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High-Altitude Ballooning Student Research with Yeast and Plant Seeds

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ABSTRACT

Weather balloon flights provide affordable access to a space-like environment for student research. Typical flights last for 2.0-2.5 hours and reach altitudes of approximately 30 km. Payloads are exposed to intense cosmic and ultraviolet radiation, temperatures below -60° C, and atmospheric pressures of approximately 0.01 atmospheres. We report on simple laboratory procedures intended primarily for high school and middle school students in studying the effects of high-altitude balloon flights on yeast and plant seeds. *Saccharomyces cerevisiae, Raphanus sativus,* and *Brassica rapa* were flown on two weather balloons inside and outside of payload containers to an altitude of approximately 27.5 km. After the flights the yeast cells were plated on YED media and incubated to assess survival and mutation rates. The seeds were planted to assess survival and variation in quantitative traits. We also discuss connections to disciplinary core ideas in the *Next Generation Science Standards* (NGSS) (NGSS Lead States, 2013) and provide an overview of further laboratory investigations designed to enhance students' understanding of the effects of radiation on living organisms.

Key words: Education; Student Research; High-Altitude Ballooning; Cosmic Radiation; Ultraviolet Radiation; K-12; Experimental Design; Mutation; DNA; NASA

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INTRODUCTION

Viruses, bacteria, fungi, yeast, tissue cultures, and plant seeds have been the subject of high-altitude balloon, rocket, and satellite based research for almost 80 years (Stevens, 1936). Microbes can serve as model organisms for evaluating biological responses to extraterrestrial conditions (Olsson-Francis and Cockell, 2010; Horneck et al., 2010). Yeast is especially important in this context because DNA repair mechanisms and many other cellular processes that occur in yeast are similar to those in human cells (Clément and Slenzka, 2006). Microbial research also plays an important role in the development of instruments designed to detect life on other planets (Olsson-Francis and Cockell, 2010), for avoiding contamination of other planets with terrestrial microflora (McCoy et al., 2012), and for understanding the origin of life on Earth and its spread throughout the solar system and universe (Raulin-Cerceau et al., 1998; Valtonen et al., 2009).

An important reason for studying plant seeds that have been exposed to the conditions of space is the fact that the ability to grow plants will likely be crucial for the success of future long-duration spaceflight and for the habitation of the Moon and Mars as a source of food and oxygen (Ferl et al., 2002). In addition, there is strong evidence that

plants have beneficial effects on the mental well-being of astronauts living in a closed environment for an extended period of time (Wheeler, 2009). Over 100 varieties of seeds have been subjected to long-term exposure to the space environment as part of the Long Duration Exposure Facility and on the outer side of the International Space Station (ISS) in order to assess the risk of genetic and physiological damage during long-term space storage (Alston, 1991; Sugimoto et al., 2011). Seeds have also been flown on satellites and high-altitude balloons to induce beneficial genetic mutations for plant breeding (Liu et al., 2009; Chen et al., 1994; Li et al., 1997). Because of the ability to resist low temperature and pressure and high ultraviolet radiation and cosmic ray intensities, seeds have been proposed as vehicles for the transport of life to and from Earth (Tepfer and Leach, 2006).

Educational Objectives

In addition to being the subject of current research, studying the effects of exposure to the space environment also supports important educational goals in middle school and high school. Inheritance and variation of traits is one of the four life science *disciplinary core ideas* emphasized in the recently released *Next Generation Science Standards* (NGSS) (NGSS Lead States, 2013). For example, middle school students should understand that genetic mutations may cause harmful, beneficial, or neutral changes to the structure and function of the organism. High school students should investigate how environmental factors such as chemicals and radiation can affect DNA. They should be able to discriminate between mutations that cause uncontrolled cell division and cancer in individual organisms and mutations that are heritable, and evaluate the role of heritable mutations as a key process in evolution.

Most existing K-12 lab activities related to the effects of radiation on microbes and plant seeds employ irradiation with ultraviolet light from the sun and germicidal light sources, irradiation with gamma rays from cobalt-60 sources, and exposure to ultraviolet light and cosmic rays during orbital flights. *A Classroom Guide to Yeast Experiments* (Manney et al., 1997) contains a set of six experiments in which students investigate the effect of ultraviolet radiation on yeast cells. For example, they measure survival rates and generate survival curves, estimate solar irradiance, and investigate DNA repair mechanisms and mitotic recombination. In the NASA Radiation Biology Educator Guide (Rask et al., 2006) students expose a UV-sensitive strain of yeast to sunlight to evaluate the protective effect of sunscreen. Sobrero and Valverde (2013) and Zion et al. (2006) discuss lab activities in which students evaluate UV radiation damage to the DNA of bacteria and DNA repair mechanisms. Yip (2007) presents an activity in which students investigate the protective effect of the antioxidants in fruit peel on *E. coli* that have been damaged by exposure to ultraviolet light.

Brassica, radish, barley, tomato, and other seeds that have been exposed to radioactive Cobalt-60 are available from many science supply companies. Using these seeds, students can measure germination and growth rates, root length and other physical parameters of seeds that have received varying radiation doses, and investigate how mutations affect subsequent generations (e.g., Rask et al., 2006; Frederiksen, 2010; Turvey, 1986).

Seeds that have orbited Earth have been available to teachers and students for over 30 years. The *Cosmic Ray Dosage* curriculum supplement (Page and Page, 1977) is based, in part, on an investigation of the effect of cosmic rays on plant seeds during the Apollo-Soyuz test project. Tomato seeds orbited Earth for 69 months as part of the Long Duration Exposure Facility (Grigsby and Ehrlich, 1991; Melton, 1991) and were distributed to 40,000 schools for science experiments after retrieval by Space Shuttle Columbia. Space Shuttle Endeavour carried cinnamon basil seeds for NASA's Lunar Plant Growth Chamber design challenge during STS-118 (NASA, 2007). In May 2013, tomato seeds that orbited Earth for almost two years aboard the ISS as part of the *Tomatosphere* project (Vuk et al., 2004) were returned to Earth in a Soyuz capsule and are currently being distributed to students in Canada and the U.S. for analysis.

High-Altitude Balloons

The Kármán line at an altitude of 100 km is generally regarded as the boundary between Earth's atmosphere and outer space. Even though

high-altitude balloons do not reach this altitude, they pass through the Pfotzer maximum and most of the ozone layer in the stratosphere, and expose payloads to high doses of cosmic and ultraviolet radiation. Because payloads are not subjected to microgravity, balloon flights can serve as controls that allow researchers to discriminate between the effects of radiation and microgravity (Dickson, 1991). Balloons are also by far the most affordable and easiest way to provide students with direct access to a space-like environment for their experiments (Larson et al., 2009). Flight hardware is readily available from several vendors and the procedures for developing a flight system and executing flights is well documented (Verhage, 2005; Montana Space Grant Consortium, 2004; Edge of Space Sciences, 1993). A typical hardware setup is shown in Figure 1.

Radiation Environments Encountered during Balloon Flights

Two of the most important environmental conditions in the stratosphere that affect balloon payloads are the increased exposure to cosmic rays and ultraviolet radiation. Cosmic rays damage cells by indiscriminately ionizing molecules throughout the cells. Ultraviolet radiation damages cells by disrupting the structure of certain parts of the DNA molecule. Microbes and seeds can be shielded from ultraviolet radiation by placing them inside of payload containers. However, because of their high energy, the thickness of the shielding material (such as lead) required to absorb the cosmic rays would make payloads prohibitively heavy. Thin shields actually increase the particle flux because they fail to absorb the showers of secondary particles that are created when the cosmic rays hit the shielding material (Parker, 2006). Other important environmental factors, such as low atmospheric pressure and temperature, can be mitigated by payload design.

Cosmic Rays

Earth's atmosphere is continuously irradiated by high-energy charged particles called cosmic rays. Most particles are galactic cosmic rays (GCR) that originate outside of the solar system. Their sources are believed to be supernova explosions. A smaller number of solar cosmic rays (SCR) originate from intermittent solar particle events, such as solar flares and coronal mass ejections. Almost 90% of cosmic rays are protons and approximately 12% and 1% are α-particles and heavier nuclei, respectively (Schlaepfer, 2003).

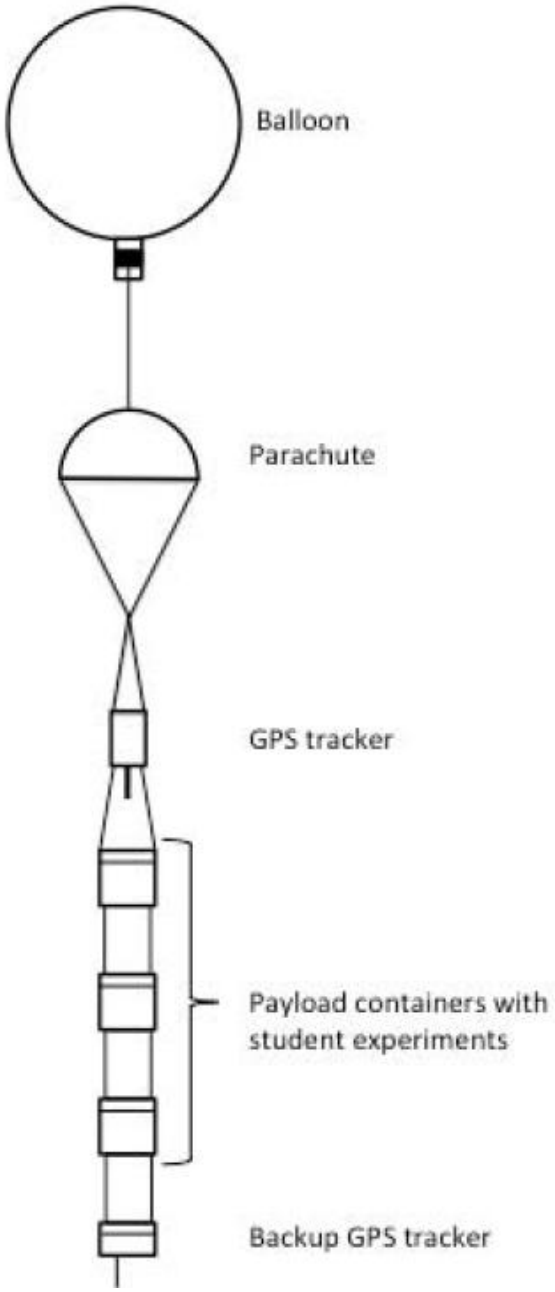


Figure 1. In addition to one or more containers with student experiments, the flight hardware consists of a helium or hydrogen-filled weather balloon to provide the necessary lift, a parachute for the controlled descent of after the balloon bursts, and one or more GPS trackers that transmit the location of the balloon to a chase vehicle on the ground.

As the particles enter the atmosphere they start colliding with atmospheric atoms and molecules, generating showers of secondary particles such as muons, positrons, neutrons, electrons, and photons (Carlson, 2012). The effect of these showers can be seen in Figure 2: the particle flux initially increases with decreasing altitude and reaches a maximum (known as the Pfotzer maximum) at an altitude of approximately 20 km. When the particle energy becomes too low the production of new secondary particles then stops and the flux decreases by about 5% for each decrease in altitude of 200 m.

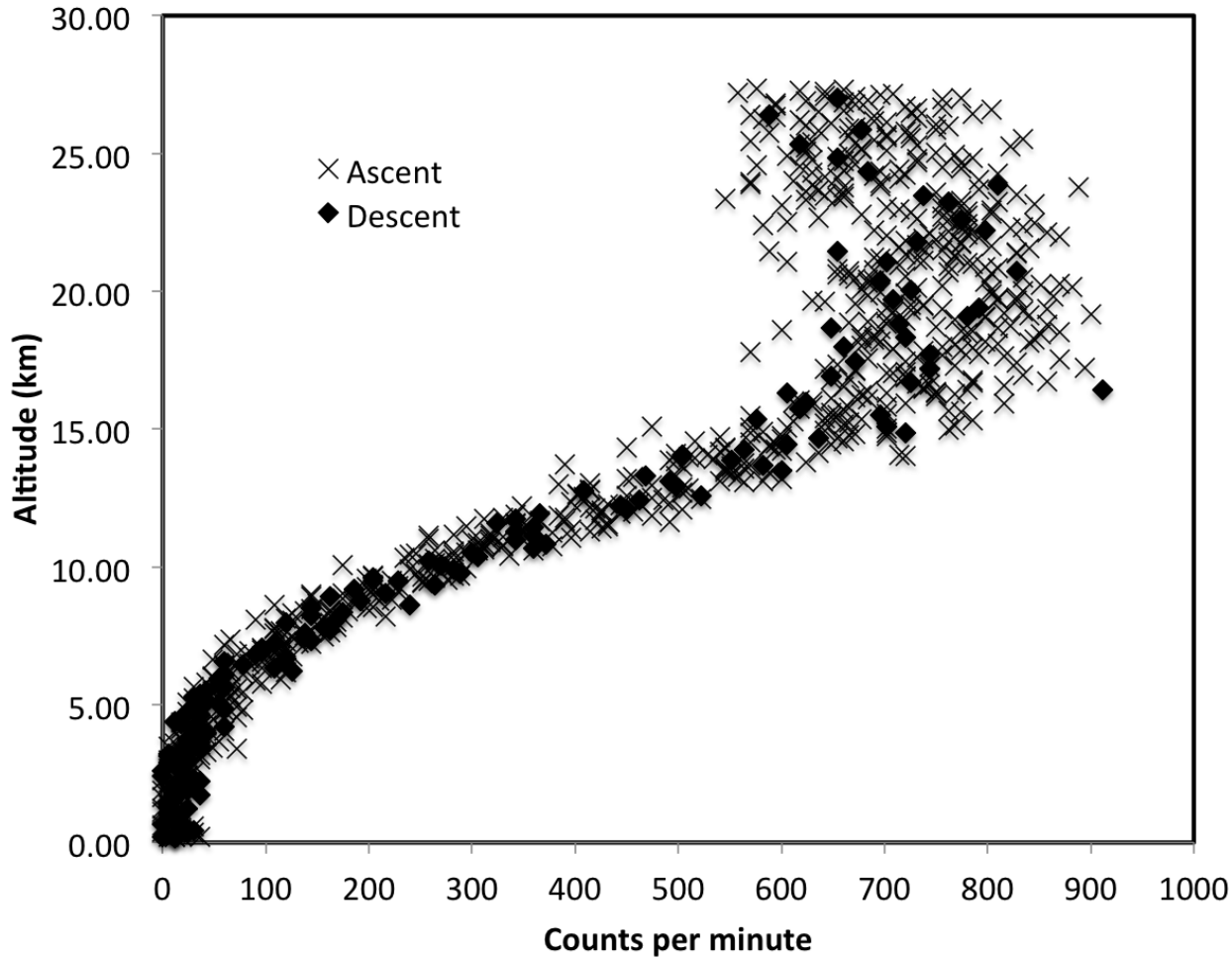


Figure 2. Cosmic ray counts measured on October 11, 2013 with an Aware Electronics RM-60 Geiger counter. The Pfotzer maximum is evident at an altitude of approximately 20 km.

Ultraviolet Radiation

The ultraviolet radiation emitted by the sun can be divided into three spectral regions: UV-a (320-400 nm), UV-b (280-320 nm), and UV-c (200-280 nm). Even though only a small fraction of the total flux of sunlight is in UV-b and UV-c, and ultraviolet photons have much less energy than cosmic rays (a few eV compared to more than 10^9 eV), these wavelengths are readily absorbed by DNA and are therefore the main cause of DNA damage (Cockell and Horneck, 2001). While most UV-a reaches the ground, most UV-b and almost all UV-c is absorbed by Earth's ozone layer, the bulk of which is located at altitudes between 20 and 30 km. Because balloons typically burst above most of the ozone layer, payloads are exposed to significantly higher doses of mutagenic UV-b and UV-c than on the ground.

METHODS

The laboratory protocols we suggest here offer a method to assess survival and mutation rates of yeast and plant seeds due to the cosmic and ultraviolet radiation encountered during high-altitude balloon flights. Samples can be flown in small (5 cm × 5 cm) zip lock bags that are attached to the inside and outside of payload containers using transparent packing tape (Figure 3). Payload containers can be constructed from Polystyrene sheets available at hardware stores (Verhage, 2005) or purchased fully assembled from StratoStar (http://www.stratostar.net). Samples attached to the outside of a container are exposed to both solar UV and cosmic rays. Samples on the inside of the containers are protected from ultraviolet light but not from cosmic rays (Figure 4). To monitor cosmic ray and UV intensity, temperature and other atmospheric variables during the flight, students should also integrate sensors and data loggers into their payload containers. These are already routinely used for science instruction at many colleges and high schools, and can be purchased from Vernier (http://www.vernier.com), Pasco (http://www.pasco.com), Onset (http://www.onset comp.com) and other vendors.

Preparation of Yeast Cultures

We used a strain of common baking and brewing yeast, *Saccharomyces cerevisiae*, containing a mutation that affects adenine biosynthesis (HA1; Manney et al., 1997). This strain turns red on yeast-extract dextrose (YED) media, a nutritionally complete medium containing a suboptimal amount of adenine. When grown on yeast-extract dextrose media with an excess of adenine (YEAD), the yeast will use adenine in the media instead of synthesizing it, and will grow into larger colonies and not turn red. HA1 (or alternatively HA2, which also turns red on YED media) may be purchased from Carolina Biological Supply Company (http://www.carolina.com). Cultures may be maintained on YEAD media, but to screen for mutants, the YED media, which can be purchased pre-poured or in powder form, is required. When grown on media with suboptimal adenine, HA1 grows until the colony exhausts the adenine and an intermediate metabolite in the biosynthesis pathway then causes the colony to turn red. The red colonies can be seen in the left half of the Petri dish shown in Figure 5. There are several mutations that can result in the red coloration and other mutations that cause slight variation in the wild-type cream color and/or size of the colony. The visible color mutations make this an ideal subject for investigating mutation rates. The genetics of yeast reproduction also allow for further research on the inheritance of new mutations (see Manney et al., 1997).

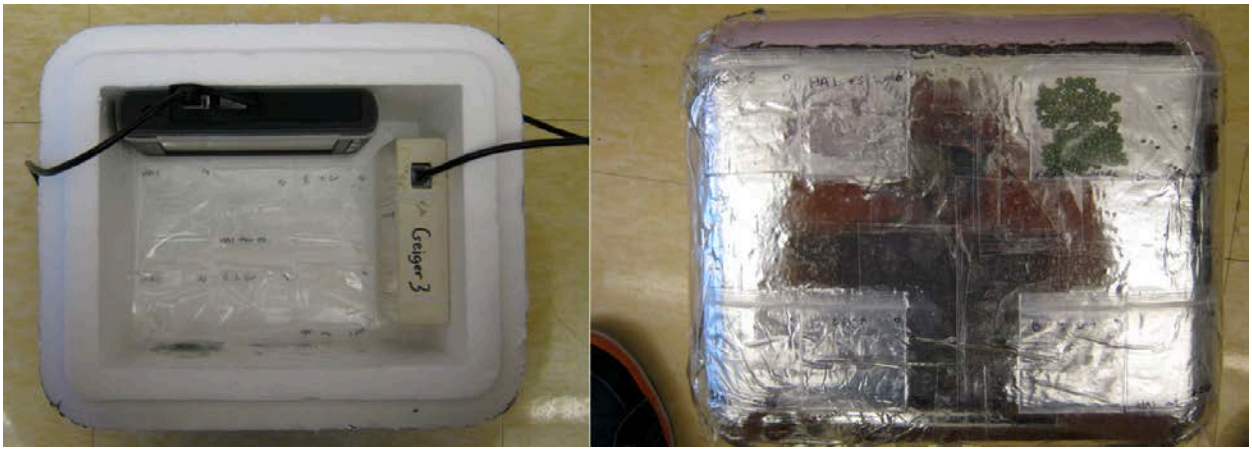


Figure 3. Yeast and plant seeds flown on the inside (left) and outside of a payload container, which also contains an RM-60 Geiger counter and Vernier LabQuest 2 data logger used to measure cosmic rays.

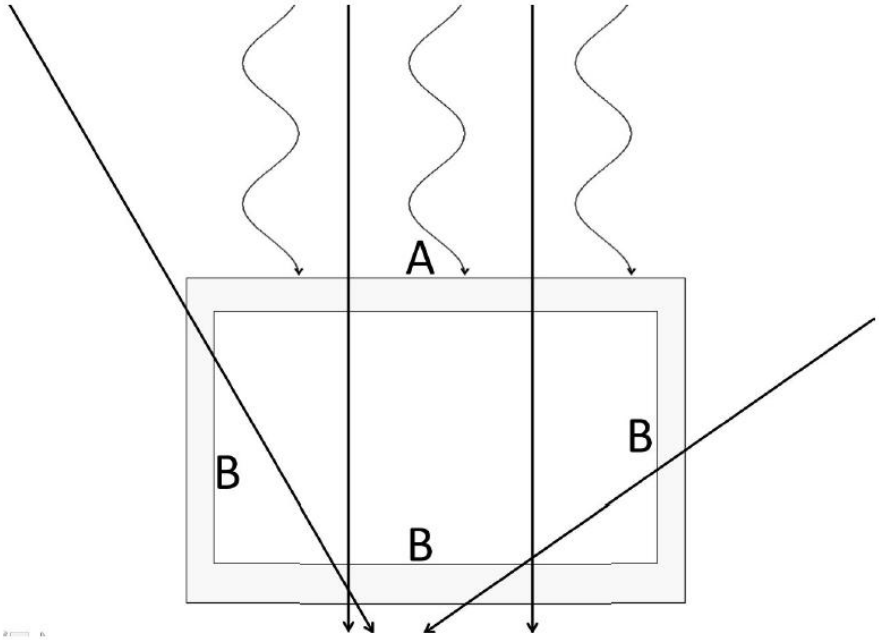


Figure 4. Illustration of the exposure of samples attached to different parts of a payload container to solar UV and cosmic rays. Samples attached to outside of the container lid (A) are exposed to both UV and cosmic rays, represented by wavy arrows and straight lines, respectively. However, solar UV cannot penetrate Polystyrene, so samples attached to the inside of the container (B) are only exposed to cosmic rays.

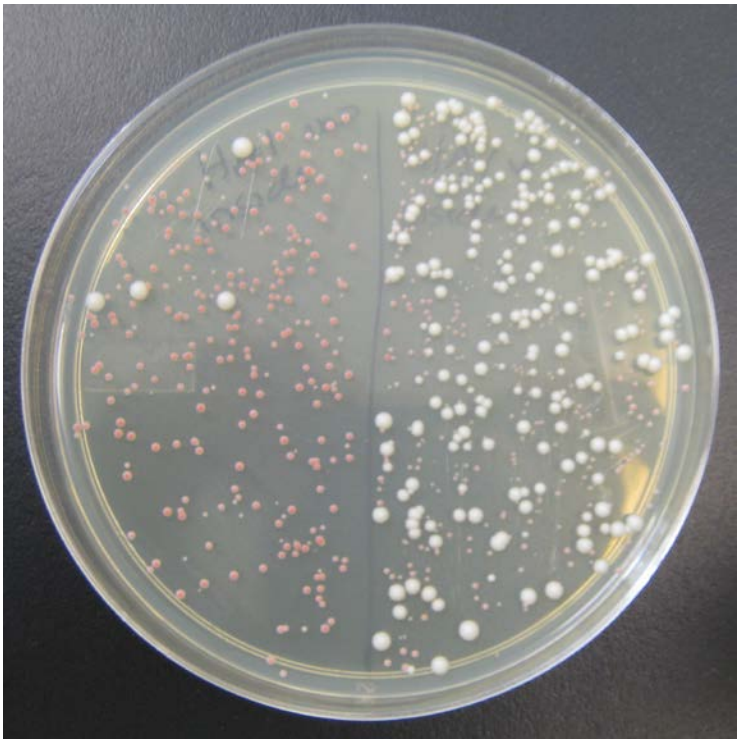


Figure 5. HA1 yeast grown on YED media show the characteristic red phenotype. Larger white colonies represent back mutations to the wild type. These colonies can manufacture their own adenine. They do not turn red and continue to grow after exhausting the small amount of adenine in the media. The right side shows a higher rate of mutation than the left.

Yeast colonies were transferred using a clean toothpick to remove a small sample of yeast into 1.0 ml of sterile water to produce a concentration of roughly 10^6 yeast cells per ml of water (Williamson, 1999). We packed 1-2 ml of yeast suspension in small zip lock bags for flight. To screen for mutants, a sterile swab was saturated in the suspension and used to spread the yeast evenly over a sterile media plate. Plates were incubated upside down at 30º C for three days and examined for colony number, color and size (Manney et al., 1997; Williamson, 1999). The yeast can also be incubated at room temperature for a slightly longer amount of time, so an incubator is not required for this experiment.

Preparation of Plant Seeds

We have used seeds of garden radish, *Raphanus sativus*, which are large and easy to handle. They germinate on damp paper within a day or two, and have primary roots easily measured for short-term germination and growth studies. For longer studies, the seeds can be planted and should mature in 20-30 days. Phenotypic traits that can be quantified include germination rate, above ground biomass, root diameter and mass, and number of flowers. Students can be challenged to quantify root taste. Other vegetable seeds can be used, but most other plants require more sunlight and longer growing times before they produce their crop. Wisconsin Fast Plants®, *Brassica rapa,* have smaller seeds that also germinate quickly. They have the advantage of producing harvestable seeds in around 40 days, thus allowing for further genetic analysis. Several dozen seeds were put into the small zip lock bags. Figure 3 shows the four bags containing Brassica and radish seeds and the 12 bags containing yeast that were taped to the inside and the outside of the payload container for the flight. After the flight, 36 seeds for each species (12 from bags inside the payload, 12 from bags outside the payload, and 12 control seeds) were planted in a standard potting mix, placed in a south-facing window and regularly watered. Figure 6 shows 24 of the plants three weeks after the seeds were planted.



Figure 6. *Raphanus sativus* and *Brassica rapa* plants grown from seeds carried on a balloon flight and from control seeds.

Table 1. Examples of quantitative measurements of reproductive rates and phenotypic traits of Brassica plants. The average number of flowers and seedpods was lowest and the average length of the longest seed pod was shortest for the seeds that were flown on the outside of payload containers, although these differences are not significant ($F_{2,33}$=2.6, 1.6, and 1.1; p=0.08, 0.22, and 0.44, respectively). In each case, the coefficient of variation (CV) was largest for the seeds flown on the outside.

Plant	Number of flowers			Number of seed pods			Length of longest pod (cm)		
	Control	Inside	Outside	Control	Inside	Outside	Control	Inside	Outside
1	3	4	2	0	1	0	1.5	3.5	4
2	3	4	3	0	2	0	2.5	3.5	3.5
3	3	5	3	1	1	0	1	2.5	2
4	3	4	4	3	1	2	1	0.5	3
5	5	3	4	2	1	1	0.5	1	1.5
6	3	3	5	1	1	1	1.5	0.5	0.5
7	4	6	3	1	0	0	4	2	0.5
8	5	4	4	1	0	1	2.5	1	0.5
9	8	3	5	0	0	0	2	1.5	1
10	6	6	4	1	0	0	2	3.5	0
11	5	5	1	2	2	0	0.5	3	0
12	4	4	0	0	0	0	2.5	3	0
Average	4.33	4.25	3.17	1.00	0.75	0.42	1.79	2.13	1.38
CV	0.36	0.25	0.44	0.95	1.01	1.60	0.56	0.56	1.04

RESULTS

Yeast flown in small bags of water or on plates outside the payload container does not survive. When flown inside, our yeast samples showed highly variable survival rates from a few percent to nearly 100%. We are still working on what causes the variation in survival. We have observed that when survival is low, mutation rates are higher, leading us to hypothesize that radiation and not temperature or pressure is responsible for the survival. This is a testable hypothesis and we will be working with Pontiac Township High School and undergraduates in our classes next spring to continue this work. We are also planning to repeat the experiment with G948-1C/U, a strain of yeast with defective DNA repair pathways, to evaluate the extent to which survival and mutation rates are affected by the DNA repair mechanisms (Manney et al., 1997). (G948-1C/U does not turn red on YED media, but morphological traits such as colony size, shape, and color can be used to assess mutation rates.)

Un-sprouted seeds have had very high survival rates in all of our balloon flight experiments. The radish and Brassica seeds in this experiment also had 100% germination. We assessed the effects of radiation by measuring a variety of quantitative traits including plant height and weight, the number of flowers and seed pods and size of the longest seed pod. Examples of these data are shown in Table 1. Reproductive rates in *B. rapa*, as measured by the number of flowers and pods, were lower for seeds exposed to both cosmic and UV radiation, than for seeds exposed just to cosmic rays; both were lower than control seeds although these differences were not significant. The radish plants above ground weight at 5 weeks did not differ between treatments.

Radiation should also increase variation in phenotypic traits. The coefficient of variation (CV = standard deviation / mean) is a useful statistic

for describing variation. For every trait measured, the CV was greater in the seeds from the balloon flights than the control seeds. It is this variation that gives us the potential to find new mutations of interest. For example, the longest seed pod was from *B. rapa* flown on the outside of the payload and the largest radish plant, flown inside the payload, was 50% larger than the largest plant grown from control seeds.

ASSESSMENT

One of our most important goals in this project is to create opportunities for students to engage in end-to-end research. We want them to take ownership of every step of the process, including formulating research questions and hypotheses, creating research plans, designing and fabricating science payloads, executing balloon flights, analyzing their data, and presenting their results. To help students with the many inherent challenges, it is important for instructors to create an organizational framework and timeline and assess student work frequently. For assignments that are designed to generate or summarize ideas, such as brainstorming ideas for investigations and note taking during presentations, group discussions, and lectures, assessments can be informal, giving students full credit simply for completing them. For more complex assignments, such as formulating well-reasoned hypotheses and preparing poster or PowerPoint presentations, assessments should be more detailed and include rubrics that specify levels of quality, accuracy, and organization. For assignments that are critically important for the safety and success of the balloon mission, such as design and fabrication of payload containers and the development of pre-launch procedures, students should be required to revise their work based on instructor feedback until it is approved by the instructor.

Integrating high-altitude ballooning projects into the science curriculum has been shown to improve students' knowledge and skills and positively impact their attitudes and beliefs about science. Snyder et al. (2009) developed a 119-item survey using a Likert Scale that is administered to undergraduates at the beginning and at the end of a course. Results show that ballooning projects can increase the students' intrinsic motivation, application knowledge, cognitive and metacognitive skills, and content knowledge. In addition, there are many other validated instruments available to instructors wishing to assess the impact of ballooning projects on their students. For example, Potosnak and Beck-Winchatz (2013) used the Colorado Learning Attitudes about Science Survey (CLASS) (Adams et al., 2006) and the Chemistry Concept Inventory (CCI) (Mulford and Robinson, 2002) to evaluate the impact of integrating a balloon project in an environmental chemistry class on undergraduates' beliefs about science and learning science, and to evaluate the improvement of their conceptual understanding of basic chemistry concepts.

DISCUSSION

We have been working with students ranging from middle school through college seniors on high-altitude ballooning science experiments in physics, astronomy, chemistry, geology, and biology for over four years and have carried out over 40 flights. Students participate as part of college classes for science and non-science majors, NASA-funded summer research programs for college and for high school students, K-12 outreach programs during the school year, and extracurricular activities of the Society of Physics Students. To ensure that every student is able to contribute to the research project and balloon flight in a meaningful way, we normally limit the number of participants per flight to 25-30. There is a consistently high level of interest in projects that involve living organisms, such as algae, terrestrial isopods and crickets, aquatic crustacea, yeast, seeds, and plants. Student-designed experiments tend to focus on shielding the organisms from the harmful effects of the environmental conditions, looking at survival as a measure of success. The most interesting experiments used containment designs that allowed them to tease apart the individual and interactive effects of cosmic and UV radiation, temperature, and pressure.

In contrast to this high level of interest, the background of most instructors who use ballooning is in the physical sciences and in engineering. This is, for example, evident in the fact that among the more than 90 papers presented

at the annual Academic High Altitude Conference from 2010-2013, not a single one focused on the life sciences. To better support our students' interest we turned to the yeast and Brassica systems to add another dimension to the study of space conditions, that of assessing mutation rates. Systems that allow for the screening of mutants provide an opportunity to see the direct effects of radiation on genetic variation and allow for experiments to tease apart the individual and interactive effect of cosmic and UV radiation. The yeast and Brassica systems also support follow-up experiments to uncover patterns of inheritance, creating opportunities for ongoing research and greatly extending the educational value of the balloon flight experience. We hope to report on the results of these new experiments in the future.

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