GENERATION OF NOVEL GENETIC RESOURCES IN THE MODEL ORGANISM *TRIBOLIUM CASTANEUM*

An Undergraduate Research Scholars Thesis

by

MAYRA GONZALEZ

Submitted to the Undergraduate Research Scholars program at
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by Research Advisor: Dr. Heath Blackmon

May 2020

Major: Biology

TABLE OF CONTENTS

ABSTRACT

Generation of Novel Genetic Resources in the Model Organism *Tribolium castaneum*

Mayra Gonzalez
Department of Biology
Texas A&M University

Research Advisor: Dr. Heath Blackmon
Department of Biology
Texas A&M University

The stored product pest, the red flour beetle (*Tribolium castaneum*), has emerged as a novel and powerful model organism over the last decade. Because these are stored product pests, the lab environment is similar to field conditions, and therefore, loss of genetic variation as the beetle strains adapt to lab conditions is minimized. Additionally, this species is an important pest with extensive impacts on US and worldwide agriculture. There is an extensive collection of mutant strains made from lab populations, but current research lacks a community genetic resource that consists of wild populations. The goal of this research is to identify phenotypically visible mutations linked to specific chromosomes and introgress these mutations into the genome of wild-type populations. The efficiency of the introgression will then be quantified through whole-genome sequencing. The genomic resources developed in this project will be publicly available and will accelerate the pace of research in labs around the world that use these species.

DEDICATION

This thesis is dedicated to my family and many friends for all of their continued love, patience and support and specially to my eight-grade science teacher, Mrs. Hannover, for sparking my love and interest for the science field.

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to my advisor, Dr. Blackmon, for providing me with the opportunity to work in his laboratory. He has been key in the development of my scientific thinking, and I would like to thank him for his advice and guidance throughout the course of my research at Texas A&M University. I also wish to extend my gratitude to my friends and colleagues from the Blackmon lab for their encouragement and support throughout the course of this project. I appreciate the time they have taken to mentor and assist me while also working on their own projects.

I also want to thank the LAUNCH Undergraduate Research Scholars program for providing me the opportunity to embark on this project. Special thanks also goes to the TAMU Writing Center and Mary for her assistance in formatting and proofreading help throughout this program, as well as the many others that have assisted in amending this paper.

NOMENCLATURE

PCR	Polymerase chain reaction, method used to make large amounts of DNA copies
Au	Aureate mutation that makes setae 2-3 times denser than normal
p	Pearl mutation that changes eye color from black to a light pearl color
F1	The first generation of offspring produced by crossing pure-breed parents
F2	The second generation produced by crossing F1 organisms with wild-type

CHAPTER I

INTRODUCTION

Model organisms have been key to building our understanding of basic biological principles (Secretariat of the Convention on & Biological Diversity, 2010). Model organisms are species where researchers have developed a variety of unique genetic strains and protocols that allow any researcher to easily explore a variety of biological questions. Today, there are many model organisms—including plants, animals, and microbes. With the help of fast-developing biotechnology, we are now able to use these genetic resources to build our understanding of the biological world and to generate products that improve quality of life (Secretariat of the Convention on & Biological Diversity, 2010). These improvements can range from life-saving medicines, to methods that improve the security of our food supplies, to a better understanding of the basic biological mechanisms that underlie all life on our planet. The purpose of this project is to create new genetic resources for the species *Tribolium castaneum* by creating a new strain of beetles with wild-type genetic variation but multiple visible mutations present on specific chromosomes.

The isolation of phenotypically visible mutations that could be mapped on to specific chromosomes and specific positions relative to other mutations was key to the development of modern genetics. Thomas H. Morgan, for example, raised many thousands of *Drosophila* flies in his famous "fly room" in search of mutant phenotypes that differed from the wild-type phenotype. The availability of these mutant strains in Morgan's lab led in part to the discovery of basic genetic concepts, such as sex chromosomes, linkage, and recombination (Morgan, 1910; Bridges, 1916; Bridges, 1921; Bridges, 1925; Bridges, 1935). Organisms that contain visible

mutations continue to serve as powerful tools in modern genetics because they allow for genotyping organisms in seconds without the necessity of Polymerase Chain Reaction (PCR) or other molecular-based approaches (Rice, 1994).

One particular species, *T. castaneum*, has rapidly emerged as a powerful model organism that offers many benefits in comparison to competing species. Unlike *Drosophila*, a common model organism, *T. castaneum* has chiasmatic meiosis in males (Park, 1934). It is also a member of the most speciose eukaryotic order, and it is more representative of other insects than *Drosophila* (Consortium, 2008). Additionally, its genome is small and well-assembled at just 250Mbp (Consortium, 2008). *T. castaneum* also has a short life cycle with a generation time of only 30 days, with a 5-day window spent as a pupa when individuals can be sexed and separated, allowing for controlled crosses (Sokoloff, 1977). Plus, *T. castaneum* has high fecundity rates and can be easily maintained on a diet of 95% whole wheat flour and 5% yeast. A final benefit to work on *T. castaneum* is that it is also a pest of stored grains, and research into fundamental aspects of *T. castaneum* biology can offer important insights into their control and eradication (Chen et al., 2015).

T. castaneum was first brought into the lab in the 1920s by Thomas Park and has been maintained and used in ecological and evolutionary studies continuously since this time (Park, 1932; Wade and Goodnight, 1998; Demuth and Wade, 2007). As a result, an extensive collection of mutant strains are available, including both spontaneous and induced mutations. However, these mutations are primarily present in highly inbred long-term lab populations which have experienced many population bottlenecks over decades of maintenance in lab settings. What is lacking in current research is strains that have recently been collected from the wild but also have visible mutations that can be employed in genetic experiments. To solve this problem, we

identified phenotypic mutations that can be introgressed into a wild-type population. Through backcrossing these mutant strains with two recently collected strains from Louisiana and Texas, we have created a valuable genetic resource that can be used by any lab studying *T. castaneum*.

CHAPTER II

METHODS

Two populations were collected from the wild, one from Marksville, Louisiana and one from San Angelo, Texas. These populations have been allowed to adapt to lab conditions for 20 generations. Each wild-type population was crossed with a mutant population containing recessive mutations that produce visible morphological changes to the beetles. A total of four different backcrosses were generated from mating the wild-type populations with the mutant populations. The mutations backcrossed into the wild-type populations are aureate (*au*), which is located on chromosome three and generates setae two-to-three times denser than normal, and pearl (*p),* which is located on chromosome nine and changes the color of the center of the eye from black to light pearl. In summary, a total of four backcross strains were generated, as shown in **Table 1**.

Table 1. Backcross Strains Generated

Starting Strains	Coordinates of Wild Source Population	Chromosome Location	Strain Identifier
Marksville x *au* Mutant Line	31.1280° N, 92.0662° W	3	Mau3
Marksville x *p* Mutant Line	31.1280° N, 92.0662° W	9	Mp9
San Angelo x *au* Mutant Line	31.4638° N, 100.4370° W	3	SAau3
San Angelo x *p* Mutant Line	31.4638° N, 100.4370° W	9	SAp9

Note: For each strain, the origin location for the wild-type strain is listed as well as the chromosomal location of the mutation that has been introgressed.

Backcross Protocol

All populations described above were maintained on standard media (95% whole wheat flour and 5% brewer's yeast) at 30°C. The following protocol below was completed for each of the four strains described in **Table 1**. All female beetles were sexed and separated during their pupal stage to ensure only virgin females were used in crosses. Fifteen virgin females of the wild-type population were individually crossed with 15 males from the mutant line. Each of the individual crosses were set up in 50 mL vials and 25 mL of standard media. The beetles were allowed to mate and lay eggs for 19 days, after which the original parents were removed from the vial, leaving behind only the new generation of beetles, known as the F1 generation.

Approximately 11 days later, the newly eclosed F1 adults, all of whom are heterozygous for the mutation of interest, were collected and combined into a single population housed in a 500 mL jar with 200 mL of standard media. These beetles were allowed to mate for 19 days, then the parents from the F1 generation were pulled out, leaving behind only the new offspring. By allowing the F1 beetles to breed with one another for one generation, we generated offspring where 25% of the population are homozygous for the mutation of interest. Males exhibiting the recessive mutation were picked out, and these beetles become the new mutant strain from which the next generation is bred.

To begin the next generation, we simply backcross these F1 males with virgin wild-type females to produce the F2 generation, and the process described above is repeated. Each time the mutant strain is backcrossed with the wild-type strain, the proportion of alleles from the mutant strain is reduced by half. This process will continue for at least seven generations, eventually yielding a strain where greater than 99% of the genome is from the wild-type strain but will carry the mutation of interest.

Figure 1 shows the crossing design and the dilution of the mutant genome with the wild-type genome by illustrating how the proportion of mutant DNA went from 50% to 25% and finally to about 12% by generation three. At the conclusion of this work, we will sequence the new strains, the mutant strain and the original wild-type strain. This will allow us to confirm the proportion of the genome that is from each original parental strain, and show that we have successfully moved these mutations from the mutant line and inserted them into the chromosomes of the wild-type population.

Figure 1: Visual representation of mutant DNA distribution in the chromosome containing the mutation. After each backcross, the amount of mutant DNA gets reduced by one half.

CHAPTER III

RESULTS

Due to the unforeseen events surrounding the COVID-19 virus in Spring 2020, complete data was unavailable at the time of publication for this URS thesis. However, this project is well under way and should be completed with two more months of backcrossing once researchers are able to return to the lab. As a result of this we have chosen to perform a simulation study of the expected results.

Crossing Results

We used a beetle line from the lab which contains both *au* and *p* mutations. This mutant line of beetles was bred with wild-type San Angelo beetles, and the San Angelo line was generated. This new line contains the crossings for both the *au* and *p* mutations. Likewise, a Marksville strain was also generated with the mutant line that contains all Marksville crossings between *au* and *p* mutations.

The San Angelo strain started with 15 individual crosses between a wild-type San Angelo beetle and a homozygous recessive beetle from the mutant line. At the 20th day, after removing the original parents, only the eggs of the next generation of beetles were left behind. By the time the entire F1 generation transitioned into adult form, there were, on average, 10-15 beetles for each vial. The total number of F1 individuals produced by the original parents was approximately 200 beetles in most generations and crosses. All of these beetles are heterozygous for both mutations, so all individuals from this generation displayed the wild-type phenotype. Backcross two was initiated, again with 15 individual crosses between these new beetles and San Angelo wild-type beetles. About 20 days after these individuals were removed, there were, once

again, about 10-15 beetles per vial for a total of approximately 200 beetles in the F2 generation. As expected, the majority of this generation was still heterozygous and displayed the wild-type phenotype, and about a quarter of this generation was homozygous for both mutations and displayed *au* and *p* phenotypes. Backcross three once more started with 15 vials containing crosses between F2 beetles and wild type San Angelo beetles. Here, only F2 beetles displaying both mutations (*au* and *p*) were used. The offspring for the F3 generation is currently pending.

Similarly, the Marksville line of crosses began with 15 crosses between wild-type beetles collected from Marksville, Louisiana and homozygous recessive individuals. At around the 25th day after the original parents were pulled out, F1 adults began to emerge. However, there were more complications with the Marksville line in comparison to the San Angelo line. First, the F1 generation had about 10 beetles for each vial, which was a lower average than the F1 generation for the San Angelo line. Once again, all of beetles from this F1 generation were heterozygous for both mutations, so all individuals displayed the wild-type phenotype. Secondly, for unknown reasons, the Marksville line ran into some complications during backcross two. As usual, backcross two was initiated with 15 crosses, and at the 30th day after removing all parental beetles, the F2 generation began to transition into adult form. Yet, out of the 15 individual crosses, there were four vials that failed to produce any offspring. Additionally, the average count of beetles per vial was once again lower, with an average of 10 beetles for each vial and slightly less than 100 total beetles for the F2 generation. Once more, this generation was mostly composed of heterozygous individuals with wild-type phenotype, and less than a quarter composed of homozygotes. However, this generation still managed to produce just enough homozygote recessives for the next backcross. The F3 generation is currently in transition into adult form.

Phenotyping Mutant Strains

T. castaneum has a total of 10 chromosomes in its genome. The first mutation identified from the generated mutant beetle lines was the *au* mutation, contained in chromosome number three of *T. castaneum's* genome. This mutation manifested itself throughout the entire surface of the cuticle. A typical wild-type beetle will display a moderate amount of setae, or body hair. On the other hand, *au* mutants will have setae on the general body surface of the beetle two-to-three times denser than normal, giving beetles with this mutation a "frosted" appearance.

The second mutation identified was the *p* mutation and is located on chromosome nine. Normal wild-type individuals have dark black colored eyes. However, since the *p* mutation changes the color of the center of the eye from black to light pearl, individuals with this mutation can be differentiated from wild-type individuals by picking out beetles that contain white-colored eyes.

Population Genetic Simulation

To explore the expected outcomes of our experiment we have used a forward time population genetic simulation. Our simulation is a diploid, 1000 loci, biallelic model with discrete generations and a constant population size of 100. In the simulation, a matrix with 1000 columns and two rows was used to represent the genome of each beetle in the simulation. Each row of the matrix represented one of the homologous chromosomes the beetles inherited from their parents. The matrix contained values of zero and one to represent the mutant and wild-type allele respectively. The initial chromosomes in the model were fixed, either for all values of zero or all values of one, to serve as the starting populations of mutant and wild-type strains respectively. The next step in the simulation was to produce gametes from these two strains and

join them, creating a chromosome that we would expect in the original F1 individual that contains one wild-type maternal chromosome and one mutant paternal chromosome.

The next step in the model is to allow the F1 individuals to mate with one another. To mimic the crossing over that occurs during gametogenesis, we allowed for a single recombination event by choosing a random location along the chromosome. After crossing-over, this gamete now contained DNA that is a mix of the maternal and paternal chromosomes. We created an excess of offspring because only 25% of the offspring are expected to be homozygous for the mutant allele. We selected 100 offspring that are homozygous at this site to move on to the next generation.

After the selfing stage of the model, we then backcrossed the beetles carrying the mutant alleles to the wild-type strain. This was done by generating gametes, as described above, from the new mutant beetles and joining these with gametes from the wild-type strain. The process of selfing followed by backcrossing can be repeated indefinitely. **Figure 2** shows the frequency of wild-type alleles for both a chromosome carrying the mutation of interest and an autosome with no mutation.

Our simulations demonstrate that for chromosomes that lack a mutation, three generations of backcrossing were sufficient to replace most mutant alleles with wild-type alleles (**Figure 2B**). In contrast, for the chromosome with our marker mutations (*au* and *p*, location is indicated as a dashed vertical line), the replacement of mutant alleles with wild-type alleles proceeded more slowly (**Figure 2A**). Our results show that by 20 generations, all mutant alleles have been purged from our strain in chromosomes lacking a marker (**Figure 2F**). However, for chromosomes with a marker mutation, a small region that contains mutant alleles will remain (**Figure 2E**).

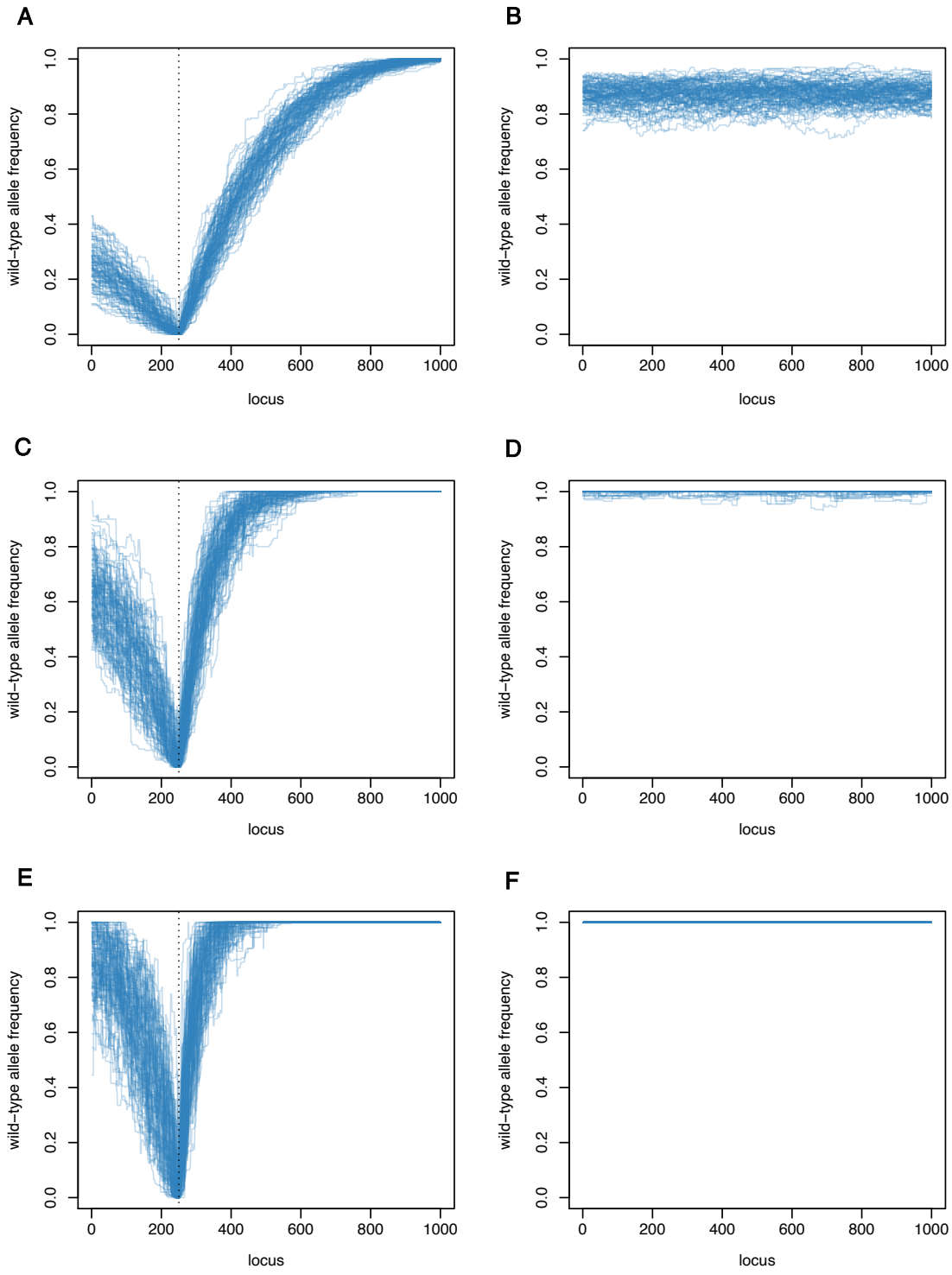

Figure 2: Each panel shows the result of 100 simulations. In all panels, chromosome position is on the horizontal axis and wild-type allele frequency is on the vertical axis. Panels A and B are after 3 generations. Panels C and D are after 10 generations, and panels E and F are after 20 generations. Panels A, C, and E show allele frequency on a chromosome with our mutation of interest, indicated with vertical dashed line. Panels B, D, and F show allele frequency on a chromosome without a marker mutation.

CHAPTER IV

CONCLUSION

My research has created a new genetic resource that will benefit my lab and other labs studying *T.castaneum*. Specifically in my own lab, these strains will be used to breed beetles in a way where we force a wild-type chromosome, homologous to the chromosome containing the introgressed mutation (*au* or *p*), to have an inheritance pattern that is the same as a Y or W chromosome (passing only through males or only through females respectively). Since Y-chromosomes are hemizygous and only found in males, we are able to create a Y-linked pattern of inheritance because the introgressed mutations are recessive and must be present in two copies to produce a visible phenotype. Therefore, if we cross females that display our mutant phenotype with males that show the wild-type phenotype for every generation, we are forcing the wild-type chromosome to pass only through males. This breeding design creates a pseudo-Y chromosome. By reversing this breeding design, breeding females that show the wild-type phenotype to males that show the mutant phenotype, we force the wild-type chromosome to become a pseudo-W chromosome. This breeding approach shown in **Figure 3** will be used to study the differences in natural selection between males and females.

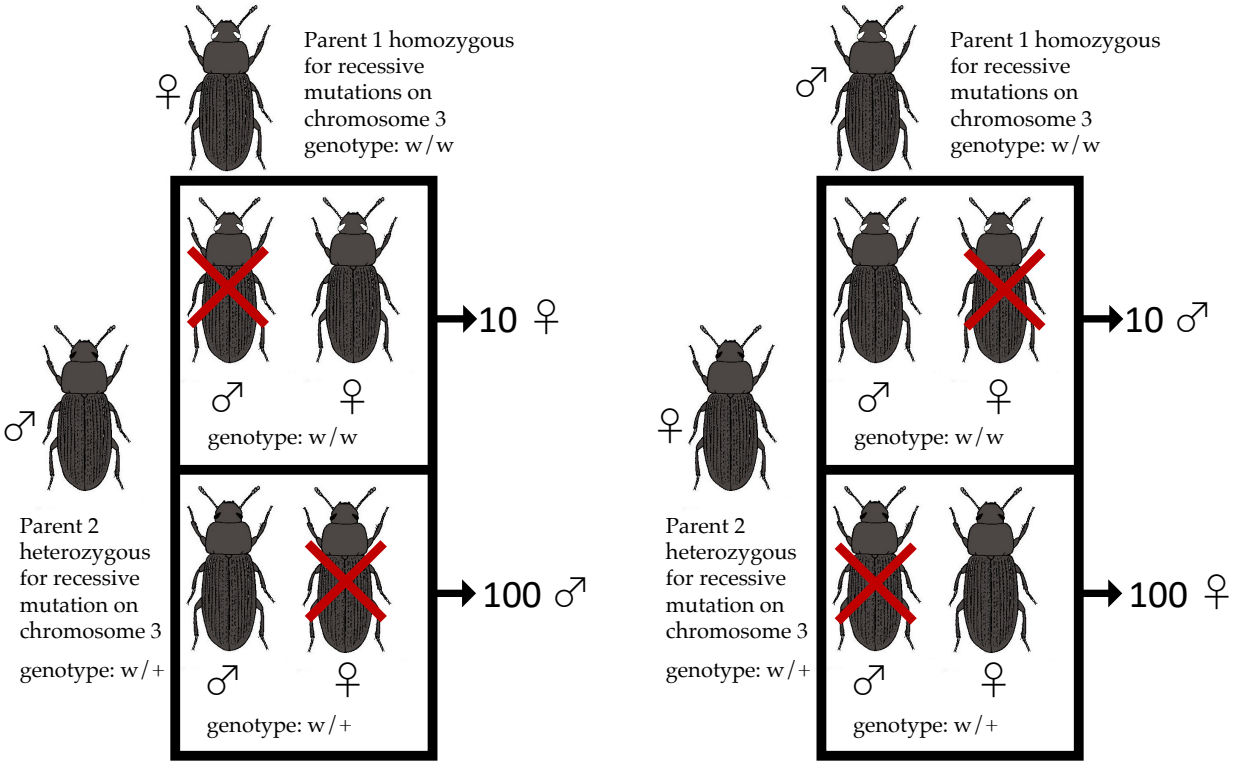

Figure 3: This breeding design facilitates feminization or masculinization the genome by biasing sex ratios and treating the two mutations (*au* and *p*) as artificial Y or artificial W chromosomes.

Throughout history, researchers, such as Thomas H. Morgan, have made groundbreaking discoveries with the aid of mutant *Drosophila* strains. However, as a field, we currently lack a community genetic resource in *T.castaneum* developed with the primary goal of revealing the nature of sexual antagonism using strains that have recently been collected from the wild but also have visible mutations. Here, we developed a genetic resource that can easily be used for this purpose. Using the breeding design shown above can allow us to investigate the frequency and characteristics of sexual antagonism across the genome.

Genetic resources have proven to be an integral component for human progress. The rapid development of modern biotechnology over the past decades has enabled scientists to use

genetic resources in ways that build our understanding of the biological world and to generate new products that improve quality of life. These improvements range from life-saving medicines, to methods that improve the security of our food supplies, to a better understanding of the basic biological mechanisms that underlie all life on our planet.

REFERENCES

Bridges, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity (concluded). Genetics 1**:** 107.

Bridges, C. B. 1921. Genetical and Cytological Proof of Non-disjunction of the Fourth Chromosome of Drosophila Melanogaster. *Proceedings of the National Academy of Sciences*, *7*(7), 186–192. https://doi.org/10.1073/pnas.7.7.186

Bridges, C. B., 1925 Sex in relation to chromosomes and genes. The American Naturalist 59**:** 127-137.

Bridges, C. B., 1935 Salivary chromosome maps with a key to the banding of the chromosomes of Drosophila melanogaster. Journal of Heredity 26**:** 60-64.

Chen, Z., Schlipalius, D., Opit, G., Subramanyam, B., & Phillips, T. W. (2015). Diagnostic molecular markers for phosphine resistance in U.S. populations of Tribolium castaneum and Rhyzopertha dominica. *PLoS ONE*, *10*(3). https://doi.org/10.1371/journal.pone.0121343

Consortium, T. G. S., 2008 The genome of the model beetle and pest Tribolium castaneum. Nature 452**:** 949.

Demuth, J. P., and M. J. Wade, 2007 Population differentiation in the beetle Tribolium castaneum. I. Genetic architecture. Evolution 61**:** 494-509.

Morgan, T. H., 1910 Sex limited inheritance in Drosophila. Science 32**:** 120-122.

Park, T., 1932 Studies in population physiology: the relation of numbers to initial population growth in the flour beetle Tribolium confusum Duval. Ecology 13**:** 172-181.

Park, T., 1934 Observations on the general biology of the flour beetle, Tribolium confusum. The Quarterly Review of Biology 9**:** 36-54.

Rice, W. R., 1994 Degeneration of a nonrecombining chromosome. Science 263**:** 230-232.

Secretariat of the Convention on, & Biological Diversity. (2010). *Convention on Biological Diversity: ABS Theme Uses of genetic resources*. Retrieved from https://www.cbd.int/abs/infokit/factsheet-uses-en.pdf

Sokoloff, A., 1977 *The biology of Tribolium with special emphasis on genetic aspects. Volume 3*. Clarendon Press.

Wade, M. J., and C. J. Goodnight, 1998 Perspective: the theories of Fisher and Wright in the context of metapopulations: when nature does many small experiments. Evolution 52**:** 1537-1553.