

Lecture 33

Agricultural Scientific Revolution: Genetic

Inheritance

The similarities and dissimilarities between parents and offspring have been commented on from the beginning of the written record. The aphorism “like begets like” is the basis of genetic wisdom. Knowledge of the genetic connection between parents and offspring is implicit in the biblical prohibitions against adultery, which results in ambiguity regarding inheritance and paternity. Similarly, insight into the function of sex in plants dates to Mesopotamia with clear knowledge of pollination in date palms (Fig. 1). Theophrastus was aware of these ancient concepts, but this information became virtually lost until the Dutch botanist Jacob Camerarius (1670) experimentally proved the sexual nature of plants (Fig. 2).

Despite the clear relation between parent and offspring there was not a basic way to predict performance. Hereditary theories were murky and the best analysis was a blending of blood although it was understood that some characters could reappear and that certain traits could be sought and maintained in certain lineages.

In the 19th century, the 1st experimental research began to confront the problem of inheritance. Thomas Andrew Knight (Fig. 3) demonstrated segregation of seed characters of the garden pea but offered no explanation.

The great Charles Darwin (Fig. 4) was the first to show the importance of genetic variation and used it to determine the evolution of species based on differential reproduction and survival of the fittest. In 1868, he collected a vast amount of information and carried out a review of experimental studies but failed to arrive at a satisfactory theory of inheritance. His concept of pangenesis involved a persistent hereditary unit, but he assumed incorrectly that units were replenished by input (gummulea) from somatic tissue. The difficulties of a genetic theory were compounded by a lack of understanding of variation both continuous and discontinuous, the interaction with environment, and of complications introduced by dominance, inbreeding, outbreeding, apomixis, and mutation. Despite his inability to account for the mechanism of inheritance, Darwin’s views on evolution was to be the dominant force of biology in the 20th century.

Yet all confusion was swept away by an obscure monk, Gregor Mendel (Fig. 5), in a backwater town of the Austro-Hungarian Empire. In a series of brilliant experiments with the garden pea (**Reading 33-1**), Mendel was able to perform precisely the correct experiment with precise correct interpretation. His evidence was presented in a scientific paper this is a model of order and lucidity. More astonishing, the hypothesis was formulated in a pre-cytological era. Mendel essentially demonstrated that characters were controlled by entities or factors that we now call genes. These genes interact to form a phenotype and segregate unaltered from one generation to the next. He demonstrated that in peas 2 forms of the gene (we now call them alleles) can interact in the formation of a visible trait (phenotype). When the alleles vary in function, one could dominate the other. Furthermore, the recessive allele although hidden, passes unaltered from generation to generation, and reappears in predictable ratios.

Genetics

The immediate impact of Mendel’s paper, presented in 1866, was nil. It was fairly widely distributed but either ignored or brushed off until its “rediscovery” in 1900. Yet the period from 1866 to 1900, the classical period of cytology, the study of cells, was to establish the basic part of structural cell biology that put Mendel’s theoretical discovery of inferred genes (*anlage*) into structures contained in each living cell. In 1866, Haeckel published his conclusion that the cell nucleus was responsible for heredity. Soon thereafter, the chromosomes, the physical framework for inheritance became the focus of attention in mitosis, meiosis, and fertilization with speculation on its relation to heredity. The issue was cloudy because the details of the meiotic process were not well understood. The pieces of the puzzle however quickly fit together only after the independent verification of Mendel’s result by Hugo de Vries, Carl Correns (a student of Nägeli, the professor who while sent Mendel’s paper, refused to understand it), and Erich von Tschermak. Neither one

of them completely understood Mendel's paper although Correns came close. It remained for W.S. Sutton to recognize, in a 1902 paper, that the association of paternal and maternal chromosomes in pairs and their subsequent separation during meiosis constituted the physical basis of Mendelian genetics. Sutton wrote 2 of the most important papers in cytology but never received his PhD; he left science for surgery. (Sutton was a student of E.B. Wilson whose famous work *The Cell* (1896) described chromosome behavior and speculated on their role in heredity).

The genetic revolution had a rapid impact on plant and animal improvement. Although breeders had unconsciously been using many appropriate procedures via crossing and selection in the 19th century, the emerging science of genetics and, especially, the fusion of Mendelism and quantitative genetics, put plant and animal breeding on a firm theoretical basis.

The relation between genetics and post-Mendelian plant breeding is best exemplified by 2 routine breeding protocols. One is the extraction and recombination of inbreds combined with selection to produce *heterozygous but homogeneous* hybrids, a procedure analogous to reforming Rubic's cube, whereby combinations are 1st disturbed to complete the final order. The other is backcross breeding, in which individual genes can be extracted and inserted with precision and predictability into new genetic backgrounds. The combination of backcross breeding to improve inbreds and hybrid breeding to capture heterosis is the basis of present-day maize and onion improvement. The elucidation of the genetics of male sterility in onions by H.A. Jones (Fig. 8) and A.E. Clarke solved a horticultural problem of hybrid seed production and brought attention to non-nuclear genetic factors.

The success of the new science of plant breeding had a substantial impact on agriculture and horticulture. Dramatic successes quickly followed: examples include hybrids and disease resistant crops. The spectacular example of plant breeding prowess was the development of short-stemmed photoperiod-insensitive wheat and rice, the forerunners of the **Green Revolution** for which Norman Borlaug (Fig. 9), a plant breeder with the Center for the Improvement of Maize and Wheat (CIMMYT) was to receive the Nobel Prize for Peace in 1970, other advances include the creation of a new crop species, triticale, from hybrids of wheat and rye (an accomplishment in which Borlaug also played a part), a host of disease resistant crops, and seedless watermelon from the production of triploids produced from intercrossing tetraploids (plants having twice the number of chromosomes) and diploids.

The Biotechnology Revolution

Dramatic advances in biology augur a 3rd agricultural revolution involving biotechnology, a catch-all term that includes both cell and DNA manipulation. A conventional baseline for the biotechnological revolution is 1953, the date of the brilliant Watson-Crick paper on the structure of DNA. However, the biotechnological revolution has no precise beginning, because science is cumulative. One pathway developed from a series of investigations into gene function and structure and another from the culture and physiology of cells using microbial techniques.

The beginnings of inquiries into gene structure can be traced to the 1860s when a young Swiss, Johann Fredrich Miescher, submitted a paper describing a substance he called nuclein that was derived from pus scraped from surgical bandages and later found in fish sperm. (The paper was held up from publication for 2 years by Hoppe-Seylers, editor of a journal that bore his name, until some of the experiments were personally repeated; editors were very dedicated in those days.) In 1889, Richard Altmann, a student of Miescher, split nuclein into protein and a substance he named nucleic acid. Two distinct kinds of nucleic acid were found in thymus and yeast. Ascoli, in 1900, and Levene, in 1903, demonstrated the presence of adenine, cytosine, guanine, and thymine in thymus nucleic acid (now known as DNA) and, with uracil replacing thymine, in yeast nucleic acid (RNA). The original assumption that those bases were present in equal amounts and thus formed a "stupid" molecule proved to be faulty, but Chargoff, in 1950, demonstrated a key equality; in molar amount, cytosine equaled guanine and adenine equaled thymine. This suggestion was basic to the complementary replication of DNA and to the discovery of the structure of the double helix.

Genetic studies of the biochemistry of gene products date to 1902, when the British physician, Sir

Archibald Garrod, demonstrated that the human disease alkaptonuria was inherited and, moreover, was due to an alteration in nitrogen metabolism. In a paper in "Lancet," entitled "Inborn Errors of Metabolism" (and published in book form in 1909), alkaptonuria was established as a gene-induced enzymatic block. This prescient study affected the course of biochemistry but remained unappreciated, if not unread, by early geneticists.

The genetic investigations of metabolism from 1900 to 1950 formed a subdiscipline: biochemical genetics. A trend developed toward the study of ever-simpler systems: from human diseases to the color of flower petals and *Drosophila* eyes and from there to the nutrient requirements of the bread mold *Neurospora*. The one-gene one-enzyme model predicted by Garrod was established as dogma in the new catechism of biochemical genetics by G.W. Beadle and E.L. Tatum. The eventual move to bacteria and bacteriophage, with new and powerful techniques for recombinational analysis, changed the concept of the particulate gene. Long considered to be analogous to a bead on a string but finally shown to be more like a long molecule first proposed by Richard Goldschmidt. There turns out to be no structural differentiation between the beads and the string but the necklace has turned out to be marvelously complex.

The emergence of the power of microbial systems and the rise of bacteriophage, with its 20-minute generation time, as a genetic subject, altered the game. A clear distinction arose between the old and the new genetics, with the "new" ruled by renegade physicists (Max Delbruck, Francis Crick, and Seymour Benzer) and "infantes terribles" (Joshua Lederberg, James Watson, and Marshall Nirenberg). Analysis of the transformation principle, in *Pneumococcus* by Oswald Avery, Colin McLeod, and Maclyn McCarty and the subsequent phage manipulations by Alfred Hershey and Martha Chase, proved that the genetic material was DNA, but details on its ability to replicate and to affect protein synthesis were unknown until the famous paper by James Watson and Francis Crick (Fig. 6 and 7). The resolution of the structure of DNA was followed by a race that unraveled the genetic code and, as if that were not sufficient, by the discovery of restriction endonucleases that snip gene sequences and plasmid vectors that transfer them across barriers considered unbreachable by even the most credulous of medieval imaginations.

In another corner of biology, plant and animal physiologists far removed from genetics were attempting to culture cells and tissues in a fully defined medium. Pioneering studies of in vitro culture of plant organs and tissues by G. Haberlandt in 1902 predicted that the notion of producing plants from cultured cells would provide final confirmation of the cell theory. There was slow but continuous progress. In 1922, procedures were introduced by W.J. Robbins for the culture of roots and L. Knudson developed the aseptic germination of the embryo-like seed of orchids. The breakthrough in plant cell and tissue culture arose from a series of physiological investigations, principally by Folke Skoog and his coworkers, on growth-regulating substances, including vitamins, hormones (particularly auxin and cytokinins), and organic complexes such as liquid coconut endosperm, and from the development of generalized tissue culture media by P.R. White in the 1930s and 1940s and most successfully by Toshio Murashige and Folke Skoog in 1962. The demonstration of asexual embryos initiated in the cultures of carrot root cells in 1958 by J. Reinert and by F.C. Steward and K. Mears (an event analogous to producing human babies from skin cells) was a confirmation of the concept of cell totipotency: that each living cell contained all the genetic information.

Plant cell and tissue culture was quickly utilized in horticulture for rapid propagation, first for orchids by G.M. Morel in 1960 and then for a number of ornamental plants. Extensive investigation continues to explore the potential of cell and tissue culture as an adjunct to crop improvement. Techniques include embryo rescue, freeing plants from virus and other pathogens, haploid induction, cryogenic storage of cells and meristems for germplasm preservation, the creation of new nuclear and cytoplasmic hybrids via protoplast fusion, and the exploitation of changes, dubbed somaclonal variation, induced by cell and tissue culture. It was recognized that cell and tissue culture technology would be required as an intermediary for recombinant DNA technology.

Recombinant DNA technology has raised great expectations for agriculture. The discovery of enzymes which cleave DNA at specific sequences and subsequently ligate to extrachromosomal DNAs of bacteria, permit gene replication in a bacterial host, a process known as gene cloning. The commercial production

of human insulin by bacteria, the first commercial achievement of gene cloning, stimulated a new industry for producing gene products for therapeutic uses such as blood clotting factors and growth hormones. The technology to describe cloned genes in terms of nucleotide sequence is available and thus manufactured genes are theoretically feasible. Finally, DNA can be inserted into the DNA of higher plants by various techniques including the gene gun. The most promising vector for dicotyledonous plants has been the tumor-inducing plasmid of *Agrobacterium tumefaciens*, a bacterium that normally incorporates its DNA in the host as part of the infection process. Even genetic engineering is not new!

The story beyond this point is still speculative because agriculturally-useful genes are not in surplus and their expression with foreign genomes is still completely unresolved, although positive results have been achieved. The ability to move new genes into old plants has led to imaginative flights of fancy: a new range of disease and stress-resistant plants, nitrogen fixation of non-legumes, and amino acid-balanced plant protein. As a result, much venture capital has been absorbed by aspiring firms, large and small. However, the concept of improving agriculture in the traditional sense by recombinant DNA technology became a reality with two dramatic discoveries. One was that soybeans could be induced to be resistant to the non-selective, environmentally benign herbicide glyphosate (Roundup). The other was that the insecticidal gene *Bt*, from the bacteria *Bacillus thuringiensis* could be transferred to the crop plant. The creation of “Roundup-Ready” soybeans was to have an extremely rapid rate of adoption, unsurpassed in agriculture. *Bt* cotton was also rapidly adopted and *Bt* maize somewhat less because the cost benefit ratio was not as high as the corn root worm incidence varied with location. By 1999, herbicide resistant soybean accounted for 57% of the crop area, *Bt* cotton 55%, and *Bt* maize 22% in the United States. Yet a number of widely predicted changes, such as the creation of blue roses, has not happened. The reasons progress has been slower than expected were perhaps too much early hype, which created unrealistic expectations; a lack of appreciation for the complexities involved, such as the need for specific promoters; unavailability of really useful genes; technical difficulties of gene transformation; and patent problems. However, the greatest roadblock was fear by the consumer, a backlash encouraged by a new class of reforming “**Luddites**,” as they were derisively termed by the *technocrats*, after a band of workman who in 1811–1816 prevented labor-saving machines in the looming industries of England. Fear of genetic manipulation emerged and restrictions imposed on research may slow advances by reducing commercial interest. Consumer resistance in Europe was intense where transgenic crops have been derisively termed “**Frankenfoods**” by their detractors, and production is essentially banned. The short-term future of **genetically modified (GM)** foods is cloudy, but the long-term future is positive. GM food is unlikely to be a problem in Asia in view of the high need for increased production and acceptance of biotechnology by China.

Despite this current backlash, tremendous advances in biotechnology continue to sweep the biological sciences involving: (1) mapping the genome (the complete set of genetic information on the chromosomes) and (2) determining gene function. A new word, **genomics**, was coined for this technology. By 2001, the DNA of a number of organisms had been completely mapped including bacteriophage, bacteria, yeast, nematode, *Arabidopsis* (mouse-ear cress)—a rapid cycling miniature plant of the mustard family, and finally humans!! Analysis of gene function indicates that all living organisms hold genes in common. Soon all our major crop plants will be mapped. The name of the next emerging field has already been coined: **proteonomics**, which will unravel the protein changes involved with gene function and development. We live in very exciting times.

Important Personages in the History of Genetics and Plant Breeding



Fig. 1. Date palm pollination



Fig. 2. Rudolph Camerarius



Fig. 3. Thomas Andrew Knight

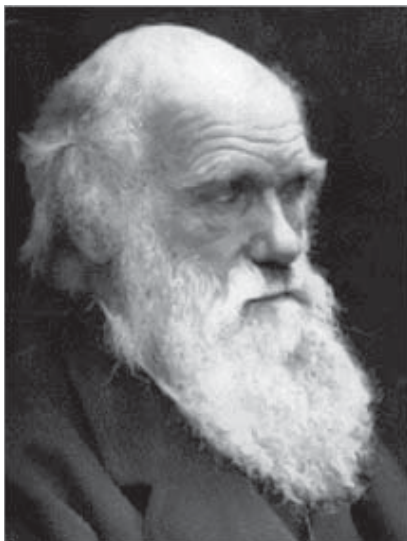


Fig. 4. Charles Darwin



Fig. 5. Gregor Mendel



Fig. 6. James Watson



Fig. 7. Francis Crick



Fig. 8. Henry Jones



Fig. 9. Norman Borlaug