Vegetated green roofs can contribute to city-wide sustainability initiatives, now critical in light of increased urban expansion and consumption of global resources. But if the plants on rooftops are essentially stuck in genetic “islands” then they run the risk of decreased population genetic diversity over time. Lack of genetic diversity in a population can eventually lead to extinction, meaning green roof could lose the very thing that makes them green: the plants. Biotic pollinators such as butterflies, moths and bees, could be the critical link needed to move pollen between plant populations on green roofs and maintain genetically diverse populations for generations to come. The aim of my ISEN-funded research was to determine how pollinators contribute to pollen movement between native plant populations on spatially-isolated green roofs within an urban environment to create the first city-wide models of green roof pollen flow.

In the summer of 2014, a controlled experiment was set up to measure gene (pollen) flow between ten green roofs in Chicago. Three focal species were used: *Penstemon hirsutus* (hairy beardtongue, Scrophulariaceae), *Asclepias tuberosa* (butterfly milkweed, Asclepiadaceae), and *Oenothera macrocarpa* (bigfruit evening primrose, Onagraceae). All species are native to the Midwest, provide pollen and nectar to insect pollinators having various foraging ranges and have been shown to survive on green roofs in Chicago. With the help of collaborators at the lakeshore campus of Loyola University, five individuals of each species were placed out in pots at evenly-spaced intervals on each of ten green roofs (50 total individuals per species). Each individual was geo-referenced and the density of all other flowering species on each green roof was recorded. I intended to measure plant fitness for all individuals of each species (number of flowers, fruit to flower ratio, fruit size and seed to fruit ratio) and record pollinator visitation rates but it quickly became apparent that the *A. tuberosa* was suffering from rotting roots due to the exceptionally wet and cooler summer weather. Unfortunately, all the *A. tuberosa* plants perished and could not be used in the experiment. Additionally, the harsh climatic conditions of the green roofs proved to be nearly insurmountable for the *Oenothera macrocarpa*, and although about a dozen of the 50 experimental plants were flowering in the greenhouse prior to the experiment, only three produced flowers once on the roof. This species could not be used in the analysis. Fortunately, 47 of the *Penstemon hirsutus* individuals produced flowers synchronously and therefore could be used to measure pollen flow between green roofs as originally intended.

Tissue was collected from all potential *P. hirsutus* pollen donors (flowering individuals) for DNA extraction and amplification through PCR. The eight markers developed for the genus *Penstemon* were tested, with four successfully amplifying regions in *P. hirsutus*. Unfortunately, likely because the experimental plants were produced from nursery stock which has traditionally low genetic diversity, the amplified regions were not highly variable. An additional 17 *Penstemon* primers known to molecular ecologists at the Chicago Botanic Garden were then tested, with another four amplifying slightly variable regions. As this low variability is still less than what is typically used to assign paternity in natural populations, I am now in the processes of optimizing an additional 20 *Penstemon* primers with the expectation that at least four to six of these will amplify variable regions to be used to fill in some of the gaps left by the former primer regions.

Once the final primer optimization is completed, I will be able to complete the paternity analysis portion of the experiment. The original plan was to use 60 offspring from each species for paternity analysis. However, due to the very low genetic diversity in my population and therefore a very low ability to detect paternity with high certainty, I needed to greatly increase the number of offspring I include. I therefore extracted enough seeds to test four germinated offspring from three mature fruits from each of the 47 maternal plants (4*3*47 = 564). Seeds from each fruit were randomly selected for
paternity determination in fall 2014 and underwent recommended pretreatments including 12 weeks in cold stratification. They were germinated this past spring on agar plates in a controlled incubator set to common Chicago spring conditions. When seedlings reached their maximum size on the agar plates, they were randomly selected to be used for further analysis, labeled and put in a -80°C freezer for storage until the primer screening is completed and this portion of the experiment can move forward.

In the future, primer optimization will be completed and paternity analysis will be conducted on tissue from all selected P. hirsutus seedlings. The program CERVUS will be used to calculate logarithm of the likelihood (LOD) ratios and paternity will be assigned to the individual with the highest LOD score. Gene (pollen) flow distance will be calculated as the straight line distance between paternal and maternal individuals as measured using ArcGIS. Flowering density and floral richness will be analyzed using Wilcoxon rank-sum test corrected for non-normal variance.

Two supporting experiments will also be set up in June 2015 to provide additional information about pollen movement in P. hirsutus populations. The 50 maternal individuals used in 2014 were overwintered and are expected to flower by the last week in June. At this time, five individuals will be set up following the same arrangement as in summer 2014 on two green roof and two ground level locations at the Plant Science Center at the Chicago (20 individuals total). Four seeds from three mature fruits from all individuals will be pretreated, germinated and used for paternity analysis, following the same procedures used for the 2014 offspring. These additional data will be used to determine not only if genes (pollen) are moving between green roofs but also from green roof to ground and from ground to green roof. Conclusions will suggest green roofs’ potential to act as both sinks and sources for genetic material in a larger urban context, which has implications for successful green roof management plans. Movement of pollen between roof and ground level locations will also be confirmed by using fluorescent dye tracking. In this experiment, all open, receptive flowers of P. hirsutus on the roofs will be brushed with different colored tracking dye before 09:00, when pollinators become most active. After dusk, a UV light will be used to detect transfer of dye to individual flowers from other populations. Transfer of powered fluorescent dye has been used as a proxy to measure the movement of pollen in other plant populations, where the probability of detecting gene flow through pollination, fruit development, seed germination and successful paternity analysis is low. Paternity analysis is a more powerful a tool that allows me to determine from which individual pollen has come, but the dye tracking will more easily allow me to confirm if pollen is likely to be moving between roof and ground level populations.

The funds from my awarded ISEN grant were essential to complete this experiment over the past year. Although molecular genetic work is now becoming increasingly “inexpensive,” especially compared to cutting edge genomic techniques, the total cost of my experiment is well over $10,000, without any direct financial support from my advisor. Critical funding from ISEN is allowing me to answer this very interesting question of how genes flow between fragmented urban plant populations. With the increasing prevalence of green roofs in many cities and their expanding uses to include tasks such as food production, an understanding of the role that biotic pollinators play in supporting plant reproduction is critical. Without continued reproduction of plants, green roofs would have to be continuously replanted, making this sustainable technology in fact very unsustainable. Thank you to ISEN and specifically the Nasaw Family Fund for Environmental Research for supporting this research. I look forward to sharing my final conclusions with the Northwestern community and beyond and will be sure to acknowledge this generous grant in all future publications.