COLLEGE OF AGRICULTURE AND NATURAL RESOURCES 2020 RESEARCH REPORT

REFLECTIONS

Fruit fly and human brains, prairie dogs and ancient grains among topics our scientists explore in this issue of Reflections.

UNIVERSITY OF WYOMING | College of Agriculture and Natural Resources

REFLECTIONS

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Adapting, evolving to meet research needs

John Ritten

Welcome to the 2020 edition of Reflections, the research magazine of the University of Wyoming's College of Agriculture and Natural Resources.

As mentioned last year, we welcomed Dean Barbara Rasco, and she has been working diligently on our behalf for almost a year now. We have also solidified more of the college's administrative roles by officially naming Kelly Crane as associate dean and director of University of Wyoming Extension and Warrie Means as associate dean and director of the Office of Academic and Student Programs. Great new faculty members have joined the college over the last year, and we have some more slated to join us in the coming months.

And, just as we thought we were getting to a new "normal," we find ourselves adapting to COVID-19. Campus certainly has a different feel compared to this time last year, but our faculty members and students are finding creative ways to adapt to what seems like a constantly evolving situation. While we are looking forward to putting this behind us, please know we are doing our part to keep the college moving forward while working to ensure the health and safety of our students and staff and faculty members.

I hope you enjoy the following examples of the breadth of research being conducted at the college. I wish we could include more, but we would need a book to fully show the research activities of everyone. And, as always, I welcome any comments, suggestions, or questions regarding work presented in this issue or about the research enterprise of our college, to aes@uwyo.edu or (307) 766-3667.

Thank you,

John Ritten
Interim Associate Dean and Director of the Wyoming Agricultural Experiment Station

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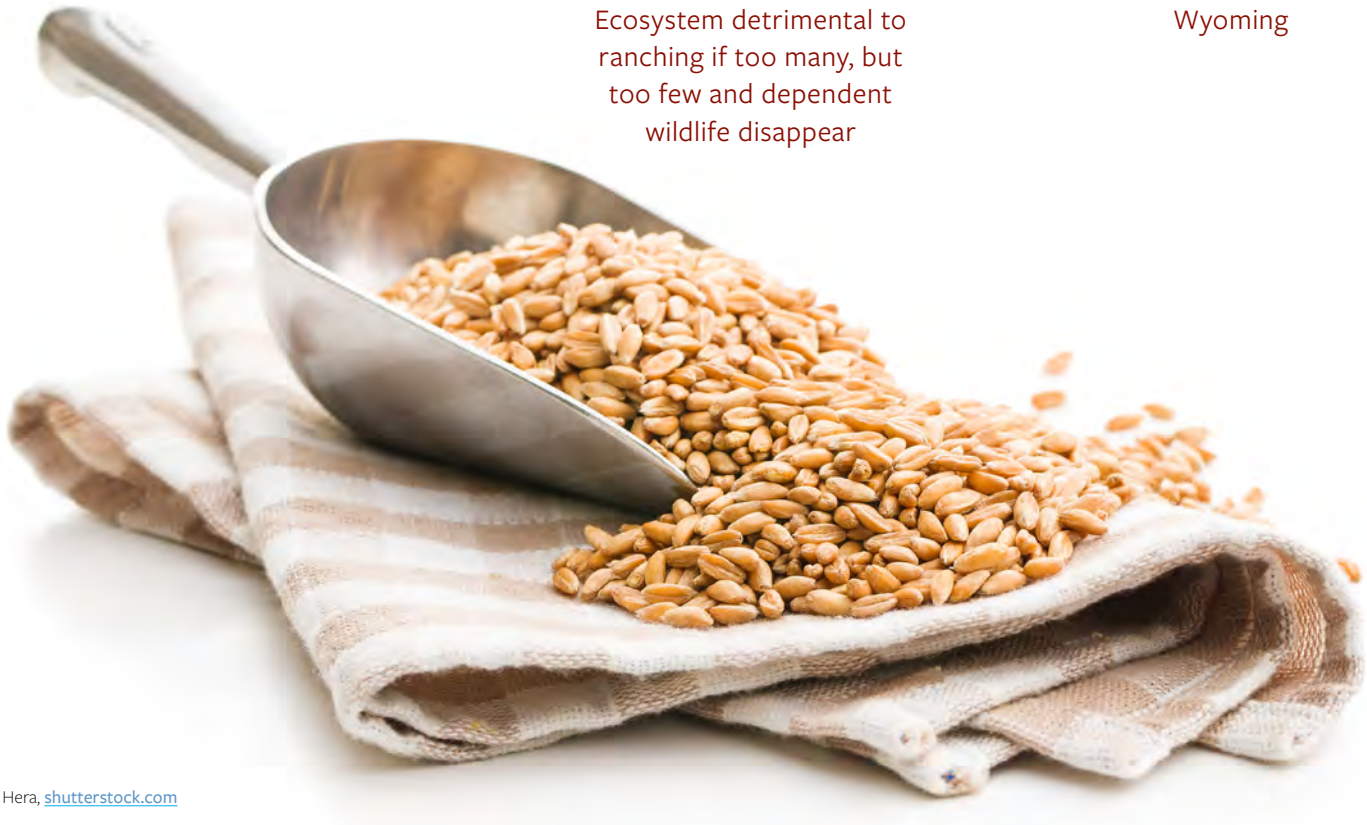
REFLECTIONS



Jiri Hera, shutterstock.com

WHAT THE FRUIT FLY CAN TELL US ABOUT HUMAN DISEASE

Todd Schoborg
Assistant Professor
Department of Molecular Biology

You've probably seen them.

Those tiny bugs that hover over your fruit bowl in the summer if your bananas get too ripe.

While your first reaction might be to smush these kitchen invaders, most folks don't realize some of the most important biological discoveries of the last 150 years were made by scientists studying this organism, including those that have had a direct impact on human health.

A biomedical revolution, led by bugs

Realizing just how much we have in common with the fruit or vinegar fly (*Drosophila melanogaster*, above), can be surprising. Sure, there are lots of differences on the outside (hey, we don't have wings), but at the level of molecules, genes and cells, fruit flies and humans share many similarities. For example, a fly's eye develops in much the same way our own eyes do, using the same set of genetic instructions, cellular machines, and cell types to make a highly complex organ. In fact, the fly eye develops just fine using either its own eye gene or one from a mouse!

This biological similarity is extremely important, because what we learn about how genes, cellular machines, and cells work in fruit flies can tell us how they work in humans, too. Since using humans for certain experiments is unethical, fruit flies are invaluable to biomedical research. People tend to not mind if you sacrifice an alien-looking, slightly annoying bug for the greater good of humankind.

Fruit flies are model organisms because they serve as a proxy for understanding human biology. My molecular biology colleagues use frogs, mice, and worms that have their own experimental advantages.

I use flies because they are easy to grow (an over-ripe banana works in a pinch), have a very fast generation time (egg to adult in 10 days), produce thousands of offspring, and have lots of genetic tools that allow us to figure out just how cellular machines work. Imagine if cattle and sheep had the same biological advantages as flies (fast generation time, simple dietary requirements). Ranch productivity would be sky-high (and more money in a rancher's pocket)!

Harnessing the power of model organisms to understand human disease

My research leverages these advantages in the fruit fly to understand the molecular basis of human disease.

My goal is to determine how mutations in genes disrupt the function of cellular machines and, in turn, alter cell behavior that ultimately leads to disease.

Once we know how this process works at its most basic level, other scientists can use this information to design better therapeutic treatments.

My lab focuses on microcephaly, characterized by a reduction in brain size, intellectual disabilities, and early death (Figure 1). While doctors understand the clinical aspects of the disorder, we don't know the molecular details: how do genes instruct the brain to develop to the correct size? We know the human gene *Abnormal Spindle-Like Microcephaly-Associated Protein* (*ASPM*) is frequently mutated in microcephaly patients, but we don't know what it does in cells that ultimately leads to small brains when it's not working.

This was the question I wanted to address as a researcher at the National Institutes of Health. Flies have their own *ASPM* gene version, so I used gene editing technology to create a mutation in the fly *ASPM* gene. This led to fruit flies that had small brains, exactly what we see in human microcephaly patients (Figure 1).

Fruit flies build brains the same way humans do

I then performed experiments to figure out what was going wrong in brain cells that had a mutant *ASPM* gene. My preferred tool is light microscopy, because we can see exactly what cells and their cellular machines are doing as a fly brain develops (Video 1, see page 5). I found the cellular machine that helps a cell divide into two cells, known as the mitotic spindle, was defective in a special population of brain cells: the neural stem cells (Video 2, see page 5).

This was an exciting finding, because that told me that *ASPM*'s job

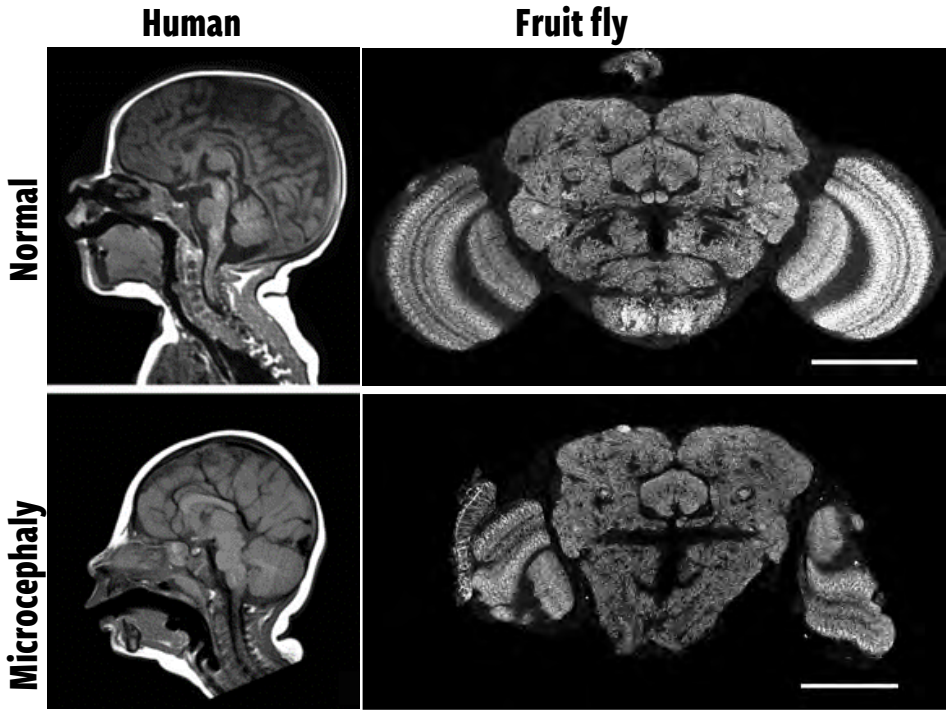


Fig. 1. Brain scans from humans and fruit flies. Microcephalic brains from both species are shown in the bottom panels. Scale bars: 200 micrometers.

What the fruit fly can tell us about cancer

The fruit fly also serves as an excellent model for understanding cancer, the second-leading cause of death in the US.

Cachexia is a muscle wasting phenotype seen in many terminal cancer patients, often directly responsible for death. Through my collaboration with Dr. Tor Erik Rusten at Oslo University Hospital in Norway, we recently uncovered how cachexia happens at the molecular level. Cancer cells can trick normal muscle cells into turning on a nutrient recycling program called autophagy, which breaks down muscle protein into amino acids.

These amino acids are then taken up by tumor cells to fuel their growth. By turning off autophagy in fly muscle cells, we could prevent muscle tissue from breaking down and tumors from growing, essentially stopping cancer in its tracks. This information can now be used to design better therapeutic strategies or drugs to treat human cancer.

was to ensure the mitotic spindle was working correctly so cells could multiply. But what was the link between this cellular defect and small brains?

The brain is a complex organ, whose size is thought to be determined by the number of cells present. In humans, there are about 170 billion brain cells! However, these cells must come from somewhere since we all start life as just a single cell. During development, cells divide to increase cell number, and the organism grows as a result. This is the job of the neural stem cells: divide multiple times using the mitotic spindle machinery to make all the brain cells.

Based on this logic, I hypothesized microcephaly was the result of mitotic spindle defects occurring in neural stem cells due to mutations in *ASPM*, which prevented them from dividing properly. Fewer cells equals smaller brains (Figure 2).

Putting the hypothesis to the test

If the hypothesis was true, then we should see small fly brains whenever we see defective mitotic spindles. To test this, I performed a "genetic rescue" experiment, where I added back different parts of *ASPM* to mutant flies and looked for both mitotic spindle and brain size defects, the latter of which I measured using microcomputed tomography (μ-CT), the perfect tool for visualizing small fly brains (Video 3, see page 5).

The result was not what I expected. Amazingly, only a small piece (~1/4) of *ASPM* was needed to make a properly sized brain – and it could still do this even though the mitotic spindle was defective in the neural stem cells. Apparently, brains don't care if the cell division machinery is not working quite right; they still find a way to grow to the correct size anyway (Figure 3)! The data simply didn't support my hypothesis.

Failure in science as a path to discovery

Failure is a common occurrence in science. We fail to support our hypotheses or get experiments to even work. Rather than being a series of successful experiments that lead you right to the answer, science is more like fumbling around in the dark, hitting dead ends along the way, until you finally emerge on the other side with only a few answers.

But that's the beauty.

Science might be the only profession where failure actually gets you anywhere. It's the combined action

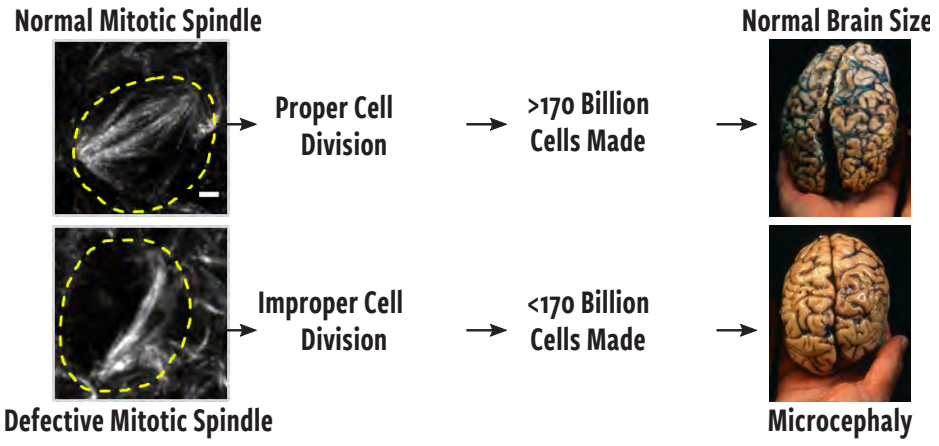


Fig. 2. Hypothesis for how defective mititoic spindles in neural stem cells cause small brains. Cell outlines are shown by the yellow dotted lines; miototic spindles are white/gray.

of many scientists, making small advances in knowledge despite these failures, that build the forest from the trees. And often, the data that failed to support your hypothesis ends up telling you more about what's going on than you could have ever imagined.

In my case, that's true. Based on the data from that experiment and a few new ones, my lab is now on the cusp of figuring out exactly what this small piece of the ASPM gene does, the cellular machines involved, and how it controls cell behavior so a brain grows to its correct size.

Who knew a tiny bug who loves rotting fruit could be so powerful?

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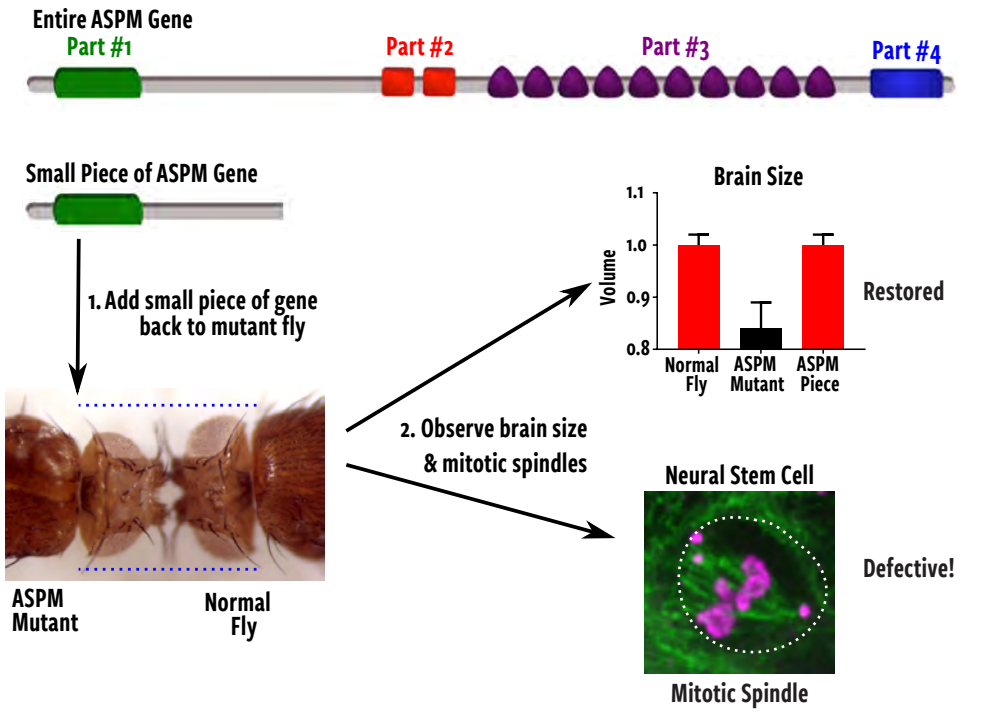

Fig. 3. Diagram of the "genetic rescue" experiment to test the hypothesis. The *ASPM* gene was split into different parts, which were introduced back into the mutant flies. I then measured brain volume and observed mitotic spindle morphology in neural stem cells from the animals that carried the small piece of *ASPM*. Even though the *ASPM* piece could restore brain size, mitotic spindles were still defective, failing to support the hypothesis.

Videos

Video 1. Video of a *Drosophila* brain developing in real time, as observed by light microscopy. The different types of cells in the brain are labelled, including neural stem cells, neurons, and glia. The neural stem cells divide multiple times to make all the neurons and glia of the brain. In this video, a neural stem cell undergoes two rounds of division to illustrate this process. Video credit: Nasser Rusan, National Institutes of Health.

Video 2. The mitotic spindle, the cellular machine that allows neural stem cells to divide and make neurons and glia, is shown in both a normal stem cell and an ASPM mutant stem cell. One cell division is shown. Note how the mitotic spindle in normal cells forms rapidly into a tight diamond shape, followed by rapid separation as it moves the chromosomes into each daughter cell. ASPM mutant mitotic spindles fail to maintain their diamond shape and there is a long delay until the chromosomes finally separate into the daughter cells. Time is shown in minutes.

Video 3. Micro-computed tomography (μ-CT) of an intact fruit fly, rendering in 3D. Most of the fly has been made "transparent" and then digitally cut away to show the central nervous system. The ventral nerve cord, like the vertebrate spine, is shown in yellow. The entire brain, located in the headcase, has been colored to show the sub-regions responsible for vision (optic lobes, blue) and learning and memory (central brain, red). This tool is used to precisely measure brain size in small flies.

Note: These videos and others are available on the Wyoming Agricultural Experiment Station's YouTube channel at bit.ly/youtube-WAES.

ONE STEP

First-grains project seeks to grow niche industries, boost Wyoming farmers and our rural economy

Wyoming has the least number of food processing facilities in the nation.

Given our climate, low population, and distance from markets, this might not seem surprising; however, even Montana and North Dakota, which face similar challenges, far outrank Wyoming.

The Wyoming First-grains Project in the Department of Agricultural and Applied Economics, with support from the Wyoming Agricultural Experiment Station, seeks to improve this with a novel approach to building food-processing capacity while contributing to rural economies. The project's ultimate goal is to spin off a profitable, stand-alone company to help create Wyoming jobs and improve producer incomes with premium pricing.

Project rallies collaborators across campus and state

The Wyoming First-grains Project is a research and economic development initiative bringing what we call "first-grains" (see sidebar, page 7) to Wyoming. The project goes several steps further than what we traditionally think of as an agricultural research project involving specialty crops. The project attempts to not just introduce the crop to farmers but build a stand-alone business and niche industry around these crops where none exist – even though we see demand in the state and in fast-growing areas of Colorado.

Malted grains and grains for flour are the first products we want to produce. There are about 25 craft breweries in Wyoming and about 340 in Colorado. This means even if we can tap only a fraction of this market, we are well-positioned to take advantage of Wyoming's location. We have partnered with Wyoming Malting Company in Pine Bluffs to malt the grain. We are developing co-branded malt products to enter this market.

Thomas Foulke
Research Scientist
Department of Agriculture & Applied Economics

AWAY FROM WILD

Research farms, producers grow grains

Our group is moving into the third year of the five-year project. We have been growing spelt, emmer wheat, and just last year einkorn (*triticum monococcum*), at UW research farms near Lingle, Powell, and Sheridan and in cooperation with several private producers in the state.

These first-grains are called "hulled grains" since their hulls do not thresh free. The extra step and extra expense of de-hulling is likely why they are no longer widely grown. De-hulling capacity also all but disappeared along with the grains early in the last century.

The project received a $50,000 grant from the University of Wyoming Institute for Innovation and Entrepreneurship (part of the Wyoming Legislature's Economically Needed Diversity Options for Wyoming [ENDOW] initiative) to purchase a de-huller. The de-huller is now at the Wyoming Seed Certification facility in Powell, with which we are working closely on this project.

Farmers, under market pressures, must continually watch costs to stay profitable in competitive commodity markets. So how can they be profitable with these grains if they are more expensive to produce?

What are first-grains?

We use the term first-grains to talk about those first domesticated cereal crops among them einkorn, emmer wheat, and spelt. These are the first plants domesticated by humans. Sometimes called ancient grains, we are trying to differentiate them from some recent crops and as a way to re-define the discussion about these crops and their nutritional value based on science as opposed to the hype surrounding ancient grains on the internet.

Tanya Engel, UW Extension

Wyoming First-grains Project team members

Plant sciences Assistant Professor **Carrie Eberle** (agronomy)

Agricultural and applied economics research scientist **Thomas Foulke**, project director

Agricultural and applied economics research scientist **Brian Lee** (costs and returns)

Agricultural and applied economics Professor Emeritus **Tex Taylor** (economics)

Assistant Professor **Jill Keith**, Department of Family and Consumer Sciences (nutrition)

Plant sciences Professor **Andrew Kniss**, agronomy (weeds)

Mike Moore, director, Wyoming Seed Certification Service (Powell)

Caitlin Youngquist, University of Wyoming Extension educator (agronomy), Worland

John Ritten, director, Wyoming Agricultural Experiment Station economics (ex officio)

If the demand is there (we have anecdotal evidence it is), then we can have some control over price. These will be premium products and as such we can command a higher price for them – at least for a while; our premium could disappear as other farmers start to jump into the market.

Branding would help us remain sustainably profitable. If we brand our products and create quality attributes customers value, then when the product becomes more commoditized, customers will still seek us out and pay a premium for our products. However, the real advantage will be our early lead in the market and our expertise in growing these premium crops.

Growing grains may not be biggest obstacle

Building the business structure is currently our greatest challenge. This project is different from the university's traditional spin-off concept with patents and intellectual property. The university's mission focuses on education **and** economic development. We are trying to springboard off the economic development part of the mission with a more proactive approach.

We call the concept we are pioneering "applied supply-chain research"– in other words, we are applying the knowledge and concepts we teach to overcome obstacles by doing. UW is applying expertise from several disciplines and assuming the early risk to solve production and supply problems and build market share to get the process up and running.

It makes sense for us to band together, to use our expertise and combined efforts to benefit Wyoming's economy. Some of that expertise comes from students in George Mocsary's legal practicum class in the UW College of Law. Through their efforts, they are helping us find the legal structure to integrate this with UW and provide a smooth transition to the private sector.

Success is never guaranteed, but we think we have put together the people and resources to set the project up for success. With a little luck, and a lot of hard work, you will be seeing our logo around Wyoming and be able to buy products made with Neolithic brand, Wyoming-grown first-grains.

Much more information on our activities and early agriculture is on our website Neolithicbrand.com.

***To contact: Foulke** can be reached at (307) 766-6205 or at foulke@uwyo.edu.*

RUMEN MICROBIOME MAY PROVE FERTILE LANDSCAPE TO IMPROVE FEED EFFICIENCY IN CATTLE AND SHEEP

There is potential to "program" the early microbiome through maternal influence

Hannah C. Cunningham-Hollinger
Assistant Professor
Department of Animal Science

Cattle and/or sheep grazing or chewing their cud is a sign of productive, healthy, ruminant animals.

These ruminants convert Wyoming forages into high-quality, Wyoming-raised beef, lamb, wool, and more. Microbial fermentation in the rumen converts even low-quality forages into high-value end products. These microbes have influences beyond digestion. The microbiome has substantial effects on host performance and long-term implications for production efficiency.

Within the rumen are many microbial species specialized in the breakdown of different feedstuffs into small components that can be utilized by other microbes. Volatile fatty acids (VFA) that serve as a primary energy source for the host (cattle or sheep), microbial protein, and other important factors for the host, are the results of this network of microbial fermentation and breakdown. These fermentation end products provide the host with energy to function – rumen fermentation provides nearly 70 percent (!) of total host energy.

Ruminating on the rumen

Our research efforts target improving efficiency by programming the rumen microbiome. Specifically, we aim to better understand how the rumen microbiome changes as the offspring develops, how maternal factors influence the early colonization of the rumen, and if any early interventions can affect host efficiency into maturity.

The rumen microbiome plays pivotal roles in feed efficiency due to the importance of these microbes in the breakdown of feed and generation of energy for the host. Many research projects from our lab and others have showed differences between high and low efficient livestock in terms of their rumen microbiome.

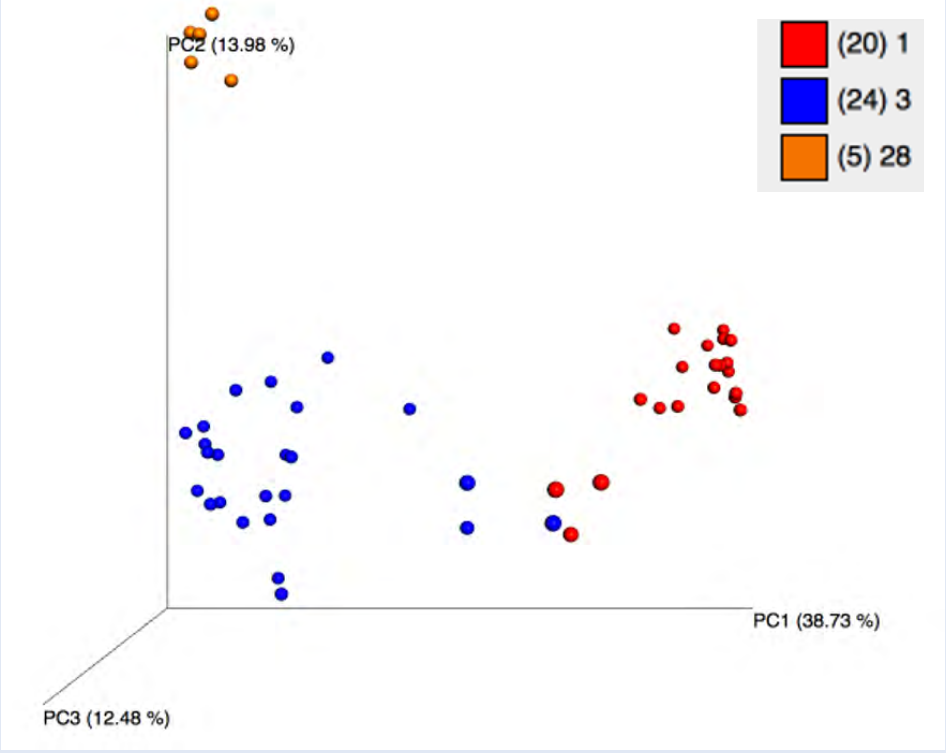

Fig. 1. Plot of diversity in the early rumen of calves of 1 day (red), 3 day (blue), and 28 day (orange) of age. The biome becomes less variable and more consistent the older the calf.

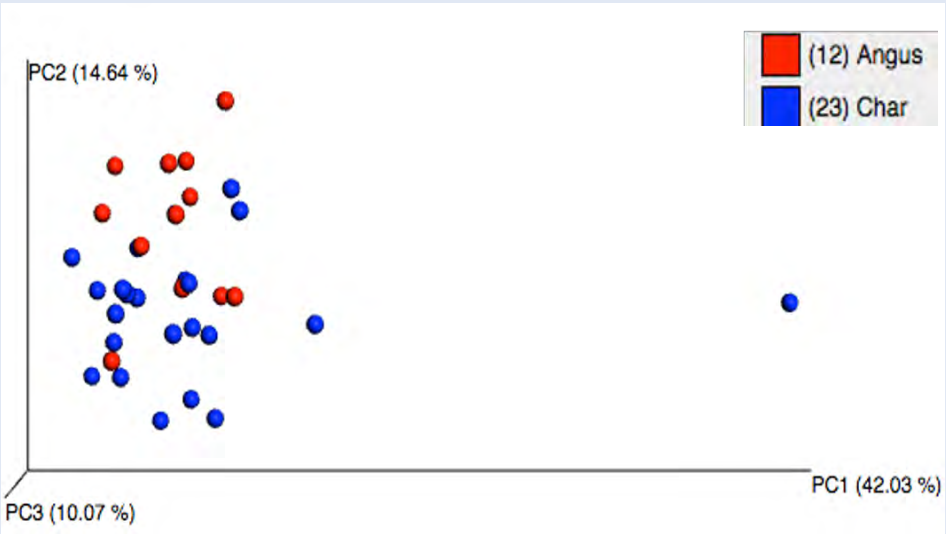

Fig. 2. Plots of beta-diversity in post-weaning calves of Angus (red) and Charolais (blue) breeds. Our research shows Charolais are more feed efficient than Angus.

Development of rumen

At birth, the rumen is essentially non-functional and very underdeveloped. The newborn calf or lamb is solely consuming milk, which allows for a more rudimentary microbiome to colonize the rumen early because the complex feedstuffs, which would require a diverse microbiome, are not present. Even so, there appears to be unique microbial profiles in the rumen from immediately after birth, which provides some evidence there may be some microbial colonization of the rumen prior to delivery.

Additionally, the meconium (or first feces) has a unique microbiome, which suggests microbial colonization during gestation. The meconium microbiome is not particularly diverse or robust; nonetheless, there are microbes present in the meconium that also appear in the mature rumen.

These early microbial species are important for proper development of the rumen and establishing the immune system. The microbes will shift dramatically as the rumen develops and the animal matures due to the consumption of more diverse feed types (hay, concentrate, etc.).

Figure 1 shows a plot of the calf rumen microbiome at day 1 (red), 3 (blue), and 28 (orange). Each dot represents an individual calf's microbiome at that respective age. Each day is distinct from another. Dots clustered tighter together indicate more similarity than those spread farther apart. Day 1 and day 3 dots are more sporadically clustered than day 28 dots, indicating that as the animal matures, the microbiome becomes more consistent and less variable than earlier in life.

How these microbes get into the meconium and early rumen to begin with remains unclear. In humans and mice, some of the main factors that can

From left, beef unit manager Travis Smith, Ph.D. student Ryan Knuth, senior research scientist Kathy Austin; master's student Gwen Hummel, and Assistant Professor Hannah Cunningham-Hollinger collect rumen fluid for microbial analysis using oral lavage. The technique is simple, easy on the animals, and effective at collecting rumen fluid, said Cunningham-Hollinger.

influence this early colonization are the nutrition status of the mother during gestation, mode of delivery, and the milk/colostrum consumed immediately following birth, among others.

Mothers, breed have strong influence

What can be concluded by the evidence and applied in our laboratory is that there is a strong influence of maternal factors on this early colonization, which means there is potential to "program" the early microbiome via management of the gestating cow/ewe.

This is the aim of our research objectives.

Some of the key maternal factors we have focused on are:

- maternal breed,
- mode of delivery,
- rearing type, and
- maternal gestational nutrition.

The rumen microbiome of Charolais calves and Angus calves differed in terms of overall microbial compositions. These differences in breed actually became more prominent as the animal matured.

Identifying breed differences in the microbiome contributes to the body of literature that emphasizes the influence of host genetics on the rumen microbiome. This may allow for genetic selection and breeding schemes that consider host genotypic influences on the calf microbiome.

Rumen differences in birth deliveries

A study was designed to deliver several calves via C-section, several via natural delivery (vaginal), and after calving another group had their calves removed at 24 hours and raised on a bottle. This alteration in management led to distinct differences in the microbiome, especially in the samples collected earlier in life (day 1, 3, and 28).

Many of the differences caused by changes in mode of delivery or rearing type went away as the animals matured with only tendencies for specific species differences persisting post-weaning.

The C-section calves did not have as "rich" a microbiome as those calves delivered through the birth canal, which can have implications on development of the rumen, immune system, and long-term health of the calf.

These data help us understand what role these maternal factors have in early

Unraveling the complexities of management practices on rumen microbiome

Ruminant livestock are incredibly valuable to our state, country, and world.

Their unique digestive systems and the tiny organisms housed inside are complex. Continuing research to understand how these organisms develop in the rumen and what influences they have on host performance and efficiency is critical.

The rumen microbiome plays pivotal roles in feed efficiency due to the importance of these microbes in the breakdown of feed and generation of energy for the host. Many research projects from our lab and others have showed differences between the high and low efficient livestock in terms of their rumen microbiome.

There is the potential to capitalize on this innate system to improve efficiencies, increase production, optimize health and performance, and have large impacts on productivity of these ruminant livestock.

Wyoming producers are already doing excellent work managing their cows and ewes to produce high-quality calf and lamb crops and optimize the land and forages available. Perhaps we can now begin to also consider the complexity of management influences on the rumen microbiome.

Seven of the 12 bottle-fed Charolais calves used in the study evaluating maternal breed, mode of delivery, and rearing-type. These calves were removed from their dam 24 hours after calving and raised on a bottle until weaning.

development and long-term impacts on calf health and performance.

Rumen effects later in life

Not only are we interested in maternal factors and early colonization, but we also want to see the impact these factors might have on feed efficiency later in life. A post-weaning feed test was conducted and residual feed intake (RFI) was calculated from the individual feed intake data generated by the GrowSafe system.

This measure of feed efficiency represents the difference in what animals are actually consuming and what their expected intake was given their metabolic body weight and rate of gain. Interestingly, RFI differed by breed but not by treatment (control, C-section, or bottle). The Charolais calves had a more efficient RFI compared to Angus calves (Figure 2), which is also found in the literature.

The microbiome of these calves were very distinct between feed efficiency class (high vs. low) and breed type. These compositional differences may be used to not only predict efficiency but also to develop targeted supplementation that may alter the microbiome and subsequently lead to improved efficiency.

There is much data for livestock species highlighting the importance of the maternal plane of nutrition during gestation on offspring development and performance; however, there has been little to no data investigating the role relative to the rumen microbiome development in offspring.

Our work shows that cows nutrient-restricted during late gestation had offspring whose rumen microbiome at day 7 were different from calves born to cows fed to requirements. This preliminary data supports our ongoing efforts to understand how maternal nutrition can alter the calf rumen development in terms of the microbiome and have potential impact on performance long-term.

What's next

The answers are still not clear as to what serves as the source of colonization in the early rumen. There is a strong maternal role, and our investigations in this area continue. Recently, we collected microbial samples from the vagina, amniotic fluid, placenta, and cow rumen to compare to the calf meconium and rumen microbiome. While we do not have these results yet, we are excited to see how different maternal microbiomes may overlap with the early calf gut microbiome.

We will continue to investigate the maternal plane of nutrition and are planning a study this year looking at targeted supplementation on rumen microbiome development in calves. Our studies should provide insights on maternal microbiome during gestation, and also on the early calf rumen microbiome, following those calves to evaluate any long-term impacts on performance and efficiency.

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Stakeholder-driven studies in bountiful Thunder Basin flip the agriculture and natural resources research model

Derek Scasta
Assistant Professor
Ecosystem Science and Management

Robert Mutch, shutterstock.com

Black-tailed prairie dog (*Cynomys ludovicianus*)

Agnieszka Bacal, shutterstock.com

Greater sage-grouse (*Centrocercus urophasianus*)

Conducting research that improves the land, and the lives of people who depend upon that land, is part of the mission of land-grant universities such as the University of Wyoming.

This has been traditionally accomplished in a researcher-driven model in which professors conduct research on university farms, ranches, and in greenhouses. Results were typically only presented to stakeholders at the end of a project – anywhere from three to five years later.

This approach is not the most effective for multiple reasons.

- First, the lack of stakeholders directing research can lead to irrelevant studies ("working on the wrong thing").
- Second, the lack of stakeholders involved in the process can lead to a lack of trusting the results ("skepticism").
- Third, research conducted only on university properties lacks local context that could enhance adoption ("it won't work on my place").

This can leave stakeholders dealing with problems for which there may not be useful information developed by a university to help guide decision-making.

The Thunder Basin region in eastern Wyoming – where ranchers face many challenges – was one such place.

Flipping the model for Thunder Basin stakeholders

An effort started in 2014 to flip the research model specifically for the Thunder Basin region. This was facilitated by the Wyoming Agricultural Experiment Station (AES) and USDA Agricultural Research Service (ARS) leadership at the direction of local stakeholders represented by the Thunder Basin Grasslands Prairie Ecosystem Association (TBGPEA).

TBGPEA includes ranchers and energy companies in addition to other partners. Local stakeholders laid out the context for researchers and highlighted their concerns.

Federal land management and complex plant-animal relationships have posed long-term issues for stakeholders. There had been petitions to list wildlife species under the Endangered Species Act (ESA), specifically black-tailed prairie dogs (*Cynomys*

ludovicianus) as recently as 2009, and greater sage-grouse (*Centrocercus urophasianus*) as recently as 2015.

Stakeholders had concerns about these two species, particularly the lack of data about their distributions, impacts on ranching enterprises, difficulty of managing the two concurrently, and how associated species (such as grassland birds) might be affected.

Stakeholders wanted to be forward-looking by identifying species that might be imperiled next and then merging agriculture and natural resources in new research projects.

Collaborative and participatory research efforts

This led to collaborative groups forming and development of initial research projects of the Thunder Basin Research Initiative. Two early projects focused on the wildlife species of concern and associated implications for ranching operations. One of these established exclosures to better understand the impacts prairie dogs were having on vegetation and agricultural production. The other sought to better quantify bird diversity and responses to disturbances in the region, particularly the disturbance of prairie dogs.

Researchers in both projects worked closely with ranchers and included research sites on private and public land.

These initial projects set the stage for strong partnerships and potential funding for future projects. For example, a new participatory research project has been funded by the USDA National Institute for Food and Agriculture titled "*Participatory research to quantify prairie dog impacts on livestock production in western rangelands.*"

In this research, we use locally owned cattle to understand cattle performance on private ranches and associated public grazing allotments. This work is measuring forage quality, animal nutrition, animal weights, and animal movements (Figures 1A-C) relative to landscape features and prairie dogs. This is a prime example of doing relevant research on private ranches with privately owned cattle to answer specific questions.

New research projects address other challenges to the region including fire and its impacts on sagebrush (*Artemisia tridentata*) and interaction with invasive species such as cheatgrass (*Bromus tectorum*).

Fig. 1. Participatory research with ranchers in the Thunder Basin using (A) privately owned cattle on private ranches and associated public grazing allotments to understand (B) cattle performance and (C) cattle movements relative to prairie dog colonies using new GPS tracking technology.

In this project, funded by the Joint Fire Science Program, titled "*Fire effects on herbaceous regeneration across an invasion gradient in grasslands and shrublands*," experimental research on wildland fire and the effects on sagebrush mortality, cheatgrass invasion, and wildfire risks is being conducted with many collaborators. They include Forest Service fire staff, ranchers, and volunteer fire departments (Figures 2A-B) and study sites include Forest Service land and private ranches.

The partnerships and infrastructures developed also allow research on emerging critical issues such as drought and its impact on productivity, sylvatic plague (*Yersinia pestis*) in prairie dogs, and vegetation response before and after the 2018 plague event.

Broader impacts

The Thunder Basin Research Initiative efforts have effectively flipped the traditional research model and produced important scientific information requested by local stakeholders:

- Important information about how drought and prairie dogs cumulatively reduce forage for livestock (Connell et al., 2019),
- How prairie dogs influence sage grouse habitat relative to well-managed livestock grazing (Connell et al., 2018), and
- How prairie dog colonies should be managed to optimize grassland bird habitat (Duchardt et al., 2019).

Through the research process, two graduate students have been closely engaged with ranchers in co-developing research and have developed a critical understanding of the challenges ranchers face in the region – in other words, training our future professionals to know the issues first-hand.

In addition, extension bulletins summarize some of the biology and ecology of the region. Results have been shared at field days in 2018 and in public meetings where researchers work alongside stakeholders at the cutting-edge of the regional issues by implementing stakeholder-driven projects in the Thunder Basin region.

Finally, the partnerships and accomplishments of the efforts in Thunder Basin are reflected in the recognition of TBGPEA with the 2019 UW College of Agriculture and Natural Resources' Research/Outreach Partner of the Year (bit.ly/thunderbasin) – stakeholders bringing the vision and resources together for long-term research in the Thunder Basin.

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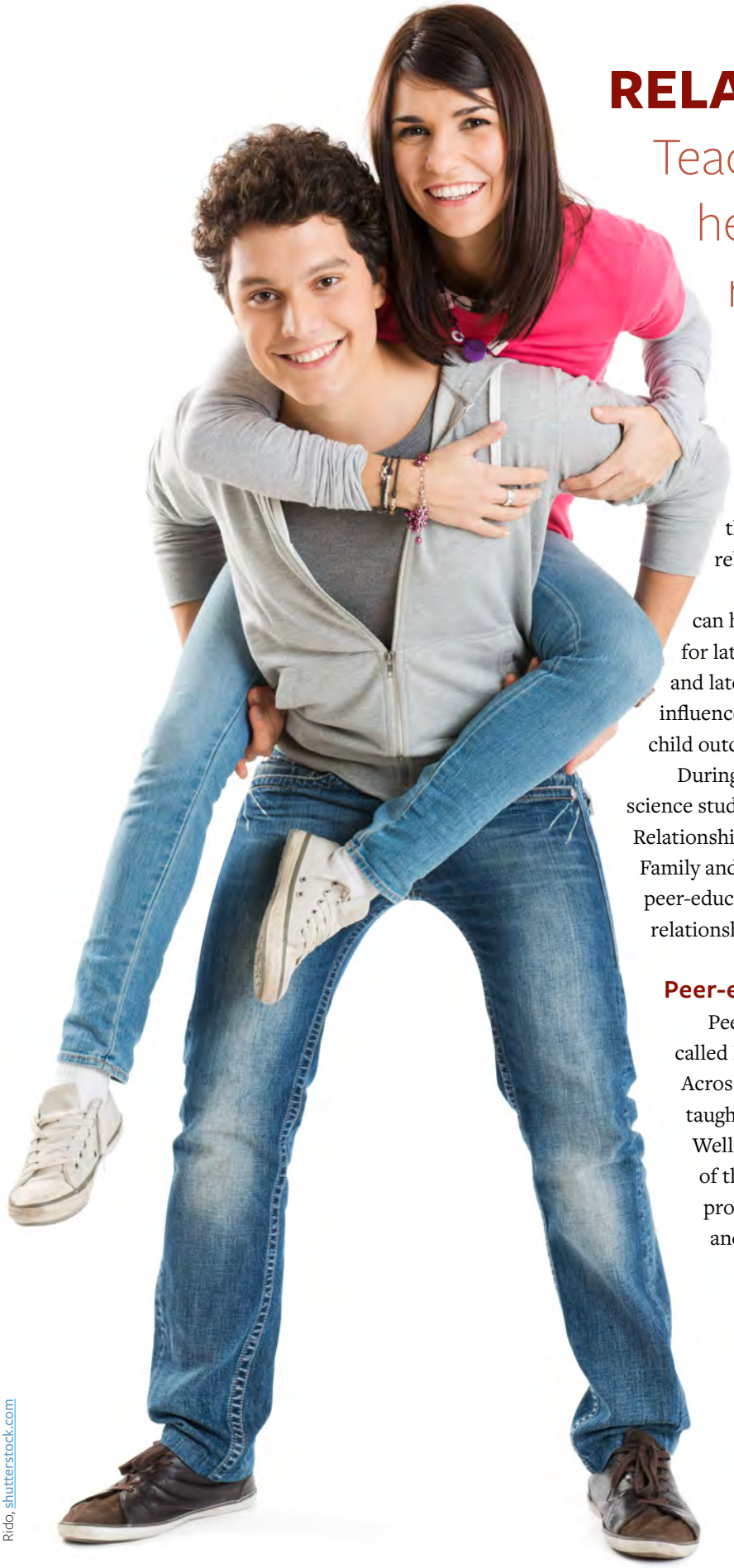
The multifaceted Thunder Basin

The Thunder Basin region consists of rangelands where prairies meet sagebrush, and ranching and energy are important economic drivers of local communities. The five counties in the Thunder Basin region – Campbell, Converse, Crook, Niobrara, and Weston – are home to more than 300,000 head of cattle and about 100,000 head of sheep.

This area also includes the Thunder Basin National Grassland, sprawling across more than 500,000 acres managed by the U.S. Forest Service and includes livestock grazing allotments. Drought and wildfire are persistent events.

Fig. 2. Research addressing fire in Thunder Basin and its impacts on sagebrush and interaction with invasive species such as cheatgrass.



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RELATIONSHIP SMARTS

Teaching young adults healthy habits for happy, romantic relationships

Romantic relationships are common sense, right? Not exactly.

Just because romantic relationships seem to come naturally does not mean we are naturally good at them. Many young people lack good examples of healthy relationships in their families, peer groups, or in the media.

More than just puppy love, early dating experiences can have important long-term effects and set the stage for later committed relationships. Romantic relationships, and later marriages, form the foundation of families and can influence personal health, worker productivity, parenting, and child outcomes.

During the last two academic years, undergraduate social science students enrolled in a service-learning course called Relationship Education and Leadership in the Department of Family and Consumer Sciences. These students were trained as peer-educators to provide educational content about romantic relationships using an evidence-based curriculum.

Peer-educators teach workshops

Peer-educators delivered a four-session workshop series called Relationship Smarts to other undergraduate students. Across three semesters, 12 undergraduate peer-educators taught the workshop series to 74 UW students. The UW Wellness Center assisted in promotion and registration of the Relationship Smarts program. Peer-educators also provided one-time educational events to student-athletes and members of fraternities and sororities on campus.

Alyssa McElwain
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Department of Family and Consumer Sciences

Course provides high-impact, community-based learning

Most young people begin dating around age 16, and the average age of marriage is now close to 30 years. This means there is a lot of "runway" for people to explore and engage in different romantic connections.

Between ages 18-29, a stage of life called emerging adulthood, romantic relationships provide opportunities for identity formation, intimacy, and companionship; however, dysfunctional relationships can lead to intimate partner violence, mental and physical health problems, and higher engagement in sexual risk behaviors.

Formal relationship education programs, although relatively uncommon, promote development of healthy romantic relationships and aim to prevent negative outcomes. Emerging adults attending college are a key audience because romantic connections may either support or hinder academic achievement.

Another important need on college campuses is the opportunity for social science students to engage in experiential, high-impact, community-based learning. Many University of Wyoming students may return to rural Wyoming communities after graduation; it is key to prepare new professionals who will work in human services. Experiential learning opportunities are mutually beneficial because they meet a community need and promote important professional skills for students who will later enter human service careers.

Peer-educators practiced key skills desired by employers in human services professions such as group facilitation, participant recruitment, marketing, development of program content, ethical practices, and program evaluation. They also gained exposure to the scholarship that informs the curriculum content.

The Relationship Smarts lessons have clear ties to research in human development and family sciences. For instance, inertia theory explains the risks associated with swift transitions to cohabitation or marriage without couples discussing their expectations. Sliding thoughtlessly into cohabitation is a risk factor for divorce. In one lesson, participants learn the phrase "decide, don't slide" to understand the benefits of taking slow, deliberate steps forward in a relationship after learning more about their partner.

Participants examine their expectations about intimacy, money, and future life choices like marriage and having children. This helps prepare them to communicate their expectations clearly and make thoughtful decisions as they establish long-term relationships.

Participants learn to identify unhealthy patterns

Have you ever said something during an argument you later regretted? In one lesson, we teach about how an "angry brain" is disempowered by stress hormones, reducing one's ability to think clearly and effectively resolve conflict. These hormones help explain why we may say hurtful words, behave poorly, and fail to see another person's perspective during an argument.

Skills are taught so participants can identify their symptoms of anger and consider ways to exit conversations when emotions consume their thoughts. They are provided tips for successfully resolving conflict when they are in a calmer state of mind.

Dating aggression is an essential topic in any relationship education program. The most common type of aggression in romantic relationships is psychological or emotional in nature. Name calling, manipulation, gaslighting (psychological manipulation to doubt your sanity), and isolation are all examples of how one person attempts to control their partner using psychologically abusive tactics.

This type of aggression may be linked to physical abuse, yet many of the early warning signs are often missed. We teach participants about the many forms of abuse and how to be on the look out for early warning signs. We play a game in which participants identify whether or not certain scenarios are warning signs. We emphasize that early signs of disrespect or control should be taken seriously and with prompt action. It is typically much easier to exit an abusive relationship early on than later when abuse has escalated.

Program outcomes

Pre- and post-program surveys collected data about the participants, and similar surveys were collected from a comparison group of non-participants. In total, 270 people completed these surveys. Several key variables were assessed. Evaluation of change within the participant group indicated participant

knowledge of relationship topics improved from before to after the program. For instance, participants had improved knowledge of healthy communication, greater awareness of warning signs of abuse, and increased understanding of how family background can influence romantic relationships.

Narrow age gap facilitates learning

What did participants think about having peer-educators lead the sessions? Peer-educators were rated highly on their group facilitation skills such as their ability to facilitate discussions, show compassion to participants, and explain content clearly.

Participants also reported peer-educators were reliable sources of information who they were more likely to learn from because of their similar age. What were the overall impressions of the program? Participants reported high satisfaction with the program with 85 percent reporting high satisfaction, 82 percent stating they would recommend it to a friend, and 67 percent reported the program exceeded their expectations.

Impacts

This work brings multiple benefits to the state of Wyoming and aligns with the University of Wyoming's strategic plan because it inspires students and impacts communities. The service learning course increases student engagement and prepares future human service professionals to meet the complex, challenging needs of individuals and families.

Relationship Smarts participants are better equipped to have happier, healthier home lives. Families serve as the foundation for rural communities, and people who are in healthy, stable, happy families are better able to contribute as citizens.

This peer-education model offers a sustainable method to prevent relational problems and impact the future health of individuals and families in Wyoming.

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Program participants

Workshop participants were, on average, 20 years old; the peer-educators were just slightly older, on average, 21 years old.

Other data: 40 percent male, 60 percent female; 86 percent heterosexual, 14 percent sexual minority; 78 percent Caucasian, 8 percent Hispanic, 3 percent African American, 4 percent Asian American, and 7 percent other ethnicity.

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Sunn hemp may prove resilient against Wyoming's formidable growing conditions

Crop could be viable alternative to alfalfa or substitution in event of crop failure

WStudio, Shutterstock.com

Carrie Eberle
Assistant Professor
Department of Plant Sciences

Sunn hemp (*Crotalaria juncea*) was grown for the first time in Wyoming in early July 2016, drilled into bone-dry powder in dryland ground at the James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC) near Lingle.

Unfortunately, an end-of-July hailstorm upended the study, but not before we saw that, despite having no water, sunn hemp germinated and grew. That first experience, seeing sunn hemp grow in Wyoming powder, cemented interest in this alternative crop.

If it could survive in those conditions, what could it do with better management?

We have been testing the agronomic potential of sunn hemp since 2017 and have continued to see sunn hemp grow well, accumulating over a ton of biomass/acre in only 60 days of growth in dryland and irrigated systems.

Graduate student Amberle Filley, who is 5-foot, 4 inches tall in comparison to the sunn hemp, uses a sensor to take crop canopy data.

Finding ideal alternative crops for Wyoming is fraught with challenges. The state stays cold too long in the spring, gets cold too early in fall, too hot in the summer, too wet to plant in the spring, too dry in the summer, has poor soil, markets are limited, and alternative crop seed isn't sold locally.

Finding novel crops that can overcome these challenges and offer farmers a viable alternative to diversify their rotations is difficult.

So far, sunn hemp looks promising.

What is sunn hemp?

Sunn hemp is in no way related to industrial hemp or marijuana. The crop is referred to as hemp because the stalks are very fibrous. Sunn hemp is a forage legume, meaning it has the ability to team up with soil bacteria to use nitrogen from the atmosphere instead of needing it supplied from fertilizer. It is a tropical crop, originating from India, which means it hates the cold and loves the heat. In Wyoming, sunn hemp should be able to grow from June through August. While three months may not seem like a lot of time, this crop can grow over 4 feet tall in that time.

Why grow sunn hemp?

Sunn hemp has many potential upsides:

- First, as a legume, it has the ability to add nitrogen to a crop rotation, which means reduced cost of fertilizer for farmers.
- Second, its rapid growth makes the crop a great option to fit into rotations or be used as a "rescue" crop if the primary crop is lost early in the season.
- Third, the legume should have very similar feed value to alfalfa and should be a viable alternative or supplement to other feeds.
- Fourth, sunn hemp is quite drought tolerant and may be an option for rainfed or deficit irrigated systems.

Sunn hemp sounds just about perfect – but nothing is perfect.

There are many questions we need to answer on both the crop and animal side of growing and using sunn hemp as a forage crop. Preliminary studies in 2017 and 2018 began to answer some of these questions, and during 2020 we will continue to test sunn hemp to evaluate its true potential for Wyoming.

Is sunn hemp a viable crop option?

Sunn hemp was grown at SAREC during the summers of 2017 and 2018 to determine the best planting time. We had irrigated and non-irrigated (rainfed) fields. We observed that sunn hemp is highly sensitive to too much water, especially in the first three weeks during establishment. This crop may be happier planted into powder than into a nicely watered field, which could be a real advantage to farmers in dry years or if they are trying to manage water allocations on their farms.

Both years we harvested sunn hemp biomass 60 days after planting (Figure 1). In the irrigated field (Figure 1a), the mid-planting yielded the highest biomass averaged over the two years, 2,820 lb/ac (Table 1). The late planting came in a close second, averaging 2,306 lb/ac, and the early planting had the lowest yield at 1,656 lb/ac. In the non-irrigated field, yield

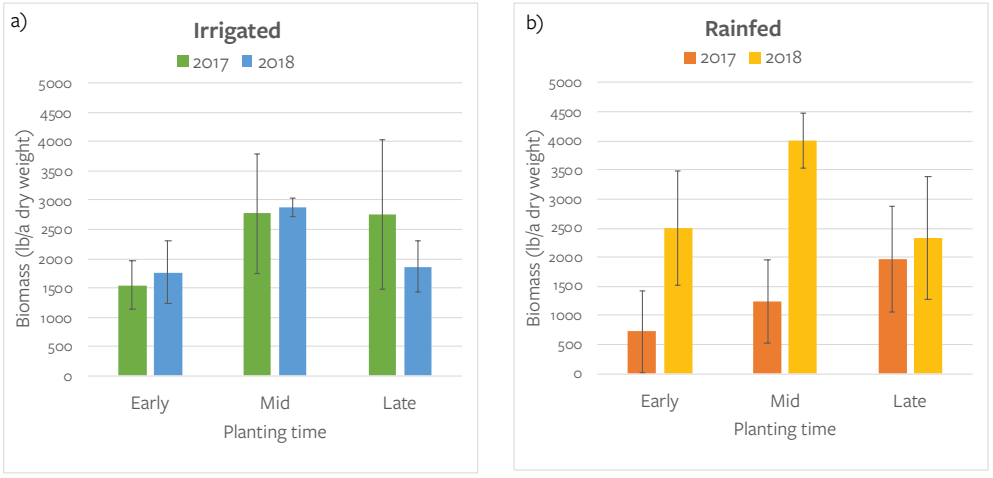

Fig. 1. Average sunn hemp biomass (lb/ac) produced 60 days after planting in 2017 and 2018 growing seasons under a) irrigated and b) rainfed conditions. Sunn hemp was planted at three times each year: early (May 26, 2017, and June 2, 2018), mid (June 5, 2017, and June 8, 2018), and late (June 21, 2017, and June 26, 2018). Error bars are standard deviation and represent the variation within the average.

varied over the two years, which makes sense because of differences in amount and time of rainfall. The early and mid-plantings yielded higher in 2018 than in 2017, but the late planting yield was the same both years (Figure 1b). When averaged over two years, the planting time with highest rainfed yield was the mid-planting at 2,624 lb/ac, followed by the late planting at 2,151 lb/ac, and then the early planting at 1,615 lb/ac.

In both rainfed and irrigated conditions, the mid-planting time produced the highest yield, indicating this may be the most reliable time of year to plant sunn hemp in southeastern Wyoming. Even more interesting is that there was no significant difference in yield over the two years (Table 1).

The sunn hemp biomass was also analyzed for feed quality, since the end use of sunn hemp is animal feed. Being a legume, the hypothesis is that sunn hemp will have feed quality similar to alfalfa. Results from 2017 and 2018 show the feed quality of sunn hemp was high, with crude protein greater than 25 percent, total digestible nutrients above 60 percent, and a relative feed value greater than 200 (Table 2).

How does sunn hemp measure up?

According to USDA-NASS, the average alfalfa yield in Wyoming for the last 10 years was 5,460 lbs./ac. While this is much higher than our highest yield of 2,624 lbs/ac, alfalfa is a perennial, full-season crop that has multiple cuttings and takes a long-term place in a crop rotation.

Meanwhile, sunn hemp is producing its biomass in a two-month period, meaning it can fit into narrow rotation windows and allow producers to intensify their rotations with winter crops like rye, brassicas, winter pea, wheat, and others. Additionally, alfalfa is not a crop that can be used as a replacement in the event of crop failure, while sunn hemp could fit well.

Sunn hemp also competed well with alfalfa on a feed quality level. The 2017 crop was comparable to supreme quality alfalfa, and the 2018 crop was comparable to premium quality alfalfa (Table 2). Results from 2017 and 2018 were very promising and indicated sunn hemp can be successfully grown in Wyoming and may be a high-quality alternative forage.

Is sunn hemp the next crop for Wyoming?

We hope to have a solid answer by the beginning of 2021, but for now

Table 1. Two-year average 60-day biomass (lb/ac) under irrigated and rainfed conditions

Planting Time	Irrigated (lbs/ac)	Rainfed (lbs/ac)
Early	1,656	1,615
Mid	2,820	2,624
Late	2,306	2,151

Table 2. Feed analysis of sunn hemp on a dry basis. Analysis was done on the mid-planted, irrigated crop in 2017 and 2018. Alfalfa guidelines for supreme and premium quality hay, as defined by the USDA AMS on Oct. 10, 2019, are presented for comparison.

Feed Nutrient	Sunn Hemp 2017	Sunn Hemp 2018	Alfalfa (supreme)	Alfalfa (premium)
Crude Protein	27%	25%	>22%	20-22%
Acid Detergent Fiber	20%	29%	<27%	27-29%
Neutral Detergent Fiber	26%	30%	<34%	34-36%
Relative Feed Value	261	205	>185	170-185
Total Digestible Nutrients	80%	61%	>62%	60.5-62%
Net Energy Maint (Mcal/cwt)	88	61		

Children pick some of the remaining flowers in the sunn hemp field in September 2019, the end of the growing season.

the crop is performing very well. The short growing season is not a problem for this rapidly growing crop. Our hot, dry summers only promote its growth. It does not get planted until June and avoids wet field conditions in the spring. Sunn hemp will fit into the same market as alfalfa, and you can already get seed from a number of local co-ops in the state. Sunn hemp seems to be a good fit as far as the challenges of growing in Wyoming.

What is next?

There are still many questions to answer. We know sunn hemp should ideally be planted around June 5 and that it can produce over a ton of biomass in 60 days with alfalfa quality feed value. What we don't know are answers to some of the more intricate production questions, like:

What is the ideal target population for an irrigated and a dryland crop?

How do we harvest on a large scale?

What is the best time to harvest for maximum yield and feed quality?

How much water does the crop need?

Will it re-grow after it has been cut?

Will livestock eat it?

How much of it is digested?

We will be trying to answer some of these questions over the next few years. A new sunn hemp study was initiated in 2019. Our team will combine our expertise in crop and animal science to provide growers with best management practices for this crop.

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Sunn hemp grown at SAREC under non-irrigated conditions in summer 2018. From left, plots are early, mid-, and late-planted.

MOVING TOWARD IMPROVED BRUCELLOSIS VACCINES

Efforts at University of Wyoming since 2005 evolve to combine basic gene discovery approaches with novel vaccine platforms

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Bruce Hoar
UW Brucellosis Research Coordinator

Brucellosis can cause elk, bison, and cattle to abort their fetuses, associated with a high risk of transmission after an animal has an abortion.

Most of the U.S. is now free of bovine brucellosis due to a decades-long eradication program; however, the disease is endemic in bison and elk in the Greater Yellowstone Area (GYA) and occasionally spills over to cattle herds.

Brucellosis vaccination has been an important part of the program to eradicate disease from domestic cattle. While vaccination of cattle with *B. abortus* strain RB51 does reduce abortions, it does not necessarily prevent infection. Also, currently available vaccines are not appropriate for use in bison or elk.

There are many other species of *Brucella* that are important to consider. *B. melitensis*, for example, is found in sheep and goats throughout the world, and this particular species can cause severe human disease. There is no effective vaccine available for *B. melitensis.*

UW scientists have applied modern molecular methods with the ultimate goal of producing a vaccine that would protect more than one species of susceptible animal hosts against *Brucella*.

For over a decade and a half, efforts to determine the molecular mechanisms that drive *Brucella* species to invade and potentially produce disease in mammals has been studied in numerous laboratories around the country, including the University of Wyoming.

These microorganisms have a propensity to colonize their animal hosts, survive for long periods of time, and remain dormant. Persistent bacterial infections in mammals abound, such as *Yersinia enterocolitica* (chronic gut colonization in elk), *Mycobacterium tuberculosis* infections (in humans and elephants), and *Helicobacter pylori*, the bacterium that causes human chronic gastritis/peptic ulcers, to name a few.

With the premise that the characteristic hallmark of *B. abortus* invasiveness (as well as other *Brucella* species) in mammals is persistence (chronic infection), the ideal pathogen would not necessarily cause disease. Epidemiological studies on *B. abortus* bear this out, as it has been difficult

to numerically correlate pathologic outcomes with *B. abortus* infection in natural host populations (domestic animals, such as cattle and/or wild herding animals [elk]) .

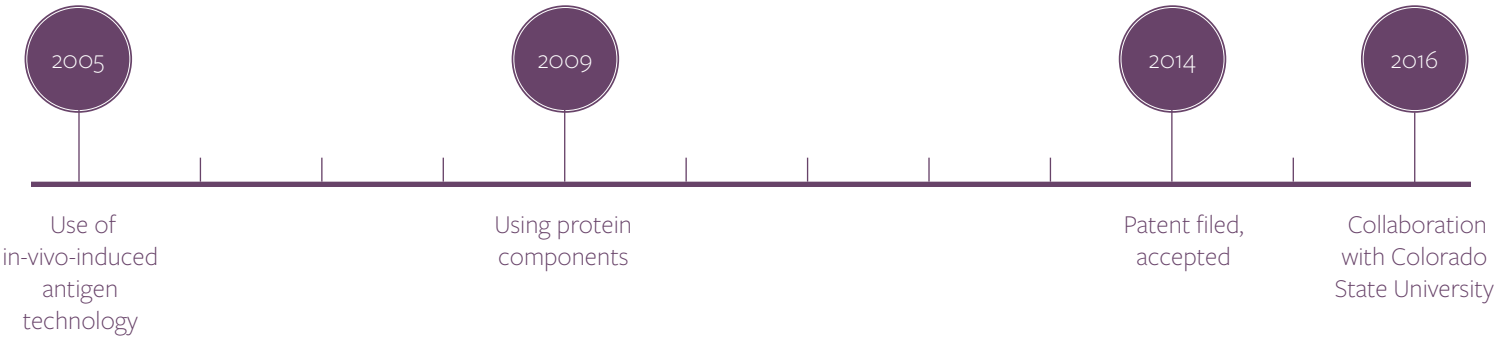
Research development timeline at University of Wyoming

2005 — Use of in-vivo-induced antigen technology

At a molecular level, persistence is likely facilitated by a number of virulence-associated molecules expressed by the pathogen. These are likely to include surface-exposed, bacterial cell wall proteins and secreted/released soluble enzymes. Research began at UW in 2005 to develop an improved vaccine that produces immunity targeting such virulence components.

At the onset, a "gene discovery" approach was used to identify new virulence-associated genes activated during brucellosis infection. In a pivotal study at UW, a laboratory methodology known as in-vivo-induced antigen technology (IVIAT) successfully identified novel *B. abortus* genes

Fig. 1. Typical *Brucella* mammalian host in the Greater Yellowstone Area – elk (*Cervus canadensis*)



expressed during infection in elk. Some of these genes were also found to be common to brucellosis infection in cattle.

The IVIAT approach to virulence-associated gene detection was also used to identify similar virulence genes up-regulated during *Yersinia pestis* (plague) infection in different animal hosts indigenous to Wyoming, including prairie dogs, rabbits, ferrets, and coyotes. This classic approach to identifying molecule factors involved in bacterial diseases is simple in practice (albeit labor intensive), safe, inexpensive, and has a long-standing track record of success.

One mechanism for persistence of *B. abortus* in the animal host is likely due to suppression of the animal's immune response to the microbial pathogen – specifically, inflammation. Reduction of inflammation by an innocuous *Brucella* enzyme, Mdh, was shown to affect the behavior of the first-line cellular defense mechanism in the mammalian host, namely macrophages (specialized cells involved in detection and destruction, among other actions, of bacteria and other harmful organisms). Although the data is not conclusive, strong evidence has suggested *Brucella* has evolved the use of several of its "housekeeping" enzymes and other maintenance proteins in facilitating its survival.

2009 — Using protein components

Since about 2009, efforts were undertaken to examine the feasibility of using such *Brucella* protein components, cloned, expressed, and purified from *E. coli*, and introduced into a variety of vaccine formulations.

These studies primarily used mice as a surrogate vaccine model to examine the clearance of the pathogen from spleen, lungs, and reproductive tissues from immunized animals after exposure to several different strains of the *Brucella* bacterium.

2014 — Patent filed, accepted

After numerous, time-intensive experiments, the results suggested the use of acellular (may contain cellular material but not complete cells) components of *B. abortus* may be usable as a suitable substitute for traditional live-attenuated (a live, but weakened, or attenuated, form of the bacteria that cannot cause disease, but can stimulate a strong immune response) platforms used in current brucellosis vaccination regimens. Subsequently, a UW patent was filed and accepted in 2014 for the continued use and development of these *Brucella* cellular components as potential vaccines and diagnostic targets.

2016 —Collaboration with Colorado State University

More recently (2016), the efficacy of experimental sub-unit vaccine formulations in an immune pregnant mouse model has been evaluated against fully virulent *Brucella abortus* infection. Additionally, a pilot study in goats was undertaken. Both of these studies represented a collaborative effort with Richard Bowen's (DVM) group at Colorado State University. While inconclusive, these experiments represented a step forward in advancing potential novel acellular vaccine candidates into applicable animal models.

Ambiguous outcomes have not thwarted efforts to continue with the identification of new virulence-associated proteins produced by the bacterial pathogen. In fact, species-distinct cell wall components have been identified at UW that may have potential in immunologic diagnostic assays. Variation between molecular components of the various *Brucella* species could lead to development of tests that differentiate between several different infectious species of the *Brucellae*. More importantly, these findings may contribute to increased specificity and sensitivity of a rapid field assay for *B. abortus* under development at UW.

While these tests are mainly serologically (blood)-based, another laboratory at UW is developing a DNA-based (PCR - Polymerase Chain Reaction) assay for *B. abortus* infection for cattle (led by Associate Professor Brant Schumaker, an epidemiologist and supervisor of diagnostic and regulatory serology at the Wyoming State Veterinary Laboratory).

Consistent with the past success of experimental sub-unit vaccine formulations against wild-type *B. abortus* infection in the mouse model, studies are being conducted in collaboration with the laboratory of Associate Professor Jeffrey Adamovicz (former department member at UW) at the University of Missouri, whereby selected vaccine candidates are augmented with immune-stimulating additives (CpG's) to enhance protection against *Brucella abortus* in a mouse model.

The way the immunized animal is infected makes these experiments unique. *Brucella abortus* has a propensity for infection by multiple routes of exposure such as oral ingestion or inhalation. In particular, airborne bacterium are highly infective, requiring a very low number of bacterium to colonize the host when inhaled.

This condition may more likely represent the more natural route of exposure in the environment. Thus far, experimental results have demonstrated equivalent efficacy of sub-unit (a fragment of a pathogen, typically a surface protein, used to trigger an immune response) vaccine formulations against the parental (injection) and inhalation route of exposure to virulent *Brucella*.

Brucella-based vaccine alternatives

Most recently, novel live vaccine platforms against brucellosis have also been explored at UW.

The probiotic bacterium (Lactococcus) has been examined for use as a "carrier" of *Brucella* genes to colonize the mammalian gastrointestinal tract and consequently immunize the animal host against *Brucella* infection. These ongoing experiments represent the potential for another alternative to the traditional live *Brucella*-based vaccine for cattle.

UW investigators have been negotiating the task of eradicating brucellosis in the United States for over 15 years. While the problem is not yet solved, the use of basic gene discovery approaches, combined with novel vaccine platforms, will facilitate a better understanding of the microorganism's biology and may ultimately lead to the end goal.

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Eric Middelkoop, shutterstock.com

Bison at Antelope Flats, Grand Teton National Park, Wyoming

Eric Isselee, shutterstock.com

Prairie dogs – Keystone species or calamity?

Prairie dogs woven into Thunder Basin Grasslands Ecosystem detrimental to ranching if too many, but too few and dependent wildlife disappear

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Black-tailed prairie dogs are one of the most charismatic and conflict-ridden critters you will ever meet. Their charisma comes in part from their volume (in numbers and decibels). A social, burrowing mammal, prairie dogs are extremely gregarious and loud, barking constantly to warn others in their colony of potential intruders.

Managing prairie dogs on landscapes meant to sustain both livestock and wildlife is a large challenge. Our piece of the puzzle is examining how prairie dogs affect bird species in the Thunder Basin National Grassland.

Because they occur at such high densities, prairie dogs are an important food for ferruginous hawks and golden eagles, species of concern in Wyoming. Burrowing owls, another species of concern, get their name from living underground in burrows created by other animals – and they are more than happy to take up residence in a prairie dog burrow.

In addition to being a food resource and digging burrows, prairie dogs also clip vegetation near their burrows to ensure they can see predators. This clipping creates perfect habitat for a lesser known bird species, the mountain plover. This small brown inland shorebird prefers short vegetation and lots of bare ground so it can see predators and blend in – in many parts of its range, plovers are only found if you look on a prairie dog colony!

That prairie dogs are so important for so many birds (as well as mammals, including the swift fox and the endangered black-footed ferret) makes them a "keystone species" – a species upon which many other members of an ecosystem depend. The disappearance of many other wildlife species is observed if keystone species are removed.

Keystone species are often the target of conservation efforts, because by

protecting one species, you can protect many. Prairie dogs, and especially the black-tailed prairie dog, have declined precipitously in the past century, making them a conservation target throughout the Great Plains.

Prairie dogs conflict with cattle

Black-tailed prairie dogs are an important keystone species, but they can also spell trouble for a rancher. Especially in dry years, prairie dogs may clip or consume vegetation to the point not enough is available for livestock. This can lead to reduced cattle weight gains and economic losses, which is why prairie dogs are controlled throughout much of their range.

Millions of dollars have been spent researching how to control and kill prairie dogs, but at least as much has gone toward reintroducing them and preventing disease in other parts of their range, especially to help other associated wildlife like the black-footed ferret, which almost went extinct due to prairie dog declines.

Finding the balance

The Thunder Basin region represents a great opportunity for bird conservation, but one challenge is understanding how we can have enough prairie dogs to provide habitat for those bird species while making sure cattle ranching is still profitable. Because some of the colonies in this area grew to many

Early effects of sylvatic plague on the Thunder Basin ccosystem

In late summer of 2017, prairie dogs in the Thunder Basin began contracting sylvatic plague (*Yersinia pestis*). Although this disease is not native to North America, it has affected prairie dog populations for decades, and often leads to drastic (>95%) fatality.

In just one year, the effects of this die-off were staggering: panels A and B show a camera trap photo at the same site in late May 2017 (A) and 2018 (B). Vegetation is noticeably taller and denser, with an almost complete elimination of bare ground.

These observations are reflected in the data which show that visual obstruction (a measure that captures both the height and density of vegetation) was much lower on colonies until plague. After plague occurred, visual obstruction increased in 2018 and in 2019 was statistically the same as areas that had never had prairie dogs.

Because prairie dogs are a keystone species, it's not surprising we saw other things changing, too: mountain plovers and some raptor species decreased precipitously, while some bird species that prefer thicker grasses increased.

Banded mountain plover

thousands of acres in size in 2016-2017, it's very literally a "big" challenge – no other known colonies currently reach these sizes.

Between 2015 and 2017 we surveyed birds and vegetation in the region to determine which birds were using prairie dog colonies and what traits of colonies were most important for these bird species.

We found mountain plovers (that little brown shorebird that likes bare ground) were less abundant on very large colonies and were actually most abundant on medium-sized prairie dog colonies with short vegetation and lots of bare ground. These colonies were still fairly large (between 250-1,235 acres each), but we found birds seemed to prefer parts of colonies fairly close to undisturbed habitat – perhaps because these areas provided cover for their chicks.

Areas on the largest colonies (up to 9,900 acres) had fairly low bird diversity as compared to smaller colonies. To conserve diverse bird species, managers may want to aim for many medium-sized colonies – potentially using non-lethal methods like visual barriers or moving them to other locations to keep colonies from getting too large. By ensuring colonies aren't too large, this also ensures enough forage for livestock – a win-win on public lands.

A plot twist

More moderate-sized prairie dog colonies are likely the best strategy for balancing bird conservation and cattle – but there is another reason to avoid having extremely large colonies in the landscape. Colony size has been linked with increased chance of sylvatic plague. Although only rarely contracted by humans, this disease can kill up to 95 percent of the prairie dogs in a given landscape, which has huge implications for associated wildlife species.

At least 90 percent of prairie dogs in the Thunder Basin died during a plague outbreak in 2017 (sidebar, page 29), although ascertaining the total mortality from the disease is difficult. In the following year, mountain plovers virtually disappeared from the landscape because vegetation became too tall. Conversely, other species that like taller grasses, like the lark bunting, were abundant. These are exactly the effects we would expect to see after the removal of a keystone species.

Boom and bust cycles

Although plague had extremely negative impacts in the short-term, remembering that grasslands are inherently ecosystems of change is important. They evolved for thousands of years with disturbances like fire, bison grazing, and prairie dogs – disturbances that may be present one year and gone the next.

Just as the vegetation recovered, so to, little by little, did the prairie dogs. While in 2018 individual prairie dogs were still scattered, in 2019 they began to coalesce into very small (less than 2.5 acres) colonies. These were the main areas where we started seeing mountain plover again – in some cases, we could tell using color bands these were the same plovers that had nested there up to four years prior. We don't know where they went when conditions were poor in 2018 – but they came back.

These extreme boom-and-bust cycles have negative ramifications for ranchers and prairie dogs and for the other wildlife species in this landscape. Even with disease control methods such as killing fleas that carry plague, disease is likely to continue to be a part of this ecosystem.

One potential way to minimize the boom-and-bust cycle is to focus on keeping many moderate-sized colonies in the landscape.

We are excited to see how others add to this research in the future – neither the conflict nor charisma of black-tailed prairie dogs is likely to decrease anytime soon, but hopefully additional research will help balance perspectives on this amazing keystone critter.

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More research-based information

The University of Wyoming Extension publication *Prairie Dog Ecology and Management in Wyoming*, B-1346, discusses the complex relationship between prairie dogs, humans, and the Wyoming rangeland landscapes they inhabit. The free publication is available for viewing or download by going to uwyo.edu/uwe and clicking on Publications. Enter the title or number in the search field.

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KEEPING IT COLORFUL:

EXPLORING SPECIALTY CUT FLOWERS FOR PROFIT IN WYOMING

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Specialty cut flower production is an opportunity to add valuable commodities for Wyoming growers, serve local economies, and diversify Wyoming's economic base with horticultural crops.

Flower production can also provide biological benefits by providing nutrition and shelter for crop pollinators and beneficial insects.

This project's purpose was to produce information for Wyoming growers and demonstrate if growing fresh cut flowers is feasible. The resurgence of locally grown goods has been a boost to the Wyoming horticulture industry. The spread and popularity of farmers markets, community supported agriculture (CSA) programs, and food cooperatives are indicative of this trend.

According to the Wyoming Business Council, farmers markets contributed more than $2.2 million to the state's economy in 2012 and at least 46 markets operated around the state in 2013. Approximately 20 CSAs serve communities and primarily provide members with vegetables but have a variety of add-on items, such as cut flowers.

The U.S. cut flower industry relies heavily on imports from countries near the equator due to lower energy and labor costs. The U.S. is not one of the major exporters but is one of the largest producers and major buyers of cut flowers. With a shift toward specialty cut flowers, local markets can gain a diverse and high-value product that can compete with imported goods.

The shift toward specialty

How specialty cut flowers are defined has evolved. Originally defined as flowers that are not roses, carnations, or chrysanthemums, specialty now describes flowers that are not regularly available or are only on the market for a short time.

Specialty cut flowers can serve local markets and provide a diversity of material. Specialty cut flowers have a short post-harvest life and do not ship well. The markets for cut flowers include wholesale, direct to florist, you-pick operations, farmers markets and stands, and subscription bases for CSAs, restaurants, etc.

There has only been one study in Wyoming for fresh cut sunflower production (please see bit.ly/wyosunflowers). Region-specific information on cut flower production is important due to unique environmental conditions in Wyoming: short-season, high-altitude environments, many plants require protection from potential frosts, high winds, and large day-to-night temperature swings. Greenhouse and high tunnel production has potential to provide season extension and quality protection in the harsh Wyoming climate and throughout the greater Rocky Mountain region.

Production practices

High tunnels are used for season extension, increasing yield, and improving quality of crops such as cut flowers. A high tunnel is a simple framed structure usually covered with clear polyethylene. High tunnels do not have active heating or cooling but instead use passive ventilation, commonly by manual roll up sides. High tunnel production has become increasingly popular for large and small scale growers to diversify production.

Greenhouse production is another popular method for producing cut flowers where sequential plantings can supply them year-round. Greenhouses can provide greater environmental control of heating, ventilation, and humidity that can give predictability to crop schedules year-round. For small-scale greenhouse operations, cut flowers can be a profitable way of generating additional income from existing greenhouse space.

The protection from inclement weather in both high tunnels and greenhouses can increase cut flower stem lengths and reduce chances of disfigurement and disease.

SPECIALTY CUT FLOWERS GROWN IN DIFFERENT ENVIRONMENTS ACROSS TWO YEARS

We grew five specialty cut flower species in two different growing environments: greenhouse and high tunnel. Flowers were grown year-round in the greenhouse and late spring through fall in the high tunnels.

The cultivars included *Calendula* 'Princess Golden', *Helichrysum* 'Double Mix', *Celosia* 'Celway Mix', *Matthiola* 'Lucinda Mix', *Daucus* 'Dara'.

The species were selected for a range of flower types from a variety of families. We harvested flowers about every other day in the mornings throughout the growing season and recorded stem lengths and days to harvest for all stems. Cut flowers are generally deemed marketable with a stem length of at least just under a foot, a desirable length for floral arrangements.

Table 1. First harvest dates, duration of harvest (days), and yield per plant for five cut flower species grown in a high tunnel and greenhouse at Laramie in summer 2018.

	First harvest date		Duration of harvest		Yield (stems/plant)	
Species	Tunnel	Greenhouse	Tunnel	Greenhouse	Tunnel	Greenhouse
Calendula officinalis	20 June	20 June	78	74	29.1	15.7
Celosia argentea	13 July	4 July	58	72	13.5	35.5
Daucus carota	11 July	11 July	50	47	24.6	24.7
Helichrysum bracteatum	29 June	20 June	72	86	13.3	10.0
Matthiola incana	27 June	23 July	6	21	1.0	1.0

Table 2. First harvest dates, duration of harvest (days), and yield per plant for five cut flower species grown in a high tunnel and greenhouse at Laramie in summer 2019.

	First harvest date		Duration of harvest		Yield (stems/plant)	
Species	Tunnel	Greenhouse	Tunnel	Greenhouse	Tunnel	Greenhouse
Calendula officinalis	3 July	28 June	66	79	22.3	37.2
Celosia argentea	31 July	28 July	42	49	12.8	30.0
Daucus carota	2 Aug	26 July	42	51	28.1	14.4
Helichrysum bracteatum	20 July	15 July	52	62	9.3	12.9
Matthiola incana	17 July	15 July	3	5	1.0	1.0

Four plots were grown in high tunnels, two plots per tunnel, and one plot was grown in the greenhouse. All plants were grown in #1 containers with 30 plants in each plot. Greenhouse and high tunnel experiments were conducted at the Wyoming Agricultural Experiment Station's Laramie Research and Extension Center greenhouse complex. This work was made possible by a specialty crop grant from the Wyoming Department of Agriculture.

Impacts and considerations

At the time of the first summer season (2018), the cut flowers were sold to the University of Wyoming's ACRES student farm, where they were incorporated into CSA shares and also sold at the Laramie Downtown Farmers Market. The second season (2019) flowers were sold to a local Laramie business, Killian Florist, where they were sold in arrangements and used in events like weddings and UW Ag Appreciation Weekend.

Generally, greenhouse conditions provided a longer duration of harvest and an earlier first harvest date with a few exceptions, most likely due to greater climate control and less exposure to environmental conditions such as wind, temperature swings, and rain.

During the 2018 season, 2,621 flowers were cut with 74 percent deemed marketable across all flower species.

The 2019 season had a later start due to weather conditions with 2,111 total cut flowers and 63 percent deemed marketable across all flower species.

Calendula, *Celosia*, and *Daucus* flowers had the highest amount of stems per plants across both summer seasons.

Daucus had the highest amount of cut stems with 900 in the 2018 summer season with 80 percent of the stems deemed marketable.

Calendula had 793 stems, with slightly over 85 percent marketable. In 2019, Calendula had the highest number of cuts with 758 and 55 percent deemed marketable.

Daucus had 575 cuts, with 91 percent deemed marketable. Most flower species had a sufficient yield based on stems per plants except for *Matthiola*, which only produced one stem per plant.

Matthiola would still have the potential to be utilized in a successional planting schedule.

We assessed beneficial pollinator visitation inside the high tunnels, and found flies (mainly syrphid flower flies), bumblebees, lepidopterans (butterflies and moths), and wasps were the most frequent flower visitors and comprised 70 percent of all insect visitation. We collected this data using timed observations every two weeks throughout the growing season to identify insects visiting open flowers. In each plot, direct observations were made for 11 minutes, spending one minute per five potted plants. Insects were generally identified to order or family, with bees being categorized as bumble bees, honey bees, or other native bees.

Daucus was the most popular for fly visitation. *Calendula* saw the most bumblebees and lepidopterans. Wasps most frequently visited Celosia. Insects provide beneficial services to the ecosystem such as pollination and biological control.

Our research suggests fresh cut flowers can be grown in Wyoming's climate under high tunnel and greenhouse production, can possibly provide necessary resources to support insect communities, and have potential as a valuable commodity for the state's growers.

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Calendula 'Princess Golden'

Helichrysum 'Double Mix'

Celosia 'Celway Mix'

Matthiola 'Lucinda Mix'

Samantha Nobes

Daucus 'Dara'

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