2010 NSF Graduate Research Fellowship Program Proposal with Proposal Reviews

Proposal by Christine Gault

Personal Statement

The midday sun beat down on my classmates and I as we hiked to the secluded bog for our Wetland Ecology field trip during my junior year of college. Branches scraped our faces while we navigated the dense thicket that surrounded the bog. A disturbed nest of bees sent us running, but the swampy ground hindered our wader-clad feet with every step. The smell of sulfur and rotting vegetation greeted us when we finally arrived at the bog. For two hours, we analyzed the soil and vegetation while walking ankle deep in a carpet of water-logged moss, and I loved every moment. That wetland was the most beautiful and unique habitat I had ever seen. During the field trip, I snacked on wild blueberries and cranberries while walking over the buoyant ground that felt like a waterbed. I was fascinated by plants I had never seen before, such as the delicate yellow and purple pitcher plants. The experience taught me the value of wild ecosystems and motivated me to conduct research aimed at reducing the environmental impacts of agriculture. In my research, I also want to improve economic and gender inequalities either in my own institution or internationally. As a result, I plan to pursue a career in agricultural biotechnology because progress in this field benefits the environment and society.

Agricultural biotechnology's potential to make farming more efficient excites me because it reduces the need to convert natural areas to farmland. Consequently, I plan to apply my knowledge of molecular biology toward research that increases crop yield. As a first year graduate student, I am completing my first of three rotations in Dr. Karen Koch's lab. My proposed graduate research in her lab will investigate a sugar processing gene called Sdh1 that affects maize kernel size. This gene seems to be directly related to the maize yield because reducing its expression causes decreased kernel weight (Sylvia de Sousa, unpublished data). By working on the Sdh1 mutant, I can explore basic genetic questions in molecular biology that have always fascinated me while investigating the processes underlying maize productivity.

By boosting agricultural yield, crop improvement research can better society. Throughout my life, I have been concerned for underprivileged communities. In my childhood, I would travel with my church to the inner city of Chicago and help wrap Christmas presents for children of struggling families. Years later in college, I volunteered for a salvage program that collected items from dormitories and distributed them to local charities. My desire to reach out to those that are less fortunate motivates me to conduct agricultural research. The development of more productive crops will help farmers in third world countries who are struggling to survive. In my career, I plan to pursue grants from the BREAD program- "Basic Research to Enable Agricultural Development" that is dually funded by the Gates Foundation and NSF. I share research goals with the BREAD program because I want to target problems that limit plant productivity for rural farmers in developing countries. By researching crop improvement, I will be acting on my desire to contribute to the global community.

My proposed work on the Sdh gene will also contribute to the global community by strengthening international collaborations while interacting with other cultures. Dr. Sylvia de Sousa's lab at the agricultural research station in Brazil (EMBRAPA) will guide me during part of my graduate project. We will analyze diverse lines of grains called "land races" that have been cultivated over centuries by geographically disparate ethnic groups and indigenous people in South America. Many lines have close cultural ties and some can even be traced back to their Mayan origins. By studying as many land races as possible, I will gain insight into the ethnic roots of farming while acquiring a better feel for grain and grass species. A deeper appreciation for Brazilian culture and farming will enable a more meaningful relationship with the de Sousa lab and others at EMBRAPA.

After earning my PhD in plant biology, I intend to continue serving the community by striving to involve women in research. As a professional scientist, I will strive to address the troubling gender disparity in scientific professions. Even though about 39% of PhD students are women, women hold only 8% of full professor positions in the geosciences, and the lack of strong female mentors largely contributes to this gender gap[1]. I feel that I can successfully serve as a mentor to young women because of my experience as a residential advisor. Sophomore year, I served as an R.A. to an all-female floor of forty-eight freshmen, organizing educational and recreational activities to ease their transition into college life. This position gave me the opportunity to individually counsel a handful of women and help guide them through their social struggles. The skills I acquired as a residential advisor directly relate to providing mentorship for young women in science. I can easily establish open lines of communication with female undergraduates and graduate students. Direct, sincere dialogue will help to quickly identify and resolve problems that usually cause women to leave scientific careers.

I will also work to address economic inequalities by mentoring underprivileged undergraduate and graduate students when I have my own lab. My experience as a math and science tutor has prepared me for this role. During the summer of 2008, I volunteered for an upstate New York high school in a poor, rural region. The school consistently underperformed on math and science sections of the state's Regent's Exam, and I witnessed first-hand the gaps in these students' education. My time spent tutoring showed me the importance of having educational opportunities early in life. As a professor, I will seek out students who have had limited education to help involve them in research early in college. By guiding them on independent projects, they will learn how to address scientific questions on their own, a skill that will prepare them for higher degree programs.

With the financial support of the NSF pre-doctoral fellowship, I will be able to conduct research in an area that addresses environmental and societal issues. This fellowship will enable me to investigate ways to make farming more land-efficient while benefiting underprivileged farmers and promoting international networks. In the process, I will serve my local community by reaching out to women and underprivileged students and mentoring them in scientific research. The prospect of working in a research area that has such far-reaching impacts truly excites me, and I am eager to begin my graduate work on the Sdh gene in maize and other grass species.

1. Holmes, MA and O'Connell, S. (2007). Leaks in the pipeline: Why do women remain curiously absent from the ranks of academia? Nature 446:15.

Previous Research Experience

I explored the fields of genetics, ecology, and conservation biology during four invaluable research experiences. Working in these diverse fields, I realized how much I enjoy using scientific processes to make new discoveries. In these projects, I loved working at the leading edge of knowledge, constantly pushing to expand the understanding of the natural world. As a result of my inherent curiosity, I chose to pursue a career in research. These four experiences prepared me to become an independent graduate student with the ability to investigate the scientific questions that I'm most passionate about.

I first learned the fundamentals of a scientific investigation while studying a mutant line of zebrafish. In the summer after my sophomore year, I worked with Dr. Elizabeth LeClair at DePaul University in collaboration with Children's Memorial Research Center. Our lab investigated mutant zebrafish that exhibited craniofacial and skeletal deformities. We chose to study these mutants because their curved spines drew comparisons to scoliosis in humans. Discoveries in the zebrafish model can be directly translated to human skeletal diseases because of the similarity of spinal formation in zebrafish and humans. Our project sought to identify the precise morphological features that were responsible for the mutant phenotype. A DePaul undergraduate and I stained the bones of wildtype and mutant fish and used photomicroscopy to record their skeletal dimensions. The data revealed that mutants had significantly shorter vertebrae and random spinal fusions. In our most meaningful contribution, we developed a system of measurement for the zebrafish skeletons that promoted consistency and effective communication throughout the project. Our system and quantitative measurements helped lay the groundwork for ongoing research at Children's Memorial Research Center. Under Dr. LeClair's guidance, I learned how to pose scientific questions that helped interpret our measurements and characterize the phenotype of the mutant zebrafish.

The skills I learned at DePaul helped me to take a more active role in my next project in ecology. In the summer after my junior year, I worked with Dr. Kristy Hopfensperger at Colgate. We studied the production of the greenhouse gases methane, carbon dioxide, and nitrous oxide in the pristine riparian wetlands of the Adirondacks. Our study investigated the relative contribution of biotic and abiotic variables to gas production. We collected gas samples, recorded plant community data, and measured several characteristics of the soil and water at each site. I analyzed our gas samples during a two-week visit to Cary Institute of Ecosystem Studies. There I learned how to calculate the rate of greenhouse gas production at all of our sites using gas chromatography. Our results, which were published in *Forest Ecology and Management*[1], showed that ecosystem-scale variables like plant cover could best predict greenhouse gas production. This project allowed me to grow as a scientist because I helped to form hypotheses and interpret results. In contrast to my project at DePaul, I conducted most experiments independently while Dr. Hopfensperger provided direction. I also learned the importance of flexibility during scientific projects because we often had to adapt our procedures when conditions in the field changed. Similarly, we developed new frameworks for interpreting our results when they contradicted our hypotheses. This experience strengthened my desire for a career that could positively impact the environment.

In my third experience, I was part of a team of four undergraduates that shared a very independent role in directing the project. As seniors, we took a year-long research course with Dr. Frank Frey. We studied pathogen dynamics in the critically endangered Ugandan mountain gorillas, which have attracted ecotourists from around the world. We wanted to know if gorilla groups that interacted with tourists had more pathogens than isolated gorilla groups because tourists may potentially be infecting gorillas. To answer this, we determined the Salmonella and Shigella infection rates across six gorilla kin groups. An individual was considered infected if pathogen-specific sequences could be PCR-amplified from its fecal sample. Surprisingly, Shigella infection rates were significantly lower in tourist groups than in isolated groups. The Salmonella infection rates did not significantly vary between groups. These data suggest that exposure to tourists does not promote Shigella or Salmonella infections in gorillas.

This study also framed pathogen infection rates in a spatio-temporal context. The GIS coordinates and date of collection were known for each fecal sample, allowing us to pose two hypotheses. We predicted that fecal samples found by rivers would have a higher infection frequency than those found far from rivers because Shigella and Salmonella are water-borne pathogens. GIS analysis determined the distance to the nearest river for each sample. We found that fecal samples near rivers contained significantly more Salmonella infections than samples far from rivers. Our second hypothesis stated that infection rates would be higher in samples found during the wet season compared to the dry season. The data supported this hypothesis for both Salmonella and Shigella infection rates. Working on this project for a course gave me valuable practice in interpreting results and working with other students to reach conclusions.

My latest experience has been the most fulfilling project of all because I took full responsibility for compiling the results. When I began graduate school at University of Florida this August, I started a ten-week rotation project in Dr. Karen Koch's lab. With the mentorship of a graduate student and research assistant, I investigated a new transposon sequence that we discovered on my first day in the lab. The purpose of my research was to determine whether the new sequence was divergent enough to be categorized as a new subclass of Mu transposons. I first used bioinformatic methods to identify and map all the new transposons on the physical map of the maize genome. We then constructed a tranposon phylogeny to analyze the relatedness of the new transposons to previously known transposons. I interpreted the phylogenetic relationships to mean that the new sequence did not differ enough to be considered a new subclass. In my project, I also wanted to determine whether this transposon sequence had been conserved over the course of maize evolution. After designing primers, I performed a series of PCR experiments to test whether the newly discovered transposons existed in five different inbred lines. I found that some of the new tranposons were not present in all inbred lines. This suggests that these transposons are not dormant and have recently moved throughout the maize genome during its evolution.

These four experiences have helped me grow into an independent graduate student. In my research, I learned how to frame novel questions and use results to guide new inquiries. My undergraduate projects gave me a background in molecular biology that has already proven to be an advantage in my graduate studies. Because of my extensive practice in conducting research, I know I will be a productive student if I am awarded the NSF graduate student fellowship. The results of my proposed research will be published and presented at the Maize Genetics Conferences and American Society of Plant Biologists Conferences. I am very excited to apply the molecular techniques I have learned toward investigating the function of the Sdh1 gene in maize.

1. Hopfensperger, K. and Gault C. (2009). Influence of plant communities and soil properties on trace gas fluxes in riparian northern hardwood forests. Forest Ecology and Management 258:2076-2082.

The role of sorbitol dehydrogenase in signaling pathways of maize and other grasses.

Sugar signaling and kernel development

Deciphering the role of sugar signaling in kernel development will greatly enhance efforts to increase maize yield. Knowledge of the sugar pathways in the maize model can provide insight into metabolic processes of other plants. Furthermore, this knowledge can stimulate progress in crop improvement research. Even though sugars have been shown to act as signals, the ability of sugars to orchestrate kernel development has been largely underestimated. Because the role of sugars is unknown, our understanding of the interplay between metabolism and kernel formation is incomplete. A maize mutant that has low levels of sorbitol dehydrogenase (SDH) provides an excellent model to test whether sugar signaling pathways are central to development.

$$\text{Fructose} + \text{NADH} \xleftrightarrow{\text{SDH}} \text{Sorbitol} + \text{NAD}^+ \qquad [1]$$

SDH reversibly converts fructose into sorbitol, which stores energy in many economically important species like maize[1] and Roseaceous species [2-7]. In Roseaceous species like apple and peach, sorbitol moves from the phloem into sink tissues, where Sdh converts it to fructose (the reverse reaction in equation [1])[6,7]. In these species, Sdh activity is associated with high sink strength[8-12]. Maize is distinct from Rosaceous species in that sorbitol is not imported into the sink tissue, but is formed in the maize kernel itself[4,5]. SDH activity in maize may establish the kernel as a sink tissue[13], but the true role of Sdh and sorbitol in kernel development remains obscure.

A maize mutant (*sdh1)* with a knocked-down *Sdh1* gene was developed by Koch and coworkers and shows a 21% decrease in dried kernel weight (de Sousa, unpublished results). Because the *sdh1* mutant has a small kernel phenotype (de Sousa, unpublished data), I hypothesize that SDH and sorbitol play a crucial signaling role in early maize kernel development. The proposed work involves three specific aims that will test this hypothesis.

1. To test if SDH activity triggers changes in kernel development.
2. To investigate whether sorbitol also acts as a signal metabolite in development.
3. To determine the conservation of the SDH signaling pathway across several grass species.

I predict that SDH activity affects the sink strength of the kernel by triggering changes in carbon transfer into the endosperm. The proposed work will test this by measuring *Sdh1* expression during different developmental stages of the following maize kernel tissues: endosperm, embryo, pedicel, and pericarp tissues. Northern blots will measure *Sdh1* transcript levels, and *in situ* hybridization experiments will measure SDH activity. I predict a peak in *Sdh1* expression at the very beginning of kernel development in the endosperm. To further test the influence of SDH, I will compare dried kernel weight and sugar content between the *Sdh1* mutant and a transgenic maize line that overexpresses *Sdh1* (in preparation, ISU). Heavier kernels with higher starch content in the overexpression line than in the wildtype or mutant line would be consistent with *Sdh1* expression triggering developmental changes in the kernel. The Koch lab's resources and expertise in sugar pathways will be invaluable to the proposed sugar analyses and *in situ* hybridization experiments.

The second aim of my graduate work will test whether sorbitol also acts as a signal in the maize kernel. Previous work in apple shoot tips has implicated sorbitol in signaling pathways[14], but maize signaling may be unique due to the deeply hypoxic environment of the endosperm and aerobic environment of the embryo. Oxygen concentrations can affect sugar signaling in maize seedling root tips[15] and may likewise affect sorbitol signaling in the kernel. The ample supply of NADH would favor sorbitol production in the hypoxic endosperm, and the high concentration of NAD^+ would favor fructose production in the aerobic embryo (equation [1]). Consequently, sorbitol may activate different genes in the endosperm than the embryo if it is a signal molecule. I propose to test sorbitol's signaling potential by measuring gene expression changes in the maize kernel after sorbitol exposure. The embryo and endosperm will be separately cultured on media containing increasing concentrations of sorbitol. I predict microarray expression patterns of these two tissues to change with sorbitol concentration. I also predict that metabolite profiles of the *Sdh1* mutant and overexpression line will vary because they have different levels of sorbitol.

Finally, I will examine whether the Sdh signaling pathway is conserved across grass species. I will analyze Sdh expression, gene evolution, and metabolic profiles in the Panicoids and related grass species. Direct enzyme assays, plus RT-PCR where possible will quantify the extent of Sdh expression in the seeds of these species. Phylogenetic analyses on the sequences of Sdh genes in these grasses will appraise the molecular evolution of this gene. The Barbazuk lab will provide expertise in these bioinformatic analyses. Finally, a targeted analysis of metabolites will be performed in these grasses, guided by the insights from the maize metabolic profiles. These genetic, phylogenetic, and metabolic methods will more fully answer whether this developmental pathway was conserved and helped shape the evolution of grasses.

My graduate research will provide insight into the sugar signaling pathways in maize development. That metabolic products can serve as signals is a relatively recent and transformative concept in plant research that can serve as a nexus between the fields of metabolism, physiology, and evolutionary biology. Data supporting sorbitol and SDH as signals can help test the centrality of metabolic pathways in early development. The proposed work will also investigate whether the evolution of the Sdh gene may have contributed to kernel development in other grasses, which can lead to breakthroughs in crop improvement research. The pathways that regulate seed size are critical to boosting yields in grasses such as millet and rice, the staples of many developing countries.The integration of the fields of metabolism, physiology, and evolutionary biology will provide an outstanding breadth of training, which will equip me for a career in scientific research.

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2. F. B. Negm and W. H. Loescher, *Plant Physiol.* **64**, 69 (1979).
3. H. Yamaguchi et al., *Plant and Cell Physiology* **35**, 887 (1994).
4. M. H. Zimmermann et al., *(Ed.).Encyclopedia of Plant Physiology, Vol.1.Transport in Plants I.Phloem Transport. Springer-Verlag: Berlin, West Germany; New York, N.Y., U.S.A.* 480 (1975).
5. R. L. Bieleski, *Encyclopedia of Plant Physiology.New Series.Volume 13 A.Plant Carbohydrates.I.Intracellular Carbohydrates [Loewus, F.A.; Tanner, W.(Editors)]*, 158 (1982).
6. W. H. Loescher, *Physiol. Plantarum* **70**, 553 (1987).
7. G. Teo et al., *Proc. Natl. Acad. Sci. U. S. A.* **103**, 18842 (2006).
8. S. W. Park et al., *Plant Science* **162**, 513 (2002).
9. W. H. Loescher et al., *Plant Physiol.* **70**, 335 (1982).
10. L. Merlo and C. Passera, *Physiol. Plantarum* **83**, 621 (1991).
11. R. Lo Bianco et al., *Tree Physiol.* **19**, 103 (1999).
12. R. Lo et al., *J. Am. Soc. Hort. Sci.* **124**, 381 (1999).
13. S. M. d. Sousa et al., *Plant Mol. Biol.* **68**, 203 (2008).
14. R. Zhou et al., *J. Exp. Bot.* **57**, 3647 (2006).
15. Y. Zeng et al., *Plant Physiol.* **121**, 599 (1999).

NSF GRFP RESULTS - Rating Sheets - Christine Gault

2010 Rating Sheet 1

Overall Assessment of Intellectual Merit: Excellent

Explanation to the applicant: The applicant clearly has the track record and intellectual abilities to succeed as a doctoral student. She has taken the initiative to plan and execute novel research at an early timepoint, works well alone and in teams, and demonstrates a strong ability to interpret and communicate her research findings.

Overall Assessment of Broader Impacts: Very Good

Explanation to the applicant: The applicant is clearly excited about her chosen field and there appears to be opportunities to fulfill one or more of the NSF broader impacts criteria. That said, this proposal would be strengthened by a more detailed discussion of how the proposed work and activities will fulfill some of the NSF broader impacts criteria.

2010 Rating Sheet 2

Overall Assessment of Intellectual Merit: Very Good

Explanation to the applicant: The applicant has adequate academic preparation and has demonstrated her ability to perform research, as evidenced by inclusion on a publication. She is described as confident, motivated, passionate and a good communicator. The application would be strengthened by evidence that the applicant has presented her work at meetings/symposia outside her home institution.

Overall Assessment of Broader Impacts: Good

Explanation to the applicant: The applicant has some experience integrating research and education. This application would be strengthened by more discussion of broader impacts, for example there could be more discussion of how the results of this research will be broadly disseminated.

2010 Rating Sheet 3

Overall Assessment of Intellectual Merit: Excellent

Explanation to the applicant: Comes from excellent undergraduate program with a very good academic record. Significant research experiences that generated one publication. No conference papers or posters. Well written, hypothesis driven plan of experimental research. Research is placed in broader context. There could be a stronger rationale for choice of graduate program. Aspects of the research will take applicant in a new direction

Overall Assessment of Broader Impacts: Very Good

Explanation to the applicant: Focus on improving crop yield and agricultural development has potential broader impact. International collaboration is a strength. Commitment to broadening access to science and community service are clear.