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Conference Theme: The Genetic Imperative: Genes and Behavior

The Department of Biology and Molecular Biology at Montclair State University has over 800 undergraduate students taught by twenty full-time faculty members with a broad range of research and teaching interests which are broadly divided into three areas: Molecular Biology, Ecology and Physiology. A number of faculty have research interests that combine two of these areas, including Molecular Ecology, Physiological Ecology and Molecular Physiology.

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Although the Department has a program, it prides itself on experience to the students.

strong emphasis is placed on hands-on learning and student-faculty interactions. All undergraduate students, regardless of major or professional interest, are required to fulfill at least one semester of research. To fulfill this requirement, students have the option of working directly with a faculty member or participating in an internship program. Student body diversity is one of the Department's strengths with over 30% of the students being of Hispanic or African-American descent. Another large portion of the students represent a vast array of nationalities ranging from Asia, Africa, South America, Russia and Europe. Students who have graduated from the program have gone on to become physicians, educators and industry leaders.

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Articles can be submitted electronically to *invivo@mec.cuny.edu* or mailed as a printed copy (preferably with a diskette that contains the file) to the Editorial Board at Medgar Evers College. All submissions should be formatted double spaced with 1 inch margins. The title of the article, the full names of each author, their academic affiliations and addresses, and the name of the person to whom correspondence should be sent must be given. As a rule, full length articles should include a brief abstract and be divided into the following sections: introduction, materials and methods, results, discussion, acknowledgments and references. Reviews and short communications can be arranged differently. References should be identified in the text by using numerical superscripts in consecutive order. In the reference section, references should be arranged in the order that they appeared in the text using the following format: last name, initials., year of publication. title of article, journal volume number: page numbers. (eg. - ¹Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* **32**: 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

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You are invited to participate in the 41st Annual Fall MACUB Conference. Proposals are now being accepted for member paper presentations and poster presentations.

Member Paper Presentations

Proposals are now being accepted for member paper presentations. If you wish to make a paper presentation (20 minutes) which will discuss the results of research or share ideas, please send an abstract to Dr. John Gaynor, Department of Biology & Molecular Biology, Montclair State University, Montclair, New Jersey 07043, 973-665-7253, gaynorj@mail.montclair.edu

Poster Presentations

If you or any of your students wish to make poster presentations, please send an abstract and notify Dr. Carlos Molina, Department of Biology & Molecular Biology, Montclair State University, Montclair, New Jersey 07043, 973-665-3302, molinac@mail.montclair.edu

An Improved Enzyme Linked Immunosorbent Assay (ELISA) to Quantify Endogenous IAA and ABA in Plant Tissues Using Polyclonal Antibodies

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Abstract

We successfully produced two types of polyclonal antibodies against indole 3-acetic acid (IAA) and abscisic acid (ABA). Using Enzyme-Linked Immunosorbent Assay (ELISA) tests with indirect competition, we were able to quantify those hormones in plant tissues. The values were in the order of 0.035 to 0.35 ng/assay and 0.0125 to 250 ng/assay, respectively. Antibodies to the hormones, coupled to protein carriers through their carboxylic groups, were induced in young rabbits. The hormones bound to the alkaline phosphatase through were used as standards. The reactivity, specificity as well as their reliabilities of these antibodies were tested. Passing plant extracts passed through a C₁₈ cartridge, a DEAE-Sephadex A-25 column and a second C₁₈ cartridge will eliminate all possible cross reaction, thus making the technique highly reliable.

Introduction

Growth hormones are known to play a major role in the development and growth of plants. They act at very minute quantities in the plant tissues. Detection of these hormones is difficult and costly in terms of equipment, material and time. The immunological methods have provided solutions to these problems by identifying, localizing and quantifying the hormones without using those costly methods¹⁻⁴. Two methods of labeling of hormones are in use, the Enzyme-Linked Immunosorbent Assay (ELISA) or the radioimmunoassay (RIA). In order to avoid the use of isotopes, ELISA is preferred over RIA⁵. When the comparison is extended to the other techniques, high-performance liquid chromatography (HPLC) and gas chromatography (GC), both ELISA and RIA are suitable for multiple sample analysis⁶. However, hormone quantification with these methods can be complicated by possible cross-reaction induced with closely related substances. The isolation of specific antibodies combined with chromatography of plant extracts resulted in highly and reproducible assays.

Material and methods

Chemicals and equipment

The chemicals used were of the highest of purity available. The synthetic hormones IAA, dimethylformamide (DMF), 1-ethyl-3-(3-dimethylaminopropyl), carbodiimide, dry dioxin,

the 2,4-D and tryptophan were provided by Merck. Bovine serum albumin (BSA) was provided by Serva. ABA ((+/- cis-trans ABA), human serum albumin (HSA), indole-3-acetoamide, indole-3-acetone, indole-3-methanol, indole-3-pyruvic acid, tryptamin, indole-3-butyric acid was obtained from Sigma, Butylated hydroxytoluene (BHT) was obtained from Jansen Chemical. The rabbits in this study used were obtained from stock of New Zeland white rabbits maintained by the university. The polystyrene plates of 32 wells each (300 μ l per well) were provided by Dynatech. The absorbances were read at 405nm using a Multiskan Titertek spectrophotometer.

Synthesis of the immunogenic complex

Due to their low molecular weight and their inability to induce the immunogenic response, IAA was coupled to BSA according to the Weiler's method⁷ and the ABA was coupled to HSA following the Daie and Wyse method⁸. After the formation of the hapten-protein complex through the carboxyl group of the haptens, coupling was complete and there is no residual uncoupled IAA and ABA.

Synthesis of markers with alkaline phosphatase

IAA and ABA were coupled through their carboxyl group to the amino group of alkaline phosphatase (AP) using the carbodiimide method (Fig.1) to form the standards^{7,8}.

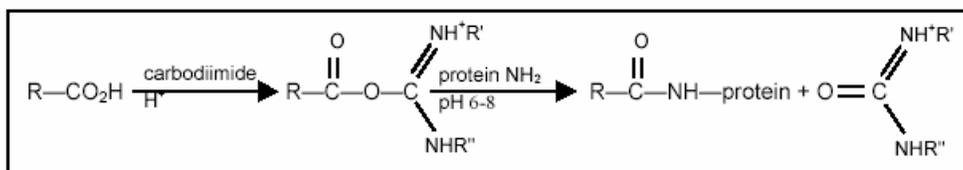


Figure 1. Principle of linkage of hormones through their carboxyl groups to the amino group of proteins (carrier: HSA or BSA and labeler: alkaline phosphatase).

Immunization and production of polyclonal antibodies

The immunization of rabbits and the subsequent serum collection were carried out following a meticulous pre-established schedule (Fig. 2, 3). The schedule continued for 4 months. The first month served as pre-immunization without collecting blood. Blood quantities collected each time varied from 1 to 5 ml. Contaminating proteins were precipitated by rivanol and the immunoglobulins (IgG) by precipitation with saturated ammonium sulfate⁹. After being concentrated through dialysis, the final concentration of IgG was determined spectrophotometrically at 280 nm absorbance. The IgG were kept in saline solution (0.9% NaCl) at -20 °C. Once the collection and purification was completed, the antibody response to the antigen was measured for each collection point, using the Elisa method without competition as described next, and the best response was selected for further use.

Methylation of standards and samples

Indole acetic acid is known to be very unstable due to the reactive carboxyl acid group; therefore it was necessary to sequester it by adding a methyl group. The methylation procedure was performed using a solution of diazomethane in ether. Diazomethane was freshly prepared from nitrosomethylurea just before the methylation step. The synthesis of nitrosomethylurea was prepared as needed and stored at -30 °C.

Preparation of DEAE sephadex a-25 column chromatography

Ten g of DEAE sephadex A-25 were suspended to react for 10 minutes in boiling water. After the gel swelled, 0.6 liter of 1 M ammonium formate was added to 10g of DEAE sephadex A-25.

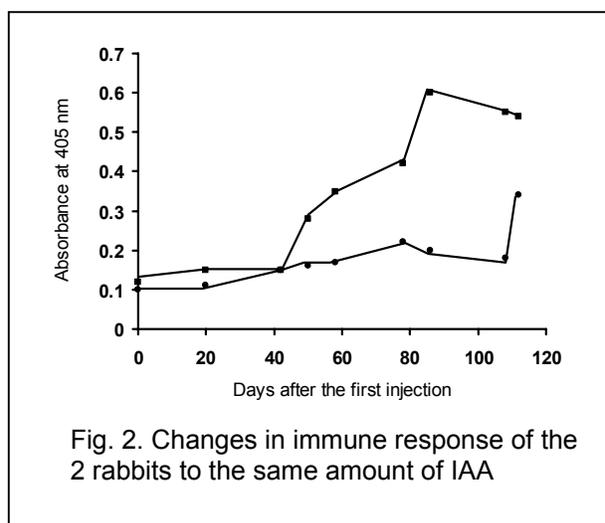


Fig. 2. Changes in immune response of the 2 rabbits to the same amount of IAA

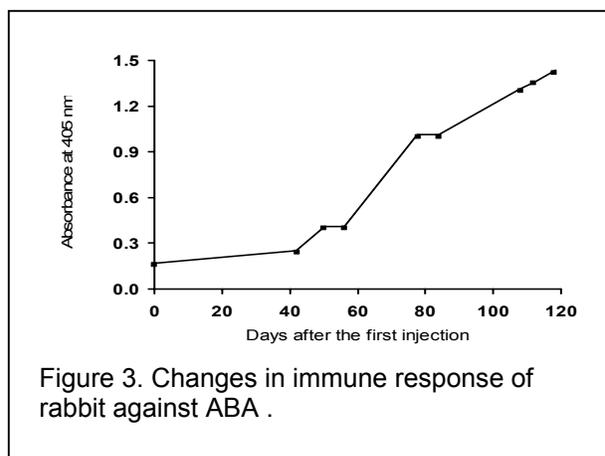


Figure 3. Changes in immune response of rabbit against ABA .

Plant material

Seedling plants of *Lycopersicon esculentum* were grown in small flower pots. After 12 days, they were transplanted to larger pots and six days later the pots were placed into a growth room where the temperature was set at 22°C during the day and at 15°C at night. A group of plants were exposed to light with an intensity of 14,000 Lux (corresponding to 4,266 microwattsc/m²) and the other group was placed in darkness by covering it with an opaque black plastic film.

Extraction and purification of plant material

The apexes above the three first leaves were cut using a razor, weighed and immediately placed in 100% methanol for three hours for extraction. After centrifugation at 27,000 g for 15 minutes at 4°C the supernatant was added to distilled water to form a 50% methanol solution. To further purify it due to the cross reaction observed during the preliminary quantification, the extracts were passed through a C₁₈ cartridge. This procedure permitted the retention of heavy molecules, thus allowing the hormones to pass with the methanol. The eluat was passed through a DEAE-sephadex A-25 column. The column was eluted with formic acid to release the hormones. This eluant was passed once more through the C₁₈ cartridge which retained the hormones because they were protonated by formic acid. The C₁₈ cartridge was washed using diethyl ether to elute the hormones. HCl diluted in methanol was added to the eluant to protonate the hormones. A methylation followed with an excess of diazomethane, prepared fresh from nitrosomethylurea. After methylation, the samples were evaporated in a rotavap and recuperated immediately in their respective buffers, half in tris pH 7.4 for IAA analysis and the other half in PBS (0.80 g of NaCl, 0.2 g of KH₂PO₄.12H₂O, 0.2 g of KCl and 0.2 g of NaN₃ dissolved in one liter of water and the pH adjusted to pH 7.4) for ABA analysis.

ELISA procedure

The immunological analysis using ELISA is based on the indirect competition between either the hormone standards or samples with the hormones marked with the enzyme, alkaline phosphatase (AP) following the principle used by Weiler⁷. The wells of a polystyrene plate were used as follows: All the wells located on the

perimeter of the plate were left empty in order to minimize the edge effect during the readings. The internal 60 wells were distributed as follows: 30 for the standards, 3 repetitions for each of the 10 different concentrations starting from 0.01 increasing to 200 picomol/50 µl for AIA and starting from 0.0125 increasing to 250ng/150 µl for ABA. The remaining 30 wells were used for samples of plant extracts in triplicate. After coating with antibodies, the plate was incubated at 37°C for 8 hours. The wells were washed with buffer followed by addition of the standards, samples and hormones marked with AP. Lastly, the substrate *p*-nitrophenol phosphate, which turns yellow upon conversion into *p*-nitrophenol, was added. Absorbance was read at 405 nm, and the data for the standards was entered into "Cricket Graph" software and the linear equation determined following the least square method. Using that equation and following a program constructed with Excel software, the quantities of the sample were determined (Fig.4).

Results and Discussion

Production of antibodies: Measuring and selecting the most reactive

The reactivity of the antibodies produced at each collection point was measured by the color intensity after the phosphatase reaction. The two rabbits both produced antibodies against IAA (Fig. 2). One rabbit was more productive reaching a peak ninety days after the first immunization. This ninety day sample of IgG was used for subsequent experiments. The other samples were stored. Of the two rabbits exposed to ABA, one died, while the other produced steadily increasing amounts of antibody (Fig. 3). The last, one hundred twenty day, IgG sample was used for all subsequent experiments. The other samples were stored as backup.

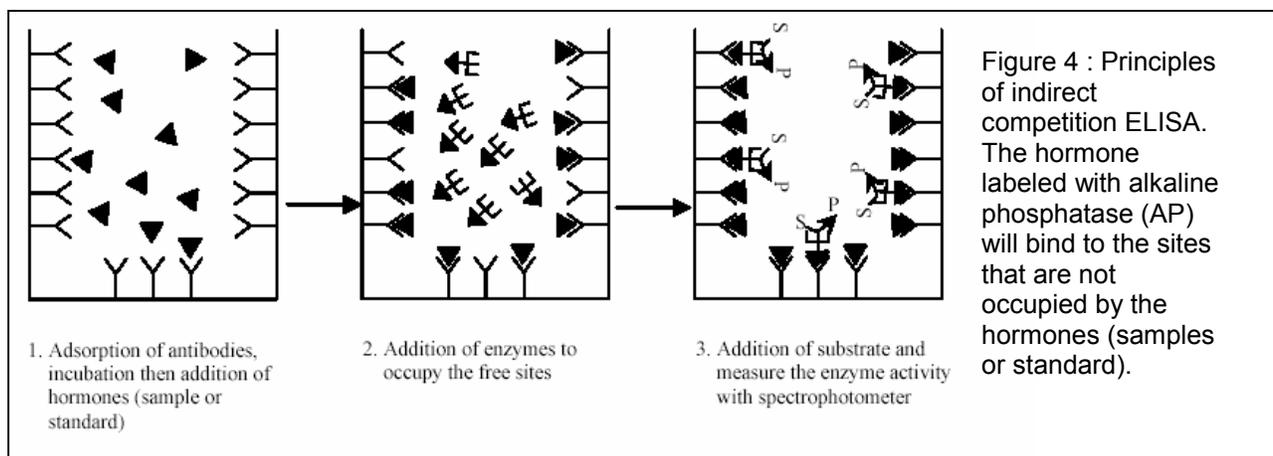


Figure 4 : Principles of indirect competition ELISA. The hormone labeled with alkaline phosphatase (AP) will bind to the sites that are not occupied by the hormones (samples or standard).

Standard curve realization

The examples of standard curves provided here for both IAA and ABA follow the description shown in Figure 4 and described in the methods section. Concentrations of IAA and ABA standards were 0.00, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 200 picomol/50 μ l of tris buffer for IAA and 0.0125, 0.025, 0.125, 0.25, 1.25, 2.5, 12.5, 25, 125 and 250 ng/150 μ l PBS buffer for ABA. The standard curves for IAA shown in Figure 5 and 6 represent the sigmoid shape curve based on the % B/B₀ and linear shape curve (Logarithmic representation). The high coefficient of regression ($R^2 = 0.99$) enabled confident quantification IAA. A high coefficient of regression ($R^2 = 0.98$) allowed a similar confidence for ABA quantification where the linear equation is based in this case directly on the absorbance (Fig. 7).

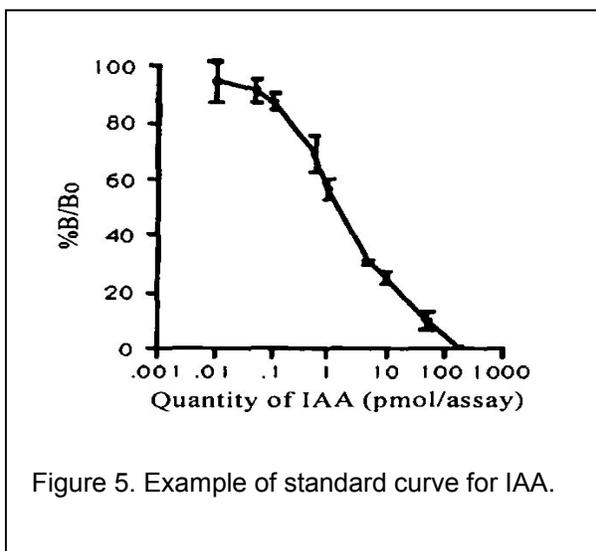


Figure 5. Example of standard curve for IAA.

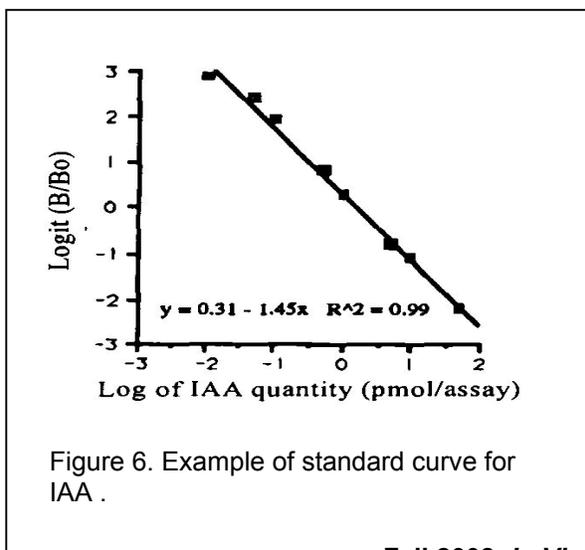


Figure 6. Example of standard curve for IAA .

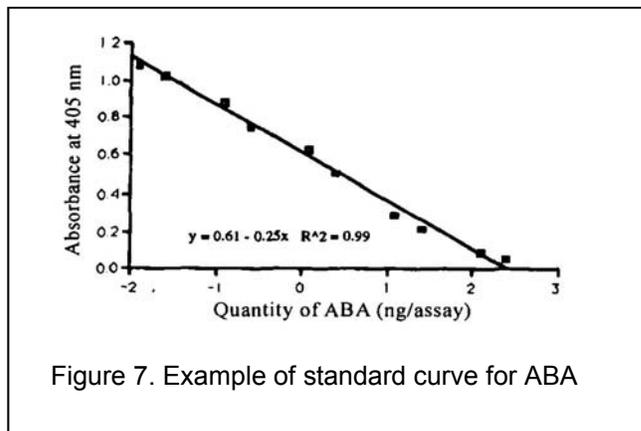


Figure 7. Example of standard curve for ABA

Evaluation of the specificity of the antibodies: verification and validation

Antibodies produced in this manner are polyclonal, and consequently, they could generate cross-reactions with substances similar to IAA and ABA, and in particular IAA is known to have many closely related molecules¹⁰. Using Crozier's method¹¹, crude extracts were serially diluted and the resulting curve was compared to the standard curve. The non-parallelism of the curves is evidence of the interference by other substances (Fig. 8 and 10). Further purification of the extracts with DEAE-Sephadex combined with C₁₈ cartridge resulted in a sample curve parallel to the standard curve for IAA (Fig. 9). Purified samples prepared by a modified Pengelly's method¹² (adding successive quantities of hormones to the diluted sample) show for ABA parallel curves for the two major dilutions (Fig. 11).

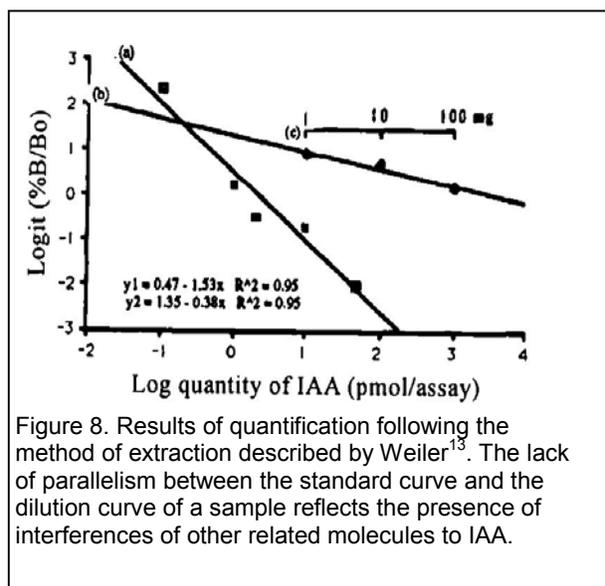


Figure 8. Results of quantification following the method of extraction described by Weiler¹³. The lack of parallelism between the standard curve and the dilution curve of a sample reflects the presence of interferences of other related molecules to IAA.

Quantification of IAA and ABA in tomato seedling exposed to the light and dark

The following experiment was conducted to verify the use of this newly developed method by quantifying the IAA and the ABA levels in the apical tissue of tomato plants grown in light compared to those grown in darkness. The results are shown in Table 1. The IAA quantity in the plants grown in darkness is four times higher than for the ones grown in light. These results are in agreement with what is known about the negative effect of light on IAA concentration by photo-oxidation reducing its normal level¹³. ABA by contrast was present in levels three times higher in the tissues of light grown plants as compared to those grown in the dark¹⁴. The results obtained may be explained by the fact that light converts xanthoxine into ABA^{15,16}.

Conclusions

Due to their very low molecular weight, plant hormones are very difficult to quantify in plant tissues. The immunological technique described is a very efficient tool to measure those minute quantities. We have successfully induced the production of antibodies directed against IAA and ABA and have been able to overcome interference from naturally occurring substances. This method can measure numerous samples in minimum amount of time with reliability similar to the monoclonal antibodies produced against IAA¹⁷ and against ABA⁸. The polyclonal antibody technique can detect IAA concentrations to 0.01pmol/assay. The DEAE-Sephadex/C18 cartridge effectively removes interfering substances. The antibodies produced against ABA can detect this substance to 0.0125ng/assay. The ABA injected into rabbits which was in the (\pm) *cis-trans* form, and the (+) *trans* form of ABA, does not exist or may be in very small quantities in plant tissues. There seem to be few molecules that could interfere with ABA. Substances such as phaseic acid and dihydrophaseic acid, do not interact with ABA antibodies as determined by Daie and Wyse⁸.

When applied to an actual measurement, tomato IAA and ABA levels shown agree with the levels found in the literature. The IAA levels found in the apices of tomato plants exposed to light are low compared to the plants grown in the dark. The IAA is known to be photooxidized by light. The ABA levels found in the apices of light grown tomato plants are higher than those grown in the dark. Light is known to convert the ABA precursor into ABA^{16,18,19}. In conclusion, the method is reliable, rapid and effective and could be of use to researchers in the field.

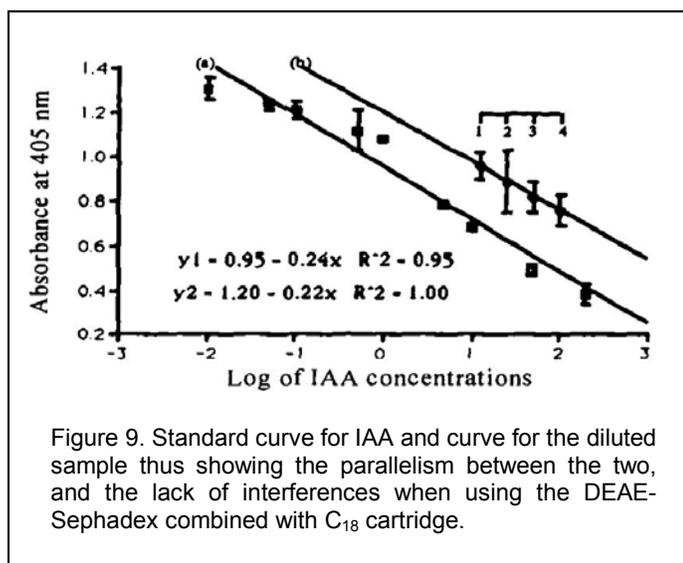


Figure 9. Standard curve for IAA and curve for the diluted sample thus showing the parallelism between the two, and the lack of interferences when using the DEAE-Sephadex combined with C₁₈ cartridge.

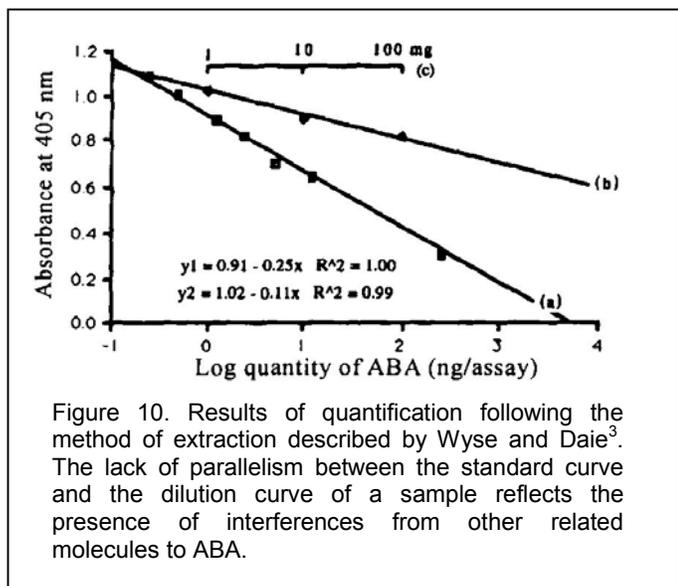


Figure 10. Results of quantification following the method of extraction described by Wyse and Daie³. The lack of parallelism between the standard curve and the dilution curve of a sample reflects the presence of interferences from other related molecules to ABA.

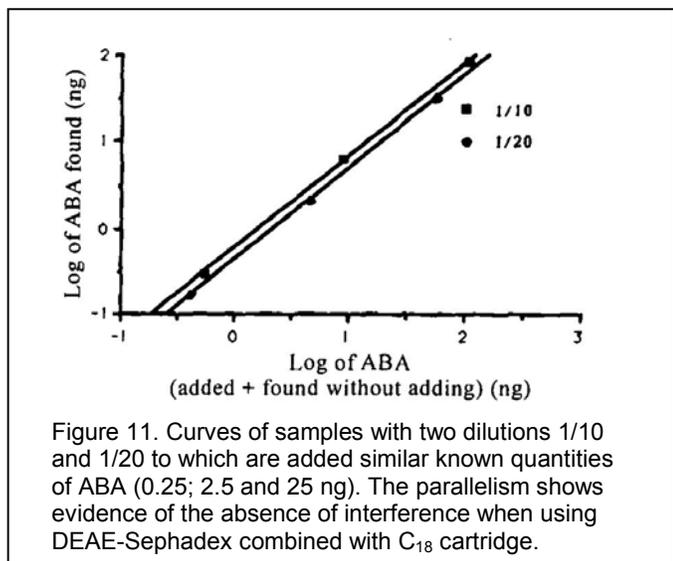


Figure 11. Curves of samples with two dilutions 1/10 and 1/20 to which are added similar known quantities of ABA (0.25; 2.5 and 25 ng). The parallelism shows evidence of the absence of interference when using DEAE-Sephadex combined with C₁₈ cartridge.

Table 1		
Treatment	IAA ($\mu\text{g}/\text{kg}$ fresh weight)	ABA ($\mu\text{g}/\text{kg}$ fresh weight)
Plants grown in light	3.9 \pm 1.21	38.26 \pm 19.89
Plants grown in darkness	15.77 \pm 8.59	13.66 \pm 6.01

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**Roles of Apolipoprotein e4, and Suprachiasmatic Nucleus:
Age Related Cognitive Decline and Sleep Disordered Breathing/Sleep Fragmentation**

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Abstract

This article evaluates the roles of apolipoprotein (APOE) e4 and the suprachiasmatic nucleus (SCN), with respect to the analysis of age related cognitive decline and sleep disordered breathing/sleep fragmentation. The interaction between these two independent physiological factors, and the non-linear analysis of physiological measurements were explored. There were significant differences between the two groups in term of total sleep time of ≤ 6 , and > 6 hours. The major findings of extra short and extra long sleepers were noted. Sleep disturbances with the related sleep fragmentation are in the vanguard to be linked by old age. Findings in 64 noninsomniacs, along with 60 insomniacs, all have indicated that SCN function declines even without insomnia. The overall analysis and impression provide a better understanding of the complex relationship presented in the title of this article.

Keywords: Sleep Disordered Breathing, Circadian rhythm, Sleep fragmentation, Aging, Alzheimer's disease, APOE e4

Introduction

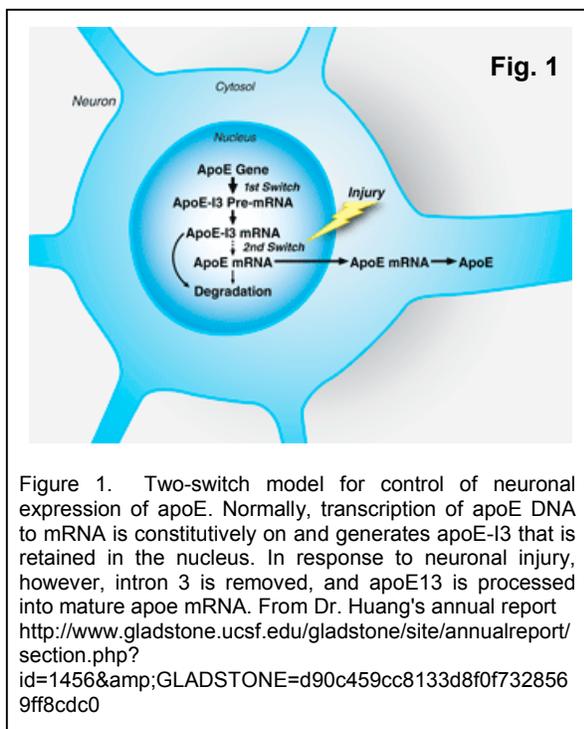
Alzheimer's Disease (AD) is a common neurodegenerative disorder affecting the elderly. It, as well, is a main cause of dementia in Western countries. Notwithstanding that the exact diagnosis of this disease is thus far only possible by postmortem pathological examination, a differential diagnosis with other types of dementia and with major depression should be attempted. People with severe clinical manifestation of AD may die earlier due to various etiologies including but not limited to sleep apnea/hypopnea. Conversely, patients who are both sleep apneics and apolipoprotein (APOE) e4 allele carriers who live long enough to old age are survivors and may have suffered from less sleep apnea in the first place. The APOE gene has three regular alleles (e2, e3 and e4). Each individual carries two copies of the allele. Individuals with one copy of the e4 allele may have enhanced risk of AD and ischemic heart disease. Individuals with two copies of e4 have an even higher risk. The association between sleep apnea and APOE e4 allele status involves a risk factor that may cause either early death or survival. The latter group who survived were censored. Censoring is a method of dealing with missing data that is common in survival analysis studies. If a subject's lifetime is known to be less than a certain duration, the lifetime is said to be left-censored. For a left-censored datum, we know that the subject exists. Obviously, some of those who survived may have suffered from AD.

With respect to neuroexpression of APOE, its controlling model is as follows. A two-switch model for control of neuronal expression is working. Normally, transcription of APOE to mRNA is constitutively on. Nevertheless, in response to neuronal injury such as stress, hypoxia, anoxia, sleep disturbance/fragmentation, etc., intron 3 is removed and APOE 13 is processed into mature APOE mRNA, according to Dr. Huang's report in Figure 1.

One of the aims of this study is to focus on SCN reduction in the function in the elderly and to determine how much SCN reduction in function could be measured in the elderly with normal cognition who had complaints of sleep disturbances, so that we only measure the effect of age and not anything else on them¹. The physiological effects studied are aging and evidences for circadian rhythm reduction in amplitude phase. This study also aimed to demonstrate the subjects' ability to stay awake in the evening or stay asleep at the end of the night, regardless of APOE e4. Another aim is to analyze age-related Sleep-Disordered Breathing/Fragmentation.

Materials and Methods

This retrospective case-series study aimed to assess 124 elderly individuals for sleep disturbance. After meeting the criteria of exclusion: age below 65 years, heart failure, and chronic obstructive lung disease, they were



admitted for nocturnal polysomnographys (PSG) from Jan.1, 2002 to Jan.31, 2003 in the Sleep Medicine Center (laboratory), Changhua Christian Hospital, Taiwan. During that period, 1,087 PSGs were performed. The Hospital Internal Research Board and Ethical Committee approved the study, which conforms to the Declaration of Helsinki. The author reports no conflict of interest. Data of 1,014 controls came from 15,798 subjects, who had participated in a health survey from 1993 to 1996. The data of age and blood pressure of these 1,014 subjects were analyzed. Among them, there were 665 with the complete data of their weights, heights, and body mass indexes (Table 1).

The protocols followed in this study are as following:

1] Dementia - The diagnosis of a dementia syndrome requires acquired, progressive impairment in two or more domains that is severe enough to cause social disability.

2] Apolipoprotein (APOE)e4, and APOE genomic DNA typing - For Alzheimer's disease, the APOE e4 allele is a genetic risk factor, nevertheless, presence of e4 allele alone is not sufficient to produce cognitive decline. The protocol of APOE genomic DNA typing is as follows. Genomic DNA is extracted from frozen whole blood samples and APOE genotyping is performed using the Hixson and Vernier restriction isotyping protocol.

3] Electroencephalography (EEG) - In this study, the EEG is incorporated in PSG. Researchers found that cerebral frontal lobe dementia (FLD) patients had more severely decreased frontal blood flow reduction and less severe parietal blood flow reduction compared to AD patients. Among patients with mild dementia the EEG changes were less severe in the FLD group².

4] Protocol of PSG - The following parameters were measured and recorded continuously by the computerized Alice-4-Polysomnography System-Respironics: (1) chest and abdominal wall motion by uncelebrated respiratory inductance plethysmography; (2) heart rate, by ECG; (3) inspired and end-tidal carbon dioxide pressure (PETCO₂); (4) combined oral nasal air flow, sampled with a three-pronged thermistor placed at the upper lip; (5) arterial oxygen saturation (SaO₂) by pulse oximetry (model N 200; Nellcor); (6) oximeter pulse wave form; (7) electro-oculogram; (8) EEG; (9) chin electromyogram; (10) actimeter (placed on the hand); and (11) microphone placed over neck. The transducers and lead wires permitted normal positional changes during sleep. PSG records were scored for sleep, breathing, oxygenation, and movement in 30-second periods. Such scoring was done by the full timed well experienced PSG annalist; (12) the Apnea-hypopnea Index (AHI) is defined as the number of events of apnea-hypopnea per hour.

5] Interpretation of PSG Data - PSG records were scored for sleep, breathing, oxygenation, and movement in 30-second periods. Sleep data were staged as stages I, II, III, and IV and rapid-eye-movement (REM sleep). An abnormal breathing event during objectively measured sleep was defined according to the commonly used clinical criterion, either a complete cessation of airflow lasting 10 seconds or more for apnea. The average scores or AHI were calculated as the summary measurement of SDB.

6] Roles of Suprachiasmatic Nucleus (SCN) - SCN declines with age and is more diminished with advanced age. Upon aging, it is theorized that there is a marked decrease in the total number of arginine vasopressin-positive (AVP) and vasoactive-intestinal-peptide neurons, and a diminishing of the SCN volume, all of which is determined by post-mortem examination, which was not performed in this study, as there were no death cases in this study.

Table 1. Standardized difference and comparison between the study cohort and control data

Characteristics	Study population Mean 2 (M2) ± SD (SD2*)	Control data Mean 1 (M1)	Standardized difference (M2-M1/SD2*)
Minimal age (years)	65 (n=124)	65 (n=1,014)	
Mean of ages (years)	71.69±4.86	71.97±5.86 (n=1,014)	-0.048
Mean of height (cms)	159±7.75	157.03±8.87 (n=665)	0.31
Mean of weight (Kgs)	65.5±11.96	58.7±10.59 (n=665)	0.64
Ratio of males to females	1.17(m) : 1(f)	50.1(m) : 49.9(f)	
Snorers/total number of subjects	88.71% (110/124)		
Diastolic blood pressure greater than 90 mmHg, and AHI greater than 5	32.7% (54/124)		
Diastolic blood pressure greater than 90 mmHg, and AHI less than 5	8.87%		
Mean of BMI	25.68±4.54	23.77±3.66 (n=665)	0.52
Mean for effect size	M2	M1	
Mean of cervical circumference (cms)	36.45±3.39		
Mean of systolic blood pressures (mmHg)	140.08	140.64±22.39 (n=1,014)	-0.025
Mean of diasystolic blood pressures (mmHg)	79.87	79.17±13.56 (n=1,014)	0.05

*(M2-M1)/SD2 between study and control populations was calculated whenever feasible. 0.1 is considered a low level, 0.25 is a medium level and 0.4 is a high level. **only 665 individuals had height and weight records available.

Table 2. Long Sleepers ≥ 7.5 hours			
		TST	AHI
TST_hr	r	1.000	-0.086
	Significance		0.872
AHI	r	-0.086	1.000
	Significance	0.872	

Spearman's Rank analysis: TST - total nocturnal sleep time, AHI - apnea-hypopnea index (the number of events per hour), r = Spearman's rank correlation coefficient rho, N=6 (see Fig.2)

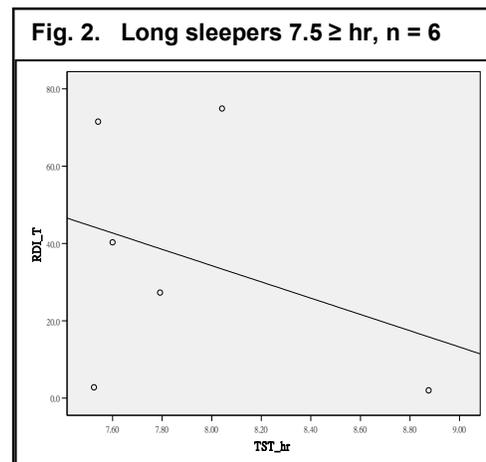


Table 3. Short Sleepers 5.5 hr to 6.5 hr			
		TST_hr	AHI
TST_hr	r	1.000	0.107
	Significance		0.483
RDI_T	r	0.107	1.000
	Significance	0.483	

Spearman's Rank analysis: TST - total nocturnal sleep time, Respiratory distress index per time is approximately equate to AHI per hour, r = Spearman's rank correlation coefficient rho, N=45 (see Fig. 3)

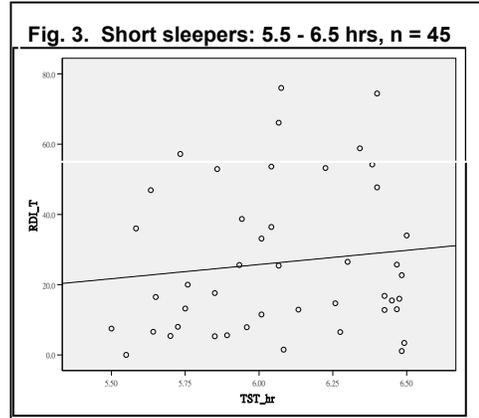


Table 4. Extra Short Sleepers 4.5 to 5.5 hr			
		TST_hr	AHI
TST_hr	r	1.000	-0.018
	Significance		.932
AHI	r	-0.018	1.000
	Significance	0.932	

Spearman's Rank analysis: TST - total nocturnal sleep time, AHI - apnea-hypopnea index (the number of events per hour), r = Spearman's rank correlation coefficient rho, N=24. (see Fig. 4)

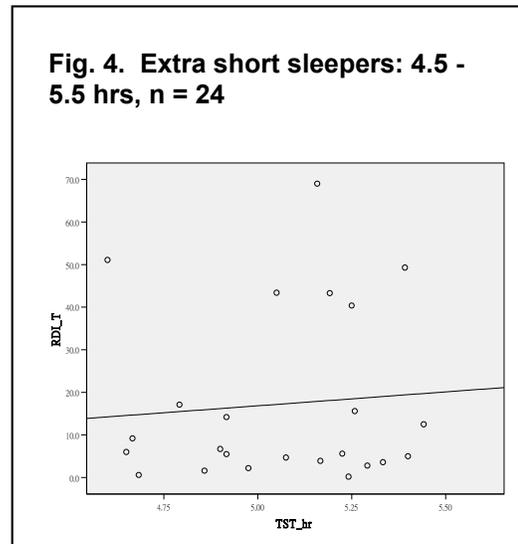
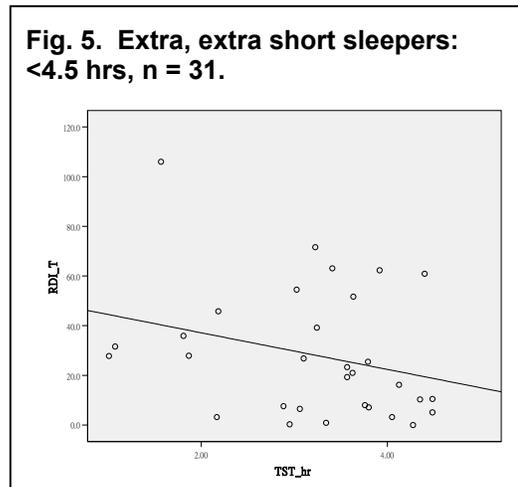


Table 5. Extra, Extra Short Sleepers < 4.5 hr			
		TST_hr	AHI
TST_hr	r	1.000	-0.269
	Significance		0.143
AHI	r	-0.269	1.000
	Significance	0.143	

Spearman's Rank analysis: TST - total nocturnal sleep time, AHI - apnea-hypopnea index (the number of events per hour), r = Spearman's rank correlation coefficient rho, N=31. (see Fig. 5)



7] Statistical analysis - Data were analyzed with statistical software (SPSS 10.0). A two-tailed p value < 0.05 was recognized as significant.

Results

There were 124 subjects who were not demented, aged from 65 to 88.5 years with no signs of mild cognitive impairment (MCI) either. Among the 124 subjects there were 60 insomniacs. There were significant differences between the two groups in term of their total nocturnal sleep time (TST) of ≤ 6 , and > 6 hours. The major findings were as follow. (1) Extra short, but as well as long sleepers, were noted. (2) There was a significant observation for the elderly who were aged 80 years and older. (Tables 2 - 5 and Figs. 2 - 5) (3) The importance of other sleep variables and circadian rhythms were also elucidated. (4) The concept of probability-based evaluation of sleep variables among insomniacs was assessed and confirmed. It is consistent with the previously published finding that poor sleep in insomniacs seems to be predictable in 2/3, while unpredictable in 1/3 of the sample³. It can thus be observed that sleep disturbances with fragmentation are in the vanguard to be linked by old age. Findings in 64 noninsomniacs with other sleep disturbance, along with 60 insomniacs, all have indicated that SCN function declines even without insomnia.

Discussion

Respiratory and sleep variables

The APOE e4 allele is a genetic risk factor for about 1/4 of general population³. Nevertheless, the genotype seems to confer as much as twofold increased risk for the development of obstructive sleep apnea/ hypopnea (OSAH). In addition, a disturbed sleep-wake rhythm is common in AD patients and well correlated with decreased serum melatonin levels⁴. Nevertheless, there are still several other mechanisms underlying non-apneic oxygen desaturation during sleep. Such mechanisms include but not limiting to decreased functional residual capacity, diminished ventilatory responses to hypoxia and hypercapnia, among other causes⁵.

Diagnostic period

Diagnostic period should be considered as a confounding factor. As in the multivariate

analyses, one can easily adjust the confounding degree of a confounding factor. Had any study been indicated with any diagnostic period, either early or late, it would have shown E (Early diagnosis), and L (Late diagnosis) respectively. For example, the serum melatonin levels to be checked in the subjects who are suspected of Alzheimer disease, then E can be established as the serum melatonin levels have altered even in the pre-clinical stage of AD. For another instance, the same principle can be applied to distinguish between cognition normal elderly (CNE) cases and minor AD neuropathological changes. Naturally, the criterion of being 'Early' or 'Late', in addition, of being "pre-clinical" or "clinical" has to be respectively defined. Conversely, a larger longitudinal study will be indicated to evaluate whether APOE e4 carriers increases vulnerability to the negative effect of OSAH on cognition in AD patients. In the light of the molecular changes underlying reduced pineal melatonin levels in AD patients, the APOE e4 allele would certainly become an additional risk variable of negative influence on cognition, especially in AD patients.

Sleep apnea

Regarding sleep apnea, subjects with APOE e4 carriers might be more likely to be overweight (according to the criteria of NIH, the range of BMI for normal is from 18.5 to 24.9, and that for overweight is from 25 to 29.9. It is noted that Asian BMI criteria have been considered independently.), and being over weight alone might have already accounted for the higher prevalence visuospatial speed of processing. It is also entirely possible that people do not perceive irregular breathing in themselves, but the fact that apnea occurs during sleep may make them lighter sleepers, and more prone to notice their own sleep disturbance symptoms, as opposed to people who are able to sleep more deeply and efficiently.

Silent obstructive apnea vs. central apnea, and preclinical AD vs. CNE

As it is not at all certain that AHI is a risk factor in subjects who are ≥ 65 years. It is noted that for those who ≥ 65 years, basically there are cases of central apnea⁶, instead of obstructive apnea. It is as well reported that central apnea is able to predict the mortality of those ≥ 65 years. Others published papers show that in the elderly, sleep apnea may not even be much of a risk factor⁷,

notwithstanding that the prevalence of OSAH is 19% in a study of 1,775 subjects (the mean age 71 years, range 40-100 years)³. The prevalence of undiagnosed sleep-disordered breathing (SDB) is high among men and is much higher than previously suspected among women. The SDB is also associated with daytime hypersomnolence⁹. Nevertheless, the focus of the dilemma is still as following: 1) in the general population, as previously mentioned, there is at least 25% APOE e4 allele carriers. The question is what should we do with those APOE carriers who are also with central type of sleep apnea and undiagnosed sleep-disordered breathing (SDB)? In addition, 2) there are pre-clinical stages of AD subjects who might as well be silent in the non-OSAH-type of sleep apnea. Such a condition has not yet affected the function of their cognition to the extent that the clinically diagnostic period concerned. Of importance in this context is the issue that how should we properly deal with such circumstances, both scientifically, and bioethically?

The cutoff of AHI

The estimated prevalence of sleep-disordered breathing has been defined as an AHI score of 5 or higher. It is 9% for women and 24% for men. Male sex and obesity were strongly associated with the presence of SDB. Conversely, habitual snorers, both men and women, tended to have a higher prevalence of AHI of 15 or even greater⁹. The cutoff point of AHI reveals that an AHI index of 10 or more would be considered pathological in young adults; whereas most agree that a pathological cutoff of 15 or even 20 is more reasonable in older adults. An AHI of 15 per hour is the proscribed cutoff for determining a high vs. low level of nocturnal sleep apnea-hypopnea in APOE e4 carriers. This may make an extensive difference in the conclusion. Likewise, the same attention should be paid to patients suffering from sleep apnea from the stage of asymptomatic progressing to that of seriously symptomatic sleep apnea.

Interaction between APOE e4 and OSAH

As for the regression context¹⁰⁻¹⁴, one can include an interaction between APOE e4 and OSAH, or between AHI and BMI on APOE e4 allele carriers. In the latter perspective, within a single model of the relationship between the variable of APOE e4 allele as opposed to either AHI or BMI variable, interactions can be studied

by examining the relationships between the dependent and independent variables one at a time while controlling for the other.

BMI

The grouping factor of BMI, and its relationship with snoring

Based on the current study of 124 subjects aged 65 year and over and all with sleep disturbance⁸, BMIs were dichotomized at the value of 25. The individuals whose BMI > 25 had more frequent snoring than those whose BMI < 25. (Kruskal-Wallis test, d.f. = 2, and p = 0.0001, while Mann-Whitney test, d.f. = 1, and p = 0.0001 respectively). Some studies report that snoring and breathing difficulties for irregular breathing is more common in the elderly with AD than those elderly CNE. Sleep problems, along with institutionalization of the elderly with AD have previously been reported¹⁶⁻²⁰.

It might be enlightening to ask participants who do snore whether they have always snored; if the researchers are paying sufficient attention in identifying whether snoring is something that precedes or results from changes in BMI then asking the participants about any changes in either snoring behavior or BMI, this way may easily clarify such an important relationship.

The relationship between BMI and performance of reasoning, as well as that between BMI and visuospatial speed of processing

A study reported how elevated BMI affects cognitive function in elderly people⁹. Overweight individuals had better cognitive performance with respect to reasoning and visuospatial speed of processing than normal-weight people. Obesity was related with better performance in visuospatial speed of processing than normal weight^{21,22}. Therefore the relationship between BMI and cognitive function ought to be investigated prospectively. In fact, monitoring the performance of reasoning, as well as that of visuospatial speed of processing will be indicated in light of the presence of overweight participants.

Age difference

It is noted that the APOE e4 allele represents a major risk factor for AD in all ethnic groups as reported in one of the studies²³, across all ages

between 40 and 90 years, and in both men and women. It is noted that this age range includes that of this study cohort.

Gender: APOE e4 allele is associated with normal age-related decline in neurocognitive functions in women only

Gender is not only an important factor, but also a significant variable especially when APOE 4 is considered. APOE 4 allele is associated with normal age-related decline in cognitive functions in women only²⁴⁻²⁷. This finding may be supportive of the evidence suggesting sex differences in APOE-associated risk for AD. The sex difference in the risk of sporadic AD may be partially explained by a sex-specific impact of the APOE 4 allele on age-related cognitive decline²⁴. In fact, it is very important to notice that many of our cognitive states and processes are hybrids, unevenly distributed across biological and non-biological realms²⁵⁻²⁷.

Comparison between the US and Taiwan on APOE

With respect to social and cultural impact, the difference between Taiwan and the US ought to be considered. Furthermore, data indicate that less than 1/5 of Taiwanese carry the e4 allele as compared to 1/4 of the Western population (15% versus 26% for Taiwanese compared to the US^{28,29}).

Limitations of animal studies being inferred to effects on humans

Kheirandish³⁰ recently reported a documented vulnerability of APOE-deficient mice to hypoxia-induced neurocognitive impairment. Gozal *et al.*³¹ reported increased susceptibility to intermittent hypoxia in the aging rat. This author envisions that the problem is mainly on whether this type of animal research is a suitable model or not for inferring the effect of ageing in the human brain. It appears now to be more skeptical than it used to be when we evaluate the decreasing volume of hippocampus to pit against a review paper of 423 studies^{32, 33}. The examination of hippocampal volume does not mean significantly specific. Such an examination does not help in making distinguishing between mild cognitive impairment and AD. Conversely, it is noted that neuroplasticity of new neurons and their network connections due to sleep deprivation leads to new

neural networks of activation³⁴. Hence, we should all pay attention "About representation; or, how to avoid being caught between animal perception and human language" just as one of the articles title speaks for itself³⁵.

The role of SCN and APOE e4 allele

There is a concern about SCN reduction in function by measuring the effect of age⁸. Studies of the physiological evidences resulting in reduced SCN function^{37,38} indicate that the SCN decreases its volume with age, and is more reduced with older age, regardless of APOE e4 carriers or not. With respect to the role of APOE e4, insufficient statistical power, study design, dissimilarities in geographic and ethnic upbringing, allele incidence, gender, along with the interrelation between latent genetic data and the environment may have their roles in the contradictory situations of various studies. Nevertheless, the APOE e4 allele is indeed a significant risk factor for coronary heart disease³⁹. It also is a major risk factor for late-onset AD.

Conclusion

On balance, hopefully we can somehow unravel the complicated biophysiological relationships rotating around dementia, AD, MCI, aging, APOE e4, stress stimuli, sleep apnea, and fragmentation. Conversely, APOE e4 with or without cerebral vascular ischemic conditions, SCN in advanced aging, SDB, and hypoxia-induced cognitive impairment merit our attention as well.

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Sunfish As Paratenic Hosts For Nematodes

by

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Abstract

Transfer of the nematode *Eustrongylides* sp. from infected *Fundulus heteroclitus* to a potential host (sunfishes) does not readily occur, even when presented as live food. However, this parasite became established in 50% of the sunfishes when gavaged, and bored into the viscera of the paratenic host.

Introduction

Sunfishes, including pumpkinseed (*Lepomis gibbosus*), and bluegill (*L. macrochirus*), may be hosts or paratenic hosts for the nematode *Eustrongylides* spp. Both sunfish are predatory and can acquire *Eustrongylides* after ingestion of the primary (oligochaetes) or secondary (other fishes) intermediate hosts infected with the larvae of this nematode¹. Fish-eating birds are usually the definitive hosts of these parasitic nematodes¹.

Infection by larval *Eustrongylides* spp. is widespread and common in numerous species of freshwater fish². For example, *Fundulus heteroclitus*, a euryhaline species, is a frequent host of the larvae of this parasitic nematode² especially in fresh water. This fish may be eaten by sunfishes where their ranges overlap.

The purpose of this study was to examine two routes of parasitic infection of sunfish including ingestion of killifish infected with *Eustrongylides* and exposure to live, encysted fourth stage *Eustrongylides* removed from killifish and presented as food to the sunfish. The study also examined the fate of the nematodes within the sunfish after the fish were gavaged with live fourth stage larval *Eustrongylides*.

Methods and Materials

Approximately 200 *Fundulus heteroclitus* (killifish), 60 to 98 mm total length, weighing one to three grams, were collected from a freshwater pond located on Sandy Hook, New Jersey, April, 2007. These were maintained in fresh water in the laboratory at 20°C. More than 50 bluegill sunfish,

Lepomis macrochirus, weighing an average of 10 grams, were obtained from the Hackettstown, New Jersey, fish hatchery on April 3, 2007. Six fish were sacrificed at the hatchery, and a necropsy performed to determine if any *Eustrongylides* spp. were present. The remaining bluegill were brought to the laboratory where 18 (11 males and 7 females) were placed into a tank of aged fresh water, without other fish. A second, and a third group, one containing 12 sunfish (6 males and 6 females), and a second containing 11 sunfish (6 males and 5 females) were placed together with 50 *Fundulus* each in their respective tanks. All fishes were fed a 24% protein mash, and fish pellets. In addition, the second group of 11 sunfish, together with the *Fundulus*, were also fed live *Eustrongylides* larvae obtained from *Fundulus* maintained in a separate tank, and subsequently sacrificed and gutted to obtain larval *Eustrongylides*. An average of eleven worms per day were fed to fish in this tank.

All of the sunfish were sacrificed after 30 days and the visceral contents examined to determine if larval *Eustrongylides* were present.

To determine if *Eustrongylides* could survive in sunfish gavaged with live nematodes, twelve sunfish, 6 pumpkinseed and 6 bluegill, were obtained from the State of New Jersey fish hatchery at Hackettstown, on October 14, 2007. Six sunfish were sacrificed at the hatchery to determine if *Eustrongylides* were present in this group of sunfishes. Sunfish were not weighed, but their total lengths were determined (100-130 mm total length). These were gavaged (Figure 1) and maintained in a fresh water tank at 20°C, and fed a 24% mash crumbles daily. Fish were sacrificed

between 50 and 75 days after gavage, and their viscera examined for the presence of *Eustrongylides*.

Results

The six bluegills sacrificed at the fish hatchery were not infected with *Eustrongylides* spp. Those fish maintained for one month in the laboratory and fed only mash had no observed *Eustrongylides* in their digestive tract or their viscera, nor did the bluegills fed mash and maintained with killifish. In the bluegills fed mash, exposed to live killifish, and fed live unencysted *Eustrongylides*, a single sunfish (a female) contained a live encysted *Eustrongylid* in its viscera.

The killifish obtained from the pond for this study had a *Eustrongylides* infection rate of 42%. In a previous study³ killifish obtained from the same pond had an infection rate of approximately 50%.

Fishes gavaged with *Eustrongylides* resulted in nematode infections in four of the six bluegills, occurring in each of the two females and in two of the four males. Two of the six pumpkinseed males had nematode infections. In all of the infected sunfishes the nematodes had bored through the intestines and encysted in the viscera (Fig. 2).



Figure 1. Method of oral gavage, using a 10 ml pipette with 4mm of its tip removed, to introduce an encysted *Eustrongylides* sp. into a pumpkinseed sunfish.



Figure 2. Fish viscera exposed indicating the presence of *Eustrongylides* in the body cavity. Fish was sacrificed 50 days after gavage.

Discussion

The 12 bluegills exposed to live killifish, and the 11 exposed to live killifish and fed live nematodes yielded only a single sunfish infected with *Eustrongylides*. However, half of those sunfishes (bluegills and pumpkinseed) gavaged with live nematodes retained the worms. The nematodes bored through the digestive tract of their second hosts and encysted in the viscera. The minimal infection of nematodes in the bluegills exposed to *Eustrongylides* parasitized *Fundulus* was probably due to the large size of the killifish relative to the size of the bluegills. Bluegills are a predatory fish but, as with pumpkinseed⁴, their fish consumption is reported to be low. In addition, third stage larvae, more likely to occur in smaller fishes prior to becoming fourth stage larvae, do not appear to be transmitted successfully to sunfish^{5,6}, but if they are, do not grow in their paratenic host⁷. In bluegills fed live unencysted fourth stage *Eustrongylides*, no remains of the nematodes were observed at the end of the feeding period. Those larvae that were eaten, with the exception of a single worm which successfully parasitized a bluegill, were possibly bitten, or mascerated during capture and ingestion. *Eustrongylids* introduced by gavage did not have to pass through the gauntlet of the fishes' jaws, pharynx, and esophagus, and 50% of these actively perforated the digestive tract of their paratenic host and became encysted in the host's viscera.

In previous studies *Eustrongylides* larvae, isolated from *F. heteroclitus* and *Perca flavescens* were introduced orally, subcutaneously, rectally⁸ and by intubation [sic]⁵ into several freshwater fish species. In most of these species reinfection of the new fish host occurred. Exceptions to reinfection included *Cyprinus carpio* and *Pomoxis nigromaculatus*. In a study that included bluegill and pumpkinseed sunfish⁵ the site of worm recovery was primarily fish musculature, unlike this study in which the worms entered the viscera.

Disposal of unused live or dead bait fish, especially of the genus *Fundulus*, obtained from fresh or low salinity waters into lakes, ponds, or streams other than their source of origin can extend the range of this parasitic nematode if definitive hosts (fish eating birds) and aquatic oligochaetes occur near or in these waters. Humans can also become infected by eating bait fish⁹, or fillet portions of infected fishes that haven't been refrigerated immediately after capture¹⁰.

Acknowledgments

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Its Time to *Step into Science* at Medgar Evers College

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Abstract

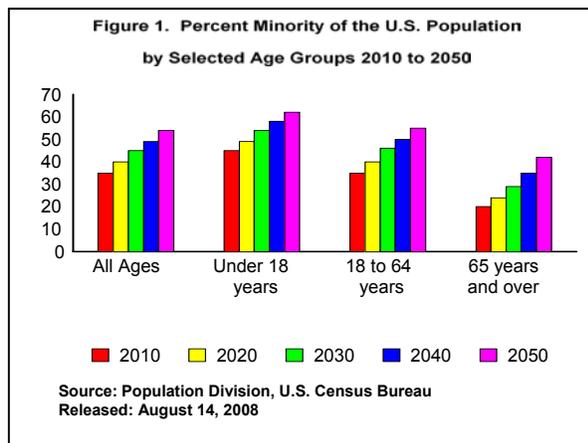
Over the next decade, demand and job opportunities in science and engineering (S&E) are expected to grow. With so many S&E “baby-boomers” retiring, questions arise as to whether America will be able to attract enough young people into Science, Technology, Engineering and Mathematics (STEM) to maintain a S&E workforce that keeps up with what is becoming a more globally technologically and scientifically advancing society. Furthermore, considering recent projections of a nation more racially and ethnically diverse by mid-century, will America’s future STEM workforce reflect the diversity projected for our growing U.S. population? In 2006, the authors received an award from the National Science Foundation (NSF) to direct a new initiative titled “*STEP into Science*.” Funded under the Science, Technology, Engineering and Mathematics Talent Expansion Program (STEP) of the NSF Division of Undergraduate Education, the main goal of the project is to increase the number of STEM majors that graduate with baccalaureate degree, specifically B.S. degrees in Biology or Environmental Science. The program has had great success implementing the use of “peer recruiters” to attract more high school, transfer, and non-science college students into STEM majors and places emphasis on the role of undergraduate research experiences as a successful strategy to increase the quality and retention of science majors through their baccalaureate degree. Since the inception of the program, total STEM enrollment has more than doubled and the number of majors actively engaged in research has risen 38% with a concurrent increase in student research presentations at scientific conference, and the number of students receiving external research internships and travel awards to attend national conferences. The number of STEM graduates (both A.S. and B.S.) has also increased and the program anticipates that these and future STEP into Science graduates will continue on to Masters and Doctoral programs in STEM and ultimately enter rewarding careers in the science enterprise.

Introduction

According to the National Science Board (2004), the events of September 11, 2001, have resulted in an increased urgency and a new focus to the changing strategic role of science and technology in this post-Cold War era. The U.S. economic performance of the 1990s has given impetus to the trend toward a knowledge-based economy, “one in which research, its commercial exploitation, and other intellectual work play a growing role in driving economic growth¹.” Emphasis has been placed on critically evaluating and improving America’s strengths especially in the areas of science, technology, engineering, and mathematics (STEM). Over the last decade the U.S. has shifted to the negative side of the high-technology trade balance, an indicator of the international competitiveness of the nation’s high-technology industries. Since 1998, trade data for a number of high-technology manufacturing industries including aerospace, pharmaceuticals and scientific instruments, indicate U.S. imports

exceeding exports. In addition, since 2002, U.S. trade for 10 high-technology product categories (including biotechnology, life sciences, aerospace and nuclear technology) has also turned negative². As economic and social development continues in science and technology-focused nations like China and India, questions arise as to whether the U.S. will be able to maintain a science and engineering (S&E) workforce that can keep up in what is becoming a more globally technologically and scientifically advancing society. With the baby-boomers retiring, we face a major loss in our S&E workforce and questions arise as to whether the need for qualified workers will be met, and perhaps more importantly will this future workforce reflect the ethnic and racial diversity projected for our growing U.S. population? The U.S. Census Bureau News released an August 2008 press statement projecting that by mid-century, the nation will be more racially and ethnically diverse, as well as much older³. Minorities, now roughly one-third of the U.S. population, are expected to become the

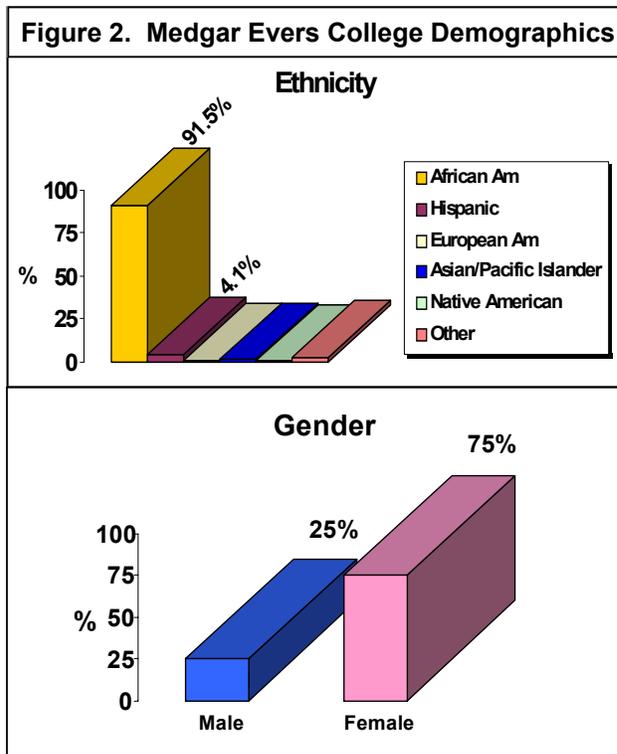
majority in 2042, with the nation projected to be 54 percent minority in 2050. Over the next 40 years, the percent minority of the U.S. population in all age groups is expected to increase (Fig. 1) and by 2023, minorities will comprise more than half of all children.



As the population demographics change, and fewer white non-Hispanic men obtain S&E degrees, the importance of women and minorities pursuing degrees in these fields rises⁴. If we are to meet the growing needs of a technologically and scientifically advanced society that fully captures the strength of America's diversity, greater effort must be placed on attracting and preparing more women and underrepresented minorities (URM) to pursue STEM careers. Many government agencies, including the National Science Foundation (NSF) National Institute of Health (NIH) and the Department of Education (DOE) are cognizant of this fact and support various programs that address the need to encourage more women and minorities to pursue science degrees.

In 2006, the authors received an award from the National Science Foundation (NSF) to direct an initiative titled "STEP into Science." This project, funded under the Science, Technology, Engineering and Mathematics Talent Expansion Program (STEP) of the NSF Division of Undergraduate Education (DUE), represents an interdisciplinary effort to recruit and graduate more students with baccalaureate degrees in Biology or Environmental Science. The program anticipates that many of these science graduates will continue on to Masters and Doctoral programs in STEM and ultimately enter rewarding careers in the science enterprise.

MEC, founded in 1970, is situated in the Crown Heights section of Brooklyn, one of the largest, most densely populated and ethnically varied sections of the borough. The college offers both Associates and Baccalaureate degrees and serves a diverse student body representing all areas of New York City, especially the surrounding Brooklyn community. Approximately 75% of the student population is female and over 95% are underrepresented minorities (URM), most of whom are of African descent (Fig 2).



While the Biology (BIO) and Physical, Environmental and Computer Sciences (PECS) departments enroll and graduate the majority of STEM majors at the College, the number of science graduates, particularly at the baccalaureate level, remains vastly inadequate and echoes the national concern of a future deficit of qualified and diverse individuals, to provide the technologically and scientifically advanced workforce that will ensure a healthy economy, respond to the demands of national security and maintain and elevate the quality of life and standard of living in the U.S.

It is common in numerous urban, public colleges across the nation that many talented and potentially successful students enter college believing they can not be successful academically or professionally as a Science major. Much of this

can be due to the fact that they: (1) have not been encouraged in their pre-college experiences to choose Science as a major; (2) are not adequately informed and encouraged when they start college about Science degree programs and the potential professional career opportunities available to B.S. graduates; (3) often enter college “at risk” with a weak high school science education and a variety of financial and other personal problems. The current retention rate at MEC for degree seeking students is inadequate and over 90% of entering freshman who earn a baccalaureate degree in the sciences require 5 or more years to do so.

Faculty in the BIO and PECS departments have a long history of initiating and participating in numerous activities to advance their programs, improve the quality and quantity of their majors, and educate non-majors and the community to the importance of science in their lives. The STEP into Science program builds upon previous STEM successes and existing articulations and research collaborations. To further increase the number of Science graduates, the STEP into Science program initiated a plan that: (1) aggressively recruits new students and non-STEM students from within the college who select majors in either the BIO or PECS departments; (2) improves retention of the science majors by providing additional academic, financial and mentoring support; (3) strengthens both academic departments with curricula that fosters the integration of research, technology and academics to better equip majors with the skills and knowledge necessary to be successful applicants to graduate/professional programs.

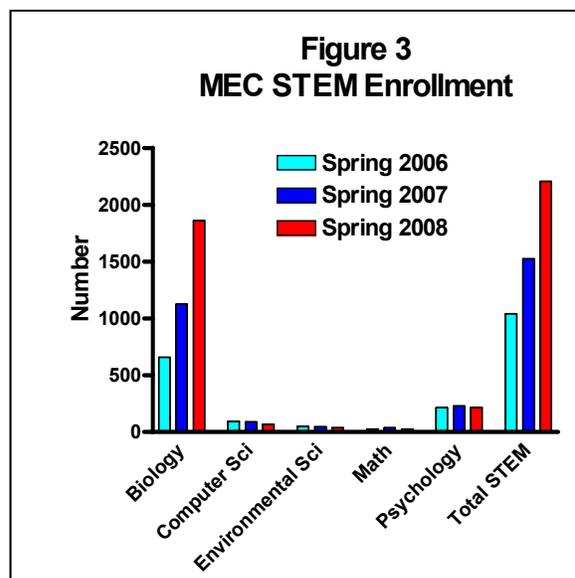
Now in its second year, STEP into Science at MEC is showing progress in furthering the number of science majors who will graduate with STEM baccalaureate degrees, and represents a logical step in the college’s continued quest to afford the highest quality science education to the urban community it serves.

Progress to Date

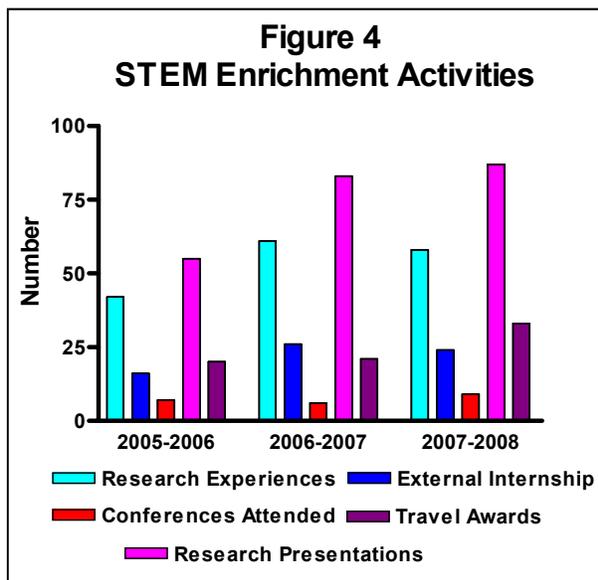
A major goal of the STEP into Science program is to recruit more students as Biology or Environmental Science majors. A comprehensive recruitment program involving brochures, fliers, web pages and open houses was begun in Fall 2006 and included the implementation of a very successful strategy that utilized “peer recruiters” to attract more non-STEM majors and transfer students into our science programs. So far, 10 STEP into Science student participants have been trained and are working as peer program recruiters. These experienced junior or senior

science majors have visited various freshman programs, non-majors science classes and campus clubs to inform MEC students of the career opportunities and “do-ability” of being a Science major. Peer recruiters have also gone to neighboring high schools and local community colleges to boost enrollment of new science majors and transfer students. STEP into Science was also able to cosponsor two major conferences at the college. The program co-hosted the Medgar Evers College Thirteenth Annual Conference on Environmental Issues. Con Edison and the PECS department were the major sponsors. About 400 students attended the conference which had a full series of activities, including a Keynote address by James Hicks on *plaNYC: A Greener, Greater New York*. The program also co-hosted the NEA Day Conference at Medgar Evers College in which representatives from the Northeast Alliance for Graduate Education and the Professoriate (NEA-AGEP), a program funded by NSF in which the University of Massachusetts, Amherst is the lead institution, were able to meet with current and potential science students to explain research and graduate school opportunities available to them. Approximately 300 students attended the events which included a Keynote address presented by Vice Admiral Adam R. Robinson, Surgeon General of the Navy.

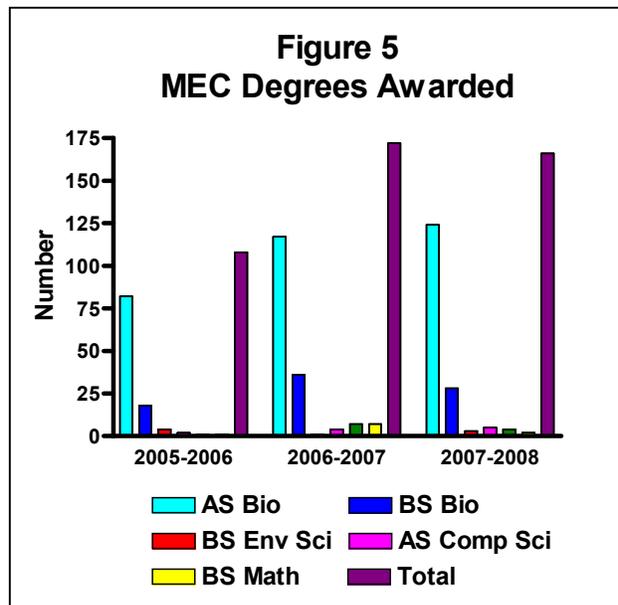
Fig. 3 indicates that program recruitment efforts have been successful in attracting more students as STEM majors. Total STEM enrollment has more than doubled since the inception of the program (Fall 2006). Most of this increase has been due to many more students electing Biology as their major.



Another major goal of the program is to increase student retention through the baccalaureate degree and to afford the academic, professional and motivational experiences necessary for them to be successful applicants into graduate STEM programs. Providing opportunities for more research to improve both student quality and retention rate is a major retention strategy of the STEP into Science program. Fig. 4 shows the significant increase in student interest and participation in research activities and external research internships since the inception of the program. The number of students actively engaged in research has increase by 38%, along with a 50% increase in external internships. Student research presentations at scientific conferences increase by 58% and the number of students receiving travel awards to attend conferences increased by 65%.



Even though the STEP into Science program is only in its second year, the college has already seen an increase in the number of students graduating with STEM degrees. Fig. 5 shows a 50% increase in the total number of students graduating with STEM degrees in AY 2007-2008 compared to AY 2005-2006, before program interventions. The most gains were seen in the number of students receiving the A.S. in Biology and B.S. in Biology degrees, 51% and 56% respectively. Many of the A.S. graduates are now enrolled in the B.S. in Biology or B.S. in Environmental Science program. So far, there has been little to no improvement in the number of graduates with other STEM degrees.



Summary

The most recent Occupational Outlook Handbook of the U.S. Bureau of Labor Statistics (2008-09) projects that the demand and job opportunities for scientists and engineers will continue to grow, with some specialties, growing far faster than the national average for all occupations. As biotechnological research and development continues to drive job growth, employment of life scientists is projected to grow 15% over the 2006-2016 decade. Even greater growth is expected for environmental scientist (25%), and biomedical and environmental engineering (21% and 25% respectively)⁵. Will America be able to attract enough young people into STEM to generate sufficient numbers of qualified, skilled scientists and science engineers to meet this need?

Implementing successful recruitment strategies is a major barrier in attracting more college students into STEM majors. Often it's assumed that high school graduates, especially women and underrepresented groups, avoid choosing STEM degree programs because their scores indicate that they are ill prepared for the rigors of college mathematics and science courses. However studies have found that African American and Hispanic college students with high grade point averages and SAT scores above 600 still may not pursue STEM college majors for reasons including poor teaching in STEM courses, lack of encouragement from teachers or parents and self-perception of their own inability to be

successful in STEM majors⁶. Most of the recruitment efforts to attract more URM students into science majors involve impersonal forms of advertising explaining various programs, or use of adult recruiters that may or may not represent valid role models to the students they are trying to attract. Wardlow, Graham, and Scott, exploring the recruitment of URM into agricultural science, noted that minority youth tended to follow the experiences of successful older youth from the community⁷. The STEP into Science peer recruiter initiative has been a very popular and engaging strategy for both recruiters and perspective majors. Upper level science majors are the best science role models to recruit the population being targeted. They can give personal insight on how they handled degree requirements, and hopefully entice students by relating their research and other enrichment experiences as well as future goals. Recruiters benefit as well as they gain in pride and self-confidence, while conveying their science accomplishments and experiences to their peers.

Even with the recruitment of more science majors, another major stumbling block is how one improves student retention through the baccalaureate degree. National statistics show that in 1998, 33% of White, Black, Hispanic and American Indian freshmen and 43% of Asian freshmen entered STEM majors⁸; however fewer than 50% completed B.S. degrees within 5 years, with URM dropping out of STEM programs at higher rates than other groups⁹. Numerous reports discuss the various academic and social factors that encourage URM science majors to “drop out” including poor high school preparation, financial problems, academic and cultural isolation, peers who are not supportive of academic success, motivation and performance vulnerability in the face of low expectations, and discrimination, whether perceived or actual¹⁰⁻¹⁹. The inability of high school teachers to properly prepare students for the rigors of college science is a serious concern. It is estimated that 29% of high school math teachers and 23% of high school science teachers never majored or even minored in these subject²⁰. The percentages are even higher for inner city school systems like NYC. The retention problem is complex for there is considerable evidence that some of the college losses in STEM areas come from a pool of capable undergraduates¹⁸. In 1997 Tinto indicated that the most important influence on students’ persistence in their studies is their ability to develop a network of support, often identified as a learning community²¹. Another major factor in student

success and retention is involvement with faculty¹⁵. When students have frequent friendly interactions with faculty members, their development of intellectual competence, sense of confidence, autonomy and interdependence, purpose, and integrity are often enhanced²². Other reports have shown increased informal student-faculty interaction results in satisfaction with the overall quality of education and persistence in obtaining the degree²³⁻²⁶. MEC is essentially a college of non-traditional, commuting students, many of whom hold full/part-time jobs and/or have families to tend to. Considering these circumstances, it is often difficult to devise successful strategies that allow for enrichment experiences fostering better faculty-student or student/student interaction outside of class time. A supportive science-learning environment involves more than just coursework. If we are to expect the students to remain as science majors and aspire to rewarding careers in the science industry, one needs to afford them opportunities that entice them to persist by informing them of the latest cutting-edge scientific advancements, by exposing them to exemplary science professionals and high-level science career opportunities, and by providing them with experiences that demonstrate the value and do-ability of scientific research. Integrating research with academics is a major focus of the STEP into Science program. The project has already made significant progress in getting more science majors involved, including freshman and sophomores, in both on-camps and external research projects. Students value and appreciate the opportunities to work on these research projects and there is great STEM retention value in engaging students in the practice of science and all the other activities that go along with practicing science, as early as possible. Students’ responses to program generated questionnaires and activity evaluations consistently indicate that their participation on research projects and having the opportunity to travel to conferences, scientific sites and various research universities are major highlights of the STEP into Science program and their undergraduate experience at MEC. These enrichment activities have generated greater science interest and science self-confidence in the majors than if the program had been designed to narrowly focused on purely academically based interventions and strategies.

Many other published reports indicate that undergraduate research experience is a successful educational tool for enhancing the undergraduate experience^{27,28}; and is particularly

effective as a strategy to increase student interest in pursuing STEM careers^{29,30}. In addition, several studies have supported the hypothesis that undergraduate research helps promote career pathways for members of underrepresented groups by increasing the retention rate of minority undergraduates³¹, increasing the rate of graduate education in minority students³² and increasing STEM graduate school enrollment for URM and women³³.

Getting undergraduate science majors to aspire to and be competitive for admission into STEM graduate programs is essential if they are to be properly prepared for rewarding careers in the science and technology enterprise. This trend towards seeking higher degrees is key because scientists and engineers with only B.S. degrees have less status and a lower earning potential than those with doctoral degrees. The current disparity among scientists from different racial/ethnic groups in educational attainment remains a serious problem. In 1997, 64% of black scientists in the U.S. labor force had a B.S. as their highest degree compared with 57% of all scientists³⁴ and the percentage of all doctoral scientist and engineers in the U.S. of African descent was only 3%³⁵. While some progress has occurred over the last decade, recent data indicate that the number of doctoral scientist and engineers in the U.S. of African descent had barely risen, to just over 4%³⁶. A quote from *Reaching the Top*, the 1997 report of the National Task force on Minority High Achievement, states:

“Until many more URM from disadvantaged, middle class, and upper class circumstances are very successfully educated, it will be virtually impossible to integrate our society’s institutions completely, especially at leadership levels. Without such progress, the United States also will continue to be unable to draw on the full range of talents in our population during an era when the values of an educated citizenry have never been greater³⁷”.

This observation remains current and provides much of the drive and momentum to the STEP into Science initiative at Medgar Evers College.

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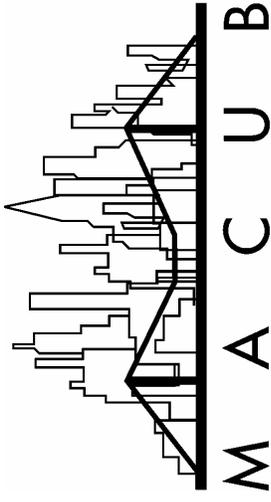
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