AN INTRODUCTION TO
Genetic Analysis

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David T. Suzuki / Anthony J. F. Griffiths

THE UNIVERSITY OF BRITISH COLUMBIA

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Preface

This book attempts to teach the reader how to do genetics. It is not designed merely to introduce genetic concepts, but rather lays out a set of ground rules and exercises that will assist the student in becoming a proficient genetic analyst.

Two basic instructive devices are used throughout. First, genetic concepts are presented for the most part in historical sequence, rather than starting with our current knowledge of molecular genetics. This is because it seems to us that a student begins much as biologists did at the turn of the century, asking general questions about the laws governing the inheritance of traits. Only after the ground rules have been established at one level, can one logically go on to pose questions about the next level of hereditary organization. In the text we have extensively used a question-and-answer format to simulate this step-by-step evolution of genetic understanding. Second, a quantitative approach is emphasized because the abstractions of genetics have been based largely on playing with numerical experimental data. However, the only mathematical knowledge assumed is a good grasp of arithmetic and basic algebra. Another instructive device that deserves mention is the use of animation sequences. These sequences occupy the right-hand margins of odd-numbered pages, starting at Chapter 2. By holding the pages in the right hand and letting them flip over at a constant rate, you will see a continuous dynamic image of four genetically important processes: meiosis, mitosis, DNA replication, and translation.

The principles of genetics have already been established to a large degree; consequently, the temptation to load the text with references to the most recent research papers has been resisted. Only a small fraction of the current literature will in time be adjudged classical: unfortunately, we do not yet know which papers will constitute that fraction, and this does not seem an appropriate place to guess. The astonishing rate at which textbooks become "out of date" results primarily from the inclusion of the latest experiments, many of which are soon found to be incorrect, incomplete, or irrelevant. The beauty and simplicity of genetic analysis are timeless, but too often lost under an avalanche of topical detail.

This book is designed for an introductory course in college genetics and grew out of the teaching of a course in fundamental genetics for third year students at The University of British Columbia. The course runs for twelve weeks with three lectures and one "tutorial" session per week. Tutorials are predominantly concerned with problem-solving assignments, which are liberally sprinkled throughout this text. Some of the problems are borrowed, but most are of our own design. They constitute an integral and absolutely necessary part of learning how to perform genetic analyses, and should be worked meticulously, starting with only a blank sheet of paper and a pencil.

In attempting to keep the book to a size that can be covered in a semester, we have restricted the material to that which best illustrates various features of genetic analysis and is generally applicable to other organisms. Consequently, only certain prokaryotes and typical haploid and diploid eukaryotes have been emphasized. A considerable amount of telescoping of subject material has also been inevitable. For example, cytoplasmic inheritance, sex determination, differentiation, and behavior are traditionally treated in separate chapters in genetics texts. Because each is a part of the basic problem of how an egg becomes a complete functional organism, they have been integrated here into a single chapter on development.

Throughout the text, material is summarized in the form of "messages." Not only does this give the reader periodic stopping points from which to orient himself within the chapter, but it also facilitates rapid review of the entire book.

Thanks are due Clayton Person and Tom Kaufman for many helpful discussions and suggestions, Joan Howley for help in proofreading, Rita Rosbergen for typing, and the students and teaching assistants of Biology 334 for their participation in testing the preliminary edition.

We hope that the book will stimulate the reader to do some first-hand experimental genetics, whether as professional scientist, student, amateur gardener, or animal fancier. Failing this, we hope some lasting impressions will be formed of the precision, elegance, and power of genetic analysis.

October 1975

David T. Suzuki
Anthony J. F. Griffiths

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Introduction

Genetics in Biology

There is an overall natural tendency in the universe to move toward disorder or disarray. However, there are some isolated eddies in this river of disorder, and one of the most interesting is called *life*. In contrast with the increasing chaos in the rest of the universe, living systems are highly ordered. In fact, life could be defined as persistent order. The word persistent is used here to emphasize the unique ability of living systems to *hand on* to their descendants the instructions necessary for the maintenance of order; in other words, persistent emphasizes the central role of hereditary phenomena in the uniqueness of life. The study of heredity is a science called Genetics.

The findings of genetics have been monumentally significant in the unification of biology into a coherent science in two basic ways: First, genetics has shown that all life forms on earth—a staggering spectrum including 286,000 species of flowering plants, 500,000 species of fungi, 750,000 species of insects, and so forth—are probably genetically derived from a common ancestor through a process known as evolution. Genetics has revealed precise mechanisms that enable us to understand how this was accomplished. Second, genetics has shown that all life forms are based on a common system of information storage, duplication, copying, and translation.

Furthermore, the science of genetics has provided the life sciences with a powerful approach for studying biological phenomena. This approach, which we will call *genetic dissection* of biological systems, constitutes an important part of this book.

Genetics and Human Affairs

Since the dawn of civilization, when nomadic man began to domesticate plants and animals, the recognition of hereditary phenomena has played a vital role at many levels of human society. Man selected grains with higher yields and vigor and animals with better fur or meat long before biology existed as a scientific discipline. In spite of this longstanding use of selective breeding, the actual basis for inheritance has only been elucidated in the last hundred years. But now, as it does in most areas of science, new knowledge brings with it not only potential benefit for mankind but an equal share of problems.

The recent, much heralded "Green Revolution," brought about by the sophisticated breeding by geneticists of high-yield varieties of dwarf wheat and rice, is a good example of this balance between good and bad. The production of these crops has been found to depend too heavily on extensive cultivation and costly fertilizers in the impoverished countries for which they were intended. Nevertheless, in the present state of overpopulation on Earth, our dependence on high-yield genetic varieties of plant crops and animals for food and other resources has become increasingly obvious. In a very practical sense the stability of society is dependent on the ability of geneticists to juggle the inherited traits that confer higher yield and keep the crops one jump ahead of destructive parasites and predators. A dramatic example of the potential effect of genetics in this connection is the breeding of Marquis wheat in Canada. This strain of high-quality wheat is resistant to disease and, furthermore, matures two weeks earlier than other commercially used strains. Consequently, millions of square miles of fertile soil in northern countries such as Canada, Sweden, and the USSR have been opened up for the growing of wheat.

Already, special genetic strains of fungi and bacteria have been isolated to greatly increase yields of antibiotics and other drugs. The potential future use of specially bred microorganisms is even wider: they may be used to clean up pollutants, to serve as food, to transfer the hereditary determinants conferring the ability to fix atmospheric nitrogen to important crop plants, to provide functions that are missing in people suffering from various kinds of disease, and a whole range of other uses that are no longer simply the wild dreams of science-fiction writers. In another example of the usefulness of genetics in applied technology, areas of heavy insect infestation are being made tolerable for human habitation by the deliberate genetic tampering with the insects' fertility.

But nowhere is the potential impact of an increased knowledge of genetics more exciting and frightening than in man himself. It is becoming increasingly obvious that the brain of man is subject to the same genetic determination as the rest of the body, resulting in certain inherited predispositions to thought and behavior. No longer can we view the mind as a clean slate at birth written upon only by experience. The extent of such inborn constraints on thought and personality, and their relevance to present sociological impasses, is being suggested in such books as *African Genesis, The Territorial Imperative,* and *On Aggression.* The same reasoning is inherent in the current claims for hereditary differences in intelligence among different racial and social groups. This concept is not new, having been debated by Lycurgus in Sparta and Plato in Athens, and reaching its zenith in Nazi Germany. With the pressure for population control mounting, the spectre of legislated sterilization looms large again. If such sterilization were to be of a selective nature, we would be essentially shaping the genetic destiny, or evolution, of our own species, an onerous responsibility that few could confidently undertake.

The sophisticated technology of molecular genetics now places a wide range of new techniques at our disposal for shaping our genetic makeup, with even more bizarre procedures undoubtedly arriving in the near future. This "genetic engineering" differs from conventional breeding procedures in that the genetic apparatus is modified essentially at a chemical level. A rash of popular books warn us of a *Genetic Fix,* a *Biological Time Bomb, Genetic Revolution,* a *Fabricated Man,* and the *Biocrats.* The extent to which such prophecy is realized will depend ultimately on political decisions based on the informed opinions of responsible citizens.

The advances made in the field of genetics have been especially useful in medicine: hereditary diseases can now be diagnosed at an early stage of life when it is possible to provide secondary cures in some cases. Refined techniques such as amniocentesis and fetoscopy (both prenatal) and a battery of postnatal chemical tests have made such cures possible. Furthermore, genetic disease can often be prevented by counseling prospective parents with the help of family pedigrees.

There is a current fear that our increased exposure to chemical food additives, and a vast array of chemicals in other commercial products, is changing our genetic makeup in a very undesirable haphazard way. Other environmental agents capable of causing this random genetic change are fallout from H-bombs, radioactive contamination from nuclear reactors, and radiation from X-ray machines. These agents may be contributing to genetic disease.

The ability to recognize the prevalence of genetic disease in our societies raises an important moral dilemma. It has been estimated that 5% of our population survives with a severe physical or mental genetic defect, and that this percentage will increase with extended exposure to the above environmental agents, and, paradoxically, with improved medical technology. As geneticist Theodosius Dobzhansky has remarked,

> If we enable the weak and the deformed to live and propagate their kind, we face the prospect of a genetic twilight. But if we let them die or suffer when we can save or help them, we face the certainty of a moral twilight.

The extent to which our society will be prepared to shoulder this genetic load will be measured by the amount of money we are prepared to spend in keeping the genetically handicapped alive. A measure of the size of this financial burden on the resources of society is seen in the estimate that 30% of the patients admitted to pediatric hospitals in North America have diseases that can be traced to genetic causes.

From this brief introduction it can be seen that genetics is relevant not only to the biologist but to any thinking member of today's complex technological society, and a working knowledge of the principles of genetics is essential for making informed decisions on many scientific, political, and personal levels. We believe such a working knowledge can come only from understanding how genetic inference is made: that is, from understanding genetic analysis, the subject of this book.

1 / Mendelism

(Or: How to deduce the existence of genes and make conclusions about their location in the cell without even seeing them.)

A basic observation about living organisms that even a child recognizes is the continuity of type from generation to generation. We know that a cat will certainly give birth to kittens and that carrot seeds will grow into carrot plants. In other words, like begets like. Yet within a species there is amazing diversity. We distinguish Rex from Fido, our brothers from our brothers-in-law, and so on, in a gratifying way that enriches all our lives. Genetics is the discipline within biology that attempts to understand how interspecific variation is maintained and how, at the same time, intraspecific variation is generated and inherited. Genetics, then, is about heredity.

Although genetics is about heredity, this is not a good definition of genetics. No geneticists existed before 1865, despite a millenial persistent interest in inheritance, because it wasn't until this time that a way of analyzing information on heredity was invented by an Augustinian monk, Gregor Mendel. His method of analysis is the one we still use today (albeit in an extended form) and call genetics. Probably the crucial advance he made was the identification or recognition of an entity we now call a *gene*. That event occurred in the brain of Gregor Mendel and marked the birth of genetics as a unique way of looking at living organisms and analyzing biological phenomena.

In this chapter we will trace the birth of the gene as a concept, and in later chapters, as a reality. We will see that genetics is an abstract science: most of its entities have begun as hypothetical ones in the minds of geneticists and later on, depending on the soundness of the reasoning that created them, have been identified in physical form.

In Mendel's time, people thought about heredity in a way that can be traced back to the ancient Greeks. Basically, their concept of procreation can be compared to making freeze-dried instant coffee. Eggs and sperm were thought to consist of many essences: of arm, of head, of hair, and so forth. These dehydrated instant building blocks needed only to come together and, with the addition of water, they would burst forth into a new individual. The main point is that it was the building blocks *themselves* that were thought to be handed on, not blueprints for making them.

Consequently, this now-discarded hypothesis is termed *blending inheritance:* just as a mixture of Maxwell House and Yuban coffee results in a blend of both types, so the union of egg and sperm was thought to produce a blend of the essences in each. Mendel was to show that this was wrong, and that inheritance does not result from the union of a teeming multitude of building blocks, but from a few very important particles (now called genes), which *direct* the synthesis of new individuals. This is called *particulate inheritance.*

Unfortunately, the importance of Mendel's work was not appreciated until thirty-five years later (by then he was dead) when it was discovered by three scientists who had independently come to the same conclusion. In a sense, then, Mendel's work was irrelevant to the development of the study of heredity, but this in no way diminishes his achievement nor the exemplary worthiness of his analysis, which we will now pursue.

Mendel studied the garden pea (*Pisum sativum*) for two main reasons: First, peas were available through a seed merchant in a wide array of different forms and colors that are very easily identified and analyzed. Second, peas left to themselves will self-pollinate (or *self*) because the male and female parts of the flower, which produce pollen and eggs respectively, are enclosed in a petal box or keel (Figure 1-1). To cross-pollinate (or *cross*) them, the anthers can be clipped off and pollen from another plant can be transferred to the receptive area with a paintbrush. Consequently, peas can be easily either selfed or crossed.

The first thing Mendel did was to choose several traits to work on and establish *pure lines.* This was a clever beginning because it amounts to a control experiment: he had to be sure of his material. A pure line is a plant pedigree that breeds true or constant for the particular character being studied. For example, he had a line that bred true for purple flowers; that is, when selfed, all the progeny seeds grew into plants that had purple flowers, and when these were selfed, *their* progeny had purple flowers too. Other lines were pure for white flowers, and others for yellow, green, wrinkled, or smooth seeds as well as many other traits.

Let's consider some specific experiments. In one of his early experiments he used a pure line with purple flowers and a pure line with white flowers. If a purple-flowered plant was pollinated by pollen from a white-flowered plant, all the progeny plants had purple flowers (Figure 1-2).

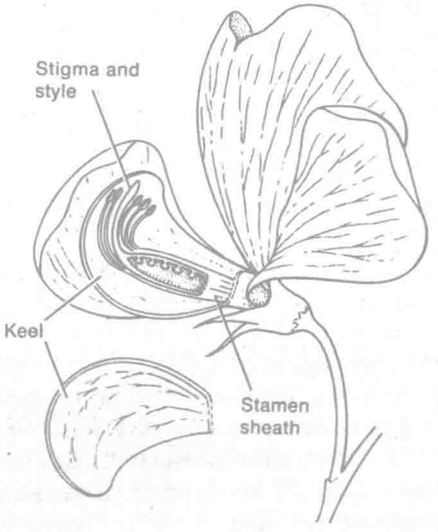

FIGURE 1-1

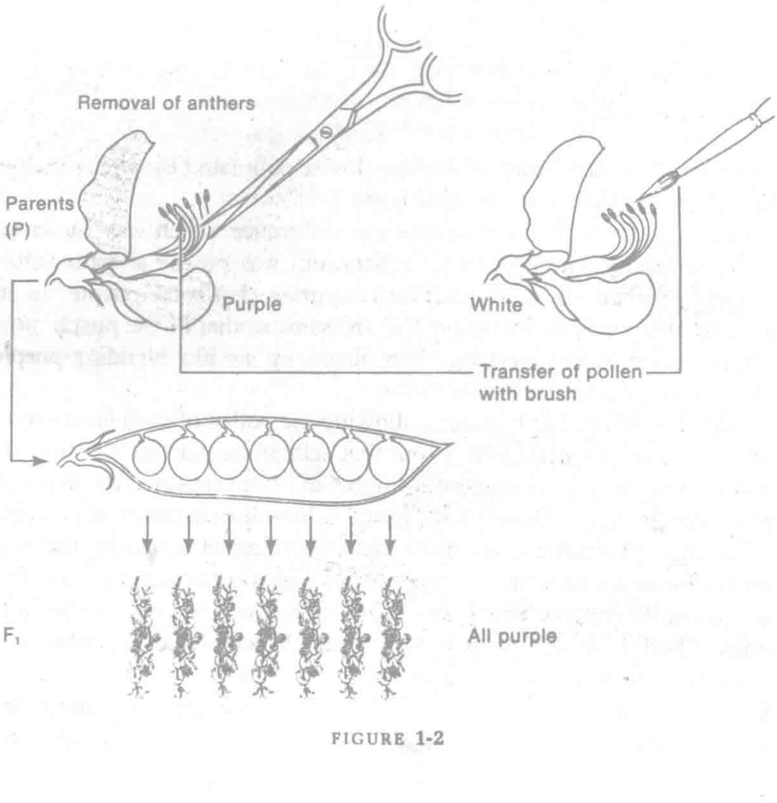

FIGURE 1-2

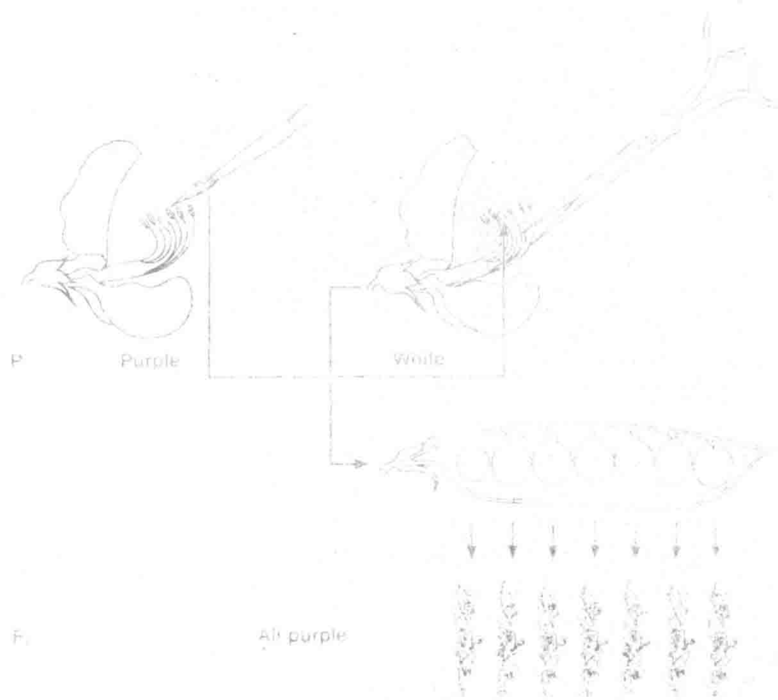

FIGURE 1-3

The *reciprocal cross*—that is, a white flower pollinated by a purple-flowered plant—produced the same result (Figure 1-3).

He concluded that it did not make any difference which way the cross was made: when one of the parents (P generation) was purple and the other was white, all the plants in the *first-filial generation* (F_1) were purple. In the F_1 generation, the purple flower color was the same as that of the purple-flowered parent; so in this case, inheritance was obviously *not* like blending purple and white paint to produce a lighter color.

Mendel then selfed the F_1 plants, allowing the pollen of each flower to fall on the stigma within its petal box. From that selfing, he planted 929 peas (the F_2 individuals). Amazingly, although as might have been expected the majority was purple, some grew up to be white-flowered. The white character had reappeared! He then did something that, more than anything else, marks the birth of genetics: he *counted* how many there were of each kind, which may seem trite in today's quantitatively oriented world, but which was the key to the future of genetics. There were 705 purple plants, and 224 white ones. He observed that this was close to a 3:1 ratio (in fact it was 3.1:1).

He repeated this for seven other pairs of pea characteristics and found the same 3:1 ratios in the F_2 generation for all of them. By this time he was undoubtedly beginning to believe in the reality of the 3:1 ratio and felt he had to explain it. Note that, even though the white coloring was completely absent in the F_1 generation, it reappeared with full expression in the F_2. So even though the F_1 flowers were purple, the plants still carried the *potential* to produce progeny with white flowers. Thus, Mendel concluded that the F_1 plants must carry "factors," received from *both* parents, that determine flower color. The factor responsible for purple is *dominant* so that, even in the presence of a factor for white, purple is expressed. The factors for white are said to be *recessive* to the dominant.

Another important observation came from selfing F_2 plants individually. Specifically, he was working in this case with characteristics of the pea seed itself. This allowed him to use much larger numbers because the characteristics can be observed without growing plants from the peas. The pea seed can be thought of as an autonomous progeny individual in its own right in this species. Its appearance is the product of its own constitution and not just that of the mother as in some seed characteristics of other plants. The two pure lines he used had yellow and green seeds, respectively. He made a cross between a plant from each line, and observed that the F_1 peas that developed were all yellow. Symbolically,

P	Yellow × Green
	↓
F_1	All yellow

Therefore yellow is dominant and green is recessive.

The F_1 peas were grown into plants and selfed. Of the resulting F_2 peas, 3/4 were yellow and 1/4 were green (the 3:1 ratio again). He then grew 519 F_2 yellow peas into plants and allowed each one to self. When the peas appeared he observed that 166 of the plants had only yellow peas and 353 had both yellow and green peas in a 3:1 ratio.

Therefore, approximately 2/3 of the F_2 yellows were like the F_1 yellows (i.e., produced yellow and green seeds when selfed) and 1/3 were like the pure-breeding yellow parent. Consequently, the 3:1 ratio could be more accurately described as a 1:2:1 ratio.

		↗ 1/4 pure-breeding yellow	1
F_2 individuals	3/4 yellow		
		↘ 1/2 impure yellow	2
	1/4 green →	1/4 pure-breeding green	1

These 1:2:1 ratios were found to be underlying all of the 3:1 ratios he tested. So the problem really was to explain the 1:2:1 ratio.

His explanation was a classical example of a "model" or "hypothesis" derived from observation, which could be subject to testing by further experiments. He deduced the following explanation:

1. There are entities called "hereditary determinants or factors" of a particulate nature. (He saw no blending of characters, so was forced to a "particulate" notion.)
2. Each adult pea plant has two determinants, one from each parent, for each characteristic. (The reasoning here was obvious: the F_1 plants, for example, must have had at least one determinant for the recessive character because it showed up in later generations, and of course they also had the determinant for the dominant character because they showed it.)
3. Each "sex cell" (pollen or egg cell) has only one determinant.
4. During sex-cell formation, either of the pair of determinants of the parent plant passes with equal frequency into the sex cells.
5. The union of sex cells (to form a new individual or *zygote*) is random.

These points can be diagrammatically illustrated, using A to represent the dominant determinant and a the recessive determinant (as Mendel did), much as a mathematician uses symbols to represent entities of various kinds (Figure 1-4).

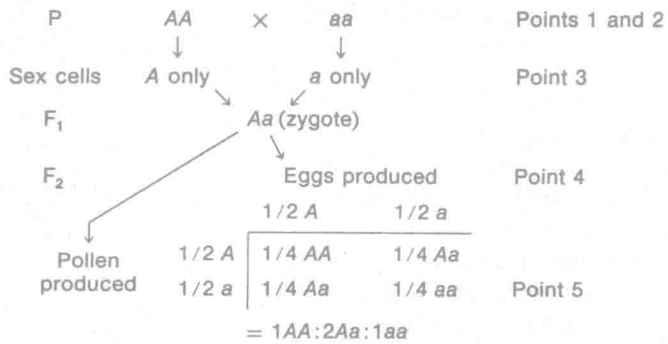

FIGURE 1-4

The whole thing fitted together very beautifully. However, many beautiful models have been knocked down under test. Mendel's next job was to test it. He did this by taking (for example) an F_1 yellow and crossing it with a green. A 1 : 1 ratio of yellow to green seeds could be predicted in the next generation. If we use Y to stand for the yellow determinant and y the recessive to show that they are a pair, we can diagram Mendel's predictions as in Figure 1-5. In this experiment he obtained 58 yellow and 52 green seeds, a very close approximation to the predicted 1 : 1 ratio.

Nowadays, the hereditary determinants or factors are called genes, and we will introduce this and some other terms at this point. The various forms of a gene, represented for example by A and a, are called *alleles.* The individuals repre-

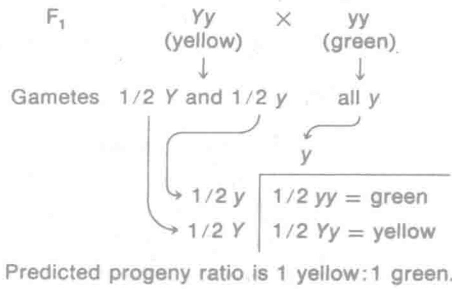

FIGURE 1-5

sented by *Aa* are called *heterozygotes* (sometimes, hybrids), whereas those in pure lines are called *homozygotes*. Thus, an *AA* plant is said to be homozygous for the dominant allele, and an *aa* plant, homozygous for the recessive. Sex cells are usually called *gametes*. The actual characteristic appearance of an organism is called its *phenotype*. Thus, yellow and green seed colors are different phenotypes. On the other hand, the designated genetic constitution is its *genotype*. Thus, *Yy* and *YY* are different genotypes even though seeds of both types are phenotypically identical (i.e., yellow).

What we have called Point 4 has been given formal recognition as Mendel's first law.

MENDEL'S FIRST LAW

Alleles segregate (i.e., separate) from each other during gamete formation into equal numbers of gametes.

Another point that should be apparent from the discussion thus far is that the starting point in any genetic analysis is the recognition of genes.

MESSAGE

Genes were originally inferred and still are today by observing precise mathematical ratios in the progeny of a cross.

So far we have considered a single gene pair or *monohybrid* system, in which alleles of only one gene affecting one characteristic are considered. The next obvious question is what happens when a *dihybrid* cross is made in which pairs of genes affect two different characteristics? We can use the same symbolism that Mendel used to indicate the genotype of seed color and seed shape. A pure breeding line of plants *yy RR*, on selfing, produces seeds that are green and round. Another pure breeding line is *YY rr*, and upon selfing it produces yellow wrinkled seeds (*r* is a recessive allele of the seed-shape gene and produces a wrinkled seed). When plants from the two lines were crossed, the F_1 seeds were