

**GENETICS IN PRACTICE:
APPLICATIONS OF QUANTITATIVE GENETICS TO
ENVIRONMENTAL AND EDUCATIONAL PURSUITS**

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PART I. DESIGN, DEVELOPMENT, AND OPTIMIZATION OF A BEEF CATTLE BREEDING SIMULATION

Overview

Active learning is a broad term for any activity that engages students in the learning process. These activities range from low-input activities, such as question breaks during lecture, to high input strategies such as the design of a computer-based simulation. Currently, educators struggle to implement active learning due to a lack of resources about proper execution and appropriateness for each technique. Therefore, understanding the nuances of active learning techniques is the logical next step in active learning research. To reach this level of understanding researchers must focus on a single technique. They must identify which aspects of that technique most effect learning as well as how those effects change across user groups and situations.

The purpose of Part I is to describe the development and optimization of a beef cattle breeding simulation that effectively improves learning across performance groups. The first chapter of this work covers current literature on active learning and simulations. The second chapter describes the design and development of the simulation. The third chapter describes the iterative approach used to determine the most effective feedback design for each performance group. Finally, a short summary on findings and future efforts concludes this part of the work.

CHAPTER 1. REVIEW OF ACTIVE LEARNING AND SIMULATIONS IN EDUCATION

Section I. Active Learning

Introduction

You don't learn to walk by following rules. You learn by doing, and by falling over.

-Richard Branson

Traditionally, undergraduate courses have been taught in an educator-centered, sage on the stage format where the professor transfers information via lecture. Even today, lecture remains a prominent method of teaching in STEM courses (Stains et al., 2018). However, the efficacy of lecture has come into question with concerns regarding engagement, student feedback, and information retention (Hake, 1998; Michael, 2006). There are educators on both sides of this debate, and many somewhere in the middle. Supporters of the lecture format cite efficiency as well as the potential to clarify difficult concepts and challenge beliefs among other benefits (Di Leonardi, 2007). Detractors say that lecture does not engage students in meaningful learning which reduces retention and transfer ability (Waldrop, 2015). Some may say the reduced effectiveness of lectures stems from today's students—the Gen Zers—and their technology though. After all, today's students are unlike any previous generation. These are the children, born after the millennials, who have never known a time without computers, internet, or smartphones. They cannot seem to stay on task for very long—just watch them scrolling on their phones—but is that really the problem?

Active learning advocates say no. Think back to when you were in school—any age really. The professor was lecturing—did you pay attention? Honestly? Probably not to all of it. Many people accept student attention spans to be only 10-15 minutes (Hartley

& Davies, 1978), but it is important to realize this is based on note-taking—an act not necessarily coordinated with attention (Hartley & Cameron, 1967), so that is probably not accurate. Other studies have attempted to quantify attention with more direct methods such as survey data and observation (Johnstone & Percival, 1976; Stuart & Rutherford, 1978). A more recent study asked students to report via clicker when they experienced lapses in attention. This work showed breaks in attention even before 10 minutes and more frequent lapses as the class proceeded (Bunce et al., 2010). There are certainly arguments to be made about the methods of each study, but all observed a similar phenomenon—attention waned later in the class. This could be remedied with short periods of interactivity however. Just a minute or two of interactivity decreased lapses and increased attention to subsequent lecturing (Bunce et al., 2010). The students in these studies were not Gen Zers—many were not even millennials. Therefore, it may be prudent to acknowledge general limitations in human attention span instead of blaming this generation—or its technology.

Background

As previously mentioned, student attention can be piqued with activities—even very brief activities (Bunce et al., 2010). Incorporation of active learning techniques has also been shown to improve information retention, increase critical-thinking skills, increase retention rates, and even reduce drop-out rates (Bonwell & Eison, 1991; Freeman et al., 2014; Prince, 2004). In fact, some argue that the discussion of ‘does active learning work’ is no longer relevant and we should instead focus on more nuanced evaluations of active learning (Streveler & Menekse, 2017). Even a response article (Hora, 2014) citing concerns about the design of active learning studies states,

This is not to suggest that active learning techniques should not be a part of instructors' pedagogical toolkits. It is simply that adequate instrumentation is rarely used to identify and control for more nuanced types of teaching beyond the crude dichotomies currently in use.

To make this shift in research it is first necessary to understand what constitutes active learning. Traditionally, active learning has been defined as an instructional method that engages students in the learning process (Prince, 2004). This sweeping definition certainly gives credence to concerns about research involving active learning as a singular topic. Therefore, it may be useful to think of active learning as an umbrella for many student engagement methods. It is the design and implementation of these methods that should be studied closely in this next generation of research. Not only will this approach elucidate what methods are most appropriate for specific situations it may also assist educators with the incorporation and implementation of active learning strategies in their own courses.

Costs and Benefits of Active Learning

To effectively integrate active learning, it is important to understand both the positives, and negatives, associated with it. Active learning can certainly increase engagement as previously mentioned, it can even help prepare students for today's digital world, but there are also challenges. Opponents voice concerns about content coverage, increased workload, and hesitation regarding student opinion (Tharayil et al., 2018).

Digital Literacy

Students have the world's information at their fingertips and will enter an increasingly digital workforce. Effective use of this information as well as adequate preparedness for the workplace requires a level of digital literacy (Di Leonardi, 2007).

Digital literacy is a broad topic that includes being able to access digital information, discern quality information, and understand digital content (Miranda et al., 2018).

However, many students do not believe they are receiving satisfactory digital training in their higher education courses (Newman & Beetham, 2017). Technology-enhanced active learning can provide these necessary digital experiences. Problem-based learning activities allow students to solve real-world problems using digital tools. For example, one educator asks students to create a treatment plan for a disease outbreak in Chicago (Prince, 2004). Not only must they solve the biological puzzle, but they must also plan the logistics. For example, if someone is flying to Chicago—how much will that cost? How can they pay for it? These types of problems give students opportunities to solve real world problems with real world tools.

Content Coverage

One major concern for active learning is the belief that active learning is not as efficient as lecture for covering content (Di Leonardi, 2007). However, is content really the question or is content retention our focus? Most likely it is retention—we want students to remember what we taught them as they go forward in their education, career, and life. The use of active learning strategies such as simulations (Weinberg et al., 2009) and in-class writing activities (Divoll & Browing, 2010; Stewart et al., 2010) have shown significant increases in content retention compared to traditional measures. Even adding collaborative questions to exams has shown increases in retention (Cortright et al., 2003).

One may argue however that a smaller percentage of more content is equal to or greater than a larger percentage of less content. This is where it becomes critical to understand that teaching is not an all or nothing. Proponents of active learning still

acknowledge the place of lecture (Faust & Paulson, 1998). Therefore, it is possible to use a combination of lecture and active learning strategies to cover more content while increasing retention.

Educator Workload

Educators also worry about the workload associated with implementing active learning. Many educators are already overworked, and, in their mind, active learning is even more work. The reality is—it can be. Thomas Menella, an educator who has implemented a flipped classroom structure states, “I still say I will never teach another way again... I’m just not sure for how much longer that can be.” (2017).

He admits he is tired—it is too much grading and too much time—and he only has 86 students. He could reduce this workload though. In his design he uses lower order questions to gauge reading completion then has students discuss higher order concepts in class. Those lower order, participation checks can easily be automated using the learning management system or some other online form-maker. Classroom response systems allow quick, automated feedback whether it be an in-class question or survey feedback. Simulations that report completion act as very effective pre-laboratory activities (Peffer et al. 2015).

In short, the incorporation of technology can certainly alleviate some, if not much, of the workload. Of course, some may have hesitations about incorporating too much technology into their classroom—technology can and does fail. That is okay too; active learning does not have to involve technology. Simply pausing during lecture and letting students check in with each other has been shown to increase understanding (Ruhl et al., 1987).

Student Response

Another concern for educators considering implementing active learning strategies is the effect it can have on their Student Evaluations of Teaching (SETs). While SETs have been shown to be poor indicators of teaching effectiveness (Lee et al., 2018; Uttl et al., 2017) affected by gender biases (Boring et al., 2015; MacNeill et al., 2015) and more associated with expected grades and satisfaction (Sitzmann et al., 2010; Stark et al., 2016), they are still important determinants of teaching ability in many university systems. Therefore, many educators are rightfully concerned about the effect new teaching styles may have on SETs. Certainly there have been reports of students resisting change in the classroom—especially with group, or cooperative work (Phipps et al., 2001). However, other work has shown that implementing active learning strategies can actually increase SET ratings but must be done so in a limited, well-structured manner (Henderson et al., 2018; Machemer & Crawford, 2007). What exactly is well-structured and limited however?

Implementation of Active Learning

Unfortunately, the literature on detailed implementation practices is limited (Auster & Wylie, 2006; Streveler & Menekse, 2017). Until now most research has focused on does active learning work compared to traditional, lecture-only approaches? Much of this literature indicates active learning strategies increase learning gains, but not always—in cases of lower gains implementation issues are typically in play (Di Leonardi, 2007; Hake, 1998). Some work has been completed that provides general guidance for implementing active learning in the classroom.

First, do not overdo it—you can have too much active learning in a course. This can actually lead to reduced effectiveness as well as lower SET scores (Hake, 1998).

Instead focus on a limited number of activities that allow students to learn higher order concepts they cannot learn from lecture alone. Second, make sure to introduce the activities and set expectations explicitly (Bonwell & Eison, 1991). Finally, remember collecting student feedback often can be very useful to informing progress about active learning implementation (Auster & Wylie, 2006). While these general guidelines can be useful they do not provide information on specifics such as which method is best for a certain class size or topic? This makes focused research on when to implement and how to implement specific strategies the logical next step for research.

Types of Active Learning

To understand when, and how to implement strategies, it is critical to first identify the specific types of active learning strategies. Active learning methods include short breaks for paired discussion in lecture, building models, using serious games, even asking students to get up and move around the room. With such a vast range of methods a brief description of different strategies is useful. We will move through methods from those requiring relatively minimal educator inputs to those requiring major development and planning.

Low-Input Strategies

One of the most basic methods for incorporating active learning is for the educator to pause during lecture and allow students to converse briefly about their notes. This method showed significant improvement in both short and long-term retention (Ruhl et al., 1987). With this method however, there is concern about students staying on task. Think-pair-share (TPS) activities can alleviate those concerns. In TPS an educator poses a question to the classroom then students respond (Lyman, 1981). TPS can be completed with or without technology (the inclusion of technology does increase planning but can

decrease time required to evaluate answers). If most students answer correctly the educator can move forward with the lesson. However, if there are many incorrect answers the teacher can have students work in pairs to submit a new answer. TPS has been shown to improve learning outcomes and increased preparedness (Fitzgerald, 2013). These methods are just a couple of examples of low input strategies for including active learning in the classroom.

Moderate-Input Strategies

Moderate-input strategies are those that require more planning but do not involve significant development inputs. Designing case studies or problem-based learning activities fall into this category. Case studies consist of students reading a scenario or dataset then answering questions or creating responses to the presented information. Case studies have been shown to be an effective method for increasing engagement and subject awareness (Manohar et al., 2015). Project based learning are typically semester long projects where students must work in groups to answer a question that may or may not have a set answer. Project based learning has been successfully implemented in animal science courses (Perry & Smith, 2004). Both strategies do require a significant amount of planning but have been shown to have positive impacts on learning and engagement.

High-Input Strategies

High-input strategies can have major developmental costs but also provide learners with experiential learning opportunities not otherwise possible due to time, money, and safety constraints. Serious games and simulations are closely related software classes designed to encourage learning. The design and development of these tools can be costly but significant improvements in learning have been observed using these tools in

diverse settings (Boyle et al., 2016; Koivisto et al., 2018). We will delve into this category of learning tools at length later in the review.

Next-Generation Active Learning Research

Now that we have a basic understanding of the costs and benefits of active learning as well as the types, we can now turn our focus to method-specific research. This requires analyzing specific active learning strategies to understand what makes them successful in certain situations and not in others. To design these studies, it is important that a researcher understands how to define success in the educational context and has a firm grasp on the design and development requirements for their strategy of choice.

Defining Success for Study Design

Success can be measured quantitatively and qualitatively but all methods have caveats that must be considered. Quantitative success is often measured as increases in knowledge, skills, attitudes or retention but typically not all (Prince, 2004). This can be due to limitations in study design as well as limitations on the measurement itself. For example, it can be difficult to measure long-term retention rates due to restrictions on time and finances. Furthermore, there is even some debate as to what is deemed a significant increase. It has been determined that standard measurements of achievement such as increases in scores are not particularly sensitive to any change in instructional approach therefore any improvement is important (Dubin, 1968). Does that really indicate significance however? According to Colliver (2000) the answer is no, only large effects, those between 0.8 and 1.0 standard deviation, should be considered significant. Albanese (2000) mentions though that these effects are extremely high and unlikely to be achieved in educational studies. Instead, he recommends effect sizes of 0.5 and greater be

considered significant. Understanding the limits associated with educational studies can help researchers set realistic, but informative, standards for success.

Qualitative success is typically measured through student responses to survey questions regarding their perceived learning. Unfortunately, perceived learning has been shown to be a poor indicator of actual learning (Sitzmann et al., 2010). The Dunning-Kruger effect has even shown where unskilled individuals rate their abilities higher than skilled individuals (Kruger & Dunning, 1999). This can certainly impact how informative survey-based feedback is to a study. There is also some question regarding students' willingness to rate novel interventions higher purely because they are new. This is known as the Hawthorne effect but has been largely disproven (Bracey, 2002; Ross & Nisbett, 2011). However, concerns regarding student perceptions of learning are very real. In fact, it has been argued that only learners who are given the opportunity to evaluate their abilities can realistically evaluate their progress (Spinath & Spinath, 2005). Therefore, it is recommended to evaluate intervention success using a combination of quantitative and qualitative measures or a mixed-methods study.

Requirements for Strategy Design, Development, and Implementation

In the next phase of this more nuanced generation of active learning research it is important to have a complete understanding of a method's background, development requirements, as well as previous work in implementation and effectiveness.

Understanding these topics can inform design, reduce production costs, and create more effective instructional tools.

Conclusion

Active learning should be considered a broad set of tools that can be used, in unison with lecture, to improve learning gains by providing students with opportunities to

engage with, evaluate, and communicate their knowledge. It should not be viewed as a single strategy meant to replace lecture in the classroom. Further, the discussion should no longer be focused on ‘does active learning work?’. Instead, we should be attempting to understand how to best design and implement specific strategies into the classroom. For this reason, the next section of this review focuses on computer-based, gamified simulations in the classroom.

Section II. Simulations in Education

Introduction

We opted to explore simulations because of their learning benefits and distinct capabilities compared to real-world experiences including: ability to visually represent otherwise obscure or invisible elements, ability to control time, ability to allow learners to engage in experimentation otherwise dangerous or impossible, and the ability to reduce cognitive load (De Freitas, 2006). Before evaluating the history of simulations, it is important to understand that interactive simulations and serious games—while both considered closely-related games in the literature—are not the same. Simulations are dynamic tools representing reality, claiming fidelity, accuracy, and validity; serious games are educational tools that include conflict, rules, and predetermined goals (Sauvé et al., 2007). This is not to say simulations cannot include gamification elements however; an attribute that lends itself to the confusion. Simulations featuring gamification elements are often referred to as gamified simulations. In this work we will focus on computer-based, gamified simulations as well as draw from literature regarding closely related serious games—particularly for information regarding design.

Background

By allowing users to test theories and manipulate variables in a controlled environment, simulations offer an excellent opportunity for experiential learning in the classroom. Simulations have been used for decades to train pilots and other military personnel. Simulations are also increasingly prevalent tools for medical and laboratory training (Bonde et al., 2014; Gelbart et al., 2009; Peffer et al., 2015). Simulations have, historically, even been well-established in collegiate agriculture courses in both Animal Science (Buchanan et al., 1988; Willham, 1970) and Agriculture Business (O'Rourke, 2001). However, challenges maintaining the simulation software and user-friendliness has led to a wane in use over time (O'Rourke, 2001).

In Education Today

This reduction in use is not common across fields however as previous work cited time efficiency, ease of use for testing hypotheses, and a support method with varying representation as some of the reasons educators incorporate simulations into their curriculum (Blake & Scanlon, 2007). Indeed, a recent review evaluating 143 studies indicated that by far the most popular educational game genre was simulation games (Boyle et al., 2016). However, the results of these studies, like the simulations themselves, were extremely diverse. In fact, due to a lack of well-accepted methods for defining and evaluating simulations, there remains much debate about their effectiveness (Dillenbourg, 2008). To gain a better sense of the effectiveness of simulations for increasing learning and learning related goals we will explore the design and findings of several studies. To begin we will look at studies focused on cognitive domain goals such as performance and learning gains. We will then look at studies focused on affective domain goals such as student satisfaction, motivation, and engagement.

Studies in the Cognitive Domain

Oftentimes, effectiveness in learning interventions is associated with increased learning gains or improved retention. To measure these outcomes it considered ideal to use a randomized control study, however this is often difficult to implement in classrooms so quasi-experimental studies tend to be most common (Boyle et al., 2016). There are also several studies which used the value-added approach to determine which elements of an intervention most affect outcomes (Clark & Jorde, 2004; Erhel & Jamet, 2013; Fund, 2007; Gelbart & Yarden, 2006; Moreno & Mayer, 2004). While these two approaches are notably different they are both useful for determining how and why simulations work, or do not work, in given scenarios.

To begin our exploration of simulation effectiveness we will focus on two studies evaluating learning gains using a bioinformatics simulation for high schoolers. These studies used an online program that featured modified versions of tools available to geneticists. In the first study (Gelbart & Yarden, 2006), students were given a short lecture, background narrative and then directions on using the simulation to solve the problem presented in the narrative. All students in the study used the simulation and were evaluated for improvement using quantitative and qualitative methods. This quasi-experimental method indicated that students working with all versions of the simulation showed positive learning gains. To determine if the tool achieved the same positive results when compared to a lecture, a quasi-experimental method was employed (Gelbart et al., 2009). In this study half of the classes used the experimental simulation and half received traditional lecture. Results indicated that students using the simulation had higher learning gains.

In a study focused on improving particle theory understanding in middle schoolers (Stern et al., 2008) half of the students only received traditional lecture whereas the other half received lecture and three additional lessons using the simulation. Learning gains were significantly higher in the simulation group. However, it is possible that the improved performance was related to longer time spent on the material rather than the simulation itself. Such weaknesses in study design must be avoided to accurately determine the effectiveness of simulations.

In a more balanced series of studies using a crime scene investigation simulation high school and undergraduate students showed significant increases in learning outcomes and motivation (Bonde et al., 2014). In this study students were assigned to one of two groups. In one group, students used the simulation and then received a lecture on the material. In the other group students received a lecture then used the simulation. A midpoint evaluation was given to measure gains from the first intervention. Students using the simulation showed significantly higher gains than those receiving lecture. After both interventions both students showed higher gains than those observed at the midpoint. This indicates a benefit in combining simulations with traditional methods such as lecture—a sentiment echoed in other research (Rutten et al., 2012). Further, this study found that the order of presentation does not matter so simulations can be used before, during, or after traditional methods to increase learning.

Studies in the Affective Domain

Looking beyond standard performance measures simulations have been promoted as mechanisms to increase affective traits such as engagement and satisfaction. In a study looking at the use of a simulation in undergraduate engineering courses an improvement

in student satisfaction was observed in the simulation group (Durán et al., 2007). Students in one course used a simulation to model concepts while the other course used traditional learning methods such as slideshow demonstrations. It should be noted however, students in the simulation group were also able to discuss their results with their partners while those in the traditional lecture were not. This adds another dimension to the study that may affect outcomes. Another study focusing on high school students found that students reported improvements in perception of the class environment and better attitudes toward the subject of biology after using a simulation (Kiboss et al., 2004). In this study no other active learning strategies were introduced to potentially bias results.

More generally, the PhET simulations—a series of single-topic, interactive physics simulations—from Colorado State University have been found to be fun, challenging, and educational. Further, it has been observed that computer simulations give students more opportunity to explore than do traditional cookbook laboratory assignments (Wieman et al., 2008). However, one must remember, just because students report higher engagement or greater willingness to explore does not automatically mean learning is improved (Bacon, 2016)—substantiating the need for mixed methods analyses.

Design and Development

Beyond incorporating a mixed-methods study, we want to test well-designed interventions. To develop a well-designed intervention, we must have a solid understanding of what elements compose an intervention and how those elements should be implemented. One of the most important challenges facing educational simulation designers is helping teachers and students connect knowledge from the simulation to the

classroom (Clark et al., 2011; Quintana et al., 2004). Furthermore, learning content should be effectively integrated with the game mechanics and narrative (Clark & Martinez-Garza, 2012). . An effective simulation makes learners actively process the educational content (Erhel & Jamet, 2013). Achieving these goals means understanding end-user needs, how to involve end-users, and how to design actual simulation features including the interface and necessary scaffolding.

End-User Requirements

The first step in designing an effective simulation is understanding end-user needs. In the case of educational simulations there are two end-users—learners and educators. Each group has individual needs that must be met by the simulation for it to be used and be effective. To gain a better understanding of these needs we will evaluate each group individually.

Students End-Users

Students, for the most part, respond favorably to simulations but there are some common issues: technical problems, simulation rigidity, time, and relatedness to the real-world (Hurst & Marks-Maran, 2011). Understanding how to fix these problems requires understanding expectations. Today’s students have been raised with technology—they have high expectations for functionality. Therefore, simulations should be tested extensively to ensure they do not feel “clunky” or have any technical issues. Another issue for students is the lack of freedom in a simulation. According to several sources, ensuring simulations are successful requires they are designed to provide a balance of guidance and freedom (All et al., 2016; Boyle et al., 2016; Peffer et al., 2015). Achieving this balance requires expertise from all disciplines involved in the development process (Winters & Mor, 2008). Students sometimes complain about the time required to

complete simulations. To this end, it is important to remember that simulations can be integrated into curriculum at different time points (Rutten et al., 2012) indicating the potential to use simulations as replacements for out-of-class activities such as pre-labs or homework. Finally, ensuring that students can see real-world applications in a simulation is critical as core ideas may not always be translated across applications (Clark et al., 2011). To confirm these ideas are translated it is imperative to make structural features and their applications explicit during training which promotes transfer (Corbalan et al., 2009).

Educator End-Users

Applicability is also a key issue for educators. A survey indicated that curriculum relatedness is a key determinant of whether or not a simulation will be used in an educators' curriculum (De Grove et al., 2012). Therefore, making curricular connections visible to educators is vital for adoption (Barzilai & Blau, 2014). Rutten (2012) suggests achieving this by designing lesson plans and curriculum guides to accompany the simulation. De Freitas (2006) recommends integrating specific lessons into the simulation and making those lessons apparent to educators. The ability to modify the simulation is also viewed favorably and therefore suggested for implementation (Blake & Scanlon, 2007). Finally, it is encouraged that developers consider methods to ensure simulations can easily be integrated in the classroom—difficulty implementing is main deterrent to adoption (Faria & Wellington, 2004). By considering end-user needs and expectations developers can design a more effective simulation.

End-User Input

The necessity of end-user input is well accepted in design of most products. User-centered design (UCD) is a process that is influenced by the end-user (Abrams et al., 2004).

UCD is a broad term that includes integration of end-user opinion anywhere in the design process. In software, agile development (Beck et al., 2001), a specific type of UCD, encourages developers to determine end-users needs and involve end-users in the development process. However, this process does not typically test effectiveness after development as this method is mostly used in commercial settings. Participatory design is a broad method of UCD that involves end-users and tests final effectiveness as part of the design process (Halskov & Hansen, 2015).

Within participatory design end-users can serve different roles as informants or codesigners. As informants the end-users are asked for feedback throughout the design process. As codesigners, end-users have equal input. Research has shown that students acting as informants for areas of design such as game dynamics and challenge are most productive (DeSmet et al., 2016). Involving students, particularly very young students, in other aspects of the design process such as character or storyline development can actually lessen effectiveness (Scaife et al. , 1997). This is not to say children should never be involved however; instead they can provide very useful insight into the design process but their inclusion must be considered carefully and thoughtfully (Druin, 2002).

Interface Design

One common complaint amongst learners regarding simulations is interface design—mainly that it is not intuitive (Hurst & Marks-Maran, 2011). It may be tempting to try to make the simulation interface mirror the real-world as much as possible, but this has been shown to negatively impact performance (Lindgren & Schwartz, 2009). Instead, it is recommended that simulation design should be a combination of concrete and idealized features which improves performance and transfer (Goldstone & Son, 2005).

Further, interfaces should be designed to lower extraneous cognitive load while increasing germane cognition by taking advantage of modality and redundancy effects (Sweller, van Merriënboer, & Paas, 1998). Blake and Scanlon (2007) suggest the integration of multiple representations and, if possible, real-time graphing to encourage redundancy. Balancing these elements can be difficult but integrating user feedback through development can alleviate some of the challenge.

Scaffolding

Scaffolding is a broad term used to describe learning supports built into a learning intervention—including simulations. Software can be designed to feature scaffolding mechanisms that support learners by structuring a task to reduce complexity and problematizing the subject matter—making learners pay more attention to ideas and connections (Reiser, 2004). Scaffolding is important, especially for novice learners, as it acts as a substitute for missing schemas which allows learners to complete tasks potentially beyond their cognitive abilities (Tuovinen & Sweller, 1999). Scaffolding should be well integrated into the simulation with relevance to the game narrative and mechanics made explicit to the learner (Barzilai & Blau, 2014). Scaffolding includes subject-matter guidance, feedback, and reflection and should be organized around three primary components of scientific inquiry: sense making, process management, and articulation and reflection (Quintana et al., 2004).

Guidance

Guidance can be thought of as a coaching element that helps learners move through the tasks of the simulation (Guralnick & Levy, 2009). There is considerable debate regarding the amount of guidance that is appropriate for different learners. According to the Expertise Reversal Effect novice learners benefit more from higher

levels of guidance than do experts (Kalyuga, 2007). However, other research indicates that novices actually lack the knowledge to make sense of highly detailed guidance (Brenner et al., 2017; Koedinger & Roll, 2012; Van Dijk et al., 2016). This negative impact may be due to cognitive overload as there is too much detail for the novice learner to process, which may suggest less detailed guidance is necessary for novice learners (Roll et al., 2018). This is further supported by the National Research Council's change in K12 standards from inquiry-based learning to science as a practice. It was determined that students cannot make inquiries without some basal level of knowledge provided (Osborne, 2014).

There may still be some benefit to allowing students to explore simulations though—particularly for advanced learners. Kapur and Bielaczyc (2012) point out that allowing students to “fail a bit” challenges existing thought processes which may support more successful knowledge transfer. It has also been shown that too much guidance can cause students to enter into an “answering hunting” mode which can reduce student's ability to see the system as a whole (McDaniel & Schlager, 1990). However, not all students will take advantage of such free exploration opportunities. Brenner (2017) found that students who were lowly active with the simulation actually benefited the most from the guidance. This indicates that different levels of guidance are necessary for different groups, but no set guidelines have been established that indicate what level is most appropriate for which group.

Feedback

There is no debate about the necessity of feedback which can provide learners with missing schemas that allow them to make sense of results (Clark et al., 2009;

Kirschner et al., 2006; Paas et al., 2003). In fact, situational feedback through authentic system reactions may be more beneficial to learning than guidance because feedback helps learners interpret their results (Wiese & Koedinger, 2017). According to Kulhavy and Wager's Feedback Triad (Kulhavy & Wager, 1993; Mory, 2004) the purpose of feedback is threefold:

- 1) Feedback serves as a motivator or incentive for accuracy
- 2) Feedback acts to provide reinforcing messages for correct answers
- 3) Feedback provides information to validate or change previous incorrect answer

To effectively serve these purposes feedback must be presented after students make a choice about how to interact with the system (Mory, 2004; Shute, 2008). It must also only be presented when actually necessary—feedback on very easy tasks can actually inhibit learning (Bangert-Drowns et al., 1991). Understanding how to design effective feedback is crucial to a simulation's success. Therefore, we will explore how feedback functions to correct errors—its most important function—as well as how to design it including complexity, timing, and tone.

Error Correction

Of the three purposes, feedback's most important function is to correct errors or misconceptions (Kulhavy, 1977). Correcting errors requires understanding how error type and certitude affect the likelihood of correction. According to Mory (2004) there are three types of error: Same, Different and New. A same error is an error transferred from the pretest to the posttest. A different error is a changed response from the pretest that is still incorrect. A new error is when the answer was correct on the pretest but incorrect on the posttest. Understanding the specific type of error(s) students are making can inform

design but one must also consider error certitude. Error certitude is a student's confidence in their answer which can greatly affect their likelihood of changing the answer (Bangert-Drowns et al., 1991; R. W. Kulhavy, 1977). Students who are confident in an answer marked incorrect will spend time trying to understand the error—often leading to a correction. Students who were not sure will not feel the same motivation and are unlikely to experience a change in their knowledge.

Understanding error characteristics can be very useful when considering designing feedback that will effectively correct misconception, or error. Feedback can give students the opportunity to identify their misconceptions (Corbalan et al., 2009) but as previously stated, students who are lowly motivated may not use this opportunity. Therefore, it is important to encourage users to heed feedback. An effective method for doing this is to design feedback that provides information crucial to success in the game or simulation context (Swanson et al., 2011; Wiese & Koedinger, 2017).

Type and Complexity

Feedback is generally categorized in two broad categories: facilitative and directive (Black & Wiliam, 1998). Facilitative guidance provides comments that direct self-revision, directive feedback informs the user of the specific issue. Generally, it is accepted that directive feedback is most useful for novice learners (Shute, 2008) and that it should contain at least an indication of the correct answer (P. B. Waldrop, Justen, & Adams, 1986). Feedback that is too complex however can negatively impact novice learners (Roll, Wiese, Long, Alevan, & Koedinger, 2014; Van Dijk et al., 2016).

Directive feedback is generally accepted as more useful for advanced learners due to the expertise reversal effect (Kalyuga, 2007). Furthermore, directive feedback can be

less detailed giving users more opportunity to explore a simulation which can lead to increased knowledge transfer skills (Belenky & Nokes-Malach, 2013). Understanding the knowledge level of the learners using a simulation is critical to its success. Overall, feedback type and complexity are most effective when suited to specific learner types as opposed to applied generally across groups. To determine this information it is recommended to conduct target group analysis prior to initial design efforts (Sweller et al., 1998).

Timing

Timing is a critical, but hotly debated, topic for feedback design. Like type and complexity, the answer may not be one-size-fits-all. Research has shown that immediate feedback is particularly useful for improving procedural knowledge (Anderson, Conrad, & Corbett, 1989) and reduces uncertainty (Nkhoma et al., 2014). However, delayed feedback is more useful for error correction (R. W. Kulhavy, 1977) and knowledge transfer (Anderson et al., 1989). These findings indicate that it is imperative to identify the intended educational goal of a simulation to determine the most appropriate feedback timing.

Tone and Delivery

Tone and delivery of feedback is also an important factor in simulation design. Research has shown that students are more responsive to verbal feedback provided by an in-game character or agent than textual feedback (Moreno et a., 2001). Ideally, however feedback is delivered simultaneously through auditory and verbal channels. This reduces mental processing requirements by allowing the materials to be processed through cognitive modes (Paas et al., 2003). Learning is further improved when the agent's messages are personalized and conversational (Moreno & Mayer, 2004). However, too

much emphasis on the individual can decrease learning (Shute, 2008). Considering this in whole, it is recommended that feedback clearly indicates correctness to the user in a personalized tone then presents additional information, likely the correct answer, in a polite and causal tone.

Feedback Summary

Like guidance, there is no universal recommendation for feedback. Simulation designers must consider the goal of their simulation and its target audience to design effective feedback. Understanding these factors can inform much more effective design decisions than attempts to make feedback fit a broad category of users or end goals.

Conclusion

Designing simulations requires input from end-users, as well as experts in subject-matter, instructional design, and software development. To encourage the development of effective simulations it is advised to spend time understanding end-user needs as well as involving them in the design process. It is then crucial to design simulation elements to meet the needs of that specific audience of end-users. Taking the time to incorporate this information will produce more valuable and useful simulations for use in today's educational settings.

CHAPTER 2. COWGAMES: INTRODUCTION TO AN ONLINE BEEF CATTLE BREEDING SIMULATION

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Abstract

Providing students with opportunities for higher order thinking is crucial. However, delivering this experience can be difficult. This is especially true in animal breeding classes where breeding animals during the semester, even mice, is not feasible. Therefore, many educators use worksheets and math problems to demonstrate genetic change. Often, the students do learn to complete the problems, but they struggle to contextualize the change they observe in the numbers. The use of computer simulations is a potential answer to this challenge. Using simulations students can manipulate modeled systems and observe results in a timely and controlled environment. To fill this need we have developed a beef cattle breeding simulation, *CowGames*. The simulation allows students to make breeding decisions in a simulated cow herd and see impacts over time. The simulation was developed according to learning objectives determined through educator interviews. Through iterative testing with students in the United States and Canada we have designed a simulation that shows increased learning gains, motivation, and engagement in undergraduate animal breeding and genetics courses.

Introduction

Animal Science students must be able to integrate and apply knowledge from multiple sources to make effective management decisions (Perry & Smith, 2004). Unfortunately, giving students the opportunity to manage livestock herds, even colonies of mice, takes too much time and money to be an effective teaching tool. Instead, many educators turn to the use of traditional teaching methods such as lectures and problem sets to teach animal breeding concepts. Unfortunately, these methods, particularly lecture, have been shown to be ineffective for engaging students which can lead to reduced retention and transfer ability (Waldrop, 2015). These methods also do not give students

opportunities to develop critical thinking skills (Lujan & DiCarlo, 2006). However, Active Learning Strategies (ALS) such as simulations, case-studies, and think-pair-share activities are potential alternatives that give students opportunities to practice those critical higher order thinking skills in the classroom (Prince, 2004). Integration of ALS in STEM courses has even been shown to reduce failure rates and improve course performance (Crouch & Mazur, 2001; Freeman et al., 2014).

Simulations are one example of ALS that is becoming more prevalent in education (Boyle et al., 2016). A simulation is defined as “a program that contains a model of a system (natural or artificial; e.g., equipment) or a process” (De Jong et al., 1998). Simulations allow students to manipulate a modeled environment and see the effects of those changes; this can lead to increased conceptual understanding (Windschitl & Andre, 1998). Simulations also allow students to observe phenomena that may not be possible otherwise due to time, money, or safety constraints (Rutten et al., 2012). Previously simulations have been used as teaching tools in Animal Science (Buchanan et al., 1988; Lewis et al., 2010; Medrano et al., 2010; Willham, 1970). Unfortunately, of the currently available simulations they are not available without webmaster intervention making their implementation difficult in many classroom settings. They also do not feature feedback which can reduce learning effectiveness. Other research-based modeling systems have also been found to be difficult to implement in genetics courses (Moulin et al., 2004).

Therefore, our objective was to develop a beef cattle breeding simulation that could be used to encourage engagement and learning for undergraduate animal breeding and genetics students. We aimed to develop a system that gave students the opportunity

to simultaneously manage their own herd and observe long-term genetic change. Through the system students would learn about general genetics topics such as Mendelian sampling and trait correlation as well as more cattle-centric topics such as Expected Progeny Difference (EPD)-based selection. The simulation would also feature scaffolding components such as data visualizations, dynamic feedback, and review questions. Finally, it would be a standalone application available 24/7. The objective of this paper is to describe the development process, features and initial learning impact data for the application.

Development Process

To develop this simulation there were several steps involved. To begin, we explored existing simulations to learn more about design and features. We then interviewed educators to learn more about what they wanted and needed in the simulation. We then employed an iterative testing approach to evaluate and optimize the simulation

Existing Simulation Exploration

CowGames is not an entirely new concept. A beef cattle breeding simulation was developed in the 1970s and became ubiquitous through the early 1990s (Buchanan et al., 1988; Willham, 1970). However, due to advancements in computational and genetic technology the simulation did not remain up-to-date and fell out of favor. Some educators had worked with this simulation and spoke favorably of its use in the classroom. To gain a better understanding of the simulation we completed a few generations of an MS-DOS version (Buchanan et al., 1988). While the simulation was not the most up-to-date regarding features or data, it did provide a general idea about design and functionality which informed early prototyping.

Pre-Prototyping Interviews

We felt that understanding educator needs and expectations was critical to the development of a useful simulation. Therefore, we interviewed 46 undergraduate animal breeding and genetics professors from across the United States. We asked them to describe their current teaching methods, content delivery, and opinions regarding the strengths and weaknesses of their methods. We then asked them to describe ways they might improve those weaknesses. If an educator mentioned the possibility of a breeding simulation or game, we asked what they envisioned for that simulation.

This interview process yielded an extensive list of potential simulation features that educators believed would benefit their courses. We selected features and topics from this list based on popularity and integration potential. For example, some educators did ask for a nutrition/feeding component, but it was deemed that this would detract too much from the genetics focus to be useful in this simulation.

Iterative Implementation

For actual application development, we employed a user-centered modification of the agile-release method common in software development (Beck, K et al., 2001). To do this *CowGames* was tested in four iterations at universities across the United States and Canada. In every iteration, students were asked to use *CowGames* for one scenario then complete a survey. Through these surveys we collected data on application functionality, ease-of-use, design, perceived engagement and motivation. Students in Iterations Two-Four were also asked to complete a pretest and posttest. This testing procedure provided us with quantitative data about simulation effectiveness for improving learning. Iterative testing was approved by the University of Missouri Institutional Review Board and all participants signed informed consent prior to study participation. This strategy allowed us

to identify strengths and weaknesses of our simulation design. In cases of weakness we were able to integrate user feedback to optimize the feature then test the update in the next iteration. This allowed us to make data-supported improvements which increased the quality of the simulation.

Statistical Analysis

To provide quantitative data about simulation performance pretests and posttests were used in the last three iterations. In those iterations, students completed a pre-test, seven years of the simulation, review questions, a post-test, and a survey. Test scores were analyzed with a Paired T-test using Proc TTest in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Students who participated in the study for more than one class only had their first attempt included in the data. This data was used to inform design choices—particularly regarding scaffolding such as subject-matter supports and feedback.

Simulation Features

Through educator interviews and iterative testing with college students we were able to build a comprehensive list of traits, concepts, and scaffolding requirements for the application. Educators were particularly interested in accessibility, traits, and curriculum alignment. They wanted a program that did not require outside intervention, so they could use it on their schedule. They also wanted an application that featured enough traits to be informative but not so many it would be overwhelming. Finally, they wanted to know exactly how the simulation would fit into their curriculum. Students were particularly interested in feedback—they wanted to know how they were doing. They also requested more subject matter supports. We also included data visualization and review components.

Standalone Application

Accessibility was a major concern for educators. They needed a program that could be used on their course schedule. Therefore, *CowGames* is a fully standalone, web application. Users can access the simulation online by going to www.cow-games.com and creating an account. Using a secured login service, Auth0 (Auth0, Bellevue, WA) and state-saving, users do not have to complete the scenario in one sitting. By making *CowGames* a standalone application with state saving capability the simulation can fit any classroom schedule.

Traits, Tools, and Concepts

Determining which traits and selection tools would be featured in *CowGames* was critical. From our educator interviews we received a list of over 25 proposed traits but ultimately settled on four traits (Table 1): Birth Weight (BW), Yearling Weight (YW), Marbling (Marb), and a lethal recessive—Curly Calf (CC). These traits were selected because when used in combination they could illustrate numerous concepts—something critical to many educators.

Expected Progeny Differences (EPDs)

Expected Progeny Differences (EPDs) are one of the most effective selection tools available to cattle producers. Unfortunately, they are not universally well-understood. This combination made EPDs a priority on many educators' lists. Therefore, we made EPDs a central part of the simulation. EPDs for each animal are generated based upon that animal's 'true' genetic value and possible change for its current accuracy. To generate the 'true' value we employ a multi-step process.

First, the average of the 'true' genetic value of each parent's yearling weight is calculated. This average, along with the standard deviation of yearling weight is used to

generate a normally distributed curve. The calf's 'true' genetic value is then chosen at random from the distribution with the Box Muller Transform (Box & Muller, 1958) which illustrates Mendelian sampling. Finally, an environmental effect is added to the 'true' value to determine the calf's phenotypic value. This environmental effect is small to encourage fidelity for illustration purposes. It is possible we may increase environmental effects based on educator input for a potential "leveling" feature in future iterations.

EPDs are then generated based on the 'true' genetic value. Each animal begins with an interim EPD which is simply the average of parental EPDs. This EPD is then adjusted based on heritability of the trait. This adjustment uses the accuracy to determine the distance between the 'true' genetic value and the current EPD. Animals that produce progeny are adjusted for higher accuracy. This process allows students to see the EPDs and accuracies change over time.

Genomic Enhanced EPDs (GE-EPDs)

EPDs are even more effective in tandem with genomic data (García-Ruiz et al., 2016). Here again though, the benefit is not universally understood. Many educators mentioned struggling to illustrate how GE-EPDs could improve herd management. In response, we implemented a genomic testing feature. Students may purchase genomic tests which improve accuracy. This feature allows students to observe the cost and benefit relationship of purchasing genomic testing for their herd.

Table 1. List of featured traits and type of trait.

Trait	Type
Birth Weight	Quantitative Growth Trait
Yearling Weight	Quantitative Growth Trait
Marbling	Quantitative Carcass Trait
Curly Calf	Mendelian Lethal Recessive Trait

Lethal Recessive Disorder

Educating students and producers about properly managing lethal recessives while maintaining genetic diversity is critical to successful livestock production (Beever, 2009; Van Eenennaam & Kinghorn, 2014). Educators mentioned they struggled to illustrate the importance of maintaining diversity however. For example, one educator mentioned that her students could calculate frequencies, but many did not understand why it would be useful to keep carriers in the herd. Therefore, we made sure to include some high-quality animals that are also carriers. This forces students to decide if they want to eliminate the gene and quality or manage it to improve their herd.

Several disorders were suggested, but we selected Arthrogryposis multiplex, or Curly Calf. We selected this disorder because it is a Mendelian trait with 100% penetrance (Beever & Marron, 2013). This means that homozygous recessive animals are always affected and can easily be tracked in the program. This allowed us to introduce our first “leveling” feature—the ability to turn the lethal status on or off. This feature can be controlled by the Educator Portal. When the status is off the system does not show if a normal animal is homozygous dominant or a carrier. This gives students the additional challenge of identifying carriers within their herd—or more motivation to purchase genomic tests.

Inbreeding

Inbreeding can be detrimental to a herd causing reductions in survivability, fecundity, and growth (Lamberson et al., 1982; Smith et al., 1998). Inclusion of this concept was therefore deemed important by educators. To illustrate the effects of inbreeding the simulation reduces survivability and growth for inbred animals. To help

students avoid producing inbred animals the application highlights inbred matings yellow on the breeding page (Figure 1).

Revenue Source

Revenue makes or breaks an operation. Therefore, educators felt it was a critical component for inclusion. Modelling systems that are too realistic or complicated however, can reduce learning gains (Moulin et al., 2004). Instead, it is recommended that a simulation feature a mixture of concrete and idealized elements which can increase learning and support transfer (Goldstone & Son, 2005). To do we created a market where students sell animals on a value-grid. This encourages the production of higher quality animals and introduces them to a relevant concept in production. Charges are made for feed, maintenance, breeding, and genomic testing. We maintained this somewhat simplistic model to help encourage students to make connections between genetic change and revenue without becoming lost in the finances.

Scaffolding

Scaffolding is learning support built into a simulation. Scaffolding can encourage learning (Tuovinen & Sweller, 1999) and help direct attention to necessary information (Reiser, 2004). We built five types of scaffolding into *CowGames*: Subject Matter Supports, Guidance, Feedback, Data Visualizations, and Review Questions.

Subject Matter Supports

Subject Matter Supports are types of scaffolding relevant to the topic of the simulation. We included two data tables and a glossary to support learners during game play. We included the Associated Possible Change Table and Percentile Rankings Table from the American Angus Association (www.angus.org). The Percentile Rankings Table

34	33.30	0.32	0.53	0.35	\$25.31	Carrier	0-7 ▼
34	29.82	0.32	0.68	0.35	\$31.30	Non-carrier	0-2 ▼
34	60.34	0.32	0.62	0.35	\$60.17	Non-carrier	0-7 ▼
34	48.39	0.32	0.65	0.35	\$43.15	Non-carrier	0-7 ▼
34	45.89	0.32	0.65	0.35	\$45.70	Non-carrier	0-7 ▼

Figure 1. Inbred mating highlighted yellow in the CowGames Breeding Stage. This was designed to help students easily identify inbred matings.

was a particularly important addition as many students indicated they did not know how to distinguish a “good” EPD from a “bad” EPD. The glossary was added at student request. This support is particularly important for students from non-agriculture or non-cattle backgrounds who may not understand some of the simulation’s terminology.

Guidance

Guidance is a coaching element that helps learners move through the tasks of the simulation (Guralnick & Levy, 2009). There is considerable debate about how much guidance should be included in a simulation. Therefore, we used student feedback to inform the provided amount of guidance. Initially, the simulation featured very minimal guidance. With each iteration we increased the detail of the guidance until we reached a point where most participants indicated they completely understood game goals (Figure 2). Guidance informs students about scenario goals, specific scenario rules, and general game guidance.

Feedback

Feedback is the support element that draws attention to progress and helps learners understand their results (Clark et al., 2009; Kirschner et al., 2006; Paas et al., 2003). Feedback was requested by students after the first iteration of testing. Specifically, students indicated they need assistance interpreting their data. Our simulation features scenario-specific, dynamic feedback that responds to each student’s progress. We are currently conducting a study on feedback optimization in this application. Preliminary information about feedback effectiveness is discussed in the Learning Impacts section.

Data Visualizations

Simulations have been shown to be very effective at helping students learn about unobservable phenomenon (Trey & Khan, 2008). However, to make these observations

simulations must include visualizations. The purpose of this simulation is to educate learners about long-term genetic change—therefore this change needed to be visible. To achieve this, we built a real-time graphing features on each module homepage. These graphs track EPD, phenotypic, market, and survival data for each scenario (Figure 3). This feature allows students to compare performance across scenarios for specific traits. Students can also compare two traits to help them visualize concepts such as correlated change.

Review Questions

An often overlooked, but critical aspect of simulations and more generally, active learning, is debriefing (Garris et al., 2002). Students need time to reflect on what they have learned. Guidance that helps them connect the material to what they learn in the classroom is particularly useful (Quintana et al., 2004). To do this we designed a set of general review questions which should be answered after completing each module. These questions are also built into the module home page (Figure 4). These questions can be custom-generated using the Educator Portal.

Simulation Flow

To understand general simulation flow, it is necessary to understand the simulation's modular design. The simulation features topic-specific modules which contain scenarios illustrating specific aspects of that module. Module features can be customized using the Educator Portal which will be released in November of 2018. Each scenario, except for Free Play, is seven years. Scenarios take about 45 minutes to complete. Each year features an introduction, selection stage, breeding stage, generation

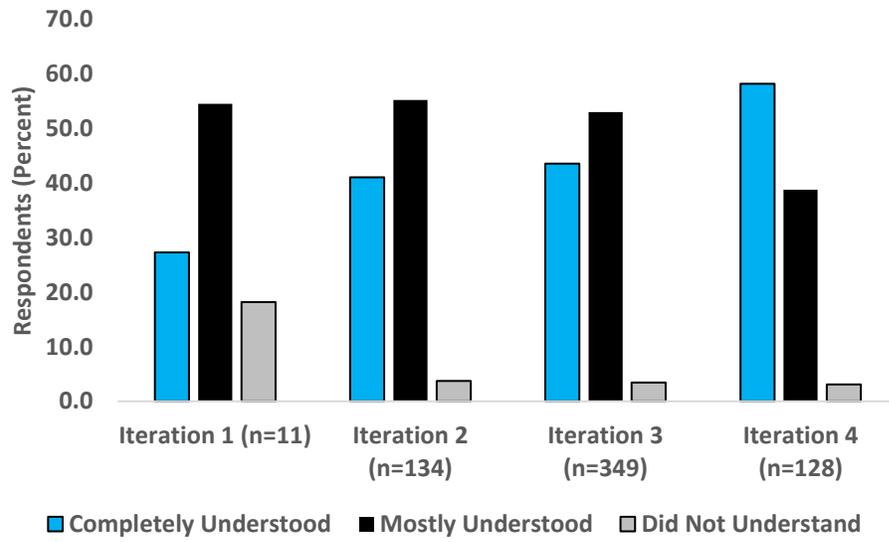


Figure 2. Student responses regarding how well they understood simulation goals for each iteration of testing.



Figure 3. Graphs showing changes in average BW and YW EPDs for each scenario in a learning module.

Think about... Save Responses

Question 1:
Which method did you think was easiest to use? Are there any limitations to this method?

Answer Here

Question 2:
Which method was the most difficult? Were there any positive aspects of this method?

Answer Here

Figure 4. Example review questions featured in a *CowGames* learning module.

summary, market stage, and financial summary. Feedback and guidance in all scenarios are provided by in-game character, Vince the Extension Agent.

Objective-Based Modules

Understanding how a simulation fits into current curriculum is a critical part of an educator's decision to adopt the tool (De Grove et al., 2012). Making the objectives of the simulation clear has been advocated as a method to ensure curricular connections (Barzilai & Blau, 2014). To do this we designed our simulation to feature topic-specific learning modules. Currently, the simulation features three modules (Table 2): Free Play, Correlated Response, and Selection Methods. Two more modules—Sire Selection and Response to Selection—are in development. Each module contains between one and four scenarios. Students receive in-game guidance and feedback during play to support their learning.

Correlated Response

The correlated response module features one scenario. In this scenario students select to increase YW then observe effects on the herd. BW and YW were selected to illustrate correlated response. Correlation between the two traits has been estimated to be around 0.55 (Koot et al., 1994). In the simulation, we raised this value to 0.7 to make sure students were able to observe the relationship. Ensuring that students can make sense of their observations in a modeling system like a simulation is critical (Moulin et al., 2004). To generate the correlated values, we employed the Cholesky Decomposition. To further elucidate the effects of correlated response dystocia is built into the simulation. There are also penalties for large or small carcasses as well as increased feed costs for large animals.

Table 2. Topic-specific modules and their objectives.

Module Topic	Objective
Correlated Response	Illustrate effects of correlated response through single-trait selection (7 years).
Selection Methods	Illustrate differences across three selection methods (7 years).
Free Play	Students can create their own rules. There is no year limit.
Sire Selection (In Development)	Illustrate the importance of effective sire selection (7 years).
Response to Selection (In Development)	Illustrate the effect of manipulating different aspects of selection on response (7 years).

To contrast correlated response we also included Mar, a carcass trait, that is negatively to lowly correlated with BW and YW respectively (J. K. Smith & Greiner, 2013). To confirm student observation of the difference in response types, we built the simulation to have a correlation of 0 with both growth traits.

Selection Methods

This module features three scenarios: Index Selection, Tandem Selection, and Independent Culling. For the Index Selection module, we developed a custom economic weighting equation for the featured traits. Current EPD values for an animal are then put into this equation to determine their index value. Students select animals based on index value. For the Tandem Selection module, students switch traits after two years of selection to illustrate the detriments of single-trait selection. They are directed in the guidance about when to change so each student follows the same selection protocol. Finally, for Independent Culling students are given a set of criteria they must follow when selecting animals. Students are then able to compare herd progress across the methods using the data visualization and review features.

Free Play

This module features a single scenario where no feedback or specific guidance is provided. There is no limit to the number of years that can be played in this module. This module is particularly useful for allowing students to test their own genetic plans.

Future Modules

Two additional modules are in development currently: Sire Selection and Selection Response. The Sire Selection module will feature three scenarios. In the first scenario, students will be assigned a single bull for use throughout the entire scenario. This will illustrate the negative effects of inbreeding on the herd. In the second scenario,

students can purchase a bull from the Bull Market to improve genetics, but only what they can afford. In the third scenario, students will be able to purchase semen from high-quality bulls but will be impacted by reduced conception rates.

The Selection Response module will feature four scenarios. In the first, students are encouraged to use genomic testing to reduce generation intervals. In the second, genomic testing is not available. The last two scenarios focus on selection intensity in a high versus low-replacement rate scenario respectively.

Yearly Stages

Each year is comprised of the same five stages: Selection, Breeding, Generation Summary, Market, and the Financial Summary. Guidance and feedback are adjusted for each stage within each scenario.

Introduction

To help students understand the purpose of each scenario they are given a short introduction. The introduction features a short description of scenario objectives, requirements, and objectives (Figure 5).

Selection

In the first year all students select from the same starting herd. This provides an even starting point for all students. Students are required to select between one and three bulls and thirty females (cows and heifers). We opted for a smaller herd to reduce redundancy since students must select individual animals. To speed up selection students can sort animals based on any column (Figure 6). Since the program generates animals based on real genetic principles the herd will never be the same for any two students after Year 1.

Index Selection Scenario



During this scenario, you will use Index Selection to make breeding decisions. This approach requires selecting animals on their *Index Value*. Index Values are calculated using economic weights on specific traits of interest. Indices are situation specific, meaning the CowGames index only works for CowGames. Think about that as you play through this scenario. Also, pay attention to Vince the Extension Agent, he'll give you some helpful advice and feedback.

Begin Scenario

Figure 5. Introduction screen giving students a brief explanation of what is to come in the scenario.

Bulls ♂ Select up to 1 more bull									
	Tag	EPD	Accuracy	EPD	Accuracy	EPD	Accuracy	Index	Carrier
		Birth Weight		Yearling Weight		Marbling			
<input checked="" type="checkbox"/>	0-9	3.44	0.43	89.50	0.35	0.75	0.28	\$88.30	Yes
<input checked="" type="checkbox"/>	0-2	3.94	0.75	66.02	0.55	0.53	0.46	\$57.22	Yes
<input type="checkbox"/>	0-7	1.28	0.80	59.43	0.51	0.91	0.73	\$74.76	
<input type="checkbox"/>	0-5	1.06	0.96	12.93	0.88	0.41	0.43	\$17.03	
<input type="checkbox"/>	0-4	2.00	0.45	10.84	0.34	0.44	0.33	\$10.24	
<input type="checkbox"/>	0-3	3.16	0.38	2.25	0.34	0.79	0.29	\$3.67	
<input type="checkbox"/>	0-8	1.94	0.77	-13.46	0.54	1.03	0.54	\$1.04	
<input type="checkbox"/>	0-4	2.55	0.87	-14.31	0.75	0.77	0.62	-\$9.85	
<input type="checkbox"/>	0-10	0.11	0.85	-22.32	0.84	0.95	0.48	\$0.79	
<input type="checkbox"/>	0-1	0.39	0.95	-58.13	0.92	0.53	0.87	-\$47.14	

Cows ♀ Select 26 more dams									
	Tag	EPD	Accuracy	EPD	Accuracy	EPD	Accuracy	Index	Carrier
		Birth Weight		Yearling Weight		Marbling			
<input checked="" type="checkbox"/>	0-26	4.56	0.40	115.83	0.28	0.76	0.20	\$108.38	Yes
<input checked="" type="checkbox"/>	0-16	4.01	0.51	89.39	0.41	0.97	0.31	\$90.38	Yes
<input checked="" type="checkbox"/>	0-15	3.42	0.47	56.92	0.38	0.72	0.24	\$55.08	
<input checked="" type="checkbox"/>	0-22	1.35	0.56	56.50	0.46	0.18	0.37	\$53.17	
<input type="checkbox"/>	0-13	2.42	0.53	56.45	0.44	0.23	0.37	\$48.16	
<input type="checkbox"/>	0-34	3.55	0.43	53.16	0.33	0.24	0.35	\$38.57	
<input type="checkbox"/>	0-37	-0.49	0.13	49.72	0.11	0.66	0.13	\$74.06	

Figure 6. Selection screen with two bulls and three cows selected; the animals are sorted by YW EPD.

Breeding

In the breeding stage, students can choose mating pairs from their selected animals. We felt it was important that users could always view the bulls to make informed decisions. To achieve this the bull table “sticks” to the top of the page as the user scrolls (Figure 7). On this page users also have the option to purchase genomic tests by selecting the test for that pair. If a bull is selected that produces an inbred mating that row will be highlighted yellow automatically.

Generation Summary

The generation summary is a tabular page showing a Generation Summary, Sire Summary, Dam Summary, Calf Summary, and Loss Summary. The Generation Summary (Figure 8) shows the average EPDs for the calves born in each year. The Sire and Dam Summaries show current EPD values for each animal, carrier status, phenotypic values and the change in EPD values (Figure 9). This feature was included to draw students’ attention to the fact that EPD values can increase and decrease while accuracies always increase. A common misconception of students is that as accuracies increase, so do EPDs. Animals highlighted in red have died. The Calf Summary shows EPDs, BW phenotype, and cause of death (Figure 10). Again, calves that have died are highlighted red. If the calf died due to dystocia or Curly Calf the cause of death is listed in the last column. Calves highlighted in yellow are the products of inbred matings. The Loss Summary shows all animals lost in that year.

Market

The Market features two sections: selection of sale animals and the sales receipt. After selecting which animals to sell a receipt showing details including carcass weight,

Bulls ♂												
i Tag	i Sire	i Dam	Birth Weight		Yearling Weight		Marbling		i Index	i Carrier Status	i Mate	i Genomic Testing
			i EPD	i Accuracy	i EPD	i Accuracy	i EPD	i Accuracy				
1-15	0-9	0-13	4.48	0.85	90.65	0.84	0.49	0.74	\$76.93	Carrier		
1-25	0-9	0-26	3.82	0.34	97.93	0.32	0.75	0.35	\$94.60	Carrier		

Cows ♀												
i Tag	i Sire	i Dam	Birth Weight		Yearling Weight		Marbling		i Index	i Carrier Status	i Mate	i Genomic Testing
			i EPD	i Accuracy	i EPD	i Accuracy	i EPD	i Accuracy				
0-13	Base	Base	2.55	0.58	56.94	0.51	0.16	0.46	\$46.18	Non-carrier	1-15 1-25	✓ \$40.00
0-14	Base	Base	1.98	0.63	13.36	0.51	0.85	0.46	\$23.12	Non-carrier	1-15 1-25	☐ Test Calf
0-15	Base	Base	2.80	0.53	52.44	0.44	0.72	0.46	\$54.08	Non-carrier	1-15 1-25	☐ Test Calf
0-16	Base	Base	3.60	0.53	91.19	0.44	1.02	0.46	\$95.83	Carrier	1-15 1-25	☐ Test Calf
0-17	Base	Base	0.95	0.53	7.95	0.44	1.11	0.46	\$30.15	Non-carrier	1-15 1-25	✓ \$40.00
0-19	Base	Base	2.42	0.45	-9.55	0.44	1.20	0.35	\$6.41	Non-carrier	1-15 1-25	☐ Test Calf
0-20	Base	Base	3.06	0.45	45.92	0.44	0.60	0.46	\$43.19	Non-carrier	1-15 1-25	☐ Test Calf
0-21	Base	Base	1.63	0.53	44.45	0.44	0.93	0.35	\$58.36	Non-carrier	1-15 1-25	✓ \$40.00

Account Balance \$ 15,310.03 [Glossary](#) [Guidance](#) [Complete Breeding >](#)

Figure 7. Breeding stage with bulls "stuck" to top of screen; three pairs were selected to have their calf genomically tested.

The values below represent average EPDs for each year's calf crop as well as the number of calves born alive that were carried full-term.

Generation Summary 					
Generation	Average Birth Weight EPD	Average Yearling Weight EPD	Average Marbling EPD	Average Index Value	Percent Live Births
1	0.85	-41.18	0.61	-\$30.83	93%
2	0.54	-17.65	0.86	\$0.65	90%
3	1.10	-31.09	0.67	-\$20.79	93%
4	1.01	-43.66	0.70	-\$32.08	90%

Figure 8. Generation Summary showing average BW, YW, Marb EPDs and Average Index Value for all calves born that year. Also shows percent of live births for each year.

Females listed below are the dams of this year's calf crop. Arrows indicate changes in EPD values.

Dam Summary ♀										
Tag	EPD	Birth Weight		Yearling Weight			Marbling		Index	Carrier
		Accuracy	Phenotype	EPD	Accuracy	Phenotype	Marbling EPD	Accuracy		
0-15	2.26 ↓ -0.01	0.52	75	46.87 ↓ -0.19	0.45	909	0.71 ↓ -0.00	0.40	\$51.53	
0-22	1.04 ↓ -0.00	0.59	74	47.40 ↓ -0.09	0.51	910	0.14 ↓ -0.00	0.46	\$44.89	
0-26	3.60 ↓ -0.01	0.47	77	115.41 ↓ -0.01	0.39	979	0.61 ↓ -0.01	0.36	\$109.87	Yes
1-11	1.98 ↓ -0.00	0.53	73	94.25 ↑ +0.32	0.49	933	1.18 ↑ +0.02	0.44	\$112.19	
1-16	4.97 ↑ +0.02	0.53	77	110.09 ↑ +0.42	0.49	959	0.88 ↑ +0.00	0.44	\$103.21	Yes
1-2	2.60 ↑ +0.01	0.53	74	53.06 ↑ +0.28	0.49	903	0.95 ↑ +0.00	0.44	\$61.75	
1-20	2.34 ↓ -0.00	0.53	73	65.78 ↓ -0.03	0.49	881	0.78 ↓ -0.00	0.44	\$71.68	Yes
1-4	1.88 ↓ -0.00	0.53	73	50.42 ↑ +0.10	0.49	879	1.07 ↑ +0.01	0.44	\$66.16	
1-8	3.38 ↑ +0.01	0.53	74	74.47 ↑ +0.10	0.49	904	0.59 ↓ -0.00	0.44	\$69.64	Yes
2-20	1.77 ↓ -0.08	0.52	72	74.84 ↑ +0.43	0.49	885	0.54 ↑ +0.00	0.43	\$78.08	

Rows per page: 10 | Prev 1 - 10 of 30 Next

Figure 9. Dam Summary showing summary information including arrows indicating the direction of change for each EPD; animal highlighted in red has died.

Calves listed below were born this year.
 Calves highlighted in red have died. Those that died due to Curly Calf or Dystocia will say so in the Cause of Death column.
 Other losses may be due to pregnancy loss or death after birth.

Calf Summary														
Tag	Sire	Dam	Sex	Birth Weight			Yearling Weight			Marbling		Index	Carrier	Cause of Death
				EPD	Accuracy	Phenotype	EPD	Accuracy	EPD	Accuracy				
7-0	4-28	5-1	Male	5.92	0.06	81	204.67	0.05	0.64	0.07	\$186.25	Yes		
7-1	4-28	5-3	Female			75								
7-10	4-28	0-26	Female			77							Curly Calf	
7-11	4-28	1-2	Female	5.78	0.06	77	198.65	0.05	0.65	0.07	\$181.47			
7-12	4-28	1-4	Female			75						Yes	Dystocia	
7-13	4-28	1-8	Female			72						Yes		
7-14	4-28	1-11	Male	5.74	0.06	79	200.71	0.05	0.67	0.07	\$184.10			
7-15	4-28	1-16	Female	5.92	0.06	76	201.50	0.05	0.65	0.07	\$183.37	Yes		
7-16	4-28	1-20	Female	5.76	0.06	74	199.30	0.05	0.64	0.07	\$181.92			
7-17	4-28	2-9	Female	5.82	0.06	77	204.33	0.05	0.64	0.07	\$186.67	Yes		

Figure 10. Calf Summary showing summary data; calves highlighted in red were lost to an unknown cause, Curly Calf, and dystocia respectively. Those highlighted in yellow were produced from an inbred mating.

marbling score, and meat grade is shown (Figure 11). Animals are sold on a value grid to encourage the production of higher quality animals.

Financial Summary

Finally, the Financial Summary gives students an opportunity to evaluate their revenue for the year (Figure 12). Costs include animal maintenance, breeding, and feed costs. Revenue is based on animals sold at market. The ledger is tabular, so students can review previous years as well.

In-Game Character

Vince the Extension Agent is the in-game guide. He guides students on gameplay and provides feedback on their progress. He delivers his messages in a casual tone via text message. Students respond to his feedback via multiple-choice questions (Figure 13).

Learning Impacts

Preliminary analysis regarding learning gains, engagement, motivation, and satisfaction indicate positive results on student learning. Since the introduction of pre and posttests in Iteration 2 cognitive gains have improved each round (Figure 14). Iteration 2 saw no difference in score likely due to the lack of feedback. Students supported this hypothesis in their surveys, with statements such as “Hard to understand what all the numbers mean”. This confirmed the need for feedback in the program. Iterations 3 and 4 had positive increases in learning gains ($P < 0.0001$, 65 ± 1 vs 70 ± 1 ; $P < 0.0001$, 66 ± 1 vs 73 ± 1). These improvements in score indicate the addition of feedback does support improved learning gains.

Livestock Bill of Sale

CowGames National Packing House

Invoice No: **1266413** Date of Sale: 4/3/2018

Account Sales of 24 Bulls, Cows and Heifers in the Amount of \$ 23,851 .00

Seller Account: amb16990 Buyer Account: CowGames Inc.
amb16990 *CowGames Inc.*

Pricing Guide

Value Grid Base Price	Grades Premium (per cwt)	Carcass Weight Discounts (per cwt)
\$150.00 / hundredweight(cwt)	Prime \$6.00 Choice \$0.00 Select -\$9.00 Standard -\$18.00	Small (< 550 lbs) -\$19.00 Large (> 900 lbs) -\$19.00

Figure 11. Sales Receipt from the market showing the value grid schedule.

Year 3 Expenses				
Expense	Description	Animals	Unit Cost	Paid
Annual Herd Upkeep	Upkeep costs including feed, land, and health expenses.	31	\$600.00	\$18,600.00
Breeding Cost	Associated breeding costs including bull upkeep and synchronization.	0	\$25.00	\$0.00
Large Cow Fee	Added cost of maintenance for cows with a mature weight over 1500 lbs.	0	\$20.00	\$0.00
Genomic Testing Fee	Cost per genetic test	0	\$40.00	\$0.00
Total Expenditures				-\$18,600.00
Revenue	Description	Animals	Unit Cost	Collected
Cattle Sales	Cattle Sold at Auction	17	-	\$17,735.00
Total Revenue				+\$17,735.00
Year End Balance				\$15,367.67

Figure 12. Financial Summary showing costs and revenues for the year.

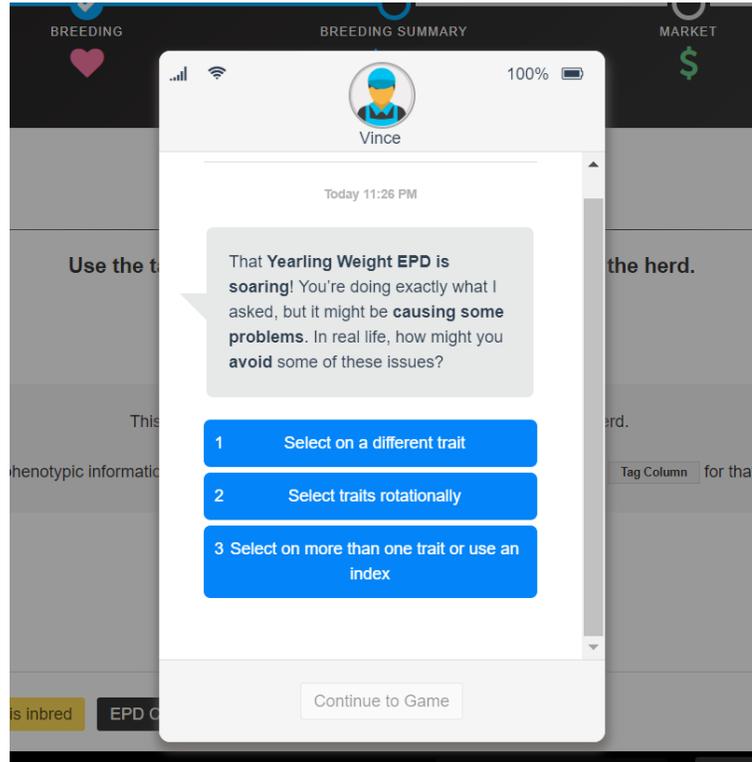


Figure 13. Vince the Extension Agent provides feedback on student progress via in-game text message.

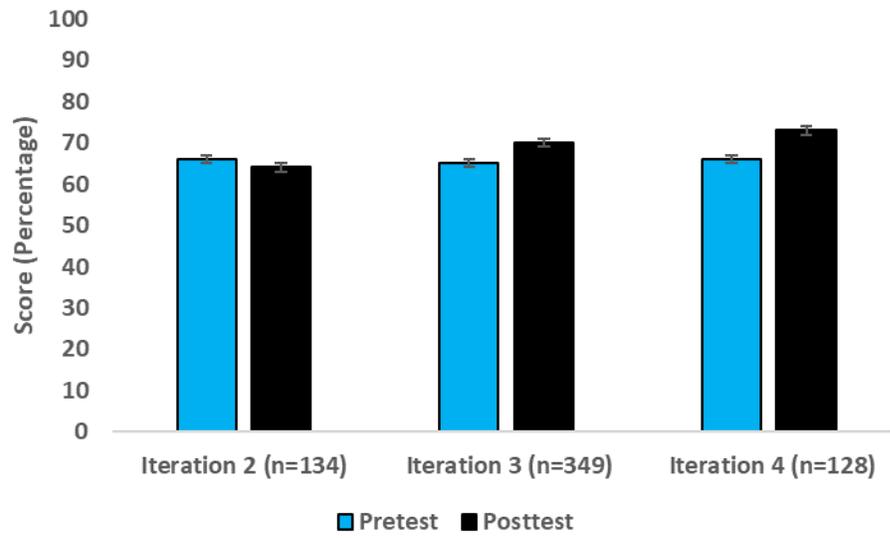


Figure 14. Pretest and posttest scores (%) \pm SE for Iterations 2-4.

We also assessed affective gains in Iterations 3 and 4. We were particularly interested in student engagement and motivation when using *CowGames* compared to traditional homework or lecture. We found that students reported higher engagement and motivation when using *CowGames* (Figure 15, Figure 16) supporting previous findings of increased affective domains when using simulations (Durán et al., 2007; Kiboss et al., 2004). Increased engagement has been associated with higher learning gains (Carini et al., 2006; Koedinger et al., 2015). Previous work has also observed increased motivation which can have a positive impact on learning (Buckley & Doyle, 2016). Students indicated reasons for increased motivation typically relating to a new understanding of how the materials in class relate to real life. For example, a student in Iteration 2 said, “I thought *CowGames* was a fun way to help me understand actual implications of the things I’m learning in class”.

Conclusions

CowGames was designed with an iterative, user-centered design approach that allowed us to work with educators and students directly. Prior to development we gathered educator requirements then used student feedback during testing to improve the simulation. The simulation has multiple modules focused on specific learning topics. Within each module are one to four scenarios illustrating specific aspects of that topic. Each scenario models seven years of production. Each year contains five stages: Selection, Breeding, Generation Summary, Market, and Financial Summary.

CowGames shows positive effects on student motivation and engagement. This research also confirmed the critical nature of feedback. For that reason, we are

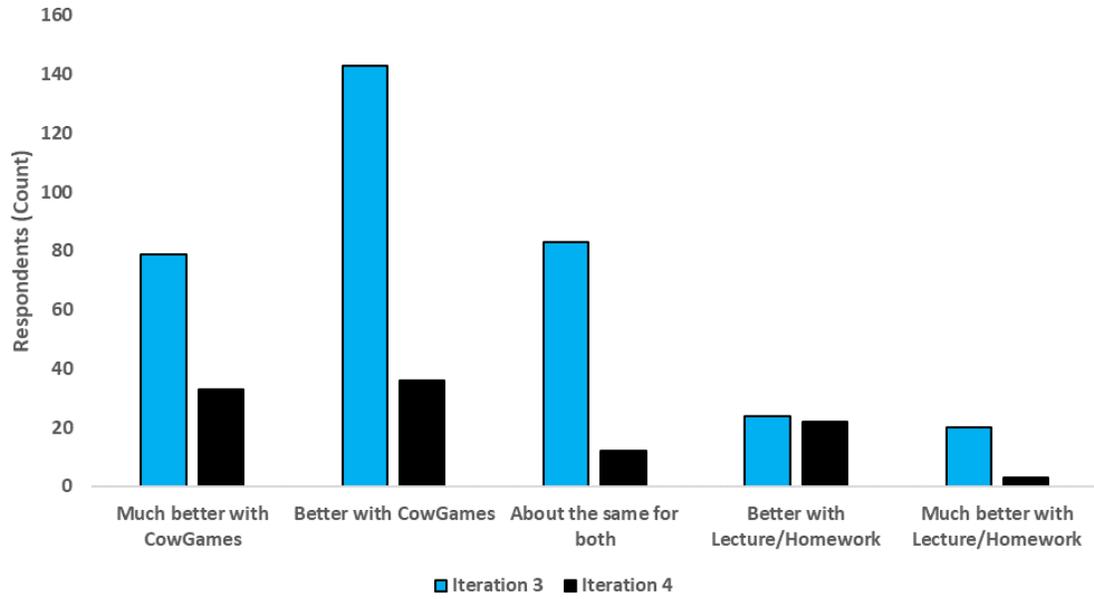


Figure 15. Reported engagement for students in Iterations 3 and 4.

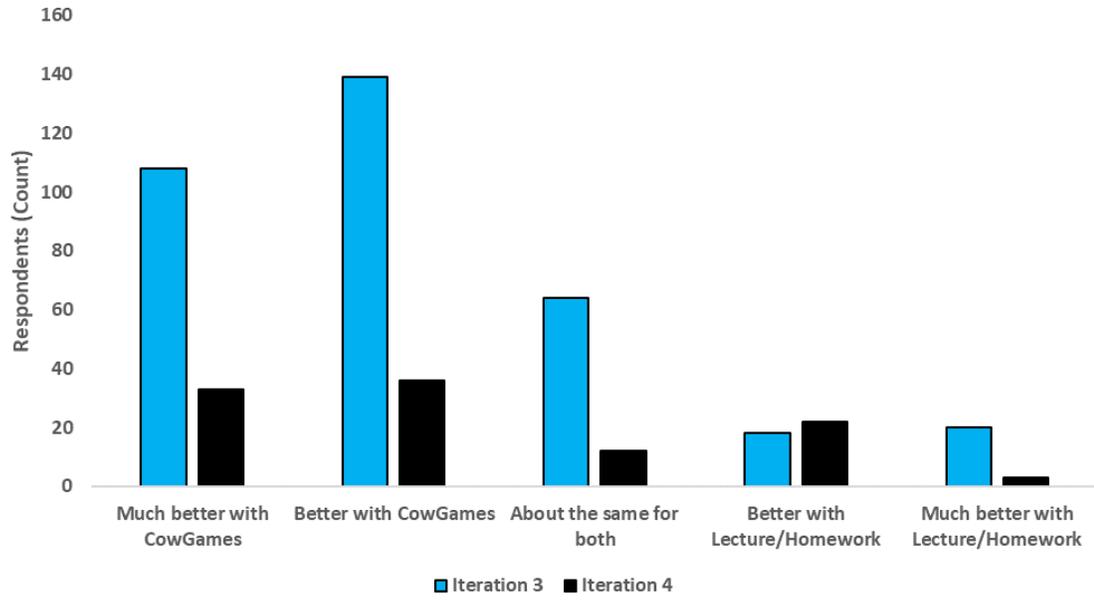


Figure 16. Reported Motivation for students in Iterations 3 and 4.

conducting a study focused on optimizing feedback for the simulation. We will also be working with high school educators to modify the simulation in the Spring of 2019.

The simulation is available at www.cow-games.com.

CHAPTER 3. FEEDBACK OPTIMIZATION: AN ITERATIVE, VALUE-ADDED APPROACH IN A BEEF CATTLE BREEDING SIMULATION

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Abstract

Feedback is a critical component of learning. Without feedback students are often unable to make sense of their results which inhibits cognitive changes required for learning. Designing effective feedback can be challenging however. Designers must understand the intricacies of feedback attributes as well as the effects of different learning characteristics. In this paper we aim to identify the most effect feedback design for a beef cattle breeding simulation using an iterative testing approach. In the first iteration we confirmed the necessity of feedback when students showed no improvement in score from pretest to posttest. In the second iteration, we explored different levels of feedback complexity. We observed an effect of pretest score but no difference across feedback types. Using survey response data, we determined that many students were not reading the feedback—a crucial factor of feedback effectiveness. In the final iteration we evaluated the effectiveness of interactive versus static feedback. We found that interactive feedback was effective for increasing scores in Low and Moderate-Low performers. Ultimately, we determined that feedback should be tailored to learner characteristics to be most effective.

Introduction

Feedback helps students understand their work—something critical to learning—especially in serious games and simulations (Wiese & Koedinger, 2017). It is particularly important for novice learners because it provides them with missing schemas which assist them in making sense of their results (Clark et al., 2009; Kirschner et al., 2006; Paas et al., 2003). However, not all feedback is effective. Providing feedback on easy tasks can actually inhibit learning (Bangert-Drowns et al., 1991). Feedback shown before students make decisions does little to change cognitive architecture (Mory, 2004; Shute, 2008).

Feedback that is too detailed can overwhelm students and reduce learning gains (Roll et al., 2014; Van Dijk et al., 2016). Therefore, understanding proper design is vital for the development of effective feedback.

Developing effective feedback requires understanding performance effects of all feedback attributes including type, timing, and delivery. There are many types of feedback but they generally can be divided into two categories: outcome-based and process feedback (Johnson et al., 2017). Outcome-based tells a learner if they are right or wrong—no additional explanation. Process feedback, or explanatory feedback, provides learners with knowledge of the outcome as well as additional information about reasoning or how to correct. Research has shown that process feedback is generally more effective for increased learning (Bangert-Drowns et al., 1991; Shute, 2008). Timing of feedback is a more disputed topic. Proponents of immediate feedback say it improves procedural knowledge (Anderson et al. 1989) and reduces uncertainty (Nkhoma et al., 2014). However, delayed feedback may be more useful for error correction (R. W. Kulhavy, 1977). Therefore, it is necessary to identify simulation goals which can then be used to inform design. Correct mode of delivery is also critical to effectiveness. Research has shown that for highly visual simulations, audio feedback is a requirement (Moreno & Mayer, 2004; Moreno et al., 2001), otherwise the simulation is likely to cause a visual overload and reduce learning. However, for simulations that are less visually stimulating text-based delivery may be equally as effective. Therefore, it is recommended to consider the overall simulation design before deciding on feedback delivery mode (Johnson et al., 2017). A general trend emerges from these different aspects of feedback design—one size does not fit all.

Feedback design is then further complicated when you consider learner characteristics. Attributes including gender and prior knowledge, can impact the effectiveness of feedback. In a study comparing explanatory feedback to percentage correct feedback, it was observed that gender affected which type of feedback was most effective (Landsberg et al., 2010). In a study using a genetics simulation, it was determined that high ability learners needed less feedback than low ability learners but do appreciate more elaborate feedback (Smits et al., 2008). Therefore, it becomes clear that understanding how different learner types respond to given feedback is critical. Unfortunately, research in this area remains relatively scarce (Johnson et al., 2017). Therefore, the objective of this work was to determine the feasibility and success of using an iterative, value-added approach to optimize feedback design for different levels of prior knowledge.

Methods

Participant Recruitment

Education studies require a fairly large number of students to identify small effect changes (Olejnik, 1984). Coupling that requirement with our iterative approach means this method of design requires a very large number of students. To recruit student participants, we contacted professors from universities and community colleges across the United States and Canada (Figure 1).

The Simulation

CowGames is a beef cattle breeding simulation. It focuses on teaching students about long-term genetic change in a herd. The simulation features topic-specific modules on subjects related to cattle breeding and genetics. Within each module are scenarios

which illustrate specific aspects of that topic. The simulation was developed using a participatory design system that involved educators and students (Haag et al., 2018).

The simulation is written in Javascript, HTML, Node.js, and CSS in the Vue.js framework. The simulation utilizes Sequelize Object Relational Mapping to transfer data between the frontend and backend of the application. User authorization is conducted and stored by Auth0 (Auth0, Bellevue, WA), an independent authorization and login firm.

The Scenarios

Students in Iteration One completed one of three scenarios in the Selection Methods module. This design was not ideal but was necessary due to educator requirements. Students in Iteration Two and Three all completed the same scenario—Correlated Response. Each scenario models seven years of production and requires about 45 minutes to complete.

In-Game Guide Character

An in-game guide character, “Vince the Extension Agent” (Figure 2) was built into the simulation to provide guidance and feedback. Vince delivers all information in a casual tone via text message. His messages and tone were designed in accordance with previous work indicating best practices for game characters (Moreno & Mayer, 2004; Shute, 2008).

Iterative Testing

We conducted three iterative tests with student groups from different institutions and education levels. The University of Missouri Institutional Review Board approved the study protocol and all participants provided written informed consent prior to participation in the study. All students were assigned to a test group then completed a

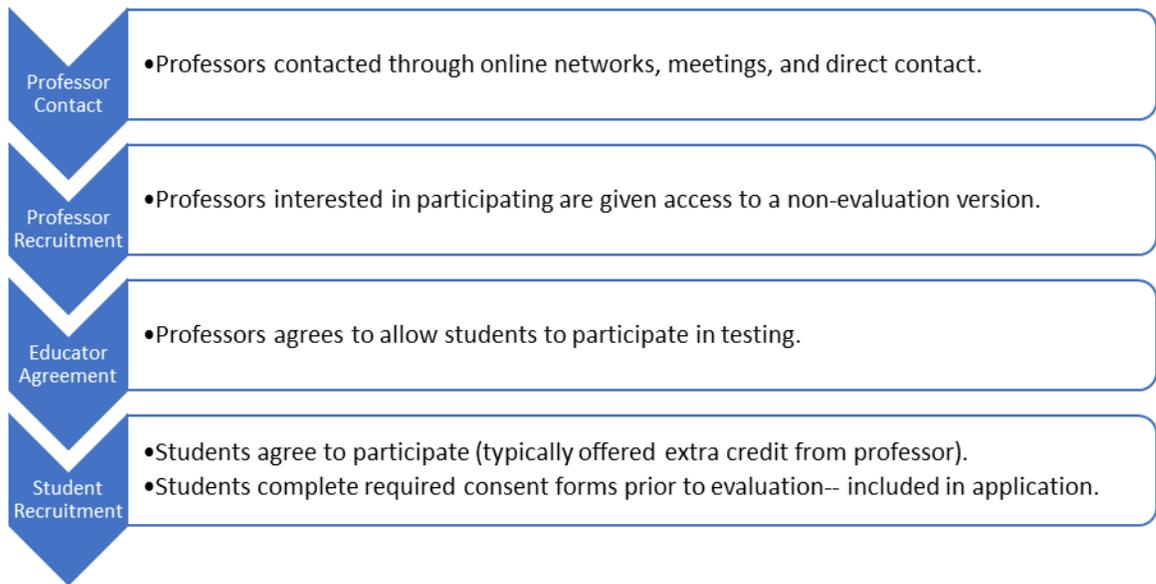


Figure 17. Diagram showing the participant recruitment process.

[Figure 1 in chapter]

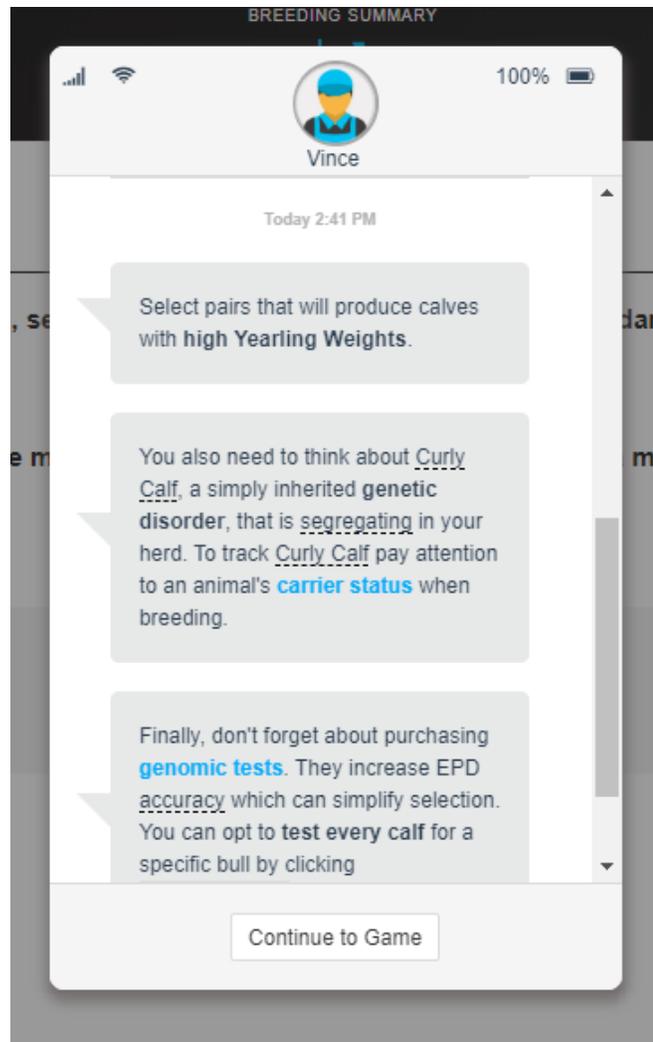


Figure 18. Screenshot showing Vince's text message guidance.

[Figure 2 in chapter]

pretest, seven generations of the simulation, review questions, a posttest, and a survey. Survey data provided qualitative measures of student perceptions of the simulation.

Students in Iterations Two were assigned to performance groups based on pretest score. Students scoring below 50% were considered low, students in the 51-84% were considered moderate, and those scoring 85% or above were considered high performers. In Iteration Three students were broken into four pretest performance groups, High (>80%), Moderate-High (70-79%), Moderate-Low (60-69%), and Low (<59%) to further elucidate patterns in the large moderate group. Test scores were reported in percentages due to differences in number of questions from Iteration Two to Iteration Three.

Iteration One

The first iteration was tested in an introductory level Animal Science course consisting of 125 students. The purpose of this iteration was to establish the necessity of feedback—therefore no feedback was built into the simulation. Since feedback was not included all students were assigned to one group but scenario differences were accounted for in the statistical analysis. A paired T-Test analysis was conducted on test score data using PROC TTEST in SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Iteration Two

The second iteration was conducted with 454 students from eight universities across the United States. In this iteration the computer randomly assigned students to one of three feedbacks: Guidance, Suggestive, or Diagnostic. The three types were increasingly complex (Table 1). Guidance provided no feedback and served as a control. Suggestive pointed at a trend and asked the student to think about potential causes or implications. Finally, diagnostic pointed out the trend and gave students information on

Table 3. Examples of Iteration Two feedback for each type.

[Table 1 in chapter]

Feedback Prompt	Guidance	Suggestive	Diagnostic
Average yearling weight increased by 5 lbs.	N/A	Yearling weight went up a lot this year. Think about why your selection method worked well then keep it up.	Yearling weight went up a lot this year. Continue picking bulls with the highest yearling weight EPD to keep up this trend.
Two or more calves affected by Curly Calf.	N/A	I see you lost some calves to Curly Calf. How could you reduce the number of affected calves while still increasing yearling weight?	Looks like you lost some calves to Curly Calf. That's never good so make sure you avoid mating carriers whenever possible.
Two or more prime-grade animals	N/A	Congratulations! You had prime-grade animals. Look at the yearling weights—do you see a relationship?	Congrats! You had prime-grade animals. This means they had high marbling scores but are not necessarily animals with high yearling weights. Marbling and yearling weight are uncorrelated traits.

the cause or implication. Test scores were analyzed with ANCOVA using Proc GLM in SAS 9.4. Posttest score as a percentage was designated as the dependent variable and fitted to a general linear model in this analysis:

$$\begin{aligned} \text{Posttest Score } (\%)_{ijkl} \\ = \mu + \text{Feedback Type}_i + \text{Gender}_j + \text{School}_k + \text{Pretest Score}_l(\%) \\ + e_{ijkl} \end{aligned}$$

In this model, posttest score is the dependent variable, μ is the mean, feedback type_i, gender_j, and school_k are fixed effects, pretest score is the covariate, and e_{ijkl} is the error term respectively. We fitted the model for gender because previous work has indicates variable effects of gender on performance and perception of online learning tools (Astleitner & Steinberg, 2005; Lal, 2002; Richards-Babb et al., 2015).

Iteration Three

The third iteration included 242 students from ten universities across the United States. In this iteration students were assigned to one of two groups: interactive and static (Table 2). In the interactive group students were required to answer a multiple-choice question as part of their feedback (Figure 3). Static feedback required no interaction like that of Iteration Two. Again, test scores were analyzed with ANCOVA using PROC GLM in SAS 9.4. Posttest score was fit to the same model used in Iteration Two.

To evaluate how much feedback each student saw during a scenario we pulled count data from the program database. The program tracks each feedback shown to a student, and in the case of interactive feedback, stores their response as well. An ANOVA using PROC GLM in SAS 9.4 was then conducted to evaluate the relationship between performance and the number of feedbacks observed. Total number of feedbacks observed was designated as the dependent variable and fitted to a general linear model in this analysis:

Table 4. Examples of Iteration Three feedback for each type.

[Table 2 in chapter]

Feedback Prompt	Suggestive	Diagnostic	
		Question	Response
Average yearling weight increased by 5 lbs.	Your Yearling Weight EPD went up this year! Continue picking animals with high YW EPDS to keep up the trend. Remember though, dramatically increasing size does have limitations like increasing feed requirements and dystocia rates.	Your Yearling Weight EPD went up this year! Continue picking animals with high Yearling Weight EPDS to keep up the trend. <i>Do you think there could be a limit to size increase?</i> <i>Yes</i> <i>No</i>	Correct: Yes, increased size can lead to things like increased feed requirements and dystocia rates. Incorrect: Sorry, there are limitations. Otherwise, we'd have giant cattle. Limitations include increased feed requirements and dystocia rates.
Two or more calves affected by Curly Calf.	Looks like you lost some calves to Curly Calf. To reduce the likelihood of producing Curly Calves you should avoid mating two carrier animals. Some people eliminate all carriers from their herd but doing this may eliminate quality animals. Instead, lethal recessive management is recommended.	Looks like you lost some calves to Curly Calf. <i>How might you reduce the likelihood of producing a Curly Calf?</i> <i>Eliminate all carriers</i> <i>Avoid mating non-carriers</i> <i>Avoid mating carriers</i>	Correct: Right, some producers choose to eliminate all carriers, but this eliminates potentially quality animals. Instead, lethal recessives should be managed by not mating carriers when possible. Incorrect: Not quite, since Curly Calf is a lethal recessive you must breed two carriers to even have the possibility of producing a Curly Calf. While some producers eliminate all carriers, this can lead to elimination of otherwise quality animals. Instead, lethal recessives

			should be managed by not mating carriers if possible.
Two or more prime-grade animals	Congrats! You had prime-grade animals! This means they had high Marbling scores but are not necessarily animals with high Yearling Weights because the traits are uncorrelated.	Congrats! You had prime-grade animals! <i>Do you think the animals with high marbling scores always have high yearling weights?</i> <i>Yes</i> <i>No</i>	Correct: Exactly, Yearling Weight and Marbling are uncorrelated traits. Incorrect: Not exactly, Yearling Weight and Marbling are uncorrelated traits so any relationship you may observe is coincidence.

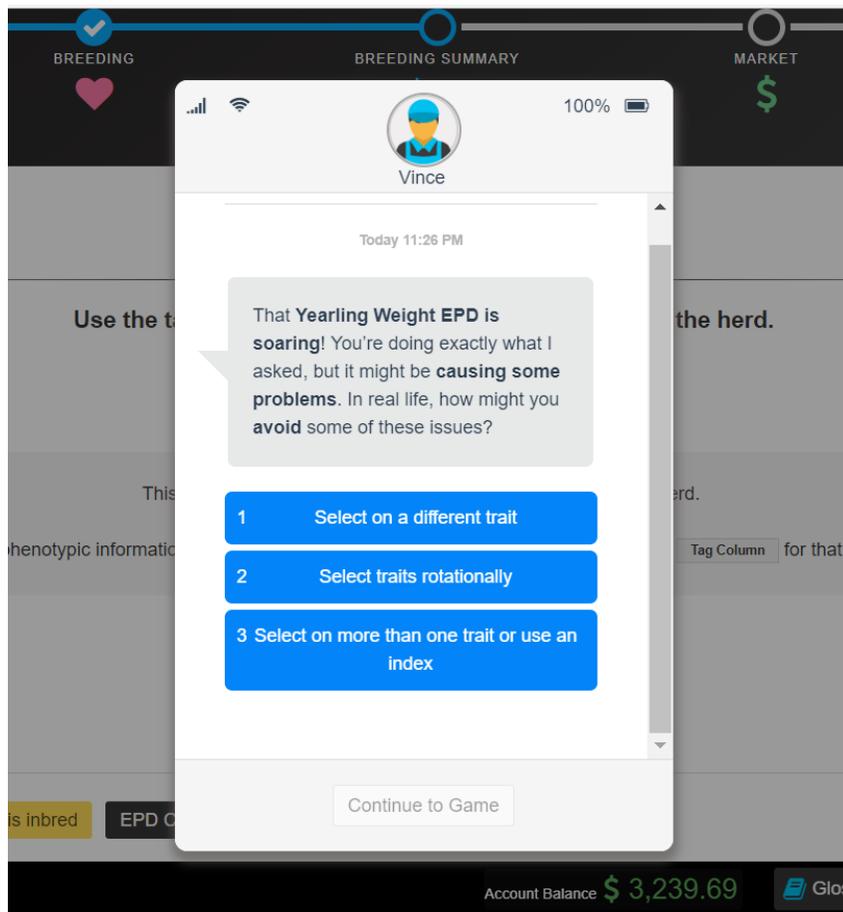


Figure 19. Screenshot of interactive feedback from Iteration Three.

[Figure 3 in chapter]

$$\begin{aligned}
 \text{Total Feedbacks Observed}_{ijk} &= \mu + \text{Pretest Performance Group}_i + \text{Feedback Type}_j \\
 &+ \text{Pretest Performance Group} * \text{Feedback Type}_k + e_{ijk}
 \end{aligned}$$

In this model, the number of feedbacks seen by a student is the dependent variable, pretest performance group_i, feedback type_j, and the interaction, performance group * feedback type_k, are fixed effects, finally e_{ijk} is the error term.

Results

Iteration One

Students in Iteration One showed no difference in score from the pretest to the posttest ($p=0.15$; 65 ± 1 vs 64 ± 1). A range of pretest scores indicated that not all learners in this iteration were novices. Survey data also showed that without feedback students were unable to interpret their results and did not effectively learn from the simulation. Responses such as “It was hard to understand what my data meant for my herd” and “I didn’t know if my herd was improving or not”, supported these findings.

Iteration Two

Students in Iteration Two scored higher ($P < 0.0001$, 65 ± 1 vs 70 ± 1) on the post-test compared to the pre-test as a group indicating an improvement in learning gains. In this iteration we compared three types of feedback: guidance, suggestive, and diagnostic. Each level provided increasing amounts of information regarding student progress. No difference in posttest score was observed within performance groups for any feedback type (Figure 4). Students in the high-performance group, regardless of feedback, scored higher than students in the moderate or low performance groups. Students in the moderate performance group scored higher than students in the low performance group. No difference in score was observed based on gender.

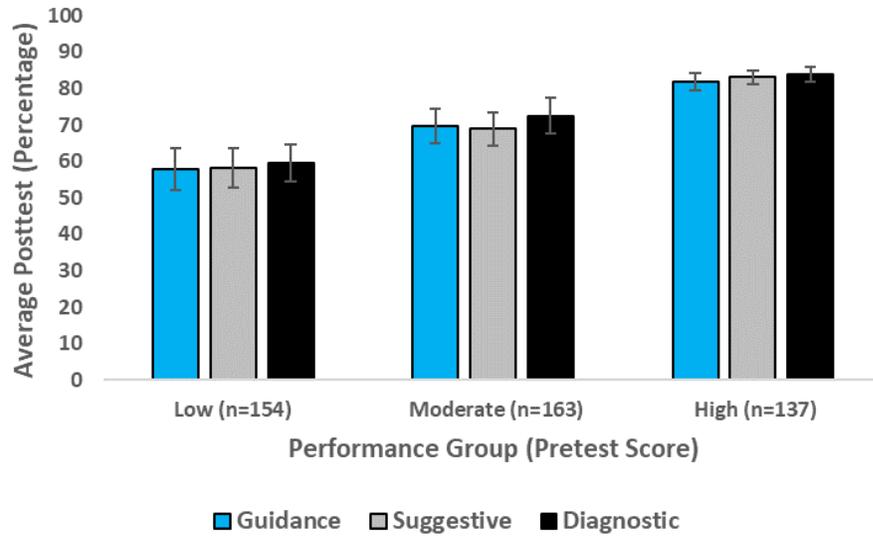


Figure 20. Posttest scores \pm SE for each feedback type and performance group in Iteration Two.

[Figure 4 in chapter]

To better understand our somewhat disappointing results we delved into the survey responses. When asked if Vince, the game guide, helped them understand their results 51% indicated that he did (Figure 5). However, 32% had a neutral response and 17% indicated he did not. To understand those neutral and negative responses we looked to the qualitative data. Some students reported that Vince's feedback was sometimes confusing as it was not completely aligned to the specific scenario, for example "Vince sometimes contradicted what my goals for my breeding operation were". Other students indicated that they did not read the feedback with statements like "I did not read Vince's suggestions, but the data was easy to figure out" and "I didn't really use Vince".

Iteration Three

In Iteration Three we saw an increase in score from the pretest to the posttest across all groups ($p < 0.0001$, 63.20 ± 1.04 vs 68.27 ± 1.23). We then evaluated the effects of the different feedback types within each performance group (Figure 6). Once again, no difference was observed due to gender. No difference in score was observed between feedback types for high performers who showed higher scores than all moderate and low performers. Moderate-high and low performers showed an increase in score using the interactive feedback ($p=0.0710$, 48.129 ± 5.500 vs 41.700 ± 5.842 ; $p=0.0596$, 77.716 ± 2.648 vs 71.583 ± 2.546). Moderate-low performers showed no difference in posttest score.

To provide a more in-depth look at student perceptions we then looked at survey responses. In this iteration 77% of students reported Vince helped them understand their results, but the remaining 23% indicated he did not (Figure 7). This was an improvement over Iteration Two but implied we still had work to do on our feedback design. When we

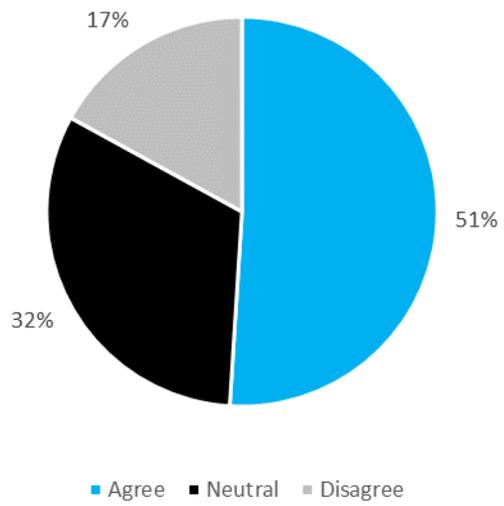


Figure 21. Respondent perceptions of Vince's feedback in Iteration Two.
[Figure 5 in chapter]

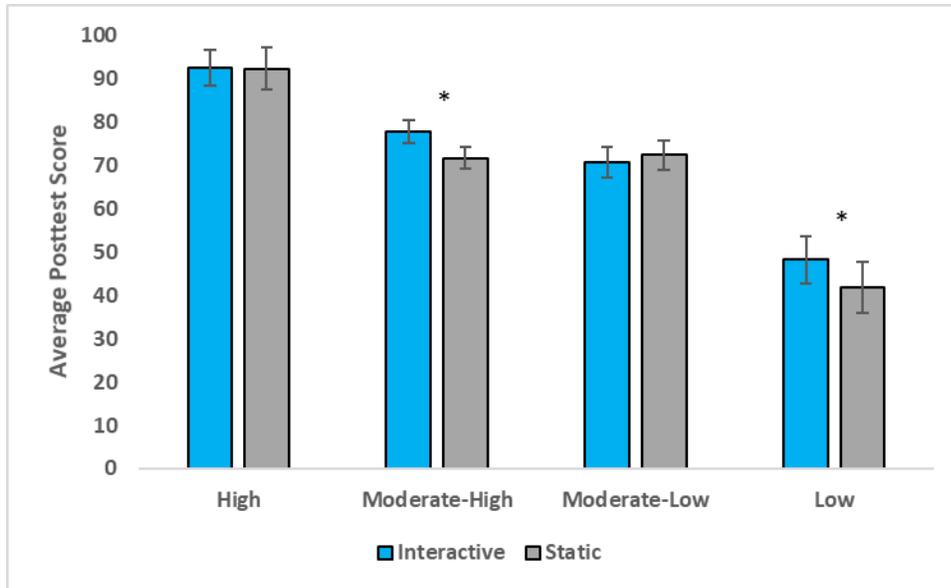


Figure 22. Posttest scores SE for each feedback type and performance group in Iteration Three¹.

[Figure 6 in chapter]

¹ * indicates a significant value ($p < 0.1$)

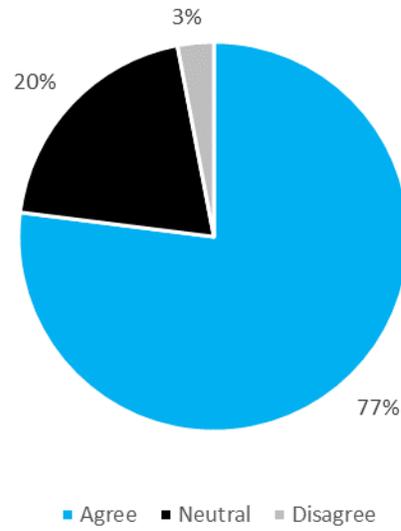


Figure 23. Respondent perceptions of Vince's feedback in Iteration Three.
[Figure 7 in chapter]

looked at the short answer responses a couple trends emerged. First, Vince helped students see things they may have missed otherwise. However, other students said they would like to see more feedback. While this first trend was encouraging we were concerned about students not seeing enough feedback.

To evaluate how much feedback students were seeing we first did a simple count. We observed that of the 21 possible feedbacks most students were observing 13 or less (Figure 8). We found no difference in total number of feedbacks shown between performance group or feedback type (Figure 9).

Discussion

Our iterative approach to feedback development allowed us to employ a value-added approach while also integrating user feedback. Through this approach we were able to identify feedback complexities that would have been overlooked with a traditional single intervention approach.

The first iteration acted as a control to confirm the necessity of feedback in an Animal Science simulation. While these students were all in an introductory course there was still a range of scores on the pretest indicating differential prior knowledge. Students showed no improvement in score from pretest to posttest without feedback. These results were expected as the necessity of feedback is well documented in other fields (Erhel & Jamet, 2013). Our data confirms that educational simulations without feedback are unlikely to be effective for increasing learning.

In Iteration Two, similar to previous work (González-Cruz et al., 2003), there was no difference in scores across feedback types within a performance group. While that

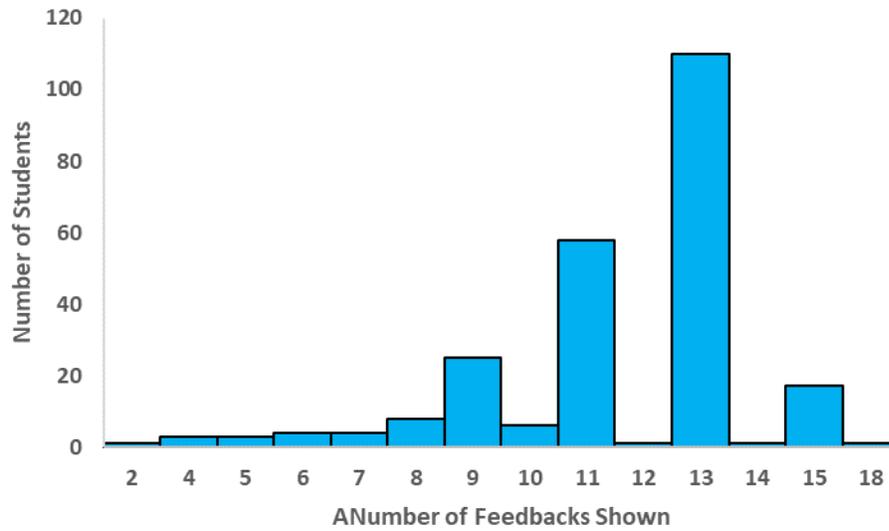


Figure 24. Histogram showing the distribution of feedbacks observed per student for Iteration Three.

[Figure 8 in chapter]

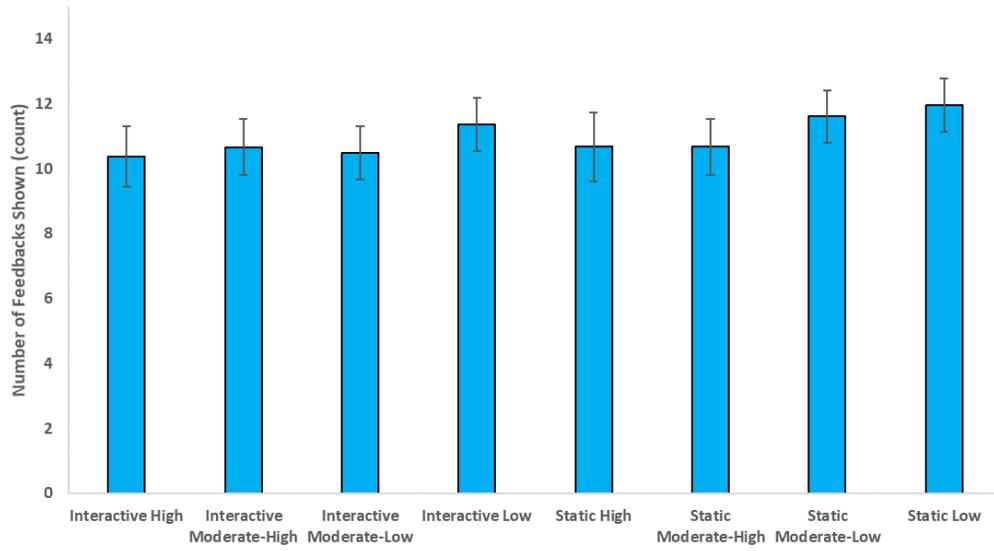


Figure 25. Average feedback count \pm SE for each feedback type and performance group in Iteration Three.

[Figure 9 in chapter]

study opted to employ a moderate feedback level we felt that did not properly address the question. Therefore, we used survey data to gain a better understanding of student perceptions of the feedback. With just over half of the students indicating the feedback helped them it was clear some issues were not resolved. Their responses indicated we needed to clarify scenario objectives as well as expectations for gameplay—an important element for learners and educators (Quintana et al., 2004).

One of the best way to build connections between learning and a simulation is through feedback that is well-integrated into the game narrative (Clark & Martinez-Garza, 2012). Students indicated they were confused by what they were being told in the feedback and what was happening in the simulation. Specifically, the scenario asks students to select animals in a manner that can be detrimental to overall herd productivity. We believed the feedback addressed the decline sufficiently, but it was clear this was not true from student responses. This was particularly problematic since it increased uncertainty instead of reducing it—a purpose of feedback.

Finally, and equally concerning to the uncertainty issue, was the problem of students not using the feedback. Many students indicated they did not read the feedback. While this is likely not an issue for high performers (Kalyuga, 2007; Landsberg et al., 2010) it was an issue for moderate and low performers. These students are those most likely to need the additional scaffolding provided by the feedback (Sweller et al., 1998). Further, it has been observed that low performers will overestimate their abilities which can be detrimental to learning (Kruger & Dunning, 1999). While we were unable to connect survey data to score data this did indicate an area of major concern for our design.

Iteration Three served as an opportunity to compare two new types of feedback as well as evaluate revisions. As a potential solution for students not reading feedback we introduced interactive feedback. In the interactive group students could not move forward in the simulation until they answered Vince's question(s). This interactive feedback was designed to encourage students to engage with the feedback—a critical component of misconception correction (Corbalan et al., 2009). Prior research has shown that students who are unsure of their answers are unlikely to consider feedback in a way that can change their thinking (Bangert-Drowns et al., 1991).

We observed students in the Moderate-High and Low performance groups had increased posttest scores using interactive feedback. Previous research has shown that increased interactivity improves performance for struggling students (Edgcomb & Vahid, 2014). Interestingly, our data indicated that of the Moderate performers, the Moderate-Low group did not benefit from the addition of interactivity. Currently, literature regarding moderate performance and feedback effectiveness is scarce, but we do have a potential hypothesis. The Dunning-Kruger effect (Kruger & Dunning, 1999) is when low-ability individuals overestimate their skill or knowledge. When confronted with information, such as a low-test score, indicating otherwise they tend to ignore it. Therefore, the need for formative feedback that forces these individuals to confront their misconceptions is critical. Since we observed no difference in feedback type for this group we can infer that neither type is forcing misconception recognition and change. Therefore, that will need to be addressed in future iterations.

To overcome the issue of confusion and uncertainty we developed feedback that was clearly connected to the scenario objectives. For example, a new feedback stated,

“That Yearling Weight EPD is soaring! You’re doing exactly what I asked, but it might be causing some problems. In real life you could avoid some of these issues by selecting animals on more than one trait or using an index”. This feedback acknowledged what was happening in the simulation while still highlighting relevant information. With over 70% of students indicating that the feedback helped them understand their results and no reports of confusion we felt we had overcome the clarity problem.

However, a new problem became evident—students not receiving equal amounts of feedback. We observed no difference in total number shown between feedback types, but we did observe a range of counts. Since previous work indicates that high amounts of feedback do not impact game flow (Barzilai & Blau, 2014) we designed a fairly extensive amount of feedback. Each feedback is designed to provide new information pertinent to the objective. Therefore, when students are not observing feedbacks they are missing out on information. This indicates we need to lower the feedback prompt requirements to ensure students are being shown the feedback.

Through this approach we were able to successfully improve feedback and identify previously unreported complexities. However, this method does require a very large number of participants. Ideally, about 150 students for each iteration.

Conclusions

Through this approach we were able to successfully optimize feedback as well as identify issues not previously identified in the literature, namely, students not reading feedback and students receiving unequal amounts of feedback. This method does require many student testers, but the outcomes justify the recruitment costs. Therefore, we would recommend the use of a value-added, iterative approach for simulation development.

PART I SUMMARY

Future Work

In future work we will be exploring the effectiveness of different complexity, interactive feedbacks. We will also work to gain a better understanding of learning characteristics in the Moderate performance groups.

Summary

CowGames was designed with an iterative, user-centered design approach that allowed us to work with educators and students directly. The simulation is designed to allow students to observe long-term genetic change quickly. Iterative evaluation has allowed for the optimization of simulation scaffolding including subject-matter supports and feedback. CowGames shows positive effects on learning gains, student motivation and engagement.

PART II. CHARACTERIZING GENETIC CONTROL OF WATER CONSUMPTION IN TWO STRAINS OF INBRED MICE

Overview

Water is a finite resource that is growing scarcer. As our population increases we must find ways to reduce water usage. One potential solution is genetic selection of animals that have low water consumption requirements. To achieve this goal, we must identify genes associated with water consumption.

The purpose of Part II is to describe the characterization and estimation of genetic effects controlling water consumption in two inbred mouse strains. The first chapter of this work includes current literature pertaining to water availability as well as background information about introgression. The second chapter describes the genetic characteristics of the trait in our experimental population. The third chapter describes the process for estimating the minimum number of genes affecting the trait. Finally, a short wrap-up and summary concludes the work.

CHAPTER 1. REVIEW OF RESOURCE AVAILABILITY AND INTROGRESSION

Water Management and Livestock Production

In 2006, the Food and Agriculture Organization (FAO) released a report, “Livestock’s Long Shadow” stating that, among other issues, livestock production had higher greenhouse gas emissions (GHG) than even livestock transportation (Steinfeld et al., 2006). Since then parts of the report have been retracted and clarified, but the issue remains—livestock production could be more efficient. Improving efficiency is especially critical when one considers projections that meat consumption will double by 2050 (Giller, 2017; Herrero et al., 2015; Shepon et al., 2016), but resource availability will continue to fall. This problem is intensified by an increasing population which will further reduce resource availability (Godfray et al., 2016). This creates a unique challenge for livestock producers that can only be met through innovation—produce more animals with less environmental impact (de Fraiture & Wichelns, 2010; Von Braun, 2007). Meeting this challenge requires understanding current livestock production systems then creating solutions.

Currently, it is estimated that livestock production uses about 10% of annual global water flows (FAO, 2018). This water is used for direct consumption, food production, cleaning, and processing. Actual water requirements vary greatly across species, environment, and operation type, but it is generally accepted that water inputs are greater for livestock than crop production (de Fraiture & Wichelns, 2010; Giller, 2017). This is not to say that animal production should be replaced by crop production however. Ruminants produce protein more efficiently than crops on low-quality land (van Zanten

et al., 2016). Economic outputs are often higher for livestock than crop production (Peden et al., 2000). Livestock also often serve purposes beyond just meat production—particularly in developing areas (Gruber et al., 2009; Schlink et al., 2010). Therefore, understanding how to reduce resource usage including water is critical.

The importance of water usage is highlighted by estimates that by 2025, two-thirds of the world’s population will live in water-scarce environments (UN-Water, 2013). This reduction in water availability will be particularly problematic for developing regions (FAO, 2018). Therefore, the question is now moving from what are the problems to how do we solve them? Eisler et al. (2014) propose numerous methods for increasing sustainability in livestock including raising regionally appropriate animals. Animals that do well in one region are often not well suited for other environments. They posit we must understand which animals perform best in each environment and use that information to create tailored sustainability plans. Many animals have already been identified that are particularly suitable to these challenging environments. In fact, adaptations of “tropical” cattle are very well characterized; however, the genetic controls of many of these adaptations are unknown (Barendse, 2017). Understanding the genetic controls of adaptations could be instrumental in producing animals that are adapted to an environment and highly productive.

Introgression

Introgression is defined as the transfer of genes between species or lines through hybridization and backcrossing. This process is distinct from hybridization in that it requires fertile hybrids that can breed with one or both parental lines. This process can be detrimental to endangered species (Fleming & Godwin, 2008), but is essential for

speciation and maintaining genetic diversity. The maintenance of diversity is particularly important when considering solutions to potential resource shortages. Diverse populations are more likely to produce types and breeds that are adapted to changing environments (FAO, 2018). In fact, introgression has been associated with increased litter size in pigs (Bosse et al., 2014), introducing yellow pigmentation into chickens (Hillman et al., 2008) and may contribute to pest resistance in cattle (Bahbahani et al., 2018). These studies demonstrate some potential benefits of introgressing genes for specific traits onto specific backgrounds.

In the process of introgression there are two parental lines—recurrent and nonrecurrent. The recurrent line is the one bred each generation to the introgressed individual(s). The nonrecurrent line is only used once—for the initial hybridization event. Over time, the recurrent line’s genome becomes more prevalent in the introgressed individuals, but backcross offspring are selected to maintain the trait(s) of interest (Figure 1). Backcrossing is continued until a line exhibits a suitable amount of the parental genome with the trait(s) of interest. At this point the backcross animals are bred *inter se* to produce animals which are homozygous for the trait(s) of interest.

To effectively use introgression, we must understand the traits of interest. Much work has been focused on identifying and understanding introgression signatures in the genomes of domesticated animals including cattle (Bahbahani et al., 2018; Zhang et al., 2018) and sheep (Rochus et al., 2017). These studies help bridge the genotype-phenotype gap but do little to introduce potential solutions for existing or future challenges—such as climate change. Today, work aiming to introduce genes often employs more modern gene-editing

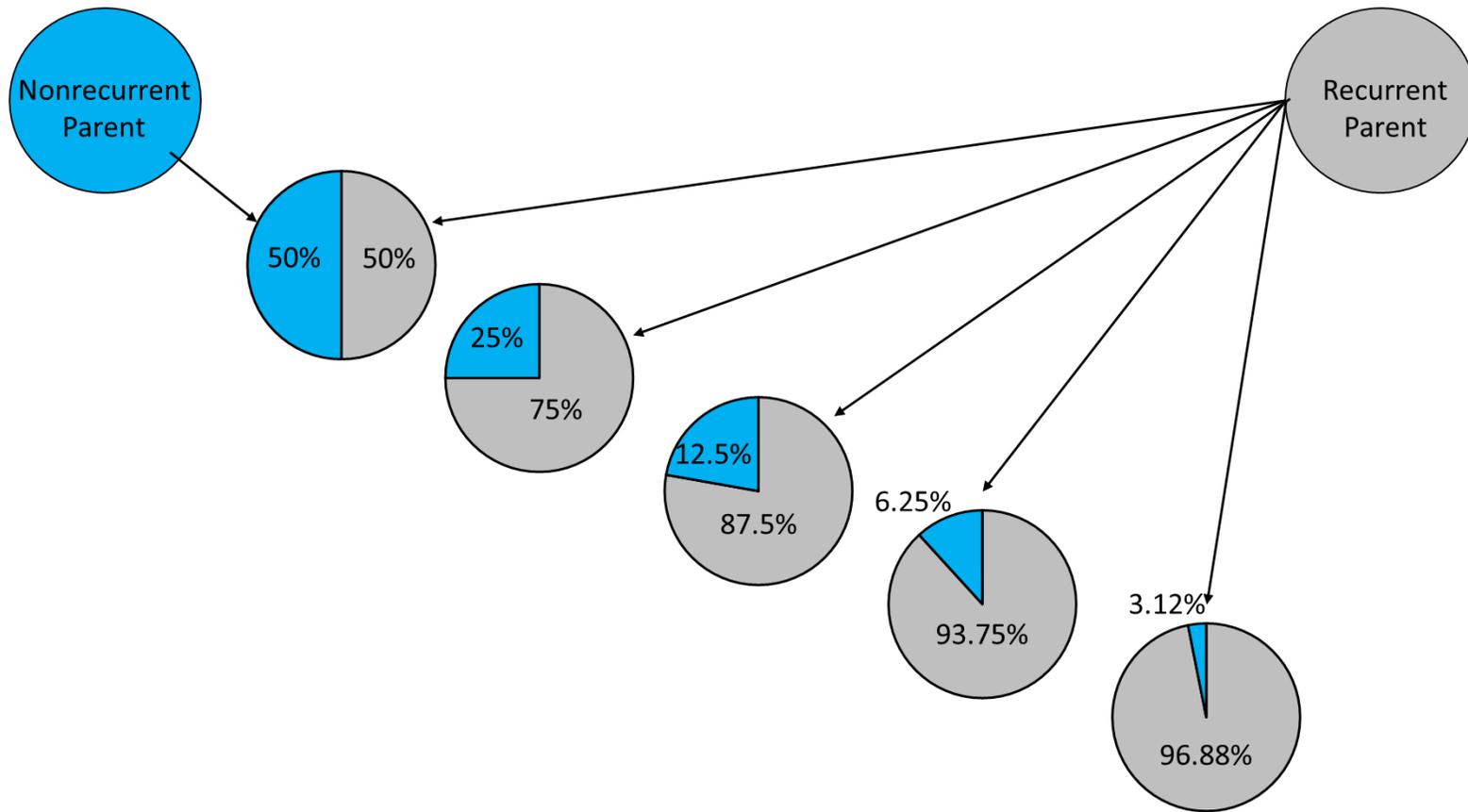


Figure 26. Schematic illustration of expected genomic influence at each generation of backcrossing.

[Figure 1 in chapter]

techniques like CRISPR and TALEN. These techniques allow researchers to efficiently edit an animal's genome by specifically targeting an area of interest and producing homozygous individuals immediately without the need to introduce genes from another species. TALENs have been shown to be especially promising for use in livestock including cattle and swine (Tan et al., 2013). For example, members of the welfare community have voiced concerns about dehorning. In 2016, Recombinetics produced polled Holsteins using TALENs (Carlson et al., 2016) eliminating the need for dehorning. In this work, researchers evaluated genomes for any signs of off-target effects—none were observed. However, consumption of animals produced with these methods is still not federally approved. Instead, the FDA plans to treat gene-edited animal like drugs—a major point of concern for researchers in the field (Maxmen, 2017). Therefore, researchers must continue to refine the technologies and produce evidence to support their benefit. Further, these techniques require knowledge of trait architecture—something still not often well-characterized in quantitative traits—meaning more work must be completed to identify potential new targets for the technology.

Traditionally, introgression studies have focused on introducing single gene traits, but they may be useful for illuminating quantitative trait architecture. Sewall Wright first suggested that these long-term backcross studies could be useful for identifying large effect genes in quantitative traits (Wright, 1952). Later work corroborated that while traditional F2 and single generation backcross (BC1) studies were effective for detecting QTLs they were not effecting for mapping QTLs (Darvasi et al., 1993; van Ooijen, 1992). Finally, in the mid to late-1990s long-term backcrossing studies became a more

commonly supported approach for fine-mapping studies (Hill, 1998; Rance et al., 1997). These studies required that the trait of interest be bred onto an inbred line's genome over multiple generations. This allowed for an increase in recombination with a simultaneous reduction in background noise—both critical for fine-mapping studies.

These studies also require that researchers select for the trait definitively over time. This can be difficult with quantitative traits so some loss of variation is expected (Hill, 1998; Wright, 1952). In plants and animals which have short generation times, selecting animals at the end of a generation is acceptable since it is possible to breed more animals if none fit the criteria. However, for slower growing animals, marker assisted selection (MAS) is often employed. The addition of MAS can also improve selection efficiency in animals such as mice by about two generations (Hospital et al., 1992). Even so however, progress in identifying quantitative trait architecture has been slow (Martin & Jiggins, 2017). Therefore, the purpose of this work was to use mice as a model for livestock to elucidate potential genetic controls of water consumption using a long-term backcross study.

CHAPTER 2. GENETIC, MATERNAL, AND HETEROSIS EFFECTS ON VOLUNTARY WATER CONSUMPTION IN MICE

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Abstract

In standard laboratory conditions, inbred mouse strains with normal kidney function show a four-fold range of daily water consumption. This study uses two strains of inbred mice identified as high and low consumers, their reciprocal F1 crosses, and *inter se* bred F2s. Daily consumption data were collected on 607 animals for four ds during the 4th, 5th, and 6th wks using custom water bottles. Animals were weighed at the beginning of the 4th, 5th, 6th, and 7th wks. Consumption data were corrected for metabolic body weight ($Bwt^{0.67}$) prior to analysis, so units of water consumption are expressed in mL consumed per g of $Bwt^{0.67}$ per d. Variables body weight and water consumption were fitted to a mixed model including the effects of sex, strain, and their interaction with sire within strain fitted as a random effect. Contrasts were designed to test the direct genetic, maternal genetic, individual heterosis, and maternal heterosis effects. An interaction ($P < 0.0001$) was observed between sex and strain so all analyses were conducted separately for each sex. C57Brown/CDJ animals (Brown) consumed more water than C57Black/10J animals (Black) ($P < 0.0001$). A maternal effect ($P = 0.036$, $P = 0.029$) was observed in males at the 4th and 5th wks as F1 animals with a Black dam (F1Black) consumed less than F1 animals with a Brown dam (F1Brown) males. No significant heterosis effect was observed for water consumption. For weight analysis, Black animals were significantly larger than Brown animals at 28 ds (males $P = 0.004$, females $P = 0.026$), but no difference was observed the remainder of the trial. Further, F1Brown females were significantly smaller than F1Black females at 28 ds ($P < 0.0001$) and F1Brown males tended to be smaller than F1Black males ($p = 0.078$). Animals from the reciprocal F1 crosses showed an increase in birth weight ($P < 0.0001$) over pure strains. These strains

form the foundation stock of an experiment designed to isolate genes influencing water consumption by reciprocal backcrossing and selection.

Key Words: direct effects, heterosis effects, maternal effects, mice, water consumption

Introduction

Today's food producers, particularly livestock producers, are facing a unique challenge: produce food which meets the demands of a growing population while maintaining a small environmental footprint (von Braun, 2007; Godfray et al., 2010). This challenge is further complicated by the reduction of available resources due to population increases. It is estimated that by 2025 nearly two-thirds of the world's population will be living under water stress conditions (UN-Water, 2013). With such resource limitations, as well as increases in consumer demands due to economic development, producing enough food to meet these requirements commands the innovation in management and production strategies.

One strategy may be to reduce daily water requirements of animals through selection or genetic modification. While direct water consumption by animals is estimated to be around only 1% of total water usage, it can have major impacts in dry areas or areas where livestock are kept for multipurpose usage (Gruber et al., 2009; Schlink et al., 2010). Furthermore, the ability to identify low consuming animals would allow producers to better select livestock for their specific operation. The usage of a model organism such as mice, with the expectation that genetic control will be conserved between species, may provide insight for larger species.

In standard laboratory conditions, inbred mouse strains with unaltered kidney function show about a four-fold range of daily water consumption (Tordoff et al., 2007).

Using this information, we selected one strain each from the high consuming and low consuming groups—C57 Brown CDJ and C57 Black 10J, respectively—for a long-term study with the objective of isolating genes that control consumption. To gain a better understanding of genetic controls of water consumption during the initial stages of this project, our objective in the present study was to estimate the genetic, maternal, and heterosis effects on voluntary water consumption in these strains of mice.

Materials and Methods

Experimental Animals

Foundation C57BL/10J (Black) and C57BR/CDJ (Brown) were purchased from Jackson Laboratories (Bar Harbor, ME). These strains were chosen based on previously quantified difference (Blacks: $0.35\text{mL/g}^{0.667}/\text{d}$; Browns: $0.80\text{mL/g}^{0.667}/\text{d}$) in water consumption (Bachmanov et al. 2002, Tordoff et al., 2007). These strains also show no difference in kidney function (Thaisz et al., 2012). These animals were bred to establish two single-strain colonies at the University of Missouri-Columbia. Females from each strain were then bred to males of the other strain to produce F1 hybrids. First cross animals with a Brown dam were denoted F1Brown and first cross animals with a Black dam were denoted F1Black to designate maternal environment. F1 animals were then bred *inter se* to produce the F2 generation. Animals in this generation were denoted F2 BLBL if both granddams were Black, F2 BLBR if the maternal granddam was Black, F2 BRBL if the maternal granddam was Brown, and F2 BRBR if both granddams were Brown.

Consumption and weight data were collected on 607 animals: 78 Black, 74 Brown, 96 F1Black, 21 F1Brown, 250 F2BLBL, 55 F2BLBR, 23 F2BRBL, and 10 F2BRBR. Differences in number of animals arose due to breeding challenges with the

Brown strain. Animals were housed in plastic tub containers with corn cob bedding per an ACUC approved protocol 8565. All males were individually housed, breeder females were group housed, and experimental animals were individually housed during water consumption measurements. The temperature was maintained at 24±1°C.

Weight and Consumption Measurement

Animals were weaned at four wks, weighed, and separated into individual cages with custom-built 25-mL serological pipette water bottles (Fig. 1) based upon a previous design (Bachmanov et al., 1996). After a 48-hour acclimation period, consumption measurements were recorded to the nearest 0.1 mL every 24 h for four ds per wk from d 28 to d 49. Spillage from this method does not typically exceed 0.2 mL over 48 h (Bachmanov et al. 2002). Consumption was corrected for body size as described below, averaged for each wk, and reported as average daily consumption (ADC) on a per wk basis. Bottles were checked daily and replaced with full bottles if the water level was at or below 14 mL to prevent bottles from becoming empty. Animals were weighed to the nearest 0.1 g at 28, 35, 42, and 49 d of age.

Statistical Analysis and Selection

Regression analysis of measured water intake on body weight, strain, and sex indicated a significant, positive relationship (Table 1). To correct for the effect of weight, intake was adjusted for metabolic body weight ($Bwt^{0.67}$), by using the equation of Tordoff et al. (2007):

$$\text{Adjusted Intake (mL/g/d)} = \frac{\text{Measured Daily Water}}{\left(\frac{\text{End Weight} - \text{Start Weight}}{7} + \text{Start Weight}\right)^{0.667}}$$

For this correction, animals are weighed at the beginning of each week, the start weight, and at the end of each week, the end weight. Water consumption is thus expressed in ml



Figure 27. Custom-built serological pipette water bottle used to measure daily water consumption of mice. To assure no leakage occurred a minimum head volume of about 3 mL was maintained in each bottle.

[Figure 1 in chapter]

consumed per g of metabolic BW per d. Regression analysis following adjustment resulted in no significant relationship between consumption and $Bwt^{0.67}$ (Table 1).

To elucidate patterns related to the effect of age on consumption, repeated measures analyses were conducted using PROC MIXED in SAS 9.4 software (SAS Institute Inc., Cary, NC, USA) with weight and adjusted consumption designated as the dependent variable in two separate analyses:

$$N_{ijkl} = \mu + \text{strain}_i + \text{sex}_j + wk_k + \text{strain}_i * \text{sex}_j + e_{ijkl}$$

In this model N_{ijkl} is the dependent variable weight or adjusted consumption, μ is the mean, strain_i , sex_j , and $\text{strain}_i * \text{sex}_j$ are designated fixed effects for strain, sex, and the strain*sex interaction respectively, and finally e_{ijkl} is the error term.

To estimate means and differences of ADC, mixed model analyses of weight and adjusted consumption were conducted using PROC MIXED in SAS software. Weight and adjusted consumption were designated as the dependent variables and fitted to a linear mixed model in two separate analyses:

$$N_{ijkl} = \mu + \text{strain}_i + \text{sex}_j + \text{sire}_k (\text{strain})_i + \text{strain}_i * \text{sex}_j + e_{ijkl}$$

In this model N_{ijkl} is the dependent variable weight or adjusted consumption, μ is the mean, strain_i , sex_j , and $\text{strain}_i * \text{sex}_j$ are designated fixed effects for strain, sex, and the strain*sex interaction respectively, $\text{sire}_k (\text{strain})_i$ is the sire within strain random effect, and finally e_{ijkl} is the error term. Contrasts (Table 2) were designed to evaluate direct strain effects (Brown-Black), maternal strain effects (F1Brown-F1Black), individual

Table 5. Regression parameters² of measured water consumption and water consumption corrected for metabolic body weight.

[Table 1 in chapter]

Variable	Water Consumption=Wt+Strain+Sex	Adjusted Water Consumption=Wt+Strain+Sex
Intercept	2.70 (2.58)	0.86 (0.36)
Weight	0.23 (0.12)	0.01 (0.02)
Strain	-3.97 (0.36)	-0.64 (0.05)
Sex	1.50 (0.44)	0.22 (0.06)

² values in bold were significant (P<0.1)

Table 6. Contrast coefficients used to estimate Strain, Maternal, Individual Heterosis, and Maternal Heterosis parameters in the analysis of weight and water consumption.

[Table 2 in chapter]

Effect	C57BR	C57BL10	F1BR	F1BL	F2BRBL	F2BLBR
Strain	0.5	-0.5	0.5	-0.5	0	0
Maternal	0	0	1	-1	0	0
Individual Heterosis	-0.5	-0.5	0.5	0.5	0	0
Maternal Heterosis	-0.25	-0.25	-0.25	-0.25	0.5	0.5

heterosis effects (F1 avg-Black/Brown avg), and maternal heterosis effects (F2 avg-F1s/Pure avg) (Dickerson, 1969; Dickerson, 1973; Koch et al., 1985).

Results

Preliminary analyses indicated a sex*strain effect for water consumption ($P < 0.0001$), but not weight ($P = 0.37$), as well as a significant strain*wk interaction. Due to our focus on water consumption, as well as prior studies noting a sex effect on water consumption (McGivern et al., 1996) we conducted all subsequent analyses separately for each sex and wk. Furthermore, a sex effect on weight in these two strains has been previously identified (Reed et al., 2007).

Weight Analysis

Repeated measures analysis indicated that animals in all strains increased in weight over the three-wk period. This increase was significant (BLBL Males: $P = 0.01$; BLBR Males: $P = 0.01$; BRBR Females: $P = 0.003$) for a few strains (Fig. 2, Table 3) from d 28 to d 35, but then diminished in the following wks (Fig. 3), likely because of slowed growth as the animals reach maturity. A similar growth pattern in mice has been previously observed and tends to be associated with maternal capabilities, resource allotment, and age (White et al., 1970).

Black animals were larger (Males: $P = 0.004$, Females: $P = 0.026$) than Brown animals indicating a strain effect at wk 4 (Table 4), but no difference was observed at the remaining timepoints (Fig. 3, Table 3). This difference may be age related as no difference in the strains was reported by Reed et al. (2007) in animals that were over 8 wks of age. This pattern of weight differences suggests an effect of maternal capability to compensate for birth size during the post-weaning growth period

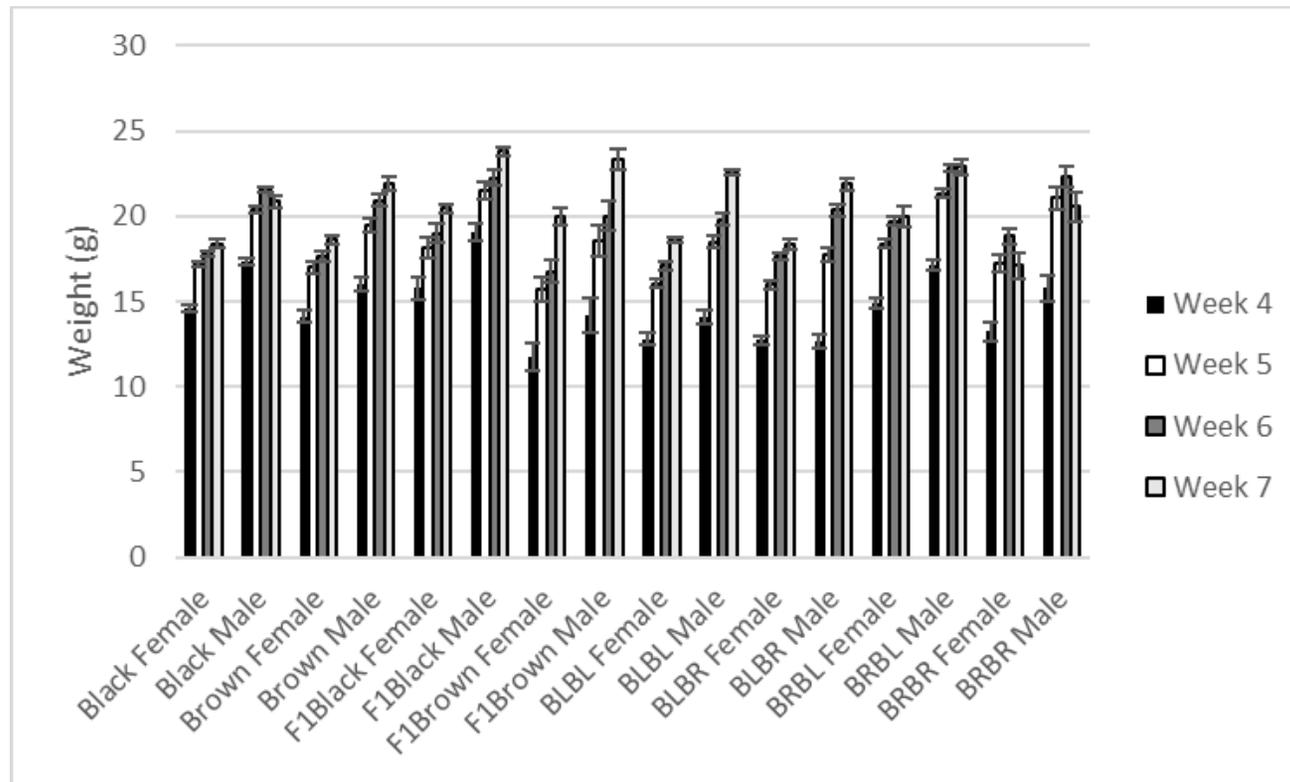


Figure 28. Least squares means SE (g) of body weight by sex and mating group at D 28, 35, 42, and 49.

[Figure 2 in chapter]

Table 7. Least squares means \pm SE (g) of body weight by sex and mating group at D 28, 35, 42, and 49.

[Table 3 in chapter]

Strain & Sex	Week 4 BW \pm SE g	Week 5 BW \pm SE g	Week 6 BW \pm SE g	Week 6 BW \pm SE g
Black Female	14.60 \pm 0.17	17.21 \pm 0.16	17.82 \pm 0.15	18.38 \pm 0.28
Black Male	17.33 \pm 0.19	20.37 \pm 0.17	21.59 \pm 0.16	20.88 \pm 0.34
Brown Female	14.11 \pm 0.37	17.00 \pm 0.34	17.66 \pm 0.31	18.63 \pm 0.25
Brown Male	15.99 \pm 0.40	19.46 \pm 0.37	20.92 \pm 0.34	21.89 \pm 0.38
F1 Black Female	15.77 \pm 0.67	18.11 \pm 0.61	18.98 \pm 0.56	20.47 \pm 0.27
F1 Black Male	19.06 \pm 0.54	21.52 \pm 0.50	22.26 \pm 0.45	23.8 \pm 0.24
F1 Brown Female	11.77 \pm 0.82	15.71 \pm 0.75	16.77 \pm 0.69	19.97 \pm 0.49
F1 Brown Male	14.15 \pm 1.01	18.6 \pm 0.92	20.00 \pm 0.84	23.36 \pm 0.62
BLBL Female	12.80 \pm 0.32	16.06 \pm 0.30	17.11 \pm 0.27	18.62 \pm 0.15
BLBL Male	14.10 \pm 0.40	18.50 \pm 0.36	19.82 \pm 0.33	22.60 \pm 0.16
BLBR Female	12.71 \pm 0.29	16.00 \pm 0.27	17.65 \pm 0.24	18.38 \pm 0.32
BLBR Male	12.67 \pm 0.43	17.75 \pm 0.39	20.34 \pm 0.36	21.87 \pm 0.35
BRBL Female	14.87 \pm 0.30	18.39 \pm 0.28	19.70 \pm 0.26	19.97 \pm 0.58
BRBL Male	17.11 \pm 0.28	21.34 \pm 0.25	22.81 \pm 0.23	22.90 \pm 0.47
BRBR Female	13.24 \pm 0.56	17.28 \pm 0.51	18.82 \pm 0.47	17.08 \pm 0.72
BRBR Male	15.76 \pm 0.71	21.05 \pm 0.65	22.34 \pm 0.60	20.58 \pm 0.89

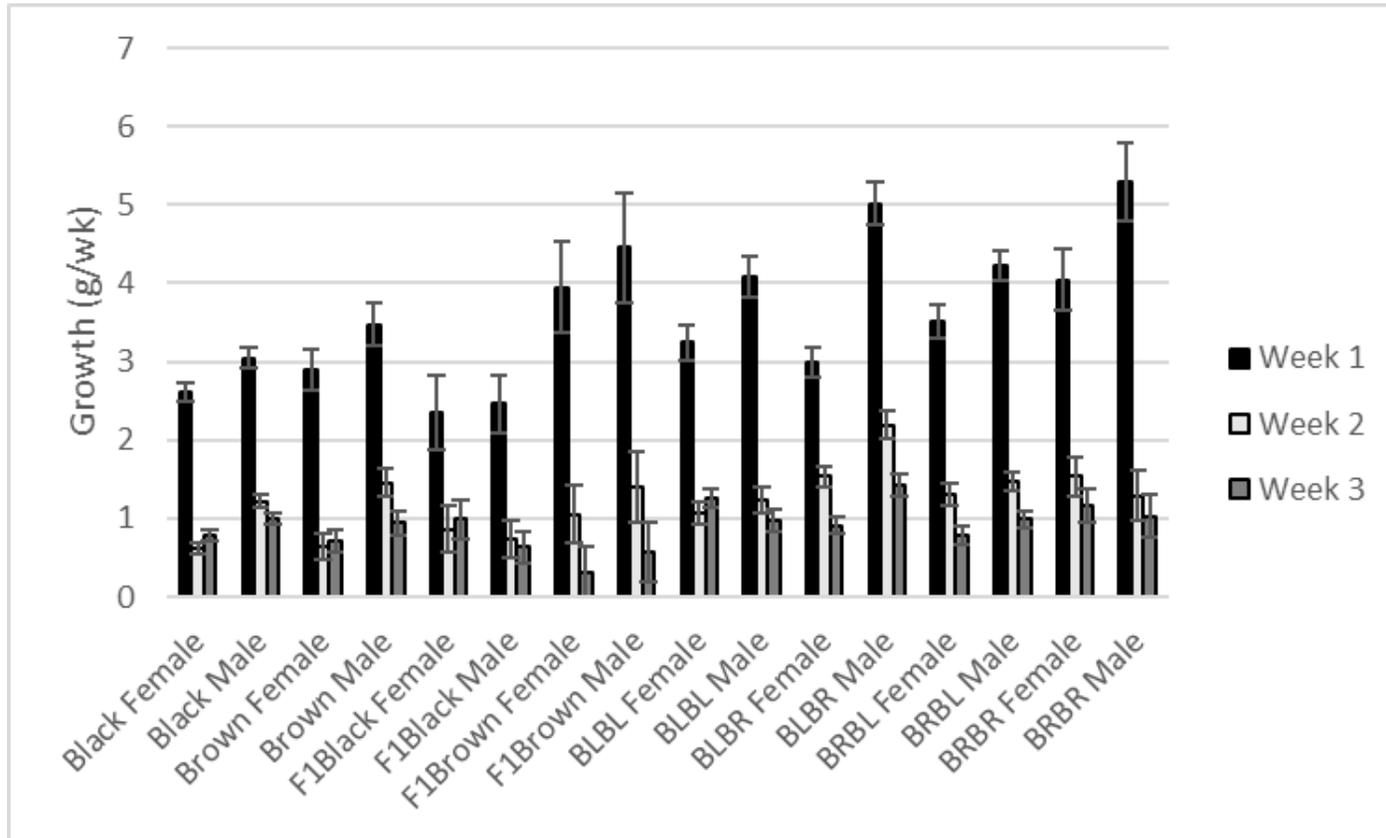


Figure 29. Least squares means of average daily gain \pm SE (g) of each mating group and sex per week of trial period.

[Figure 3 in chapter]

Table 8. Strain, Maternal, Individual Heterosis and Maternal Heterosis estimates SE (g) for weight by sex and mating group at D 28, 35, 42, 49³.

[Table 4 in chapter]

Age (Ds)	Strain Effect, g		Maternal Strain Effect, g		Individual Heterosis Effect, g		Maternal Heterosis Effect, g	
	Male	Female	Male	Female	Male	Female	Male	Female
28	-1.39 (0.48)	-0.86 (0.39)	-1.35 (0.77)	-1.64 (0.64)	3.06 (0.48)	1.30 (0.39)	3.25 (0.42)	1.30 (0.36)
35	-0.52 (0.44)	-0.59 (0.35)	-0.29 (0.70)	-1.12 (0.58)	3.07 (0.44)	1.81 (0.35)	1.87 (0.39)	0.98 (0.33)
42	0.02 (0.40)	-0.17 (0.32)	-0.48 (0.64)	-0.88 (0.53)	2.50 (0.40)	1.88 (0.32)	1.12 (0.35)	0.43 (0.30)
49	0.29 (0.42)	-0.17 (0.34)	-0.44 (0.67)	-0.51 (0.55)	2.20 (0.42)	1.71 (0.34)	0.60 (0.37)	0.00 (0.31)

³ Each value in bold is significant (P<0.05).

(Metcalf and Monaghan, 2001). Further, F1Brown females were smaller ($P < 0.0001$) than F1Black Females (Fig. 3, Table 3) at d 28. F1Brown males showed a trend ($P = 0.078$) for decreased body weight (Fig 3, Table 3) compared to F1Black males. No difference in body weight was observed at any other timepoint again, supporting the compensatory growth theory.

A positive heterosis effect (Table 4) on body weight ($P < 0.0001$), was observed in both sexes at all time points in F1 compared to pure strain animals (Fig. 3, Table 3). This increase was expected based on previous literature regarding size of F1 hybrids in mice due to heterosis effects (Lippman and Zamir, 2006; White et al., 1970). Significant positive maternal heterosis (Males: $P < 0.0001$; Females: $P = 0.0003, 0.0029$) was observed in both sexes at d 28, but only in males ($P = 0.0018$) on d 42 (Table 3). This indicates no long-term effect on weight due to having a hybrid dam.

Consumption Analysis

Repeated measures analyses indicated that most mating groups did not show change in consumption from wk to wk during the trial; however, some exceptions did occur (Fig. 4). F1 females showed significant increases in consumption during the trial, but F1Black males showed a significant decrease in consumption during the same period. This trend became less defined in the F2 generation with males still exhibiting a decrease, but female patterns becoming less consistent (Fig. 4).

Brown animals consumed more ($P < 0.0001$) water than Black animals for all three wks of the trial (Fig. 4, Table 5) indicating a significant strain effect (Table 6). These results confirmed previously observed phenotypes reported by Tordoff et al. (2007). Black females did not consume significantly more than Black males; however, Brown

females consumed significantly more ($P < 0.0001$) than Brown males during wks 5 and 6 (Fig. 4, Table 5). These data supports previous findings of increased consumption in Brown females compared to males (Reed et al., 2007). This observation is further supported by the comparison of growth rate to consumption (Figs. 5,6,7) where Brown animals consistently exhibit the highest consumption and Black animals the lowest.

Maternal effect analysis indicated that F1Brown males consumed significantly more ($P = 0.036$, $P = 0.029$) water than F1Black males (Fig. 4, Table 5) during wks 4 and 5; F1 females showed no difference in water consumption (Table 5), indicating a significant maternal effect only for males (Table 6). This increase in water consumption only observed in males may be related to maternal environment prior to birth. It has been observed that litters born to Brown females do tend to skew toward female pups (Hansen and Judge, 1973).

At wk 6 a significant decrease ($P < 0.0001$) in consumption was observed in both sexes of F2 animals (Fig. 4, Table 5). This may be related to an increased maturation rate in F2 animals compared to pure strain animals which can affect consumption (Maxwell and Kleeman 1980; McGivern et al., 1996).

Discussion

In this study we have estimated genetic, maternal, and heterosis effects on voluntary water consumption as well as body weight in two inbred strains of mice. Body weight was added to the study to reduce confounding caused by size—larger animals are likely to consume more water.

Our data indicate Brown animals are smaller than Black animals at 4 wks, but no difference is observed by wk 5. This period of compensatory growth

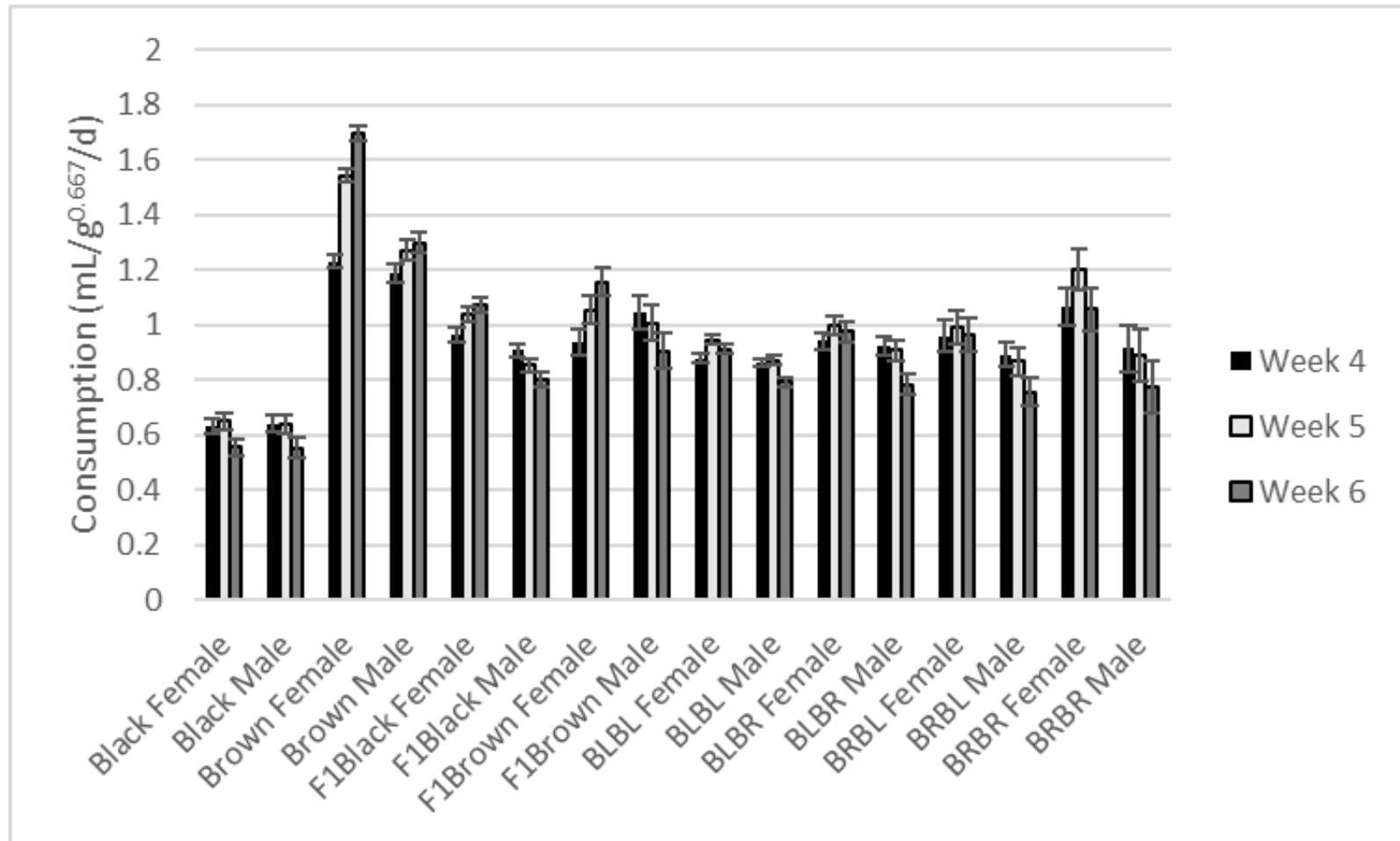


Figure 30. Least squares means \pm SE of water consumption by mating group and sex per week of trial period.

[Figure 4 in chapter]

Table 9. Least square means \pm SE of water consumption by mating group and sex per week of trial period.

[Table 5 in chapter]

Strain & Sex	Week 4	Week 5	Week 6
	Consumption \pm SE mL/g0.667/d	Consumption \pm SE mL/g0.667/d	Consumption \pm SE mL/g0.667/d
Black Female	0.63 \pm 0.03	0.65 \pm 0.03	0.55 \pm 0.03
Black Male	0.64 \pm 0.03	0.64 \pm 0.03	0.55 \pm 0.04
Brown Female	1.23 \pm 0.02	1.54 \pm 0.03	1.70 \pm 0.03
Brown Male	1.19 \pm 0.03	1.27 \pm 0.03	1.30 \pm 0.04
F1 Black Female	0.97 \pm 0.03	1.04 \pm 0.03	1.07 \pm 0.03
F1 Black Male	0.91 \pm 0.02	0.85 \pm 0.03	0.80 \pm 0.03
F1 Brown Female	0.94 \pm 0.05	1.05 \pm 0.05	1.16 \pm 0.05
F1 Brown Male	1.05 \pm 0.06	1.01 \pm 0.07	0.91 \pm 0.07
BLBL Female	0.88 \pm 0.01	0.95 \pm 0.02	0.91 \pm 0.02
BLBL Male	0.86 \pm 0.02	0.87 \pm 0.02	0.79 \pm 0.02
BLBR Female	0.94 \pm 0.03	1.00 \pm 0.03	0.97 \pm 0.03
BLBR Male	0.92 \pm 0.03	0.91 \pm 0.04	0.78 \pm 0.04
BRBL Female	0.96 \pm 0.06	0.99 \pm 0.06	0.96 \pm 0.06
BRBL Male	0.89 \pm 0.05	0.87 \pm 0.05	0.74 \pm 0.05
BRBR Female	1.07 \pm 0.07	1.20 \pm 0.08	1.06 \pm 0.07
BRBR Male	0.91 \pm 0.09	0.89 \pm 0.09	0.78 \pm 0.09

Table 10. Strain, Maternal, Individual Heterosis and Maternal Heterosis estimates SE (mL/g/wt^{0.67}) for water consumption by sex and mating group per week of the trial period.⁴

[Table 6 in chapter]

Wk	Strain Effect, (mL/g/wt ^{0.67})		Maternal Strain Effect, (mL/g/wt ^{0.67})		Individual Heterosis Effect, (mL/g/wt ^{0.67})		Maternal Heterosis Effect, (mL/g/wt ^{0.67})	
	Male	Female	Male	Female	Male	Female	Male	Female
4	0.34 (0.04)	0.28 (0.03)	0.14 (0.07)	-0.03 (0.05)	0.06 (0.04)	0.02 (0.03)	-0.01 (0.03)	0.03 (0.03)
5	0.39 (0.04)	0.45 (0.04)	0.15 (0.07)	0.02 (0.06)	-0.03 (0.04)	-0.05 (0.03)	-0.07 (0.04)	-0.06 (0.03)
6	0.43 (0.04)	0.61 (0.04)	0.11 (0.07)	0.09 (0.06)	-0.07 (0.04)	-0.01 (0.04)	-0.15 (0.04)	-0.15 (0.03)

⁴ Values in bold are significant (P ≤ 0.0001).

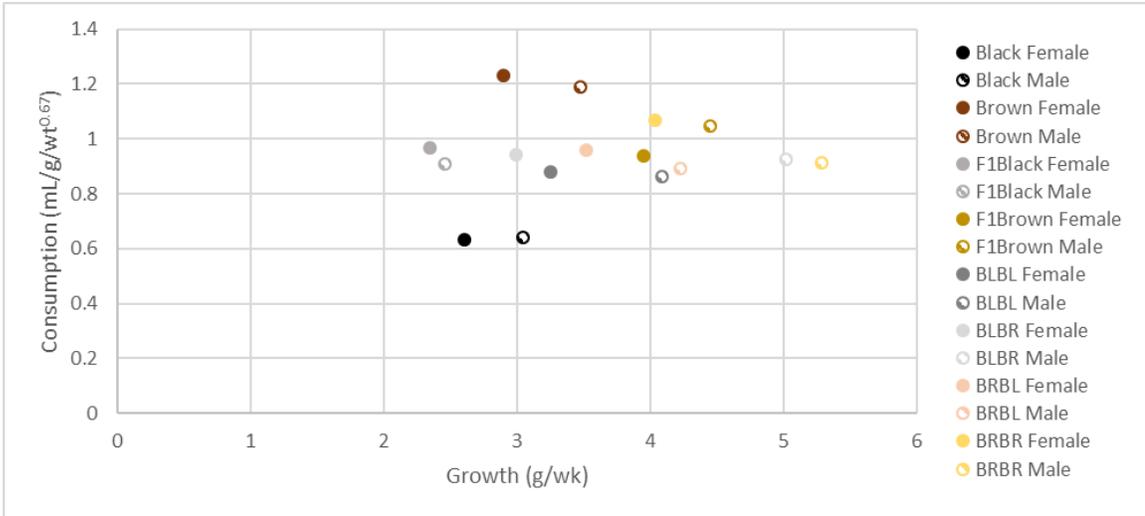


Figure 31. Scatterplot showing relationship between growth (g/wk) and consumption (mL/g/wt^{0.67}) for Week 4.

[Figure 5 in chapter]

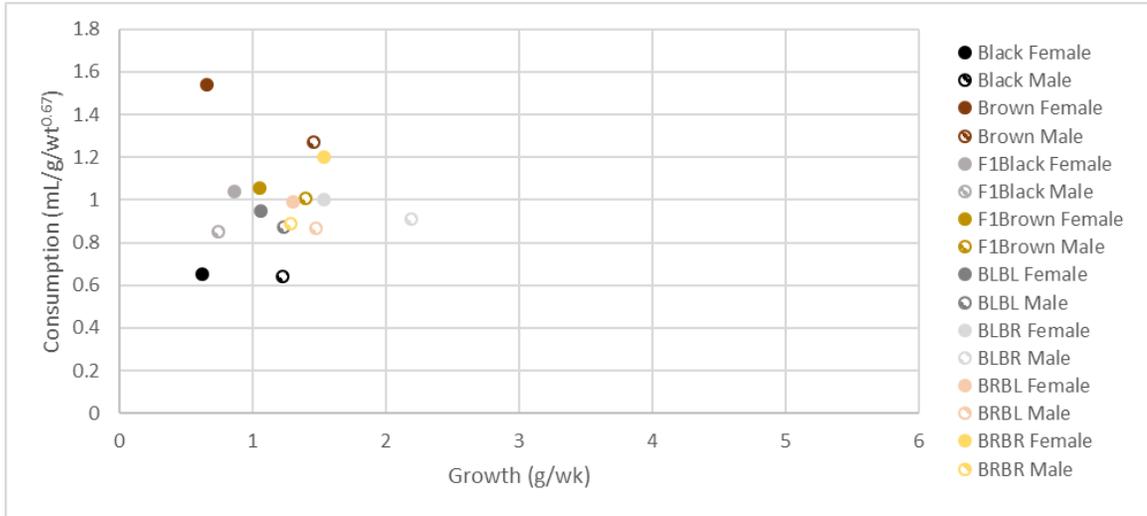


Figure 32. Scatterplot showing relationship between growth (g/wk) and consumption (mL/g/wt^{0.67}) for Week 5.

[Figure 6 in chapter]

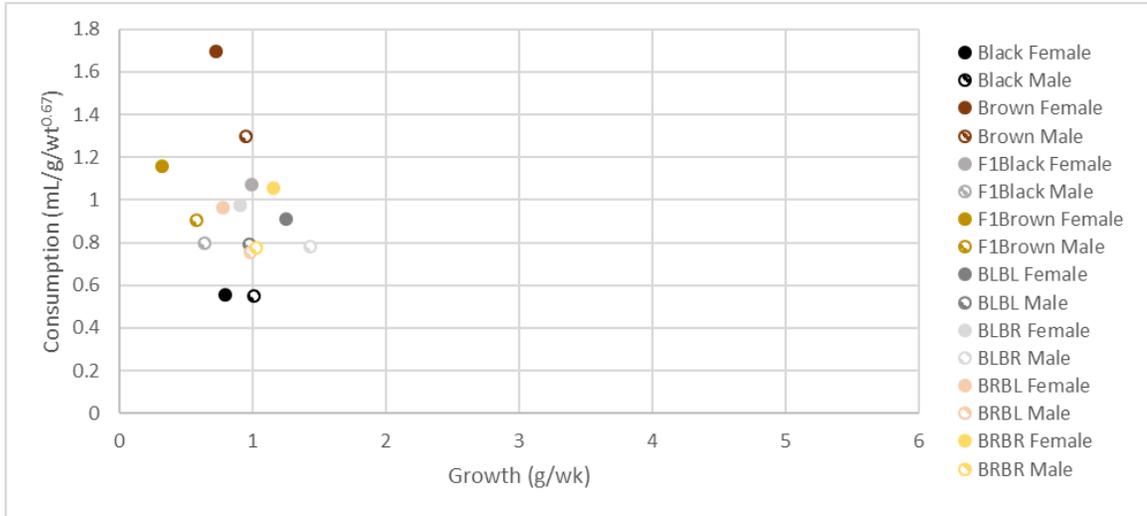


Figure 33. Scatterplot showing relationship between growth (g/wk) and consumption (mL/g/wt^{0.67}) for Week 6.

[Figure 7 in chapter]

(Metcalf and Monaghan, 2001) likely occurs at this point due to pups no longer needed to compete for resources with their littermates. Previous work has shown poor reproductive performance in the Brown strain (Hansen and Judge, 1973). Further, unpublished observations from this study show a decreased maternal capability in the Brown strain. About two-thirds of litters are lost due to abandonment or cannibalization. Abandoned litters tend to have mothers who are producing, but not letting down milk. Litters that do survive to weaning tend to be much larger than black litters. These differences are certainly reflective of the resource allotment limitations observed by White (1970).

On average F1 animals have significantly heavier body weights than pure strain animals at all time points due to heterosis. Previous research in a number of species, including mice, have identified increases in body size and weight in the F1 generation as a result of heterotic effects (Bhuvanakumar et al., 1985; Gordon E. Dickerson, 1973; Koch et al., 1985; Kurnianto et al., 1998; White et al., 1970). Interestingly however, observations at weaning in F1 animals further supports the theory of poor mothering with F1Brown animals being smaller than F1Black animals. Finally, F2 animals with an F1 dam were significantly larger at d 28 and d 35 in both sexes and d 42 in males. However, by d 49 neither sex is significantly larger than the pure strain animals. This indicates no long-term effects on body weight as a result of a hybrid dam; something previously identified in cattle as well (Gregory et al., 1987).

Our analysis of water consumption supports previous work showing a significant difference in consumption between the Black and Brown strains (Bachmanov et al., 2002) at all time points. We also confirmed a previously reported difference in

consumption between sexes in the Brown strain (McGivern et al., 1996; Tordoff et al., 2007). The mechanism of this difference is not fully understood, but may be related to differences in hypothalamic production of vasopressin or oxytocin (Cooper and McGivern, 1983). Further work in Browns have also shown an increase in sodium consumption (Bachmanov et al., 2002) which has been associated with oxytocin deficiency (Puryear et al., 2001). This deficiency may also explain the poor maternal care observed in this study and Hansen (1973).

No difference between F1 water consumption and pure strain consumption was observed indicating no individual heterosis effect on water consumption. However, a significant increase in consumption between F1Brown and F1Black males was identified indicating a potential maternal effect. However, no such difference was observed in female F1s. Since this difference was only observed in males it may indicate a greater effect of maternal care levels on males than females which has been shown previously (Stinson, 1985; Trivers & Willard, 1973). Therefore, this difference in consumption likely occurs due to the compensatory growth occurring during this period (Metcalf and Monaghan, 2001) as evidenced by the lower weaning weights of F1Brown males rather than a maternal genetic effect. A decrease in consumption was observed at wk 6 in F2 animals compared to pure strain animals. It is possible this is a maternal heterosis effect as trends for decreased consumption were observed at all timepoints but determining this would require a larger number of animals. With our current results, this is more likely related to an increased maturation rate in F2 animals compared to pure strain animals which can affect consumption as body weight tends to be a lower percentage of water at maturity (Maxwell and Kleeman, 1980; McGivern et al., 1996). In

conclusion, water consumption shows genetic effects due to strain, but no other effects.

There does seem to be an effect related to dam maternal care levels however, particularly in the males. To better elucidate these patterns conducting this experiment with two strains that have a similar level of maternal care would be useful.

CHAPTER 3. GENETIC BASIS OF VOLUNATRY WATER CONSUMPTION IN TWO DIVERGENTLY SELECTED STRAINS OF INBRED MICE

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Abstract

Background

Water availability is a global concern. Currently, many efforts to reduce water usage are underway. We explore the possibility of using genetic selection to identify genes associated with low water consumption. Under the premise of genetic control being conserved across species, we designed a long-term backcross study to isolate genes that control water consumption in two divergently selected strains of mice. Inbred mouse strains with normal kidney function show a substantial difference in daily water consumption across strains. This study uses two strains of inbred mice C57BR/CDJ (BR) which are high consumers and C57BL/10J (BL) which are low consumers, their reciprocal F1 crosses, *inter se* bred F2s, and backcrosses produced by breeding high consuming F2 animals to the low consumer parent strain and low consuming F2 animals to the high consuming parent strain. Consumption was corrected for body prior to analysis. The effective number of genes controlling water consumption was estimated using the Castle-Wright estimator. Additive and dominance genotypic values as well as the degree of dominance were calculated using estimated strain means.

Results

According to Castle-Wright a minimum of ten factors were estimated to affect the difference in consumption across the two strains. Between seven and eight are expected to be high effect factors. Using the Zeng adjustment, we estimate the potential of 30-40 factors affecting the difference in consumption. A negative degree of dominance indicated the BL strain has more dominant factors.

Conclusions

These number of genes affecting consumption between these two strains was surprisingly high but may be related to several sources of variation present in the BR strain. This

information leads us to suggest replacing the C57BR/CDJ with a less variable strain such as C57BL/6J.

Keywords: Water consumption; divergent selection; mice; C57BL/10J; C57BR/CDJ

Background

It is estimated that by 2025 more than two-thirds of the world's population will be living in a water-scarce environment (UN-Water, 2013). The issue is intensified by increased demands for water due to a growing population, changing diets, and development (de Fraiture & Wichelns, 2010; Godfray et al., 2016; Schlink et al., 2010). This shortage demands innovative techniques for reducing water usage. While direct consumption of water by livestock only accounts for about 1% of water usage (Maupin et al., 2010) there is certainly merit in its reduction. This is particularly true in developing countries, such as those in northern Africa, where water is scarce and demand for animal products is on the rise (Allan, 2001). These areas need animals that can consume limited amounts of water and still show maximized growth and meat production. There is also a need for animals to perform draught work as crop production increases in developing countries (Schlink et al., 2010). Again, animals that are less affected by water scarcity would certainly be beneficial.

Many animals already show adaptations that make them more suitable to these challenging environments. In fact, adaptations of “tropical” cattle are very well characterized however the genetic controls of many of these adaptations are unknown (Barendse, 2017). Under the premise of genetic control being conserved across species, we designed a long-term backcross study to isolate genes that control water consumption in two divergently selected strains of mice.

In standard laboratory conditions, inbred mouse strains with unaltered kidney function show about a four-fold range of daily water consumption (Tordoff et al., 2007). Using this information, we selected a high-consuming strain and a low-consuming strain. The objective of the present study is to estimate the effective gene number controlling the difference in consumption between these two strains and estimate additive and genotypic values for the trait.

Methods

Experimental Animals

Foundation C57BL/10J (Black) and C57BR/CDJ (Brown) were purchased from Jackson Laboratories (Bar Harbor, ME). These strains were chosen based on previously quantified difference (Blacks: $0.35\text{mL/g}^{0.667}/\text{d}$; Browns: $0.80\text{mL/g}^{0.667}/\text{d}$) in water consumption (Bachmanov et al.; 2002, Tordoff et al., 2007). These strains show no difference in kidney function (Thaisz et al., 2012). These animals were bred to establish two single-strain colonies at the University of Missouri-Columbia. In accordance with the suggested design for diallel crosses (Griffing, 1956) females from each strain were bred to males from the opposite to produce F1 reciprocal crosses. F1 animals were then bred *inter se* to produce the F2 generation. To produce backcross animals the two highest consuming and two lowest consuming male, F2 animals were selected for breeding. They were then bred to the opposite parental strain—high consumers to Blacks and low consumers to Browns. Backcrosses produced from the high consumer were referred to as High Backcross 1s (HB1s). Those from the low consumer were referred to as Low Backcross 1s (LB1s).

Consumption and weight data were collected on 1001 animals: 68 Black, 81 Brown, 117 F1, 338 F2, 129 HB1, and 115 LB1. Animals were housed in plastic tub

containers with corn cob bedding per ACUC approved protocol 8565. All males were individually housed, breeder females were group housed, and experimental animals were individually housed during water consumption measurements. The temperature was maintained at $24 \pm 1^\circ\text{C}$.

Weight and Consumption Measurement

Animals were weaned at four wks, weighed, and separated into individual cages with custom-built 25-mL serological pipette water bottles based upon a previous design (Bachmanov et al., 1996). To see a more specific description of data collection methods refer to Haag et al. (2018).

Statistical Analysis

Regression analysis of measured water intake on body weight, strain, and sex indicated a significant, positive relationship. To see more specific information about the weight correction please see Haag et al. (2018). Only adjusted consumption was evaluated in this study because weight did not meet the divergency requirements for further analysis.

To estimate means, variances, and standard errors mixed model analysis of adjusted consumption were conducted using PROC MIXED in SAS software. Adjusted consumption was designated as the dependent variables and fitted to a linear mixed model in the analysis:

$$N_{ijkl} = \mu + \text{strain}_i + \text{sex}_j + \text{sire}_k(\text{strain}_l) + \text{strain}_i * \text{sex}_j + e_{ijkl}$$

In this model N_{ijkl} is the dependent variable, adjusted consumption, μ is the mean, strain_i , sex_j , and $\text{strain}_i * \text{sex}_j$ are designated fixed effects for strain, sex, and the strain*sex

interaction respectively, $sire_k$ (strain)_i is the sire within strain random effect, and finally e_{ijkl} is the error term.

To estimate effective gene number, we employed the Castle-Wright estimator (Castle, 1921; Cockerham, 1986; Lande, 1981; Wright, 1968):

$$n_e = \frac{(\bar{z}(P_1) - \bar{z}(P_2))^2 - \text{Var}[\bar{z}(P_1)] - \text{Var}[\bar{z}(P_2)]}{8 * \sigma_S^2}$$

In this equation N_e is the estimated number of factors affecting a trait of interest, $\bar{z}P_1$ and $\bar{z}P_2$ are the observed means of the trait for each parental strain, $\text{Var}[\bar{z}P_1]$ and $\text{Var}[\bar{z}P_2]$ are the sampling variances for each parental strain estimated by squaring observed standard errors, and σ_S^2 is the estimated segregation variance for each trait. We will describe segregation variance estimation later in this section. The number of large effect factors was estimated using the square root of the variance of n_e .

To yield a less biased estimation we then used our calculated N_e to evaluate in Zeng's adjustment equation (Zeng, 1992):

$$n = \frac{2\bar{c}n_e + C_\alpha(n_e - 1)}{[1 - n_e(1 - 2\bar{c})]}$$

To evaluate assumptions associated with the Castle-Wright Estimator we evaluated adjusted consumption data for epistasis and additivity. To test for epistasis, we used the following equation (Lynch & Walsh, 1998):

$$\Delta = z(F_2) - \left(\frac{z(P_1) + z(P_2)}{4} + \frac{z(F_1)}{2} \right)$$

Δ represents the epistatic estimate, $z(F_2)$, $z(P_1)$, $z(P_2)$, and $z(F_1)$ represent the observed line means for the F2, Black, Brown, and F1 animals respectively. Since the observed Δ

was not zero for the dataset we estimated the sampling variance of Δ with the following equation:

$$\text{Var}(\Delta) = \text{Var}[z(F_2)] + \frac{\text{Var}[z(F_1)]}{4} + \frac{\text{Var}[z(P_1)] + \text{Var}[z(P_2)]}{16}$$

$\text{Var}(\Delta)$ is the sample variance for estimated Δ value, $\text{Var}[z(F_2)]$, $\text{Var}[z(F_1)]$, $\text{Var}[z(P_1)]$, and $\text{Var}[z(P_2)]$ are the observed sampling variances for F2, F1, Black, and Brown animals respectively. The ratio of $|\Delta|/\sqrt{\text{Var}(\Delta)}$ then provides a t-test for evaluation of significance.

To evaluate the fit of the additivity model, we used the Joint-Scaling test (Cavalli, 1952; Gale et al., 1977; Mather & Jinks, 1971) which was designed to evaluate the increased variance observed in the F2 generation. The test begins by fitting data to the simplest, additive model:

$$M = \begin{bmatrix} 1 & 1 \\ 1 & -1 \\ 1 & 0 \\ 1 & 0 \\ 1 & 0.5 \\ 1 & -0.5 \end{bmatrix}$$

Matrix M represents the coefficients of effects for μ and α_c for Brown, Black, F1, F2, HB1, and LB1, respectively. A chi-squared test ultimately determines the adequacy of the model for the data.

There are several methods to estimate segregation variance however these methods can produce highly variable results (Lande, 1981; Lynch & Walsh, 1998). For this reason, we chose to use the Least-Squares analysis method (Lynch & Walsh, 1998):

$$M = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0.5 & 0.5 & 0 \\ 0.5 & 0.5 & 1 \\ 0.75 & 0.25 & 0.5 \\ 0.25 & 0.75 & 0.5 \end{bmatrix}$$

The matrix M contains the coefficients of the variance components for Black, Brown, F1, F2, HB1, and HB2 respectively. Iterative analysis eventually results in the final least-squares parameter estimates for $\sigma^2(P_1)$, $\sigma^2(P_2)$, and σ_s^2 .

Additive and dominance genotypic values were calculated in our population with means from Black, Brown, and F1 animals (Falconer & MacKay, 1996). To determine the additive genotypic value (a), we used the equation:

$$a = \frac{\mu_{P2} - \mu_{P1}}{2}$$

In this equation, the parental line with the highest phenotypic value should be first in the numerator, hence why we subtract P1 from P2, or Black from Brown. To estimate the dominance genotypic value (d), we used the following equation:

$$d = \mu_{F1} - \frac{\mu_{P1} + \mu_{P2}}{2}$$

In this equation, the mean of the parental strains is subtracted from the mean of the F1 strain. To estimate the degree of dominance we divided the dominance value by the additive value.

Results

A significant sex*strain interaction was observed in the dataset. This previously observed interaction (Haag et al., 2018; McGivern et al., 1996; Reed et al., 2007), was accounted for by analyzing each sex separately for all analyses in the study. Brown

animals consumed more water ($P < 0.0001$) than Black animals (Table 1). Significant difference in phenotype between the parental lines is a requirement for the Castle-Wright Estimator. F1 animals had significantly higher consumption but were much closer to that of the Black animals (Table 1). F2 animals showed a range of phenotypes encompassing both parental phenotypes as well as higher variance than the F1 animals. However, the variance in the F2 was still not as high as that observed in the Brown animals. In fact, observed variance in Brown animals was higher than that observed in any other line (Table 1). Consumption means for backcross lines trended toward their respective parental strain. Variances also trended toward parental strains with LB1 animals having higher variance than F2s and HB1s lower than F2s.

Prior to factor number estimation, we evaluated the epistatic, additive, and dominance effects to determine how well the data fit the assumptions for Castle-Wright. Both sexes showed significant indication ($P < 0.0001$) of epistatic effects (Table 2) indicating a violation of assumptions that would minimize the number of factors identified. Both sexes were however, adequately fit by the additive model as evidenced by the significant chi-square value (Table 2). Degree of dominance estimations indicated that Black alleles are dominant over Brown alleles in this cross.

Segregation variances (σ_s^2) determined using Least Squares were used to estimate factor number (n_e). The estimated factor number was higher in females than males, which indicates some factors may be X-linked which causes an overestimation in females and an underestimation in males (Otto & Jones, 2000). The square roots of the variances were used to estimate the number of large effect factors for each sex. Finally, n_e was

Table 11. Number of animals per sex and strain, least squares means + SE (mL/g/wt^{0.67}) of each sex and strain, and the estimated variance for each sex and strain.

[Table 1 in chapter]

Line	N	$\mu \pm SE$	σ^2
Black Female	40	0.6189 \pm 0.0385	0.0072
Black Male	28	0.5819 \pm 0.0419	0.0086
Brown Female	53	1.4586 \pm 0.0503	0.0805
Brown Male	28	1.1362 \pm 0.0443	0.0651
F1 Female	56	1.0344 \pm 0.0217	0.0193
F1 Male	61	0.8743 \pm 0.0216	0.0103
F2 Female	179	0.9635 \pm 0.0124	0.0289
F2 Male	159	0.8756 \pm 0.0130	0.0157
HB1 Female	72	0.7406 \pm 0.0316	0.0202
HB1 Male	57	0.6903 \pm 0.0322	0.0111
LB1 Female	64	0.9335 \pm 0.0306	0.0300
LB1 Male	51	0.9236 \pm 0.0329	0.0200

Table 12. Significance test values for epistasis and additivity by sex, estimated degree of dominance by sex.

[Table 2 in chapter]

	Male	Female
Epistasis Test (t-value)	15.64	127
Additivity Test (χ^2)	0.44	4.00
Degree of Dominance	-0.593	-0.010

used to estimate a more unbiased number of factors (n) (Table 3). Again, differences in effective factor number and n are likely related to estimation biases based on sex.

We also conducted a similar analysis on weight data however we observed a negative value for the Castle-Wright Estimator. This was likely due to the strains not being differentiated enough for weight. This lack of divergence was expected as we had selected for similarly sized animals. We did this to reduce the number of potential factors affecting the difference in consumption across the two strains.

Discussion

Our results indicate many genes control the difference in water consumption between these two strains. In fact, our estimation is likely minimized due to violations of Castle-Wright estimator assumptions. To produce an unbiased prediction with the Castle-Wright estimator several assumptions must be met (Castle, 1921; Wright, 1968):

- 1) All alleles increasing the value of the phenotype are fixed in one line and all those that lower it are fixed in the other line.
- 2) Allelic effect differences are equal at all loci.
- 3) All loci are unlinked.
- 4) All alleles interact additively—no dominance or epistasis.

To evaluate our data for violations we tested for epistasis, dominance, and the fit of the additivity model. Expectedly, our data showed significant epistatic effects due to the quantitative nature of the trait. We also observed dominance effects that moved the F1 and F2 phenotypes more near the Black parent than the mid-parent value. However, Joint-Scale Testing indicated our data was adequately fit by the additive model indicating we could proceed with our analysis using the Castle-Wright Estimator. It should be noted though, research (Huang & Mackay, 2016) has indicated that data, regardless of genetic architecture, can typically be fitted to the additive model. Previous comparisons of

Table 13. Estimated segregation variance by sex, estimated number of factors using the Castle-Wright estimator by sex, estimated number of large effect factor by sex, and estimated number of factors using the Zeng adjustment by sex.

[Table 3 in chapter]

	σ_S^2	n_e	Large Effect Factors	n
Male	0.00372	10.20	6.99	29.17
Female	0.00681	12.87	8.10	43.21

Castle-Wright Estimators and Quantitative Trait Loci analyses have yielded similar results indicating the robustness of the estimator reducing concerns about the additivity of the data (Wu et al., 1997).

The number of genes controlling the difference in consumption may be surprisingly high from two closely related strains (Beck et al., 2000). However, previous work has indicated higher than expected genetic variance and divergence in the strains, particularly in regard to copy number variation (Cutler et al., 2007). Further, the Brown strain has been noted for its high degree of genetic distinctiveness potentially related to mutation rate (Taylor, 1972) as well as a higher level of haplotypic introgression than typically observed in inbred strains (Yang et al., 2011). Regarding water consumption specifically, previous research has indicated differential androgen regulation in males (Melanitou et al., 1987); however no differences in androgen receptor or affinity (Kemp & Drinkwater, 1989). Brown females have also been noted for lowered ovarian hormone production (Maronpot, 2009) which can increase water intake (McGivern et al., 1996; Tarttelin & Gorski, 1971). This level of phenotypic diversity in the strain could explain the high levels of variance observed in the Brown strain.

Conclusions

In conclusion, the difference in water consumption between these two strains is controlled by many genes. This indicates a requirement for a very high number of animals to conduct a QTL analysis. It may be advisable to instead evaluate this trait using less genetically diverse strains such as C57L/J and C57BL/10J which still have a sufficient difference in water consumption (Tordoff et al., 2007).

PART II SUMMARY

Estimated Gene Effects

In our study, we aimed to identify genetic control of and demonstrate the potential for introgression of genes associated with reduced water consumption in mice. Ultimately, we estimated that the difference in water consumption between the C57BL/10J (BL) and the C57BR/CDJ (BR) was controlled by many genes. While this impacted our ability to accurately select for the trait, it did not eliminate the possibility of at least partial introgression. To explore this prospect, we determined it would be useful to quantify predicted effect of each gene based on effect size. This would allow us to estimate how many genes were potentially still segregating in the population after the second generation of backcrosses.

To do this we first estimated the mean and standard deviation of consumption using Proc Means in SAS 9.4 for all the BL, BR, F1, F2, first-generation backcrosses (BC1), and second-generation backcrosses (BC2). BC1 and BC2 were further split into high backcross (HB) and low backcross (LB) lines. Since our previous work has identified an effect of sex on this trait each sex was evaluated separately. Based on consumption data HB animals did retain some level of increased consumption while LB animals did not retain the phenotype (Figures 1,2). We presume the phenotype was lost in LB animals due to insufficient numbers of animals. While this was somewhat disappointing since we were certainly more interested in genes that reduce consumption we moved forward with the analysis.

We used these means to estimate effect size between strains of interest. We opted to use Glass' delta due to the large standard deviation observed in the BR animals. To

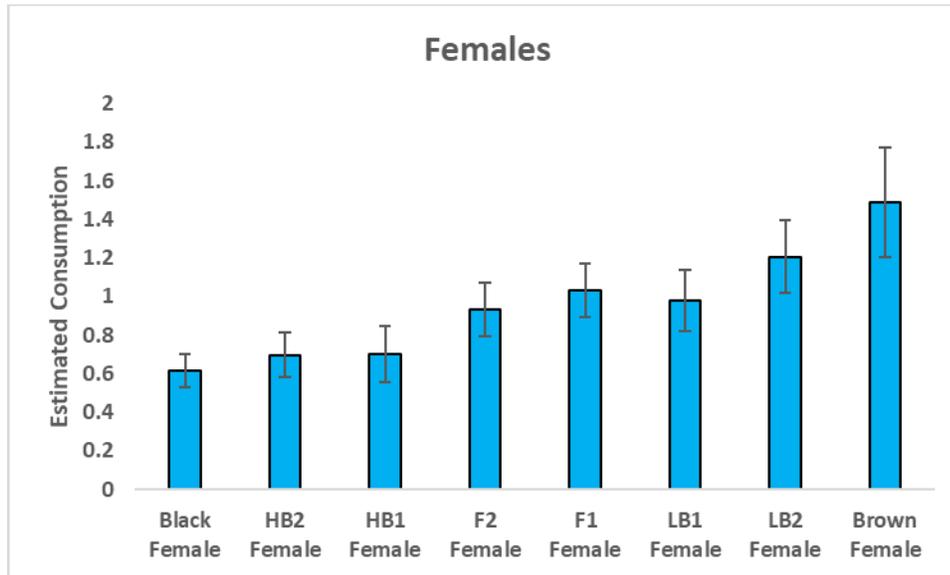


Figure 34. Mean \pm SD of average water consumption for female mice from 4 to 6 weeks of age.

[Figure 1 in chapter]

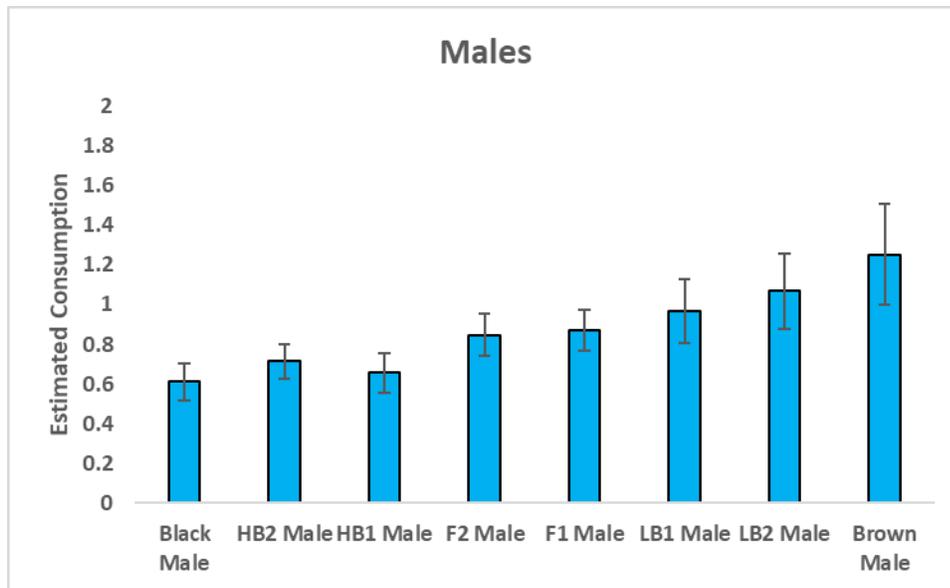


Figure 35. Mean \pm SD of average water consumption for male mice from 4 to 6 weeks of age.

[Figure 2 in chapter]

begin we evaluated the effect size between BL and BR animals. Using this calculation, we estimated a female effect of -3.085 SD and a male effect of -2.516 SD. Based on our predicted gene number we estimated each gene has an effect of -0.237 SD in females and -0.194 SD in males (Table 1).

To estimate how many genes may still be segregating in our population we first estimated the effect size between HB1 and BL animals then HB2 and BL animals. We did not estimate effect sizes for LB and BR animals due to the high variance observed in the BR strain. For BL and HB1 animals we observed small to moderate effects of -0.620 SD in females and -0.450 SD in males (Table 2). In BL and HB2 animals we observed moderate to large effects of -0.726 SD in females to -1.167 SD in males (Table 2).

Based on estimated effect size for BL and HB male animals we determined about 6 genes were still segregating in population. This is supported by probability estimates for producing an animal that is heterozygous at all genes of interest considering the number of males produced each generation in our study. We bred 159 F2 males, based on probability this gave us the power to select at least one individual that was heterozygous for between 7 and 8 genes (Table 3). These estimates would also support our supposition that we did not produce enough animals to capture both the high and low phenotype. It is worth noting that since we opted to use F2s for our backcrosses selected individuals may be homozygous for some genes of interest. These animals would require fewer offspring to produce heterozygotes at all loci of interest, so our estimations are still reasonable.

In the first generation of backcrossing we selected two males from 75 individuals. At this number of animals, it realistic to assume at least of one of those was heterozygous

Table 14. Estimated effect sizes for individual genes based on calculated effect size for trait.

[Table 1 in chapter]

Number of Genes	Female Effect Size (SD)	Male Effect Size (SD)
1	-3.085	-2.516
2	-1.543	-1.258
3	-1.028	-0.839
4	-0.771	-0.629
5	-0.617	-0.503
6	-0.514	-0.419
7	-0.441	-0.359
8	-0.386	-0.315
9	-0.343	-0.280
10	-0.309	-0.252
11	-0.280	-0.229
12	-0.257	-0.210
13	-0.237	-0.194

Table 15. Estimated effect size for female and male mice between BL and BR, BL and HBI, and BL and HB2, respectively.

[Table 2 in chapter]

Strains	Female Effect Size (SD)	Male Effect Size (SD)
BL BR	-3.085	-2.516
BL HB1	-0.620	-0.450
BL HB2	-0.726	-1.167

Table 16. Probability of producing an individual heterozygous for all genes of interest at an increasing number of genes.

[Table 3 in chapter]

Number of Genes	Probability
1	$\frac{1}{2}$
2	$\frac{1}{4}$
3	$\frac{1}{8}$
4	$\frac{1}{16}$
5	$\frac{1}{32}$
6	$\frac{1}{64}$
7	$\frac{1}{128}$
8	$\frac{1}{256}$
9	$\frac{1}{512}$
10	$\frac{1}{1024}$
11	$\frac{1}{2048}$
12	$\frac{1}{4096}$

for 6 genes (Table 3). This holds true for the second generation of backcrosses where we produced 77 individuals. Therefore, we estimate that 6 genes are still segregating in the selected male population.

Due to differences in segregation as well as previous observations that BL genes tend to be dominant, it is reasonable to expect more genes are still segregating in the population. To have maintained the trait in its entirety we would have needed to breed about 4100 males each generation. That number of animals would not have been practical; however, our study does demonstrate the potential for successful introgression for at least 6 genes associated with increased consumption. While successful introgression of low consumption would have been favored this does confirm the method, we employed does have the capacity to assist in identifying genes associated with water consumption.

Summary

The ability to elucidate quantitative trait architecture and introduce potentially beneficial traits makes introgression a useful tool. Unfortunately, due to the nature of inheritance there are certainly limitations to the usefulness of this approach for introducing quantitative traits to a recipient population. In this project we attempted to introduce genes associated with water consumption to strains with opposite consumption profiles. Our two strains were selected based on previously quantified differences in consumption. In our first study, we confirmed differences in consumption based on strain. We also observed a potential impact of maternal care on consumption—particularly in males. In the second study, we estimated the difference in consumption was due to a minimum of about 10-12 genes. Such a high number of genes with little

information on architecture makes reliable phenotypic selection difficult. Ultimately, we determined the phenotype was lost in LB animals but about six genes were still segregating in HB animals. This indicates that this method may be effective for determining a limited number of high-impact genes after a short number of generations. To further resolve the architecture of the difference between these two strains it would likely be useful to continue backcrossing then produce a generation of HB intercrosses. These individuals would provide a higher resolution population for mapping work.

REFERENCES

- Abras, C., Maloney-Krichmar, D., & Preece, J. (2004). *User-Centered Design. Encyclopedia of Human-Computer Interaction*. Sage Publications.
<https://doi.org/10.3233/WOR-2010-1109>
- Albanese, M. (2000). Problem-based learning: Why curricula are likely to show little effect on knowledge and clinical skills. *Medical Education*, *34*(9), 729–738.
<https://doi.org/10.1046/j.1365-2923.2000.00753.x>
- All, A., Nuñez Castellar, E. P., & Van Looy, J. (2016). Assessing the effectiveness of digital game-based learning: Best practices. *Computers & Education*, *92–93*, 90–103. <https://doi.org/10.1016/j.compedu.2015.10.007>
- Allan, J. A. (John A. (2001). The Middle East water question: hydropolitics and the global economy. *Choice Reviews Online*, *39*(04), 39-2423-39–2423.
<https://doi.org/10.5860/CHOICE.39-2423>
- Anderson, J. R., Conrad, F., & Corbett, A. (1989). Skill acquisition and the LISP tutor. *Cognitive Science*, *505*(4), 467–505. https://doi.org/10.1207/s15516709cog1304_1
- Astleitner, H., & Steinberg, R. (2005). Are there gender differences in web-based learning? An integrated model and related effect sizes. *AACE Journal*, *13*(1), 47–63. Retrieved from
<https://pdfs.semanticscholar.org/4139/0b3bcff0bd083ea6c300f411d6f55255fe01.pdf>
- Auster, E. R., & Wylie, K. K. (2006). Creating active learning in the classroom: A systematic approach. *Journal of Management Education*, *30*(2), 333–353.
<https://doi.org/10.1177/1052562905283346>
- Bachmanov, A. A., Beauchamp, G. K., & Tordoff, M. G. (2002). Voluntary consumption of NaCl, KCl, CaCl₂, and NH₄Cl solutions by 28 mouse strains. *Behavior Genetics*, *32*(6), 445–457. <https://doi.org/10.1023/A:1020832327983>
- Bachmanov, A. A., Tordoff, M. G., & Beauchamp, G. K. (1996). Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice. *Alcoholism: Clinical and Experimental Research*, *20*(2), 201–206. <https://doi.org/10.1111/j.1530-0277.1996.tb01630.x>
- Bacon, D. R. (2016, April 8). Reporting Actual and Perceived Student Learning in Education Research. *Journal of Marketing Education*, *38*(1), 3–6.
<https://doi.org/10.1177/0273475316636732>
- Bahbahani, H., Afana, A., & Wragg, D. (2018). Genomic signatures of adaptive introgression and environmental adaptation in the Sheko cattle of southwest Ethiopia. *PLoS ONE*, *13*(8), e0202479.
<https://doi.org/10.1371/journal.pone.0202479>
- Bangert-Drowns, R. L., Kulik, C.-L. C., Kulik, J. A., & Morgan, M. (1991). The Instructional Effect of Feedback in Test-Like Events. *Review of Educational Research*, *61*(2), 213–238. <https://doi.org/10.3102/00346543061002213>

- Barendse, W. (2017). Climate Adaptation of Tropical Cattle. *Annual Review of Animal Biosciences*, 5(1), 133–150. <https://doi.org/10.1146/annurev-animal-022516-022921>
- Barzilai, S., & Blau, I. (2014). Scaffolding game-based learning: Impact on learning achievements, perceived learning, and game experiences. *Computers and Education*, 70, 65–79. <https://doi.org/10.1016/j.compedu.2013.08.003>
- Beck, K; Beedle, M; Bennekum, A; Cockburn, A; Cunningham, W; Fowler, M; Greening, J; Highsmith, J; Hunt, A; Jeffries, R; Kern, J; Marick, B; Martin, R; Mellor, S; Schwaber, K; Sutherland, J; and Thomas, D. (2001). Manifesto for Agile Software Development. Retrieved from <http://www.agilemanifesto.org>
- Beck, J. A., Lloyd, S., Hafezparast, M., Lennon-Pierce, M., Eppig, J. T., Festing, M. F. W., & Fisher, E. M. C. (2000, January 1). Genealogies of mouse inbred strains. *Nature Genetics*. <https://doi.org/10.1038/71641>
- Beever, J. (2009). Managing Genetic Abnormalities background. In *Range Beef Cow Symposium*. Casper, WY. Retrieved from <http://rangebeefcow.com/2009/documents/Beever2009RBCS.pdf>
- Beever, J., & Marron, B. (2013). Screening for arthrogryposis multiplex in bovines. US Patent Office. Retrieved from <https://patents.google.com/patent/US8431346B2/en>
- Belenky, D. M., & Nokes-Malach, T. J. (2013). Mastery-approach goals and knowledge transfer: An investigation into the effects of task structure and framing instructions. *Learning and Individual Differences*, 25, 21–34. <https://doi.org/10.1016/j.lindif.2013.02.004>
- Bhuvanakumar, C. K., Lynch, C. B., Roberts, R. C., & Hill, W. G. (1985). Heterosis among lines of mice selected for body weight - 1. Growth. *Theoretical and Applied Genetics*, 71(1), 44–51. <https://doi.org/10.1007/BF00278252>
- Black, P., & Wiliam, D. (1998). Assessment and Classroom Learning. *Assessment in Education: Principles, Policy & Practice*, 5(1), 7–74. <https://doi.org/10.1080/0969595980050102>
- Blake, C., & Scanlon, E. (2007). Reconsidering simulations in science education at a distance: Features of effective use. *Journal of Computer Assisted Learning*, 23(6), 491–502. <https://doi.org/10.1111/j.1365-2729.2007.00239.x>
- Bonde, M. T., Makransky, G., Wandall, J., Larsen, M. V., Morsing, M., Jarmer, H., & Sommer, M. O. A. (2014). Improving biotech education through gamified laboratory simulations. *Nature Biotechnology*, 32(7), 694–697. <https://doi.org/10.1038/nbt.2955>
- Bonwell, C. C., & Eison, J. A. (1991). *Active learning: Creating excitement in the classroom*. ERIC digest. ASHE-ERIC Higher Education Reports. Retrieved from www.eric.ed.gov
- Boring, A., Po, O.-P.-S., Badgett, L., Al-Bahrani, A., Brown, J., Cattan, S., ... Wasmer, E. (2015). *Gender Biases in Student Evaluations of Teachers Gender Biases in Student Evaluations of Teachers and their Impact on Teacher Incentives*. Retrieved

from <http://www.econjobrumors.com/topic/tenure-standards-at->

- Bosse, M., Megens, H. J., Frantz, L. A. F., Madsen, O., Larson, G., Paudel, Y., ... Groenen, M. A. M. (2014). Genomic analysis reveals selection for Asian genes in European pigs following human-mediated introgression. *Nature Communications*, 5(1), 4392. <https://doi.org/10.1038/ncomms5392>
- Box, G. E. P., & Muller, M. E. (1958). A Note on the Generation of Random Normal Deviates. *Ann. Math. Statist*, 29(2), 610–611. <https://doi.org/10.1214/aoms/1177706645>
- Boyle, E. A., Hailey, T., Connolly, T. M., Gray, G., Earp, J., Ott, M., ... Pereira, J. (2016). An update to the systematic literature review of empirical evidence of the impacts and outcomes of computer games and serious games. *Computers and Education*, 94, 178–192. <https://doi.org/10.1016/j.compedu.2015.11.003>
- Bracey, G. W. (2002). Tips for readers of research: beware the classic study. *Phi Delta Kappan*, 83?(April), 642–643. <https://doi.org/10.1177/003172170208300814>
- Brenner, D. G., Matlen, B. J., Timms, M. J., Gochyyev, P., Grillo-Hill, A., Luttgen, K., & Varfolomeeva, M. (2017). Modeling Student Learning Behavior Patterns in an Online Science Inquiry Environment. *Technology, Knowledge and Learning*, 22(3), 405–425. <https://doi.org/10.1007/s10758-017-9325-0>
- Buchanan, D. S., Burditt, L. G., & Willham, R. L. (1988). COWGAME: a beef cattle selection simulation program. *Journal of Heredity*, 79(3), 215. <https://doi.org/10.1093/oxfordjournals.jhered.a110497>
- Buckley, P., & Doyle, E. (2016). Gamification and student motivation. *Interactive Learning Environments*, 24(6), 1162–1175. <https://doi.org/10.1080/10494820.2014.964263>
- Bunce, D. M., Flens, E. A., & Neiles, K. Y. (2010). How long can students pay attention in class? A study of student attention decline using clickers. *Journal of Chemical Education*, 87(12), 1438–1443. <https://doi.org/10.1021/ed100409p>
- Carini, R. M., Kuh, G. D., & Klein, S. P. (2006). Student engagement and student learning: Testing the linkages. *Research in Higher Education*, 47(1), 1–32. <https://doi.org/10.1007/s11162-005-8150-9>
- Carlson, D. F., Lancto, C. A., Zang, B., Kim, E. S., Walton, M., Oldeschulte, D., ... Fahrenkrug, S. C. (2016). Production of hornless dairy cattle from genome-edited cell lines. *Nature Biotechnology*, 34(5), 479–481. <https://doi.org/10.1038/nbt.3560>
- Castle, W. E. (1921). AN IMPROVED METHOD OF ESTIMATING THE NUMBER OF GENETIC FACTORS CONCERNED IN CASES OF BLENDING INHERITANCE. *Science (New York, N.Y.)*, 54(1393), 223. <https://doi.org/10.1126/science.54.1393.223>
- Cavalli, L. L. (1952). An analysis of linkage in quantitative inheritance. In *Quantitative inheritance. Papers read at a colloquium held at the Institute of Animal Genetics Edinburgh University under the auspices of the Agricultural Research Council April*

- 4th to 6th, 1950.* (pp. 135–144). HM Stationery Office. Retrieved from <https://www.cabdirect.org/cabdirect/abstract/19541603514>
- Clark, D. B., & Martinez-Garza, M. (2012). Prediction and explanation as design mechanics in conceptually integrated digital games to help players articulate the tacit understandings they build through game play. In C. Steinkuehler, K. Squire, & S. Barab (Eds.), *Games, Learning, and Society: Learning and Meaning in the Digital Age* (pp. 279–305). Cambridge: Cambridge University Press. <https://doi.org/10.1017/CBO9781139031127.023>
- Clark, D. B., Nelson, B. C., Chang, H.-Y., Martinez-Garza, M., Slack, K., & D'Angelo, C. M. (2011). Exploring Newtonian mechanics in a conceptually-integrated digital game: Comparison of learning and affective outcomes for students in Taiwan and the United States. *Computers & Education*, *57*(3), 2178–2195. <https://doi.org/10.1016/j.compedu.2011.05.007>
- Clark, D., & Jorde, D. (2004, January 1). Helping Students Revise Disruptive Experientially Supported Ideas about Thermodynamics: Computer Visualizations and Tactile Models. *Journal of Research in Science Teaching*. Wiley-Blackwell. <https://doi.org/10.1002/tea.10097>
- Clark, D., Nelson, B., Sengupta, P., & Angelo, C. D. (2009). Rethinking Science Learning Through Digital Games and Simulations : Genres , Examples , and Evidence. In *National Academies Board on Science Education Workshop on Learning Science: Computer Games* (pp. 36–41). Washington, D.C. Retrieved from https://sites.nationalacademies.org/cs/groups/dbassesite/documents/webpage/dbasse_080068.pdf
- Cockerham, C. C. (1986). Modifications in estimating the number of genes for a quantitative character. *Genetics*, *114*(2), 659–664. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/3770473>
- Colliver, J. A. (2000). Effectiveness of problem-based learning curricula: Research and theory. *Academic Medicine*, *75*(3), 259–266. <https://doi.org/10.1097/00001888-200003000-00017>
- Cooper, S. J., & McGivern, H. (1983). Effects of naloxone and naltrexone on the increased water intake and drinking duration in phenobarbitone-treated rats. *Psychopharmacology*, *79*(1), 25–28. <https://doi.org/10.1007/BF00433011>
- Corbalan, G., Kester, L., & van Merriënboer, J. J. G. (2009). Dynamic task selection: Effects of feedback and learner control on efficiency and motivation. *Learning and Instruction*, *19*(6), 455–465. Retrieved from <http://dspace.ou.nl/handle/1820/2142>
- Cortright, R. N., Collins, H. L., Rodenbaugh, D. W., & DiCarlo, S. E. (2003). STUDENT RETENTION OF COURSE CONTENT IS IMPROVED BY COLLABORATIVE-GROUP TESTING. *AJP: Advances in Physiology Education*, *27*(3), 102–108. <https://doi.org/10.1152/advan.00041.2002>
- Council, N. R. (2011). *Learning Science Through Computer Games and Simulations*. Washington, D.C.: National Academies Press. <https://doi.org/10.17226/13078>

- Crouch, C. H., & Mazur, E. (2001). Peer Instruction: Ten years of experience and results. *American Journal of Physics*, 69(9), 970–977. <https://doi.org/10.1119/1.1374249>
- Cutler, G., Marshall, L. A., Chin, N., Baribault, H., & Kassner, P. D. (2007). Significant gene content variation characterizes the genomes of inbred mouse strains. *Genome Research*, 17(12), 1743–1754. <https://doi.org/10.1101/gr.6754607>
- Darvasi, A., Weinreb, A., Minke, V., Weller, J. I., & Soller, M. (1993). Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. *Genetics*, 134(3), 943–951. <https://doi.org/10.1038/nrg1407>
- de Fraiture, C., & Wichelns, D. (2010). Satisfying future water demands for agriculture. *Agricultural Water Management*, 97(4), 502–511. <https://doi.org/10.1016/j.agwat.2009.08.008>
- De Freitas, S. (2006). Learning in Immersive worlds A review of game-based learning Prepared for the JISC e-Learning Programme. *JISC ELearning Innovation*, 3.3(October 14), 73. <https://doi.org/10.1111/j.1467-8535.2009.01024.x>
- De Grove, F., Bourgonjon, J., & Van Looy, J. (2012). Digital games in the classroom? A contextual approach to teachers' adoption intention of digital games in formal education. *Computers in Human Behavior*, 28(6), 2023–2033. <https://doi.org/10.1016/j.chb.2012.05.021>
- De Jong, T., & Van Joolingen, W. R. (1998). Scientific Discovery Learning with Computer Simulations of Conceptual Domains. *Review of Educational Research*, 68(2), 179–201. <https://doi.org/10.3102/00346543068002179>
- DeSmet, A., Thompson, D., Baranowski, T., Palmeira, A., Verloigne, M., & De Bourdeaudhuij, I. (2016). Is participatory design associated with the effectiveness of serious digital games for healthy lifestyle promotion? A meta-analysis. *Journal of Medical Internet Research*, 18(4), e94. <https://doi.org/10.2196/jmir.4444>
- Di Leonardi, B. C. (2007). Tips for facilitating learning: The lecture deserves some respect. *The Journal of Continuing Education in Nursing*, 38(4), 154–162. <https://doi.org/10.3928/00220124-20070701-09>
- Dickerson, G. E. (1969). Experimental Approaches in Utilising Breed Resources. *Animal Breeding Abstracts*, 37(191), 191–202.
- Dickerson, G. E. (1973). Inbreeding and Heterosis in Animals. *Journal of Animal Science*, 1973(Symposium), 54–77. <https://doi.org/1973.1973Symposium54x>
- Dillenbourg, P. (2008). Integrating technologies into educational ecosystems. *Distance Education*, 29(2), 127–140. <https://doi.org/10.1080/01587910802154939>
- Druin, A. (2002). The role of children in the design of new technology. *Behaviour and Information Technology*, 21(1), 1–25. <https://doi.org/10.1080/01449290110108659>
- Dubin, R. T. C. (1968). *A Comparative Analysis of College Teaching Methods*. Retrieved from <https://eric.ed.gov/?id=ED026966>

- Durán, M. J., Gallardo, S., Toral, S. L., Martínez-Torres, R., & Barrero, F. J. (2007). A learning methodology using Matlab/Simulink for undergraduate electrical engineering courses attending to learner satisfaction outcomes. In *International Journal of Technology and Design Education* (Vol. 17, pp. 55–73). Kluwer Academic Publishers. <https://doi.org/10.1007/s10798-006-9007-z>
- Edgcomb, A., & Vahid, F. (2014). Effectiveness of online textbooks vs. interactive web-native content. *American Society for Engineering Education Annual Conference and Exposition*. Retrieved from <http://zybooks.com/wp-content/uploads/2016/06/Effectiveness-of-Online-Textbooks-vs.-Interactive-Web-Native-Content.pdf>
- Eisler, M. C., Lee, M. R. F., Tarlton, J. F., & Martin, G. B. (2014, March 5). Steps to sustainable livestock. *Nature*. <https://doi.org/10.1038/507032a>
- Erhel, S., & Jamet, E. (2013). Digital game-based learning: Impact of instructions and feedback on motivation and learning effectiveness. *Computers and Education*, 67, 156–167. <https://doi.org/10.1016/j.compedu.2013.02.019>
- FALCONER, D. S., & MACKAY, T. F. . (1996). Introduction to quantitative genetics. *Introduction to quantitative genetics*, 463. <https://doi.org/10.1002/bimj.19620040211>
- FAO. (2018). *Water use of livestock production systems and supply chains Guidelines for assessment (Draft for public review)*. Retrieved from www.fao.org/publications
- Faria, A. J., & Wellington, W. J. (2004). A survey of simulation game users, former-users, and never-users. In *Simulation and Gaming* (Vol. 35, pp. 178–207). Sage PublicationsSage CA: Thousand Oaks, CA. <https://doi.org/10.1177/1046878104263543>
- Faust, J. L., & Paulson, D. R. (1998). Active learning in the college classroom. *Journal on Excellence in College Teaching*, 9(2), 3–24. <https://doi.org/10.5926/arepj1962.47.0>
- Fitzgerald, D. (2013). Employing think–pair–share in associate degree nursing curriculum. *Teaching and Learning in Nursing*, 8(3), 88–90. <https://doi.org/10.1016/j.teln.2013.01.006>
- Fleming, P., & Godwin, M. (2008). Lifestyle interventions in primary care: Systematic review of randomized controlled trials. *Canadian Family Physician*, 54(12), 1706–1713. [https://doi.org/10.1016/S0169-5347\(01\)02290-X](https://doi.org/10.1016/S0169-5347(01)02290-X)
- Freeman, S., Eddy, S. L., McDonough, M., Smith, M. K., Okoroafor, N., Jordt, H., & Wenderoth, M. P. (2014). Active learning increases student performance in science, engineering, and mathematics. *Proceedings of the National Academy of Sciences*, 111(23), 8410–8415. <https://doi.org/10.1073/pnas.1319030111>
- Fund, Z. (2007). The effects of scaffolded computerized science problem-solving on achievement outcomes: A comparative study of support programs: Original article. *Journal of Computer Assisted Learning*, 23(5), 410–424. <https://doi.org/10.1111/j.1365-2729.2007.00226.x>

- Gale, J. G., Mather, K., & Jinks, J. L. (1977). Joint scaling tests. *Heredity*, 38(1), 47–51. <https://doi.org/10.1038/hdy.1977.6>
- García-Ruiz, A., Cole, J. B., VanRaden, P. M., Wiggans, G. R., Ruiz-López, F. J., & Van Tassell, C. P. (2016). Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. *Proceedings of the National Academy of Sciences*, 113(28), E3995–E4004. <https://doi.org/10.1073/pnas.1519061113>
- Garris, R., Ahlers, R., & Driskell, J. E. (2002). Games, motivation, and learning: A research and practice model. *Simulation and Gaming*, 33(4), 441–467. <https://doi.org/10.1177/1046878102238607>
- Gelbart, H., Brill, G., & Yarden, A. (2009). The impact of a web-based research simulation in bioinformatics on students' understanding of genetics. *Research in Science Education*, 39(5), 725–751. <https://doi.org/10.1007/s11165-008-9101-1>
- Gelbart, H., & Yarden, A. (2006). Learning genetics through an authentic research simulation in bioinformatics. *Journal of Biological Education*, 40(3), 107–112. <https://doi.org/10.1080/00219266.2006.9656026>
- Giller, K. (2017). *Climate Change, Agriculture and Food Security*. Retrieved from www.fao.org/publications
- Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Muir, J. F., Pretty, J., ... Toulmin, C. (2016). The Challenge of Feeding 9 Billion People. *Science*, 327(5967), 812–818. <https://doi.org/10.4337/9780857939388>
- Goldstone, R. L., & Son, J. Y. (2005, January). The transfer of scientific principles using concrete and idealized simulations. *Journal of the Learning Sciences*. Lawrence Erlbaum Associates, Inc. https://doi.org/10.1207/s15327809jls1401_4
- González-Cruz, J., Rodríguez-Sotres, R., & Rodríguez-Penagos, M. (2003, March). On the convenience of using a computer simulation to teach enzyme kinetics to undergraduate students with biological chemistry-related curricula. *Biochemistry and Molecular Biology Education*. Wiley-Blackwell. <https://doi.org/10.1002/bmb.2003.494031020193>
- Gregory, K. E., Dearborn, D. D., Cundiff, L. V., & Koch, R. M. (1987). Maternal Heterosis and Grandmaternal Effects the in Beef Cattle: Postweaning Growth and Carcass Traits². *Journal of Animal Science*, 65(5), 1180–1194. <https://doi.org/10.2527/jas1987.6551180x>
- Griffing, B. (1956). Concept of General and Specific Combining Ability in Relation to Diallel Crossing Systems. *Australian Journal of Biological Sciences*, 9(4), 463. <https://doi.org/10.1071/BI9560463>
- Gruber, I., Kloos, J., & Schopp, M. (2009). Seasonal water demand in Benin's agriculture. *Journal of Environmental Management*, 90(1), 196–205. <https://doi.org/10.1016/j.jenvman.2007.08.011>
- Guralnick, D. A., & Levy, C. (2009). Putting the education into educational simulations:

- pedagogical structures, guidance and feedback. *International Journal of Advanced Corporate Learning*, 2(1), 10–15. <https://doi.org/10.3991/ijac.v2i1.693>
- Haag, M., Le Tourneau, J., Marra, R., & Lamberson, W. (2018). *CowGames: Introduction to an Online Beef Cattle Breeding Simulation*. Columbia, MO.
- Haag, M. T., Wells, K. D., & Lamberson, W. R. (2018). Genetic, maternal, and heterosis effects on voluntary water consumption in mice. *Journal of Animal Science*, 96(8), 3055–3063. <https://doi.org/10.1093/jas/sky218>
- Hake, R. R. (1998). Conceptual Physics for Parents and Teachers: Especially Elementary School Teachers, Who, After Parents, Provide the Learning Foundation for Children-Mechanics. *Citation: American Journal of Physics*, 66, 595. <https://doi.org/10.1119/1.18809>
- Halskov, K., & Hansen, N. B. (2015). The diversity of participatory design research practice at PDC 2002-2012. *International Journal of Human Computer Studies*, 74, 81–92. <https://doi.org/10.1016/j.ijhcs.2014.09.003>
- Hansen C. T., Judge F. J., and W. R. A. (1973). Catalog of NIH Rodents. *DHEW Publication (NIH)*, 74–606.
- Hartley, J., & Cameron, A. (1967). Some observations on the efficiency of lecturing. *Educational Review*, 20(1), 30–37. <https://doi.org/10.1080/0013191670200103>
- Hartley, J., & Davies, I. K. (1978). Note-taking: A critical review. *Programmed Learning and Educational Technology*, 15(3), 207–224. <https://doi.org/10.1080/0033039780150305>
- Henderson, C., Khan, R., & Dancy, M. (2018). Will my student evaluations decrease if I adopt an active learning instructional strategy? What do we know about student evaluations as a measure of teaching quality? *American Journal of Physics*, (86). Retrieved from <https://drive.google.com/file/d/179ejdWZNrb6FsKZvtpBRSipDpeLdtIDh/view>
- Herrero, M., Wirsenius, S., Henderson, B., Rigolot, C., Thornton, P., Havlík, P., ... Gerber, P. (2015). *Livestock and the Environment: What Have We Learned in the Past Decade? SSRN* (Vol. 40). Annual Reviews. <https://doi.org/10.1146/annurev-environ-031113-093503>
- Hill, W. G. (1998). Selection with recurrent backcrossing to develop congenic lines for quantitative trait loci analysis. *Genetics*, 148(3), 1341–1352. Retrieved from <http://www.genetics.org/content/genetics/148/3/1341.full.pdf>
- Hillman, C. H., Erickson, K. I., & Kramer, A. F. (2008). Be smart, exercise your heart: exercise effects on brain and cognition. *Nature Neuroscience*, 9(1), 58–65. <https://doi.org/10.1038/nrn2298>
- Hora, M. T. (2014). Limitations in experimental design mean that the jury is still out on lecturing. *Proceedings of the National Academy of Sciences of the United States of America*, 111(30), E3024. <https://doi.org/10.1073/pnas.1410115111>

- Hospital, F., Chevalet, C., & Mulsant, P. (1992). Using markers in gene introgression breeding programs. *Genetics*, *132*(4), 1199–1210. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/1459437>
- Huang, W., & Mackay, T. F. C. (2016). The Genetic Architecture of Quantitative Traits Cannot Be Inferred from Variance Component Analysis. *PLoS Genetics*, *12*(11), e1006421. <https://doi.org/10.1371/journal.pgen.1006421>
- Hurst, H. M., & Marks-Maran, D. (2011). Using a virtual patient activity to teach nurse prescribing. *Nurse Education in Practice*, *11*(3), 192–198. <https://doi.org/10.1016/j.nepr.2010.08.008>
- Johnson, C. I., Bailey, S. K. T., & Van Buskirk, W. L. (2017). Designing Effective Feedback Messages in Serious Games and Simulations: A Research Review. In *Instructional Techniques to Facilitate Learning and Motivation of Serious Games* (pp. 119–140). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-39298-1_7
- Johnstone, A. H., & Percival, F. (1976). Attention breaks in lectures. *Education in Chemistry*, *13*, 49–50. Retrieved from <https://eric.ed.gov/?id=EJ136799>>
- Kalyuga, S. (2007). Expertise reversal effect and its implications for learner-tailored instruction. *Educational Psychology Review*, *19*(4), 509–539. <https://doi.org/10.1007/s10648-007-9054-3>
- Kapur, M., & Bielaczyc, K. (2012). Designing for Productive Failure. *Journal of the Learning Sciences*, *21*(1), 45–83. <https://doi.org/10.1080/10508406.2011.591717>
- Kemp, C. J., & Drinkwater, N. R. (1989). Genetic Variation in Liver Tumor Susceptibility, Plasma Testosterone Levels, and Androgen Receptor Binding in Six Inbred Strains of Mice. *Cancer Research*, *49*(18), 5044–5047. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/2766275>
- Kent Divoll, & Sandra Browning. (2010). The Classroom Ticket to Concept Retention. *International Journal for the Scholarship of Teaching and Learning*, *4*(2), 1–15. <https://doi.org/10.20429/ijsotl.2010.040210>
- Kiboss, J. K., Ndirangu, M., & Wekesa, E. W. (2004). Effectiveness of a Computer-Mediated Simulations Program in School Biology on Pupils' Learning Outcomes in Cell Theory. *Journal of Science Education and Technology*, *13*(2), 207–213. <https://doi.org/10.1023/B:JOST.0000031259.76872.f1>
- Kirschner, P. A., Sweller, J., & Clark, R. E. (2006). Why Minimal Guidance During Instruction Does Not Work. *Educational Psychologist*, *41*(March 2015), 87–98. <https://doi.org/10.1207/s15326985ep4102>
- Koch, R. M., Dickerson, G. E., Cundiff, L. V., & Gregory, K. E. (1985). Heterosis retained in advanced generations of crosses among Angus and Hereford cattle. *Journal of Animal Science*, *60*(5), 1117–1132. <https://doi.org/10.2527/jas1985.6051117x>
- Koedinger, K. R., Kim, J., Jia, J. Z., McLaughlin, E. A., & Bier, N. L. (2015). Learning is

- Not a Spectator Sport. In *Proceedings of the Second (2015) ACM Conference on Learning @ Scale - L@S '15* (pp. 111–120). New York, New York, USA: ACM Press. <https://doi.org/10.1145/2724660.2724681>
- Koedinger, K. R., & Roll, I. (2012). Learning to Think: Cognitive Mechanisms of Knowledge Transfer. In *The Oxford Handbook of Thinking and Reasoning*. Oxford University Press. <https://doi.org/10.1093/oxfordhb/9780199734689.013.0040>
- Koivisto, J. M., Haavisto, E., Niemi, H., Haho, P., Nylund, S., & Multisilta, J. (2018). Design principles for simulation games for learning clinical reasoning: A design-based research approach. *Nurse Education Today*, *60*, 114–120. <https://doi.org/10.1016/j.nedt.2017.10.002>
- Koots, K. R., Gibson, J. P., & Wilson, J. P. (1994). Analyses of published genetic parameter estimates for beef production traits. 2. Phenotypic and genetic correlations. *Animal Breeding Abstracts*, *62*(11), 825–853. <https://doi.org/10.1177/1088868315615450>
- Kruger, J., & Dunning, D. (1999). Unskilled and unaware of it: How difficulties in recognizing one's own incompetence lead to inflated self-assessments. *Journal of Personality and Social Psychology*, *77*(6), 1121–1134. <https://doi.org/10.1037/0022-3514.77.6.1121>
- Kulhavy, R. W. (1977). Feedback in Written Instruction. *Review of Educational Research*, *47*(2), 211–232. <https://doi.org/10.3102/00346543047002211>
- Kulhavy, R., & Wager, W. (1993). Feedback in Programmed Instruction: Historical Context and Implications for Practice. In J. Dempsey & G. Sales (Eds.), *Interactive Instruction and Feedback* (pp. 3–19). Educational Technology Pubns. Retrieved from https://books.google.com/books?hl=en&lr=&id=ss3fz-5WC6gC&oi=fnd&pg=PA3&dq=Feedback+in+programmed+instruction:+Historical+context+and+implications+for+practice.&ots=uz0MnvIaM0&sig=JFxH4LvYh5PNu_j94zXbTraGRkg#v=onepage&q=Feedback+in+programmed+instruc
- Kurnianto, E., Shinjo, A., & Suga, D. (1998). Average Direct and Maternal Genetic Effects and Heterosis Effects on Body Weight in Two Subspecies of Mice. *American Journal of Animal Science*, *11*(5), 475–479.
- Lal, V. (2002). *Gender and the impact of computer-based technologies*. Retrieved from <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.84.9332&rep=rep1&type=pdf>
- Lamberson, W. R., Thomas, D. L., & Rowe, K. E. (1982). THE EFFECTS OF INBREEDING IN A FLOCK OF HAMPSHIRE SHEEP 1 ' 2 ' 3. *Journal of Animal Science*, *55*(4), 780–786. <https://doi.org/10.2527/jas1982.554780x>
- Lande, R. (1981). The minimum number of genes contributing to quantitative variation between and within populations. *Genetics*, *99*(3–4), 541–553. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/7343418>
- Landsberg, C. R., Van Buskirk, W. L., & Astwood, R. S. (2010). Does Feedback Type

- Matter? Investigating the Effectiveness of Feedback Content on Performance Outcomes. *Proceedings of the Human Factors and Ergonomics Society Annual Meeting*, 54(27), 2304–2308. <https://doi.org/10.1177/154193121005402718>
- Lee, L. J., Connolly, M. E., Dancy, M. H., Henderson, C. R., & Christensen, W. M. (2018). A comparison of student evaluations of instruction vs. students' conceptual learning gains. *American Journal of Physics*, 86(7), 531–535. <https://doi.org/10.1119/1.5039330>
- Lewis, R. M., Lockee, B. B., Krishnamurthy, L., & Meese, J. A. (2010). Experiential Learning On-line : CyberSheep. In *Proceedings of the World Congress on Genetics Applied to Livestock Production*. Retrieved from <http://www.wcgalp.org/system/files/proceedings/2010/experiential-learning-line-cybersheep.pdf>
- Lindgren, R., & Schwartz, D. L. (2009, February 17). Spatial learning and computer simulations in science. *International Journal of Science Education*. Routledge. <https://doi.org/10.1080/09500690802595813>
- Lippman, Z. B., & Zamir, D. (2007). Heterosis: revisiting the magic. *Trends in Genetics*. <https://doi.org/10.1016/j.tig.2006.12.006>
- Lujan, H. L., & DiCarlo, S. E. (2006). Too much teaching, not enough learning: what is the solution? *Advances in Physiology Education*, 30(1), 17–22. <https://doi.org/10.1152/advan.00061.2005>
- Lyman, F. (1981). The responsive classroom discussion. In *Mainstreaming Digest* (pp. 109–113).
- Lynch, M., & Walsh, B. (1998). Genetics and Analysis of Quantitative Traits. *Sinauer, Sunderland, Mass.* <https://doi.org/10.1086/318209>
- Machemer, P. L., & Crawford, P. (2007). Student perceptions of active learning in a large cross-disciplinary classroom. *Active Learning in Higher Education*, 8(1), 9–30. <https://doi.org/10.1177/1469787407074008>
- MacNell, L., Driscoll, A., & Hunt, A. N. (2015). What's in a Name: Exposing Gender Bias in Student Ratings of Teaching. *Innovative Higher Education*, 40(4), 291–303. <https://doi.org/10.1007/s10755-014-9313-4>
- Manohar, P. A., Acharya, S., Wu, P., Hansen, M., Ansari, A., & Schilling, W. (2015). Case Studies for Enhancing Student Engagement and Active Learning in Software V&V Education. *Journal of Education and Learning*, 4(4). <https://doi.org/10.5539/jel.v4n4p39>
- Maronpot, R. R. (2009). Biological Basis of Differential Susceptibility to Hepatocarcinogenesis among Mouse Strains. *Journal of Toxicologic Pathology*, 22(1), 11–33. <https://doi.org/10.1293/tox.22.11>
- Martin, S. H., & Jiggins, C. D. (2017). Interpreting the genomic landscape of introgression. *Current Opinion in Genetics & Development*, 47, 69–74. <https://doi.org/10.1016/J.GDE.2017.08.007>

- Mather, K., & Jinks, J. L. (1971). *Biometrical Genetics: The Study of Continuous Variations*. Chapman and Hall.
- Maupin, M. A., Kenny, J. F., Hutson, S. S., Lovelace, J. K., Barber, N. L., & Linsey, K. S. (2010). *Estimated Use of Water in the United States in 2010 Circular 1405*. *US Geological Survey*. (Vol. 1405). <https://doi.org/http://dx.doi.org/10.3133/cir1405>
- Maxmen, A. (2017). Gene-edited animals face US regulatory crackdown. *Nature*, *January*. <https://doi.org/10.1038/nature.2017.21331>
- Maxwell, M. H., Kleeman, C. R., & Narins, R. G. (1994). *Maxwell and Kleeman's Clinical Disorders of Fluid and Electrolyte Metabolism*. McGraw-Hill. Retrieved from https://books.google.com/books/about/Clinical_disorders_of_fluid_and_electrol.htm?id=kflrAAAAMAAJ
- McDaniel, M. A., & Schlager, M. S. (1990). Discovery Learning and Transfer of Problem-Solving Skills. *Cognition and Instruction*, *7*(2), 129–159. https://doi.org/10.1207/s1532690xci0702_3
- McGivern, R. F., Henschel, D., Hutcheson, M., & Pangburn, T. (1996). Sex difference in daily water consumption of rats: Effect of housing and hormones. *Physiology and Behavior*, *59*(4–5), 653–658. [https://doi.org/10.1016/0031-9384\(95\)02017-9](https://doi.org/10.1016/0031-9384(95)02017-9)
- Medrano, J. F., Ahmadi, A., & Casellas, J. (2010). Dairy Cattle Breeding Simulation Program: a simulation program to teach animal breeding principles and practices. *Journal of Dairy Science*, *93*(6), 2816–2826. <https://doi.org/10.3168/jds.2009-2640>
- Melanitou, E., Cohn, D. A., Bardin, C. W., & Janne, O. A. (1987). Genetic variation in androgen regulation of ornithine decarboxylase gene expression in inbred strains of mice. *Mol Endocrinol*, *1*(3), 266–273. Retrieved from https://watermark.silverchair.com/mend0266.pdf?token=AQECAHi208BE49Ooan9kKhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAhowggIWBgkqhkiG9w0BBwagggIHMIICAwIBADCCAfwGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMm7yPZ7BR_lhpWApJAgEQgIIBzeCQsLbINPV3KjQCUE1bN_U1k1CgokxarjgXxbvmgWWV66
- Menella, T. (2017). The Hidden Costs of Active Learning. Retrieved from <https://campustechnology.com/articles/2017/04/05/the-hidden-costs-of-active-learning.aspx>
- Metcalf, N. B., & Monaghan, P. (2001). Compensation for a bad start: Grow now, pay later? *Trends in Ecology and Evolution*. [https://doi.org/10.1016/S0169-5347\(01\)02124-3](https://doi.org/10.1016/S0169-5347(01)02124-3)
- Michael, J. (2006). How We Learn Where's the evidence that active learning works? *Adv Physiol Educ*, *30*, 159–167. <https://doi.org/10.1152/advan.00053>
- Miranda, P., Isaias, P., & Pifano, S. (2018). Digital Literacy in Higher Education. In *International Conference on Learning and Collaboration Technologies* (pp. 71–87). <https://doi.org/10.1057/9781137476982.0019>

- Moreno, R., & Mayer, R. E. (2004). Personalized Messages that Promote Science Learning in Virtual Environments. *Journal of Educational Psychology*, 96(1), 165–173. <https://doi.org/10.1037/0022-0663.96.1.165>
- Moreno, R., Mayer, R. E., Spires, H. A., & Lester, J. C. (2001). The case for social agency in computer-based teaching: Do students learn more deeply when they interact with animated pedagogical agents? *Cognition and Instruction*, 19(2), 177–213. https://doi.org/10.1207/S1532690XCII902_02
- Mory, E. H. (2004). Feedback research revisited. *Handbook of Research on Educational Communications ...*, 2, 745–784. <https://doi.org/10.1007/s00127-009-0052-2>
- Moulin, C.-H., Blanc, F., Ezanno, P., & Bocquier, F. (2004). Modelling as a tool for the teaching of livestock dynamics. *Anim. Res*, 53, 439–450. <https://doi.org/10.1051/animres:2004027>
- Newman, T., & Beetham, H. (2017). Student digital experience tracker 2017 : the voice of 22000 Uk learners, (June), 2017–2018. Retrieved from <http://repository.jisc.ac.uk/6662/1/Jiscdigitalstudenttracker2017.pdf>
- Nkhoma, M., Calbeto, J., Sriratanaviriyakul, N., Muang, T., Tran, Q. H., & Cao, T. K. (2014). Towards an understanding of real-time continuous feedback from simulation games. *Interactive Technology and Smart Education*, 11(1), 45–62. <https://doi.org/10.1108/ITSE-03-2013-0005>
- O ’rourke, P. D. (2001). Developments in Business Simulation and Experiential Learning CURRENT USE OF AGRIBUSINESS SIMULATION GAMES: SURVEY RESULTS OF UNIVERSITY AGRIBUSINESS AND AGRICULTURAL ECONOMICS PROGRAMS. *Developments in Business Simulation and Experiential Learning*, 28. Retrieved from <https://journals.tdl.org/absel/index.php/absel/article/viewFile/823/792>
- Olejnik, S. F. (1984). Planning educational research: Determining the necessary sample size. *Journal of Experimental Education*, 53(1), 40–48. <https://doi.org/10.1080/00220973.1984.10806360>
- Osborne, J. (2014). Teaching Scientific Practices: Meeting the Challenge of Change. *Journal of Science Teacher Education*, 25(2), 177–196. <https://doi.org/10.1007/s10972-014-9384-1>
- Otto, S. P., & Jones, C. D. (2000). Detecting the undetected: Estimating the total number of loci underlying a quantitative trait. *Genetics*, 156(4), 2093–2107. <https://doi.org/10.1534/genetics.104.029686>
- Paas, F., Renkl, A., & Sweller, J. (2003). Cognitive Load Theory and Instructional Design: Recent Developments. *Educational Psychologist*, 38(1), 1–4. https://doi.org/10.1207/S15326985EP3801_1
- Peden, D., Amede, T., & Haileslassie, A. (2000). Opportunities for water-efficient livestock production, 1–4. Retrieved from <https://cgspace.cgiar.org/bitstream/handle/10568/10602/IssueBrief5.pdf?sequence=1>

- Peffer, M. E., Beckler, M. L., Schunn, C., Renken, M., & Revak, A. (2015). Science Classroom Inquiry (SCI) Simulations: A Novel Method to Scaffold Science Learning. *PLOS ONE*, *10*(3), e0120638. <https://doi.org/10.1371/journal.pone.0120638>
- Perry, G. A., & Smith, M. F. (2004). A simulation exercise to teach principles of bovine reproductive management. *Journal of Animal Science*, *82*(5), 1543–1549. <https://doi.org/10.2527/2004.8251543x>
- Phipps, M., Phipps, C., Kask, S., & Higgins, S. (2001). University Students' Perceptions of Cooperative Learning: Implications for Administrators and Instructors. *Journal of Experiential Education*, *24*(1), 14–22. <https://doi.org/10.1177/105382590102400105>
- Prince, M. (2004). Does active learning work? A review of the research. *Journal of Engineering Education*, *93*(3), 223–232. <https://doi.org/10.1002/j.2168-9830.2004.tb00809.x>
- Puryear, R., Rigatto, K. V., Amico, J. A., & Morris, M. (2001). Enhanced salt intake in oxytocin deficient mice. *Experimental Neurology*, *171*(2), 323–328. <https://doi.org/10.1006/exnr.2001.7776>
- Quintana, C., Reiser, B. J., Davis, E. A., Krajcik, J., Fretz, E., Duncan, R. G., ... Soloway, E. (2004). A scaffolding design framework for software to support science inquiry. In *Journal of the Learning Sciences* (Vol. 13, pp. 337–386). Lawrence Erlbaum Associates, Inc. https://doi.org/10.1207/s15327809jls1303_4
- Rance, K. A., Heath, S. C., & Keightley, P. D. (1997). Mapping quantitative trait loci for body weight on the X chromosome in mice. II. Analysis of congenic backcrosses. *Genetical Research*, *70*(2), 125–133. <https://doi.org/10.1017/S0016672397002929>
- Reed, D. R., Bachmanov, A. A., & Tordoff, M. G. (2007). Forty mouse strain survey of body composition. *Physiology and Behavior*, *91*(5), 593–600. <https://doi.org/10.1016/j.physbeh.2007.03.026>
- Reiser, B. J. (2004). Scaffolding complex learning: The mechanisms of structuring and problematizing student work. In *Journal of the Learning Sciences* (Vol. 13, pp. 273–304). https://doi.org/10.1207/s15327809jls1303_2
- Richards-Babb, M., Curtis, R., Georgieva, Z., & Penn, J. H. (2015). Student Perceptions of Online Homework Use for Formative Assessment of Learning in Organic Chemistry. *Journal of Chemical Education*, *92*(11), 1813–1819. <https://doi.org/10.1021/acs.jchemed.5b00294>
- Rochus, C. M., Tortereau, F., Plisson-Petit, F., Restoux, G., Moreno-Romieux, C., Tossier-Klopp, G., & Servin, B. (2017). High density genome scan for selection signatures in French sheep reveals allelic heterogeneity and introgression at adaptive loci. *BioRxiv*, 103010. <https://doi.org/10.1101/103010>
- Roll, I., Butler, D., Yee, N., Welsh, A., Perez, S., Briseno, A., ... Bonn, D. (2018). Understanding the impact of guiding inquiry: the relationship between directive support, student attributes, and transfer of knowledge, attitudes, and behaviours in

- inquiry learning. *Instructional Science*, 46(1), 77–104.
<https://doi.org/10.1007/s11251-017-9437-x>
- Roll, I., Wiese, S., Long, Y., Aleven, V., & Koedinger, K. R. (214AD). Tutoring Self- and Co-Regulation with Intelligent Tutoring Systems to Help Students Acquire Better Learning Skills. In R. Sottolare, U. A. R. Laboratory, A. Graesser, X. Hu, & B. Goldberg (Eds.), *Design Recommendations for Intelligent Tutoring Systems: Volume 2* (pp. 169–182). U.S. Army Research Laboratory.
- Ross, L., & Nisbett, R. E. (2011). *The Person and the Situation: Perspectives of Social Psychology*. Retrieved from
https://books.google.com/books/about/The_Person_and_the_Situation.html?id=s_p2cgAACAAJ
- Ruhl, K. L., Hughes, C. A., & Schloss, P. J. (1987). Using the Pause Procedure to Enhance Lecture Recall. *Teacher Education and Special Education: The Journal of the Teacher Education Division of the Council for Exceptional Children*, 10(1), 14–18. <https://doi.org/10.1177/088840648701000103>
- Rutten, N., Van Joolingen, W. R., & Van Der Veen, J. T. (2012). The learning effects of computer simulations in science education. *Computers and Education*, 58(1), 136–153. <https://doi.org/10.1016/j.compedu.2011.07.017>
- Sauvé, L., Renaud, L., Kaufman, D., & Marquis, J. S. (2007). Distinguishing between games and simulations: A systematic review. *Educational Technology and Society*, 10(3), 247–256. Retrieved from <http://www.savie.qc.ca/BaseConnaissances/>
- Scaife, M., Rogers, Y., Aldrich, F., & Davies, M. (1997). Designing for or designing with? Informant design for interactive learning environments. In *Human Factors in Computing Systems (CHI)* (pp. 343–350). <https://doi.org/10.1145/258549.258789>
- SCHLINK, A. C., NGUYEN, M. L., & VILJOEN, G. J. (2010). Water requirements for livestock production: a global perspective. *Revue Scientifique et Technique de l'OIE*, 29(3), 603–619. <https://doi.org/10.20506/rst.29.3.1999>
- Shepon, A., Eshel, G., Noor, E., & Milo, R. (2016). Energy and protein feed-to-food conversion efficiencies in the US and potential food security gains from dietary changes. *Environmental Research Letters*, 11(10). <https://doi.org/10.1088/1748-9326/11/10/105002>
- Shute, V. J. (2008). Focus on Formative Feedback. *Review of Educational Research*, 78(1), 153–189. <https://doi.org/10.3102/0034654307313795>
- Sitzmann, T., Ely, K., Brown, K. G., & Bauer, K. N. (2010). Self-assessment of knowledge: A cognitive learning or affective measure? *Academy of Management Learning and Education*, 9(2), 169–191.
<https://doi.org/10.5465/AMLE.2010.51428542>
- Smith, J. K., & Greiner, S. P. (2013). *Selection for Marbling and the Impact on Maternal Traits Understanding the implications of selection for marbling in a cowherd*. Retrieved from [https://www.cabpartners.com/articles/news/2681/Smith and Greiner](https://www.cabpartners.com/articles/news/2681/Smith%20and%20Greiner)

- Smith, L. A., Cassell, B. G., & Pearson, R. E. (1998). The Effects of Inbreeding on the Lifetime Performance of Dairy Cattle. *Journal of Dairy Science*, *81*(10), 2729–2737. [https://doi.org/10.3168/jds.S0022-0302\(98\)75830-8](https://doi.org/10.3168/jds.S0022-0302(98)75830-8)
- Smits, M. H. S. B., Boon, J., Sluijsmans, D. M. A., & van Gog, T. (2008). Content and timing of feedback in a web-based learning environment: Effects on learning as a function of prior knowledge. *Interactive Learning Environments*, *16*(2), 183–193. <https://doi.org/10.1080/10494820701365952>
- Spinath, B., & Spinath, F. M. (2005). Longitudinal analysis of the link between learning motivation and competence beliefs among elementary school children. *Learning and Instruction*, *15*(2), 87–102. <https://doi.org/10.1016/j.learninstruc.2005.04.008>
- Stains, M., Harshman, J., Barker, M. K., Chasteen, S. V, Cole, R., DeChenne-Peters, S. E., ... Young, A. M. (2018, March 30). Anatomy of STEM teaching in North American universities. *Science*. American Association for the Advancement of Science. <https://doi.org/10.1126/science.aap8892>
- Stark, P., Ottoboni, K., & Boring, A. (2016). Student Evaluations of Teaching (Mostly) Do Not Measure Teaching Effectiveness. *ScienceOpen Research*. <https://doi.org/10.14293/S2199-1006.1.SOR-EDU.AETBZC.v1>
- Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, D. H. C. (2006). Livestock’s long shadow, environmental issues and options. *Food and Agricultural Organisation, Rome*, 390. <https://doi.org/10.1007/s10666-008-9149-3>
- Stern, L., Barnea, N., & Shauli, S. (2008). The effect of a computerized simulation on middle school students’ understanding of the kinetic molecular theory. *Journal of Science Education and Technology*, *17*(4), 305–315. <https://doi.org/10.1007/s10956-008-9100-z>
- Stewart, T. L., Myers, A. C., & Culley, M. R. (2010). Enhanced Learning and Retention through “Writing to Learn” in the Psychology Classroom. *Teaching of Psychology*, *37*(1), 46–49. <https://doi.org/10.1080/00986280903425813>
- Stinson, S. (1985). Sex Differences in Environmental Sensitivity During Growth and Development. *Yearbook of Physical Anthropology*, *28*, 123–147. <https://doi.org/10.1002/ajpa.1330280507>
- Streveler, R. A., & Menekse, M. (2017). Taking a Closer Look at Active Learning. *Journal of Engineering Education*, *106*(2), 186–190. <https://doi.org/10.1002/jee.20160>
- Stuart, J., & Rutherford, R. J. D. (1978). MEDICAL STUDENT CONCENTRATION DURING LECTURES. *The Lancet*, *312*(8088), 514–516. [https://doi.org/10.1016/S0140-6736\(78\)92233-X](https://doi.org/10.1016/S0140-6736(78)92233-X)
- Swanson, E. A., Nicholson, A. C., Boese, T. A., Cram, E., Stineman, A. M., & Tew, K. (2011). Comparison of Selected Teaching Strategies Incorporating Simulation and Student Outcomes. *Clinical Simulation in Nursing*, *7*(3), e81–e90.

<https://doi.org/10.1016/j.ecns.2009.12.011>

- Sweller, J., van Merriënboer, J. J. G., & Paas, F. G. W. C. (1998). Cognitive Architecture and Instructional Design. *Educational Psychology Review*, *10*(3), 251–296. <https://doi.org/10.1023/A:1022193728205>
- Tan, W., Carlson, D. F., Lancto, C. A., Garbe, J. R., Webster, D. A., Hackett, P. B., & Fahrenkrug, S. C. (2013). Efficient nonmeiotic allele introgression in livestock using custom endonucleases. *Proceedings of the National Academy of Sciences*, *110*(41), 16526–16531. <https://doi.org/10.1073/pnas.1310478110>
- Tartelin, M. F., & Gorski, R. A. (1971). Variations in food and water intake in the normal and acyclic female rat. *Physiology & Behavior*, *7*(6), 847–52. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/5167385>
- Taylor, B. a. (n.d.). Genetic relationships between inbred strains of mice. *The Journal of Heredity*, *63*(2), 83–86. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/5031317>
- Thaisz, J., Tsaih, S.-W., Feng, M., Philip, V. M., Zhang, Y., Yanas, L., ... DiPetrillo, K. (2012). Genetic analysis of albuminuria in collaborative cross and multiple mouse intercross populations. *AJP: Renal Physiology*, *303*(7), F972–F981. <https://doi.org/10.1152/ajprenal.00690.2011>
- Tharayil, S., Borrego, M., Prince, M., Nguyen, K. A., Shekhar, P., Finelli, C. J., & Waters, C. (2018). Strategies to mitigate student resistance to active learning. *International Journal of STEM Education*, *5*(1), 7. <https://doi.org/10.1186/s40594-018-0102-y>
- Tordoff, M., Bachmanov, A., & Reed, D. (2007). Forty mouse strain survey of water and sodium intake. *Physiology & Behavior*, *91*(5), 620–631. Retrieved from <http://www.sciencedirect.com/science/article/pii/S0031938407001187>
- Trey, L., & Khan, S. (2008). How science students can learn about unobservable phenomena using computer-based analogies. *Computers and Education*, *51*(2), 519–529. <https://doi.org/10.1016/j.compedu.2007.05.019>
- Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science*, *179*(4068), 90–92. <https://doi.org/10.1126/science.179.4068.90>
- Tuovinen, J. E., & Sweller, J. (1999). A comparison of cognitive load associated with discovery learning and worked examples. *Journal of Educational Psychology*, *91*(2), 334–341. <https://doi.org/10.1037/0022-0663.91.2.334>
- UN-Water. (2013). Water Scarcity. *World Water Day 2013: International Year of Water Cooperation*, 2025. Retrieved from http://www.unwater.org/fileadmin/user_upload/watercooperation2013/doc/Factsheets/water_scarcity.pdf
- Uttl, B., White, C. A., & Gonzalez, D. W. (2017). Meta-analysis of faculty's teaching effectiveness: Student evaluation of teaching ratings and student learning are not

- related. *Studies in Educational Evaluation*, 54, 22–42.
<https://doi.org/10.1016/j.stueduc.2016.08.007>
- Van Dijk, A. M., Eysink, T. H. S., & De Jong, T. (2016). Ability-related differences in performance of an inquiry task: The added value of prompts. *Learning and Individual Differences*, 47, 145–155. <https://doi.org/10.1016/j.lindif.2016.01.008>
- Van Eenennaam, A., & Kinghorn, B. (2014). Use of Mate Selection Software to Manage Lethal Recessive Conditions in Livestock Populations. In *10th World Congress on Genetics Applied to Livestock Production*. Vancouver, BC. Retrieved from https://www.researchgate.net/publication/268109949_Use_of_Mate_Selection_Software_to_Manage_Lethal_Recessive_Conditions_in_Livestock_Populations
- van Ooijen, J. W. (1992). Accuracy of mapping quantitative trait loci in autogamous species. *Theoretical and Applied Genetics*, 84(7–8), 803–811.
<https://doi.org/10.1007/BF00227388>
- van Zanten, H. H. E., Mollenhorst, H., Klootwijk, C. W., van Middelaar, C. E., & de Boer, I. J. M. (2016). Global food supply: land use efficiency of livestock systems. *International Journal of Life Cycle Assessment*, 21(5), 747–758.
<https://doi.org/10.1007/s11367-015-0944-1>
- Von Braun, J. (2007). *The world food situation: new driving forces and required actions. Food Policy Reports 18. International Food Policy Research Institute*. International Food Policy Research Institute. <https://doi.org/10.2499/0896295303>
- Waldrop, M. M. (2015, July 15). Why we are teaching science wrong, and how to make it right. *Nature*. <https://doi.org/10.1038/523272a>
- Waldrop, P. B., Justen, J. E., & Adams, T. M. (1986). A comparison of three types of feedback in a computer-assisted instruction task. *Educational Technology*, 26(11), 43–45. Retrieved from https://www-jstor-org.proxy.mul.missouri.edu/stable/44424764?newaccount=true&readnow=1&seq=3#metadata_info_tab_contents
- Weinberg, E. R., Auerbach, M. A., & Shah, N. B. (2009). The use of simulation for pediatric training and assessment. *Current Opinion in Pediatrics*.
<https://doi.org/10.1097/MOP.0b013e32832b32dc>
- White, J. M., Eisen, E. J., & Legates, J. E. (1970). Sex-Heterosis Interaction, Heterosis and Reciprocal Effects among Mice Selected for Body Weight. *Journal of Animal Science*, 31(2), 289. <https://doi.org/10.2527/jas1970.312289x>
- Wieman, C. E., Adams, W. K., & Perkins, K. K. (2008). Physics. PhET: Simulations that enhance learning. *Science*. <https://doi.org/10.1126/science.1161948>
- Wiese, E. S., & Koedinger, K. R. (2017). Designing Grounded Feedback: Criteria for Using Linked Representations to Support Learning of Abstract Symbols. *International Journal of Artificial Intelligence in Education*, 27(3), 448–474.
<https://doi.org/10.1007/s40593-016-0133-9>
- Willham, R. L. (1970). Beef genetic simulation program.

- Windschitl, M., & Andre, T. (1998). Using Computer Simulations to Enhance Conceptual Change: The Roles of Constructivist Instruction and Student Epistemological Beliefs. *Journal of Research in Science Teaching*, 35(2), 145–160. [https://doi.org/10.1002/\(SICI\)1098-2736\(199802\)35:2<145::AID-TEA5>3.0.CO;2-S](https://doi.org/10.1002/(SICI)1098-2736(199802)35:2<145::AID-TEA5>3.0.CO;2-S)
- Winters, N., & Mor, Y. (2008). IDR: A participatory methodology for interdisciplinary design in technology enhanced learning. *Computers and Education*, 50(2), 579–600. <https://doi.org/10.1016/j.compedu.2007.09.015>
- Wright, S. (1952). The genetics of quantitative variability. *The Genetics of Quantitative Variability*. Retrieved from <https://www.cabdirect.org/cabdirect/abstract/19541603507>
- Wright, S. (1968). The genetics of quantitative variability. In *Evolution and genetics of populations* (Vol. 1, pp. 373–420). HM Stationery Office. Retrieved from <https://www.cabdirect.org/cabdirect/abstract/19541603507>
- Wu, R., Bradshaw, H. D., & Stettler, R. F. (1997). Molecular genetics of growth and development in Populus (Salicaceae). V. Mapping quantitative trait loci affecting leaf variation. *American Journal of Botany*, 84(2), 143–153. <https://doi.org/10.2307/2446076>
- Yang, H., Wang, J. R., Didion, J. P., Buus, R. J., Bell, T. A., Welsh, C. E., ... De Villena, F. P. M. (2011). Subspecific origin and haplotype diversity in the laboratory mouse. In *Nature Genetics* (Vol. 43, pp. 648–655). <https://doi.org/10.1038/ng.847>
- Zeng, Z. B. (1992). Correcting the bias of Wright's estimates of the number of genes affecting a quantitative character: A further improved method. *Genetics*, 131(4), 987–1001. Retrieved from <https://brwebportal.cos.ncsu.edu/zeng/Genetics-92-Z.pdf>
- Zhang, Q., Calus, M. P. L., Bosse, M., Sahana, G., Lund, M. S., & Gulbrandsen, B. (2018). Human-mediated introgression of haplotypes in a modern dairy cattle breed. *Genetics*, 209(4), 1305–1317. <https://doi.org/10.1534/genetics.118.301143>

VITA

Maria Therese Haag was born August 13, 1990 in Terre Haute, Indiana. Maria grew up showing a variety of animals including horses, goats, pigs, and rabbits. During high school she also worked at a local pet store. These early experiences solidified her interest in pursuing a career related to animal production.

Maria attended DePauw University from 2009-2013 where she received a BA in Biology and Political Science. Her initial goal was to become a science lawyer focused on agriculture and genomics. However, a brief internship made her reconsider. She then became more serious about pursuing a research career. Fortunately, as a member of the Science Research Fellows program at DePauw she had many opportunities to learn about research. Through this program, Maria spent the first two years of her undergraduate career conducting ecology research then the last two in physiology research. During her senior year, Maria won two national undergraduate research awards for her work in cardiac regeneration. During her time at DePauw she also completed two external internships—both at the University of Missouri. As a Miller Intern, Maria was able to gain firsthand experience conducting research into animal genomics.

These internships drove Maria to enter a master's program with Dr. Bob Schnabel in 2013. She completed her degree in 2015 where she stayed to pursue a PhD with Dr. Bill Lamberson. During her time at MU, Maria has published numerous peer-reviewed journal articles and presented at many conferences in the United States and abroad.