plant and Soil Sciences Dept, MSU) is the chair for the 2006 meetings and Dr. Evelin J. Cuadra was elected as the Vice-Chair, to be chair in 2007.

The meeting was closed by thanking Dr. Juan Silva (past chair) for his invaluable leadership, support and commitment to the Division and MAS. The chair also acknowledged the support of all the judges and vice chair, Dr. William L. Kingery and all the members.—O.P. Vadhwa



Judges for the competition in Agriculture and Plant Sciences (from left): Dr. Frank Matta, Mississippi State University, Mr. Earl Gordan, USDA-ARS, Stoneville, and Dr.William L. Kingery, Mississippi State University, with division chair Dr. O.P. Vadhwa.

#### Cellular, Molecular and Developmental Biology

Summary of meeting events. The meeting of the CMDB Division during the MAS Annual meeting was quite successful despite some unavoidable but distracting room noise. All of our platform presenters are to be commended for performing admirably under less than ideal conditions. Amazingly, no one seemed to be overly bothered by the distractions and the noise forced everyone to pay close attention throughout the meeting. We had a total of 17 oral presentations and 24 poster presentations. Among the oral presentations, 3 were given by undergraduate students. We were quite pleased to have 3 poster presentations given by high school students who were very impressive and professional.

List of awards and awardees. This year's awards were sponsored by VWR (Best Graduate Student Presentation, \$50 and Best Undergraduate Presentation, \$50) and Fisher (\$50 Best High School Presentation). Judge committee members are: Dr. Ross Whitwam (Mississippi University for Women), Dr. Mary Haasch (University of Mississippi), and Dr. Naila Mamoon (University of Mississippi Medical Center), Dr. Hattie Spenser (Mississippi Valley State), Dr. P. C. Yang, Dr. Elgenaid Hamadain and Dr. Stephen Ekunwe (Jackson State University). The CMDB hopes to be able to solicit additional sponsors for next year and all CMDB Division members are encouraged to contact potential sponsors as soon as possible so that we can offer more awards to deserving students. VWR is a new sponsor this year and CMDB is thankful for their generous support. The CMDB Division is very grateful for the long-standing support of Fisher and is especially thankful to Susie Emfinger who over the years has at times provided support out of her personal funds. Any members who see Susie or Ben Tull, please let them know how important their support is to the success of the division.

#### Best Graduate Presentation:

Antony Schwartz – University of Southern Mississippi (Elasri Lab)

Best Undergraduate Presentation:

Amrita Balachandran – Mississippi University for Women (in collaboration with the University of Pittsburgh)

#### Best High School Presentation:

Jonathan Priester - Murrah High School, Jackson, MS (UMMC – Stanley V. Smith)

Future meeting, new Chairs elected for next year. At the business meeting, Dr. Stephen I.N. Ekunwe of Jackson State University was nominated and elected as the Chair and Dr. Lidija Halda-Alija of University of Mississippi Biology Department in Oxford was nominated and elected as the Vice Chair for the CMDB division. For the first time in recent memory, several others expressed interest in taking an active role in division governance and it is hoped that these individuals continue to participate.—Mary L. Haasch



Amrita Balachandran (right) accepts the CMBD award for best undergraduate presentation from division chair Mary Haasch.

#### **Chemistry and Chemical Engineering**

The Chemistry and Chemical Engineering Division had a highly successful meeting. Forty six oral presentations were made as well as twenty-three posters. Attendance at the sessions was the best in years with a consistent audience over twenty. Presentations from University of Mississippi, Mississippi State, Mississippi Valley State, MUW, USM, Jackson State, Delta State and Millsaps College. The Division Chair for next year is Professor Dionne Fortenberry of MUW. The Vice Chair is Professor Mudlagiri Goli of Mississippi Valley State University—John Pojman

#### **Ecology and Evolutionary Biology**

The Division of Ecology and Evolutionary Biology has been growing in size since it was formed three years ago. At the 2005 meeting there were 6 poster presentations and 13 oral presentations. Presentations were made by graduate students and faculty. Schools represented by presentations were Jackson State University, Mississippi College, Mississippi State University, the University of Mississippi, and the University of Southern Mississippi. Topics of presentations ranged from microbial ecology to ecosystems ecology to systematics. We expect the interest and growth in this division will continue with more presentations at next year's meeting. Dr. Clifford Ochs, of the University of Mississippi, was elected as division chair for another year. Mr. Tom Mohrman, of the University of Southern Mississippi, is Vice-Chair.—Cliff Ochs

#### **Geology and Geography**

The Geology and Geography Division hosted 13 presentations and two posters at the 2005 MAS meeting in Oxford, MS on February 17<sup>th</sup>. Presenters were from the State Office of Geology (7), The University of Southern Mississippi (4), University of Mississippi (2), Millsaps College (1), and Mississippi State University (1). Four of the presentations were done by students; Amy Seiter from the University of Southern Mississippi won the best student paper award sponsored by the Mississippi Geological Society. The session attendance ranged from 8 to 16.

The Chair for the 2006 meeting is Barbara Yassin, Office of Geology (barbara\_yassin@ deq.state.ms.us). The Vice-chair is David Ufnar, University of Southern Mississippi (david.ufnar@usm.edu).—Stan Galicki

#### **Marine and Atmospheric Sciences**

This year the division held both a theme session and an open session. The theme was "The Influence of Climate Variability and Anthropogenic factors on Biota and Biochemical Processes in Coastal Waters." The theme session was held on Thursday and included 12 oral presentations and 2 posters. One talk was cancelled due to illness. Eleven of the presentations were prepared and presented by students.

The open session was held Friday morning, when 6 talks were given, 4 by students. Two talks were cancelled.

The division meeting was held at the close of talks on Thursday afternoon. Dr. Judith Williams of USM-Gulf Coast was nominated and elected to serve as vice chair of the division during the upcoming year. Dr. Paulinus Chigbu, vice chair this year, will assume the duties of chair.—Charlotte Brunner

#### Mathematics, Computer Science and Statistics

All the 20 presentations listed in the program and errata, except the poster by Colin Osterman and Cesar Rego were presented as scheduled. Thursday morning the poster presentations were given. Then, afterwards, several interesting talks on the application of graph theory to enumerating chemical molecular formations were presented. On Thursday afternoon, a divisional business meeting was held to elect a new chairperson and vice-chairman for next year's convention. The vice-chair, Dr. Lixin Yu stated that for personal reasons he could not serve next year. Dr. Andrew Harrell of the Engineering Research and Dev. Center in Vicksburg was re-elected chair for 2005-2006 and later on Friday Dr. Elgenaid Hamadain of Jackson State U. was elected vice-chair. On Friday morning, the special subsession on supercomputing was well attended by the heads of computing of many of the State Universities. During this session, an additional presentation by representatives of Silicon Graphics Company was made having to do with the new computer the University of Mississippi is purchasing. Also, a discussion was held about what to do concerning the recent budgetary cutbacks involving the computing departments of the State's Universities. At the end of the main session, on Friday four certificate awards for 1<sup>st</sup> and 2<sup>nd</sup> place best presentations were presented in the student and professional categories. The winners were 1) 1<sup>st</sup> place professional division: Dr. Laura Sheppardson, for her talk on "Generating Molecular Graphs for Predictive Chemistry" and 2) 2<sup>nd</sup> place professional division: Dr. Gregory Tschumper, for his talk, "Toward AB Initio Theoretical Predictions of Chemical Reactions", 3) 1<sup>st</sup> place student division: Frank Mathew for his talk, "A New Ramp Algorithm for the Capacitated Minimum Spanning Tree Problem", and 4) 2<sup>nd</sup> place student division: Susan Lukose, for her talk: "Effective Information Extraction Using Natural Language Processing Techniques". As the division did in 1995,1996, and 1997 paper and poster presenters were invited to submit expanded versions of their talks for publishing in a divisional transactions and also the Journal of the Academy.—Andrew Harrell

#### **Physics and Engineering**

There were six professional and eighteen student oral presentations, and six student posters presented. Mr. James Hill, Director of Rainwater Observatory and NASA ambassador, conducted a symposium in which he described the telescopes and programs at Rainwater Observatory and NASA's Cassini-Huygens mission to Saturn's moon Titan. We learned that, due to light pollution, French Camp is the in the best location in the southeastern United States for dark sky observing and astronomical research. The symposium, which was given in three 45-minute sessions, was well attended by members of other conference divisions with attendance increasing for each subsequent session.

#### Graduate Student Awards

Oral Competition (18 presentations) 1<sup>st</sup> Place Manish Hiranandaani \$100.00 2<sup>nd</sup> Place Matthew Inman 50.00 3<sup>rd</sup> Place Yizhe Zhang 25.00

Poster Competion (Six Posters) 1<sup>st</sup> Place Tezesw Tadepalli \$25.00

The \$100.00 cash award for first place in oral competition was donated by the School of Engineering at The University of Mississippi. Tennessee Valley Authority donated \$350.00 to our program: of this, \$100.00 was used for student competition awards and \$250.00 was paid as an honorarium to Mr. Jim Hill of Rainwater Observatory. Mr. Hill

indicates that he will use the honorarium to help pay for the new Research telescope being acquired from Colorado.

The meeting was chaired by Mr. Robert Fritzius of Shade Tree Physics and Dr. Alexander Yakovlev of the Electrical Engineering Department of The University of Mississippi. Division Officers elected for 2005-2006 are: Chairman: Dr. Alexander Yakovlev, The University of Mississippi; Vice Chmn: Dr. S. Kant Vahpayee, The University of Southern Mississippi.—Robert Fritzius

#### **Science Education**

The Division of Science Education had a very successful meeting with two full days of presentations. There were 26 platform and five poster presentations with three workshops scheduled for Friday afternoon. These 34 presentations indicate growth of the Division. During the business meeting Dr. Joe Sumrall of the University of Mississippi was elected as Division Chair for the 2005–2006 year. The elected Vice Chair is Mr. Todd Adams of the J. L. Scott Marine Education Center and Aquarium, Gulf Coast Research Laboratory of The University of Southern Mississippi.

For the first time awards were presented to student participants. A certificate and cash award of \$25 was awarded to an undergraduate and graduate student. Ms. Erica Peterson of Mississippi Gulf Coast Community College, Jackson County Campus, Gautier, MS received the undergraduate student award for her presentation entitled *Defining Walter Anderson's Influence On Linoleum Block Printing In the United States At The Walter Anderson Museum Of Art.* Ms Jana J. Causey of The University of Southern Mississippi, Hattiesburg, MS was the graduate student recipient. Ms Causey's presentation was entitled *Design And Implementation Of An Interpretive Science Trail For Grades K-12.*—Sheila Brown

#### **Psychology and Social Science**

In 2005, the Division of Psychology and Social Science continued its steady growth with a full day of excellent presentations. Posters and papers were presented by undergraduates, graduate students, and faculty from The University of Mississippi, Tougaloo College, Jackson State University, The University of Southern Mississippi, Millsaps College, Delta State University, University of Mississippi Medical Center, University of North Carolina-Fayetteville and the University of Phoenix. Again, the breadth of psychological and social scientific research was highlighted with papers focusing on such varied topics as using movies to teach principles of logotherapy, trait anger and spirituality, the effects of calcium enriched diets on weight gain, a study of skeletal materials from Chickasaw Bayou, casinos as workplaces, a comparison of in-class and online student evaluations, and a study of Garnett's bushbabies.

Each year divisional awards for best undergraduate and graduate poster and oral presentation have been sponsored by Dr. Pamela Banks, Department of Psychology, JSU. The decision was made to designate the annual awards in honor of Dr. Banks' mother, Mrs. Thelma Gipson. Mrs. Gipson was a life-long advocate of education and took great pride in the educational advance she witnessed in the state of Mississippi during her lifetime. This year's Thelma Gipson Best Undergraduate Paper Presentation Award went to Minervia Scott of Jackson State University, for her paper, *The Role of Religiosity on*  Alcohol Consumption and Alcohol Expectancies Among African-American College Students. The Best Graduate Student Paper Presentation Award went to Stacy Curry, The University of Southern Mississippi, for her paper, The Use of Anthropometric Data in Otolemur garnettii Subspecies The Best Undergraduate Poster Identification. Award went to Theresa Woodard, Jackson State University, for her poster, Trait Anger and Spirituality. The Best Graduate Student Poster Award went to Evan Lee Garner, The University of Southern Mississippi, for his poster, A Study to Determine Traits of Asian Ancestry through Measurement of the Mandible. Each student received a certificate and a monetary award.

At the divisional business meeting, Dr. Sheree Watson (USM–Psychology) was elected chair of the division for 2005-2006. Dr. David Swanson (UM – Sociology) was elected vice-chair. Finally, as usual, special thanks go to Dr. Marie Danforth (USM) who was a great help in transporting equipment for this year's meeting.—Ann Marie Kinnell and Sheree Watson

#### MISSISSIPPI ACADEMY OF SCIENCES Sixty-ninth Annual Meeting—February 2005 Late and Corrected Abstracts

#### Cellular, Molecular and Developmental Biology

#### PROTEOMIC ANALYSIS OF COTTON FILLER PROTEINS AT DIFFERENT DEVELOPMENTAL STAGES

Meng-Hsuan Ho and Din-Pow Ma, Mississippi State University, Mississippi State, MS 39762

Cotton fibers are epidermal cells differentiated from the outer integument of a developing seed. Fiber development can be divided into four stages: initiation, elongation (primary wall synthesis), secondary wall deposition, and maturation. Many genes expressed in fibers cells at different developmental stages have been isolated and characterized. The functional role of these genes that control fiber development is the major goal in our cotton research project. As an alternative approach to understand the molecular mechanism(s) of fiber development, we have used the two-dimensional (2-D) SDS-P AGE method to study fiber proteins that are differently expressed during the elongation and secondary wall synthesis stages. The stained 2-D protein profiles are imaged with Bio-Rad Versa 3000 and analyzed with

the PDQuest 2-D analysis software. The protein spots that are differentially expressed are excised from the gels and analyzed with the Thermo Finnigan Proteomix Workstation (housed in LSBI, MSU), a capillary-column liquid chromatography (LC)-electrospray-ion trap tandem mass (MS/MS) spectrophotometer.

HARNESSING THE *PLASMODIUM FALCIPA-RUM* GENOME DATA-BASE FOR NOVEL DRUG TARGETS

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The morbidity and mortality attributable to malaria are staggering (approximately 500 million new infections and 1 to 3 million deaths per year). Almost half of the world's population is currently under the risk of getting malaria infection. Although the incidences of malarial infection are mostly centered in tropical regions, the impacts, especially economic, of the disease are global. Considerable efforts have been devoted towards understanding the

molecular peculiarities and genome structure of *Plasmodium falciparum*, the causative organism for the most deadly form of malaria. A fully sequenced and annotated genome map of P. falciparum is available now. The parasite genome data-base may be utilized for identification of novel drug targets. Through analysis of PlasmoDB (www.plasmodb. org), expressed sequence-tags data and oligonucleotides/cDNA microarray expression profiles of P. falciparum several potential target genes have been discovered in the malaria parasite genome. Studies were undertaken to clone the target genes, their overexpression in heterologous prokaryotic organisms and purification of the target proteins for functional and structural characterization. A unique proteobacterial-type malate dehydrogenase, an enzyme associated with carbohydrate and energy metabolism pathways, was characterized from Plasmodium falciparum. The enzyme was suggested to complement the function of NAD/NADH coupling in the malaria parasite. A novel adenosine deaminase, an important enzyme of purine salvage pathway, was found to be a peak function enzyme in P. falciparum. Also a novel pathway for biosynthesis of thiamine was discovered in the malaria parasite. The unique molecular, structural and functional characteristics of the malarial enzymes may be useful for design and development of selective inhibitors as potential antimalarial agents.

#### **Ecology and Evolutionary Biology**

#### PRELIMINARY PHYLOGENY OF THE SOUTH-EASTERN *MATELEA* SPECIES

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Matelea (Apocynaceae) is a genus of over one hundred species of mostly twining vines found in tropical and subtropical North and South America. In the southeast United States, eight species are found, and of these, six are in need of taxonomic revision. Within the Gonolobinae, the subtribe to which Matelea belongs, genera are not cleanly delimited, and morphological features defining genera are few. A phylogenetic analysis of Matelea, and the Gonolobinae as a whole, has never been conducted. Thus, there exists a great deal of controversy over the circumscription of these species. A phylogeny of southeastern Matelea was inferred from *rpoB-trnC* intergenic spacer and *rpl16* intron sequences. Taxon sampling included 25 species of Matelea, as well as species of Gonolobus, Phero*trichus, Schubertia*, and *Macroscepis*. Hypotheses tested with this data set included the monophyly of southeastern *Matelea*, as well as the monophyly of Matelea and Gonolobus. Results support the mono phyly of southeastern *Matelea* and *Gonolobus*. Most *Matelea* are placed in a single clade; *Pherotrichus* may belong to the same clade. In addition, the closest relatives to southeastern *Matelea* in the data set are identified.

#### History and Philosophy of Science

## GUNTER LIBRARY: CELEBRATING 50 YEARS OF SERVICE

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Gunter Library was established by Dr. Gordon Gunter in September 1955. As the library celebrates 50 years of service to the Gulf Coast Research Laboratory, a special effort is being made to organize archival materials relating to the history of the library. Archival materials include: newspaper and magazine articles, photographs, scrapbooks. quarterly and annual reports, brochures, maps, correspondence, and reprints of scientific and popular works written by GCRL staff. To highlight the 50 years of service, a poster illustrating library services and activities will be displayed at the 2005 Mississippi Academy of Sciences annual meeting. Included in the discussion is the role of libraries in marine laboratories and their mission in preserving the history of science.

#### **Psychology and Social Science**

## USING MOVIES TO TEACH PRINCIPLES OF LOGOTHERAPY

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Logotherapy is a way of thinking that emphasizes the importance of meaning in life. There are many books and articles that illustrate principles of Logotherapy. However, one potentially useful way of learning about the importance of purpose in life is through contemporary cinema. Movies often address issues related to life purpose. Examples of meaningful movies include *Cast Away* and *Life as a House* (Schulenberg, in press). Despite the existence of meaningful movies, little has been written on using films to teach principles of Logotherapy. With the aid of graduate and undergraduate psychology students, this issue was examined. After being exposed to readings in Logotherapy, students were asked to watch Life is Beautiful, a movie with meaning-laden content (Paden-Levy, 2000). It is the story of a man's love for his family and his passion for life in the midst of their imprisonment in a concentration camp during World War II. Students were then asked the following questions: 1) What Logotherapy themes became evident to you upon viewing the film? 2) What are the pros and cons of using film clips from such movies to teach principles of Logotherapy? Many examples of Logotherapy were noted that involved choice, responsibility, and finding meaning in unavoidable suffering. Many pros of using movies to teach Logotherapy were noted: 1) Modern cinema, with its popularity, can make learning enjoyable; 2) Movies demonstrate concrete examples of very abstract concepts. They apply the principles to life situations that people can see and grasp more clearly than just being taught the principles themselves; and 3) Movies may create an impression that lasts longer than other methods of instruction. Several cons of using movies were noted: 1) Movies may not seem as scientific as other methods of instruction; and 2) The same movie may affect people differently.

## THE MEANING IN SUFFERING TEST (MIST): A FACTOR ANALYTIC INVESTIGATION

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The Meaning in Suffering test (MIST; Starck, 1985), grounded in the Logotherapy framework, was designed to assess the amount of meaning people have discovered in unavoidable suffering experiences. The part of the MIST used in research due to ease of quantification is composed of 20-items using a Likert-type response format ranging from 1 to 7. Items are summed to arrive at a total score that ranges from 20 to 140. The MIST has a proposed structure consisting of three subscales (Starck, 1985): A (subjective characteristics of suffering, items 5, 11, 12, 16, 18, 19), B (personal responses to suffering, items 1, 3, 4, 8, 10, 13, 15, 20), and C (meaning of suffering, items 2, 6, 7, 9, 14, 17). In a

study of the psychometric properties of the MIST and other measures in a sample of undergraduate students (N = 341), Schulenberg (in press) reported that two of the three MIST subscales (A and B) had unacceptably low reliabilities. Concerns over the proposed factor structure led to the current investigation, a follow-up study examining the internal structure of the MIST with the original data set. We attempted to use Amos 5.0 to confirm the MIST subscale structure. However, the fit of the model with the data was so poor that the analysis failed. Next, exploratory factor analyses were performed using SPSS 11.5. A theoretically useful and statistically viable factor structure was not found. Given the current data, the MIST appears to be a unitary measure.

## THE INFLUENCE OF PANIC ON ANXIETY SENSITIVITY

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The purpose of this study project was to examine whether experience with panic attacks influences anxiety sensitivity in an African American sample. Anxiety sensitivity is the fear of anxiety related bodily sensations, arising from beliefs that the sensations have a harmful somatic, social or psychological consequence (Reiss, 1991; Reiss & McNally, 1985). The Anxiety Sensitivity Index (ASI) (Taylor & Cox, 1986) which measures the fear of the symptoms of anxiety was administered. Participants whose scores fell one S.D. above the mean (cut-off score = 55+) and one S.D. below the mean (cut-off score = 25) on the ASI were placed in the high anxiety sensitivity (N=61) and low anxiety sensitivity groups(N = 65), respectively. Personal experience with panic attacks was assessed on the Panic Attack Questionnaire (PAQ) (Cox, Norton, & Swinson, 1992. As predicted, high anxiety sensitivity scores were significantly greater for the panickers (92%)than for the non-panickers (42%) Fisher's exact probability test = 23.46, df = 1, p < 0.001). This suggests anxiety sensitivity is a clear characteristic of persons who experience panic attacks. Because the sample consisted of African Americans, these findings expand the literature regarding African Americans and their panic experiences.

#### Mathematics, Computer Science and Statistics

## ANALYTICAL STUDIES OF THE $N^{\rm TH}$ ROOT OF 2

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Have you ever considered the massive amount of numbers between 1 and the square root of 2? Given the fact that the interval between 1 and the square root of 2 is less than  $\frac{1}{2}$ , it might seem unfathomable that the numbers in this interval contain an uncountably infinite set of irrationals. First, we show that the nth root of 2 is irrational. Second, we show that there is a 1-1 correspondence between decimal approximations to the nth root of 2 and the set of natural numbers, thus showing that the set of these approximations is a countable, infinite set. Also, by taking the derivative and the limits of these approximations as a function of n, we show that the function decreases and converges to 1. We conclude that this set of numbers which approximate the n<sup>th</sup> root of 2 is an infinite, decreasing, irrational function that ranges from 1 to the square root of 2.

#### AUGMENTED WORD FREQUENCY MATRICES FOR TEXTUAL DATA MINING

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Textual data mining typically formulates word frequency matrices to help locate the knowledge desired. In this paper we combine word frequency matrix matching with a part-of-speech tagger to provide finer grained textual-based data mining. The part-of-speech tagger is used to further classify word usage. For example, if the root word "match occured in text A in the sentence "she matched the pieces together," it would be entered into the augmented word frequency matrix as an entry for "match verb". If the sentence "He lit the match" was encountered in text B, the frequenc};' entry will be for "match - noun." The noun, "match" and the verb, "match" describe fundamentally different concepts, and if text A is compared with text B it will be clear that two separate concepts were being discussed. Thus, including the part- of-speech tagger in the data mining operation allows for a more precise results set. The result is a better ordering of 'Google'-like searches.

#### A COMPLEMENTARY ERROR MODEL FOR A FINITE DIFFERENCE LINEAR BAROTROPIC OCEAN MODEL

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In this paper we present a way to objectify grid truncation error. The eventual goal of this objectification is to build a software tool to assist numerical modellers assessment of the impact of their specific grid on their model forecasts. We use a multi-dimensional Taylor Series expansion for all numerical approximation terms within a modeling system. We then transform these to Frequency space, and reformulate the equations with the goal of separating the grid dependent terms from their individual frequency components. This allows us to construct a complementary error model where the evolution of the errors may be approximated in parallel with the forecast model. A complementary Linear Barotropic Ocean Model will be presented.

#### PROGRAMMING A DIGITAL SIGNAL PRO-CESSING CHIP

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Signal processing is the lynch pin of most electronic systems. Historically such si~nal processing has been provided by analogue devices. However, chip speciallzation is now sufficiently inexpensive to allow the manufacture of a processor designed specifically to perform digital signal processing (DSP) in near real time. The processor is available, but the operations are not yet built in. In this project we present several programs to alter a signal. A Fast Fourier Transform, convolution, and several filters are presented.

#### A PEDAGOGICAL STRUCTURE FOR A UNI-VERSITY NETWORK LABORATORY

Ted Stringfellow and A. Louise Perkins, University of Southern Mississippi, Hattiesburg MS 39406

We present the design of a networking laboratory that we have constructed to reinforce the lecture portion of the university of Southern Mississippi computer Science Department's graduate networking course. projects have been constructed that cover all 7 levels of the 150 standard network. Disparate operating systems families of computers and networking equipment are included to provide the widest exposure to the students.

#### **Physics and Engineering**

#### ANALYSIS OF INVERTED-F ANTENNAS US-ING THE FINITE DIFFERENCE TIME DOMAIN METHOD

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This study applies the three-dimensional finite difference time domain (FDTD) method to analyze the Inverted-F antenna, which can be used for wireless local area network (WLAN). The antenna is fabricated on Rogers FR4 printed-circuit-board substrate. The Berenger perfectly matched laver absorbing boundary condition (PML-ABC) is used to truncate the FDTD computational space. The proper choice of the source time domain waveform parameters allows for the antenna frequency response to be obtained using the discrete Fourier transform (DFT) technique. The FDTD results of the designed antenna are compared with the results obtained using the commercial software package ADS Momentum of Aligent Technologies and the measurements conducted at our lab. The good agreement between measurement and simulations is observed and confirmed the suitability of the antenna for WLAN applications.

#### **Science Education**

#### MIDDLE SCHOOL STUDENTS INVOLVED IN RESEARCH OF WATER QUALITY ENVIRON-MENTAL ISSUES

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No environmental issue is a study unto itself. The issue of water quality is, by far, one of the most pressing environmental issues today. Educators agree that children should be taught and encouraged to learn to manage the delicate ecological balance of man and nature. It is from this premise that this Power Point presentation was created. It is concerned with the impact of sustaining the quality water, which is an integral part of al living things on Earth. Middle school student are involved in a demonstration of a series of experiments, oral presentation and display of their findings on how they view their responsibility to contribute to water quality processes and sustain the future of all living things. They explored and discovered the natural processes that created it, display their interpretation of how nature uses it to sustain living things and how nature purifies and recycles it to be reused time and time again. More importantly, students discovered and appreciated their role within this dynamic cycle. Our middle school students will take the center stage in the future. Understanding the process now, we believe, will enhance their active and influential involvement throughout their adult life. Special acknowledgements go to the Saturday Science Academy (SSA) who provided the Middle School students depicted in this instructional demonstration. SSA is a Title III federally funded project of Alcorn State University, providing enrichment in science and mathematics to the surrounding Claiborne and Jefferson county Middle Schools.

#### **Zoology and Entomology**

THE VARIEGATED MUD-LOVING BEETLES (INSECTA: COLEOPTERA: HETEROCERIDAE) OF MISSISSIPPI

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Thirteen species of Heteroceridae representing two genera (Heterocerus and Tropicus) were found to occur in Mississippi, including one new species in the genus Tropicus. Collection data has been compiled from material housed at the California Academy of Science, the University of Mississippi and Mississippi State University. Collecting efforts were focused on riparian habitats throughout Mississippi during the summers of 2003 and 2004. Geographic and seasonal distributions are presented for each species. Of the species occurring here, two are generally distributed throughout the US, seven are widely distributed in the East, one is primarily midwestern in distribution, one is known almost exclusively from Florida and another almost exclusively from Mississippi. The undescribed species of Tropicus occurs in Mississippi, Florida and South Carolina.