**Genetic Modification and the Power of CRISPR-Cas9**

* 1. **Gregor Mendel**

Genetic modification is a process that has gone on since the beginning of time and is present in nearly every species of organism on the planet. **Genetic modification** is described as the altering of the genotype of an organism through some specific means such as selection, biochemical means or engineering. Genetic modification of organisms has occurred since the ancient times. Gregor Mendel, best known for his experiments regarding inheritance, utilized different characteristics of pea plants to record and observe genetic traits and how they could be altered via selection (Wakim et al., 1). His pea plants usually included two distinct characteristics whether it was the seed form of round or wrinkled, the **cotyledons** color of yellow or green, the flower color of white or pink or the pod type.

 He was able to influence these characteristics by selectively breeding said species and observing the genetic differences that followed. The alteration of a species offspring are regarded as an **F1** generation which often showed the results of genetic alteration. The second generation of plants were dubbed the **F2** generation and were the result of breeding the altered F1 generation with themselves, which often resulted in differing observable traits from the original F1 generation. This is known as the **Law of Segregation**, which describes two distinct factors that control a genetic expression, where one often overrides the other (Wakim et al,. 2). These are seen or can be observed in a **punnet square** which allows a user to observe the possible genetic combinations of two different species via their gene identification and level of heredity, as well as the probability of each gene combination. Repeats of Mendel’s experiments led to him creating his second law or the **Law of Independent assortment**, which describes the factors of different genetic characteristics often work independently of the other (Wakim et al,. 2). These laws and the observations of Gregor Mendel have paved the way for an understanding of DNA and the power of genetics that have influenced so much in our modern culture today.

* 1. **DNA**

In the 1860’s Swiss scientist Friedrich Miescher was considered the first man to discover DNA while observing human white blood cells (Pray et al., 1). Years later in 1919, Russian scientist Phoebus Levene proposed of a polynucleotide model of DNA composed of nucleotides, nitrogen bases, sugars, and phosphates. Years later in the 1950’s, scientists Francis Crick and James Watson would conduct their own research and publish the study “*Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid*” in which they describe a three-dimensional structure for said DNA that followed a twisting double helical structure where individual nucleotide bases were held together by bonds surrounded by a sugar phosphate backbone (Prayet al., 2). This discovery put a structure and form to the ideal of genetic differentiation in place, through the bases of A, T, C, and G. More importantly it was discovered that these bases were universal in all forms of organism across the globe, by extension Watson and Crick had described the keys that are responsible for **genotypes** of all living things.

The result of this revelation shed light on the genetic makeup of every organism and allowed a specific look into the universal instructions present in them. The official name of DNA is **Deoxyribonucleic Acid**, lending itself to the repeating nucleotides linked as chains (Deoxyribonucleic Acid Fact Sheet, 1). Nitrogen bases are the specific distinctions that determines DNA, these bases are Adenine, Thymine, Guanine and Cytosine (Pray, 2). The genetics of an organism can be broken down into a universal sequence such as ATATCGTG that may account for a specific phenotypic expression of said trait, for example coat color in an animal or skin tone in humans. Each nucleotide base will only pair with its specific compatible base, so Adenine will only pair with Thymine and Guanine will only pair with Cytosine. These simple characteristics are what determine the complexity of life seen around us today. The discovery of the building blocks of life have lend itself to be one of the most important scientific advancements of the twentieth and twenty first century.

* 1. **Genetic selection**

As Mendel formally reviewed, genetic selection for specific traits in animals has been going on for thousands of years. The agricultural industry is a prime example we can look to today. From the beginnings of society, mankind has used breeding and genetics to alter and or select for more desirable traits in farm and plant species without even recognizing the full story of genetics. Breeds of cattle that are raised and produced today all began with the goal in mind of taking a like set of characteristics in said animal and breeding it with others to select for those desirable traits, such as the milking capacity of the Holstein cow or the fat content of the Jersey dairy cow.

Plant species are no different, today we have selected and shaped breeds of plants such as corn to be very different from their formally grown characteristics dating back to the 1700’s in America. Watermelon is another good example, compare the looks of watermelon today from the species of watermelon formally grown in the 1800’s and you will see just how drastically a species **phenotype** can change. We still use these techniques today although with a greater understanding of the potential and power of said techniques. Artificial Insemination in the cattle industry has allowed producers to select the F1 offspring of their cows to be influenced by the genetic traits of different breeding bulls, and their specific traits. If a producer is looking to introduce a higher milking component in his calves, then he can select a parent with those characteristics and apply those genetics to the F1 generation of his calf crop. The advent of this technology has allowed for producers to be extremely flexible in their ability to reach and comply with market demand for traits in our food animals. The same thing occurs in plant agriculture where we have crossbred different species of plants to select for desirable traits in said generations. The development of drought and insect resistant species of plants, seedless fruits or the selection of lodging resistant grain species have all come about from genetic selection and have helped to secure food for countless people across the globe. Specialization of agriculture today and the availability of so many different types of food present in our modern- markets has been possible, in large part due to genetic selection in our food species, plant, and animal alike.

* 1. **CRISPR-Cas9 microbial adaption**

In the early nineties, researcher Francisco Mojica, at the University of Alicante, Spain was one of the first individuals to research and develop working theories on the CRISPR biological system (Broad Institute, 1). Research of adaptive immunity revealed parts of sequenced DNA of **bacteriophages** shared common sequences present in adaptive immunity of biological systems.

Cas9 is a Cas gene system present in homology of certain genes that share a specific protospacer adjacent motif sequence (Barrangou et al., 1). Cas 9 was discovered in 2005 by the French scientist Alexander Bolotin at the French National Institute for Agricultural Research, after experimenting with a Streptococcus thermophilus bacterium DNA sequence (Broad Institute, 1). The Cas9 was discovered in the CRISPR **locus** of the bacterium. **Protospacer Adjacent Motif** is required by the target sequence to be recognized fully. Cas9 is ultimately a component of DNA cleavage in immune systems. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) DNA segments are often extremely simple and have allowed for scientists to hijack them to edit genes.

CRISPR has become an extremely effective form of adjusting specific genes in organisms from creating drought resistant crops to eliminating genetic diseases in animals. CRISPR is possible by utilizing **Ribonucleic acids**, as a way to read the genetic DNA of a specific organisms. The RNA allows for a scientist to identify a specific gene compartment of a nucleus of cells, by bringing the Cas9 DNA cleavage protein to bear on the specific location of the gene. The Cas9 then cleaves the DNA section and creates a break in the DNA double helix strand. The cleaved DNA section is automatically repaired by the cell, which is where scientists come in. Researchers have been able to modify the repair in such a way that they can do things like disable certain genes, and in many cases insert a specific nucleotide into the code. The CRISPR has taken the form of scissors able to cut and later paste certain genes into specified DNA strands. The utilization of the CRISPR by an organism is a natural part of **adaptive immunity**, as an organism will utilize the CRISPR-Cas9 to cleave off specific strands of DNA from pathogenic bacteria and viruses and copy those DNA strands for storage in an immune response. Cells can recreate a specific antibody stored from a previous encounter with a pathogen through this method, allowing the body to mass produce the specified antibody for the pathogen in a rapid setting.

This contributes to a quick immune response. In essence the utilization of CRISPR-Cas9 by scientists is a hijacking of the body’s immune systems for the purpose of genetic edits. Furthermore, the availability and simplicity of the technology has allowed for many extreme advancements of genetic editing and tracking in recent years, making it a widespread technology with far reaching applications.

* 1. **Modern Genetic Editing**

Today gene editing has taken center stage in technological and medical advancements. Gene editing via the CRISPR-Cas9 method is being explored for it is potential in fighting disease. The human genome is extremely complex hosting over three billion base nucleotide pairs of DNA with over twenty two different **chromosomes** and the average chromosome containing nearly 120 million nucleotide DNA pairs. The reach of genetics on our bodies is understated, with each human genetic code responsible for protein, RNA, DNA, synthesis, growth, bodily maintenance, and many other things. The complexity of genomic makeups is not without apparent errors as DNA sequences can be miscopied, deleted, mutated, and added causing their own slew of potential problems for a living organism. **Monogenic diseases** such as Huntington’s Disease, Cystic Fibrosis, Sickle Cell disease and Hemophilia, as well as types of cancer, down syndrome and autoimmune disease are caused by problems in the genetic code of individuals.

With the mapping of the human genome in the early 2000’s, researchers can get a better look at how these diseases occur as well as their origin points on the genetic code. CRISPR-Cas9 has also allowed researcher to identify **epigenic** markers of specific diseases. Specifically, in the past, the use of electroporation – or a pulsed voltage current to edit genes more effectively has been used with success to cure HIV in some patients **in vivo** by genetically mutating the HIV virus along the CCR5 Delat32 cell linage, rendering the virus inoperative (KC et al., 1). Other techniques such as hydrodynamic injection of a bolus type gene editing substance into a subject’s bloodstream has been shown to relieve certain liver diseases in laboratory tests. Gene therapy treatment has made significant bounds with clinical trials of monogenic diseases such as Hemophilia in patients involved in said studies. Treatment of Cystic fibrosis and Sickle Cell disease have also conducted several trials via gene therapy where in genetically modified stem cells were injected into patients suffering from these diseases. The results of said subjects showed a direct result on patients through several infusions and subsequent checkups where the patients’ bodies, accepted the modified blood cells and began to reproduce them naturally following injections. The subsequent results of the experiments showed that subjects were relatively symptom free owing to the success of the treatment.

Gene therapy in cancer treatment has occurred lately relating to chimeric antigen receptor **T cell** adjustment where said T cells of patients are made to express antigen binding and then are reinjected into patients. The results of T cell response show that the modified cells can recognize specific tumors by antigen receptors (B-cell antigens) and has been used in **lymphoblastic** leukemia treatments.

Gene editing in agriculture has been used for some time as well. Genetic breeding and editing of specific crops around the world have been responsible for development of insect and drought resistant species of crops that are able to be grown at a more efficient rate and with fewer chemical means of pest control, leading to less potential ecological damage, and greater overall food production in developing countries.

* 1. **Concerns in Gene editing**

The power of gene editing cannot be underestimated, as the ability to individually select and edit specifics of a person’s genetic code unlocks a whole potential gray area in which science may be unethically used. The subject regarding the use of gene editing in disease treatment is generally agreed upon by those having the conversation but questions remain as to how far science should be allowed to go regarding the genomes of humans. It will soon be possible to alter the genetic traits of people to feature more favorable traits during embryonic development, dubbed “designer babies”. The fear of genetically superior humans being introduced into today’s society is a real quandary that politicians and leaders must face in the future. Unregulated use of genetic altering can also be used in unique circumstances to alter and recreate extinct species present such as certain experiments undergoing in Russia to clone Wooly mammoths from DNA collected in the ice. Genomic cloning of animals from collected stem cells have already been performed successfully such as Dolly the sheep, the first cloned animal. Subsequent cloning procedures have been done each resulting in successful results.

Today Chinese scientists have claimed to have successfully genetically edited twin human embryo’s that were carried to term, causing outrage among the scientific and political community (Normile et al., 1). Ultimately the future of genomic editing and allowable space that this technology can expand will be a point of major debate for future generations. One thing is for certain, that the advent of easily useable technologies like CRISPR – Cas9 has pushed the science of genomics to the brink of new territories.

**Key Words:**

GENETIC MODIFICATION; changing the genetic makeup of a specific organism through means of breeding, or DNA alteration.

COTYLEDON; plant embryo.

F1; the first generation of two separate breeds.

F2; the offspring of the F1 generation following interbreeding.

LAW OF SEGREGATION; Law proposed by Gregor Mendel that gene pairs will separate when creating gametes but will unite following fertilization.

PUNNET SQUARE; tool used to identify and predict the probability of recessive and dominant genes in an organism.

LAW OF INDEPENDENT ASSORTMENT; refers to how different genes will separate when developing independent of each other into gametes, and that alleles received for one gene will not influence the alleles received for another.

PHENOTYPE; The physical expression of the genes of an organism.

BACTERIOPHAGE; A virus that reproduces within and targets bacteria.

LOCUS; The point of a genetic code that will determine a trait via multiple genes.

PROTOSPACER ADJACENT MOTIF; Classified by dual – 6 pair bases of DNA that are targeted by Cas 9.

RIBONUCLEIC ACID; single stranded ribose backbone with a phosphate group and differs from DNA in which Thymine amino acid is replaced by Uracil.

ADAPTIVE IMMUNITY; Characteristic of the immune system to fight infection by adapting to viral exposure of a specific antigen.

CHROMOSOME; Nucleic acid structure of the nucleus of cells that is responsible for carrying the genetic information.

MONOGENIC DISEASE; Diseases that are caused by the modification of a single gene in the genetic code.

DEOXYRIBONUCLEIC ACID ; dual polynucleotide chains on a double ribose strand. Differs from RNA in which Thymine amino acid replaces Uracil.

EPIGENIC; influences on genetic expression that are not the result of genetics ex: cancer causing agents.

IN-VIVO; some process that occurs within an organism.

T-CELL; bodily lymphocyte that is a primary cell in the immune system.

LYMPHOBLASTIC; Refers to white blood cells – generated within the bone marrow.

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