Canine Genetics for Dog Breeders: Part 1

By Dr. Matthew Breen
Jun 15, 2018 | 4 Minutes

Genetics is a fundamental field of biology that covers the passage of genetic information to successive generations (inheritance). More broadly, genetics covers the study of genes, how they are organized in cells, how they are regulated, and how they interact with each other and the environment. Genetics is a key component of genomics, a much broader area of the life sciences that is highly interdisciplinary and considers the structure, function, mapping, and both natural and induced alterations of genomes.

Over the course of a series of short articles, we will cover the basics of what DNA is and how it is organized in cells, how cells divide and pass genetic information to the next generation, the laws of inheritance, the molecular basis of disease, how purebred dogs can help advance genetic research, and how genetic variation (inherited and sporadic) impacts disease.

What is DNA and how is it organized in cells?

Cells are the basic structural and functional units of all living organisms. Inside all animal cells (with the exception of red blood cells) is a nucleus that contains the genetic material, deoxyribonucleic acid (DNA). A key component of DNA is the nucleotide. There are four nucleotides: adenine (A), thymine (T), guanine (G), and cytosine (C), and it is the specific order of these nucleotides that determines the genetic code.

- *The genome of a dog contains approximately 2.4 billion nucleotides.*
- *If all the DNA inside one dog cell was laid out end to end, it would stretch over 6 feet in length.*
- *If all the DNA in the cells of one adult dog was laid out end to end, it would stretch to the sun and back many times.*

A genome can be thought of as an instructional manual. The letters in the manual represent the nucleotides of the DNA, the words represent the genes, and the chapters represent the chromosomes. When the words are spelled correctly, and in the right order, they make sense and tell a genetic story.

To allow all the DNA from a single cell to fit inside a nucleus, a special protein scaffold allows the DNA to wind tightly into bundles. These bundles of protein and DNA, referred to as chromosomes, can then fit inside the nucleus. Chromosomes behave as nature's biological filing cabinets, with animals of the same species having the same number of chromosomes in each cell and the same DNA within each chromosome.

In all animal cells (except egg and sperm cells), each chromosome is present twice, with one full set inherited from the mother and the other full set inherited from the father. Organisms with two copies of each chromosome are referred to as diploid. In reproductive cells (egg and sperm), each chromosome is present once. In all mammals, there are two chromosomes, X and Y, that determine the sex of an individual. Females have two copies of the X chromosome; males have one X and one Y chromosome.

The rest of the chromosomes in a nucleus are called autosomes and are generally numbered, according to their size and shape, with the largest autosome called number 1, the second largest number 2, and so on.

The organization of the canine genome.

For many years, it has been known that the DNA of the canine genome is organized into 78 chromosomes per somatic (non-gamete) cell (39 in gametes; egg and sperm cells), present as 38 pairs of autosomes with either two copies of the X chromosome in females or one X and one Y in males [1]. Only males have the Y chromosome, and all female eggs have an X chromosome. This means that the sex of offspring is determined by whether the sperm that fertilizes the egg carries an X or a Y chromosome. From Affenpinscher to Yorkshire Terrier, all healthy dogs have 78 chromosomes, and under a microscope the chromosomes from each cell within a single dog and between dogs look the same. It is the actual DNA sequence where differences between breeds are detected.

Chromosomes can be stained with a dye that allows them to be seen under a specialized microscope when at a stage of cell division called metaphase (Figure 1). Specific dyes can be used to induce a banding pattern for each and then observed with a powerful microscope. Chromosomes of a dog cell are shown in Figure 2 below. Since each chromosome is present twice (maternal and paternal sets), the chromosome can be aligned into pairs, numbered accordingly, and displayed as shown in Figure 3.

Figure 1. Metaphase chromosomes of the domestic dog as they appear when stained with a blue fluorescent dye that allow them to be seen with a high-powered microscope. Each of the 78 rod-shaped structures are separate chromosomes. The two sex chromosomes (X and Y) are labeled, indicating that this cell is from a male.

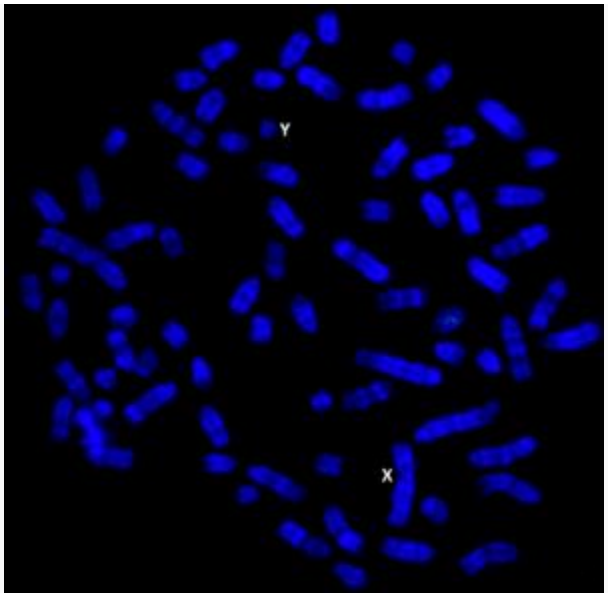


Figure 1

Figure 2. Metaphase chromosomes of the domestic dog when stained with a specific dye and viewed with a high-powered microscope to reveal banding patterns along the length of each chromosome. The banding patterns are the same for each pair, allowing the pairs to be identified. This is a male cell, as indicated by the presence of an X and Y chromosome (labeled).

Figure 2

Figure 3. Chromosomes of the domestic dog from Figure 2 organized into 38 pairs, according to their banding pattern. In each pair, one is from the mother (egg) and one is from the father (sperm).

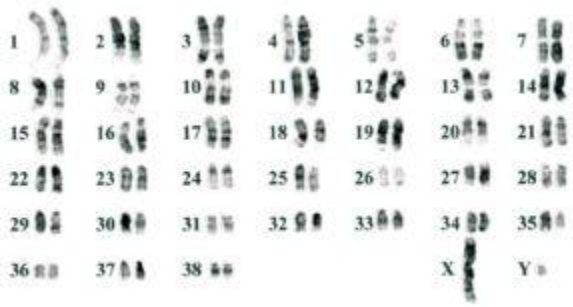


Figure 3

This arrangement of the chromosomes is referred to as a karyotype and serves as a common format for researchers and veterinary professionals to identify and refer to the individual chromosomes.

The precise order of the nucleotides that make up the canine genome sequence was determined in 2005, revealing a total of approximately 2.4 billion nucleotides [2]. The amount of DNA per chromosome was determined to range from approximately 126 million nucleotides for chromosome 1, to just 27 million for chromosome 38.

Dr. Matthew Breen is a professor of Genomics and the Oscar J. Fletcher Distinguished Professor of Comparative Oncology Genetics in the Dept. of Molecular Biomedical Sciences at the NC State University College of Veterinary Medicine.

Cited publications.

1. Breen, M., J. Bullerdiek, and C.F. Langford, *The DAPI banded karyotype of the domestic dog (Canis familiaris) generated using chromosome-specific paint probes.* Chromosome Research, 1999. **7**(5): p. 401-6.

2. Lindblad-Toh, K., et al., *Genome sequence, comparative analysis and haplotype structure of the domestic dog.* Nature, 2005. **438**(7069): p. 803-19.

Canine Genetics for Dog Breeders: Part 2

By Dr. Matthew Breen
Oct 09, 2018 | 4 Minutes

This article is the second in a two-part series. Read part one.

Genetics is a fundamental field of biology that covers the passage of genetic information to successive generations (inheritance). More broadly, genetics covers the study of genes, how they are organized in cells, how they are regulated, and how they interact with each other and the environment. Genetics is a key component of genomics, a much broader area of the life sciences that is highly interdisciplinary and considers the structure, function, mapping and both natural and induced alterations of genomes.

Over the course of a series of short articles, we will cover the basics of what DNA is and how it is organized in cells; how cells divide and pass genetic information to the next generation; how genetic variation (inherited and sporadic) impacts disease; and how Here

How do cells divide?

Understanding the processes of cell division requires the definition of some key terms.

- **Mitosis** – Process of cell division of somatic cells in which a series of steps leads to the formation of two daughter cells that each has the same number of chromosomes as the parent cell
- **Meiosis** – Process of cell division with two stages, leading to the production of gametes in which the number of chromosomes is reduced to one half of the number in a somatic cell
- **Haploid** – Single set of chromosomes. In the dog, this is 38 autosomes plus one sex chromosome (either X in all females, or X or Y in males).
- **Diploid** – Double set of chromosomes. In the dog, this is 76 autosomes plus two sex chromosomes (either XX or XY).
- **Somatic cell** – Cell of a multicellular organism not associated with reproduction – (e.g. skin, bone, lung, liver, etc.)
- **Gamete –** Cell of a multicellular organism associated with reproduction (i.e. egg and sperm cells)
- **Zygote –** Cell formed by a fertilization event between an egg and sperm cell, combining the genetic material of these gametes.

From a single cell to the playful puppy that becomes part of our family and, for the rest of the dog's life, the process of cell division is ongoing and carefully controlled. When a cell divides, it makes two cells (daughter cells), which then divide to make four cells, which divide to make eight cells, and so on. It is this exponential increase that leads to adult dogs comprising trillions of cells. During fetal development, the cells of a puppy are directed to form different types of cells, such a heart, brain, skeleton, kidney, lung, skin, nerve, blood, etc.

Once an adult, the extent of cell division alters, to repair injuries and replace dead cells. There is a very strict mechanism that signals when cells need to start and stop dividing. Different cell types divide at different rates. For example, millions of skin cells die each day and so the need to replace them means skin cells divide regularly. Other cells, such as nerve cells, divide much less frequently.

There are two types of cell division, referred to a *mitosis* and *meiosis*. Mitosis is the process by which somatic cells (non-reproductive) divide to replicate themselves. Meiosis is restricted to the germ cells (reproductive cells, egg and sperm) and is the process by which genetic variation is introduced into the next generation. The major difference between the processes of these alternate forms of cell division is the number of copies of each chromosome that the daughter cell each have. At the start of cell division, each cell has two copies of each chromosome and is referred to as diploid. In mitosis, each of the two daughter cells is diploid, whereas there are four daughter cells at the end of meiosis with each having half the number of chromosomes as the parent cell. These cells are referred to as haploid.

Mitosis.

Each time a cell divides, it advances through the cell cycle (Figure 1), a process comprising a series of well-defined steps that need to happen for a cell to duplicate. While many somatic cells are generated daily, cells spend most of their time in interphase, where the cells gather all they need to divide, duplicate their DNA content to have sufficient to pass to each daughter cell. The actual process of mitosis (Figure 2) is a series of well-defined steps that each cell must take to divide into two daughter cells.

During the first three stages, prophase, prometaphase and metaphase, the chromosomes become progressively more condensed within the cell nucleus. By the end of metaphase, the chromosomes are aligned randomly along a structure called the metaphase plate, which spans the center of the nucleus. It is at the stage of metaphase that scientists trained at observing chromosomes will evaluate cells for changes to the expected number and structure of these elements. This is how many chromosomal aberrations are detected.

As the cell enters anaphase, special structures called spindle fibers have become attached to the centromeres of each chromosome. The fibers then pull apart the two halves (sister chromatids) of each chromosome, taking each half to opposite ends of the cell nucleus and the cell elongates. The chromosomes continue to condense during telophase and an enclosure (nuclear envelope) forms around the separated sets of chromosomes. The opposite ends of the cell continue to be pushed apart, and the cell then cleaves into two daughter cells.

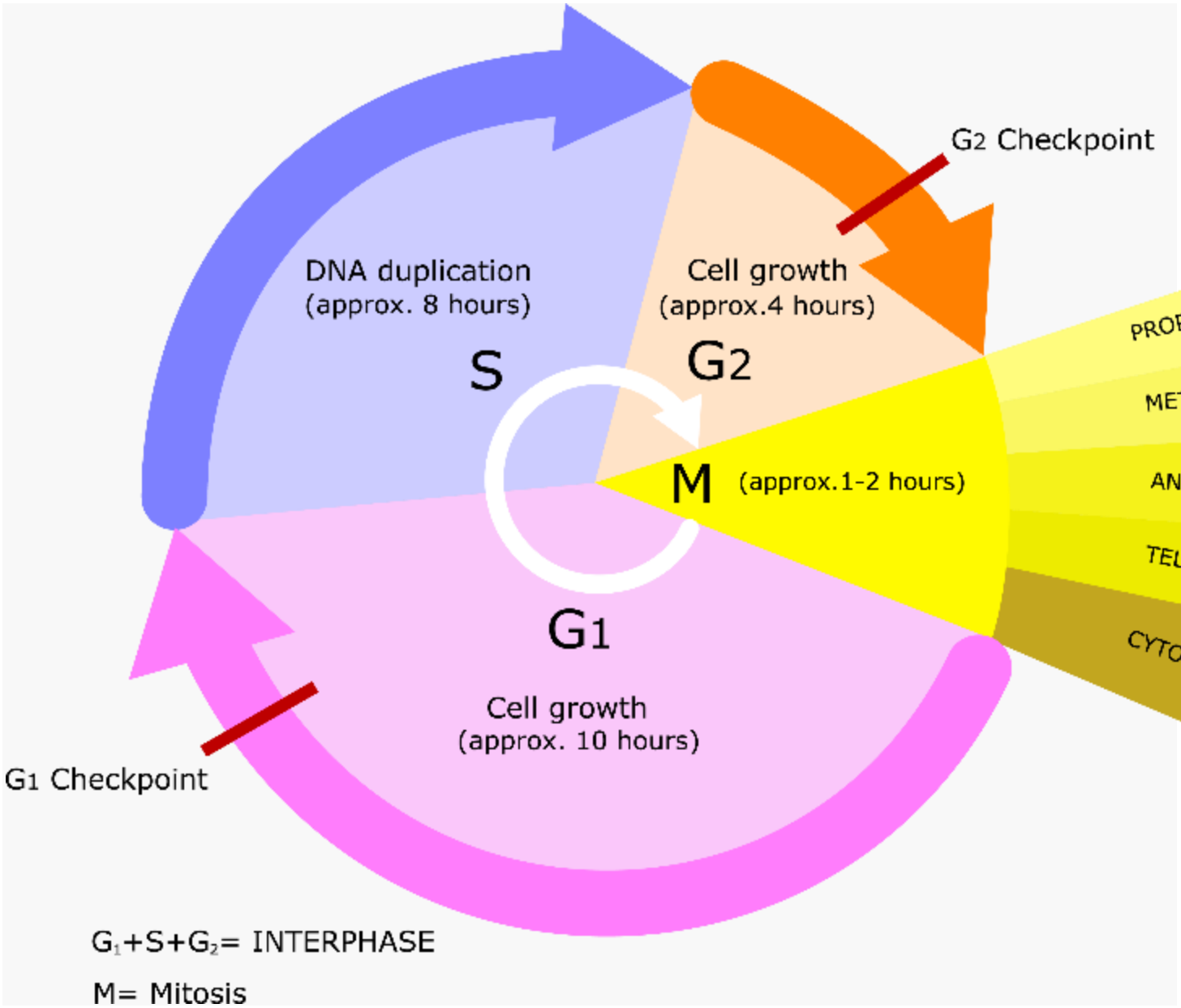


Figure 1. The cell cycle. Cells spend most of their time in interphase, during which they grow and duplicate their DNA. The actual process of dividing is the M (mitosis) phase, which has five major steps as shown.

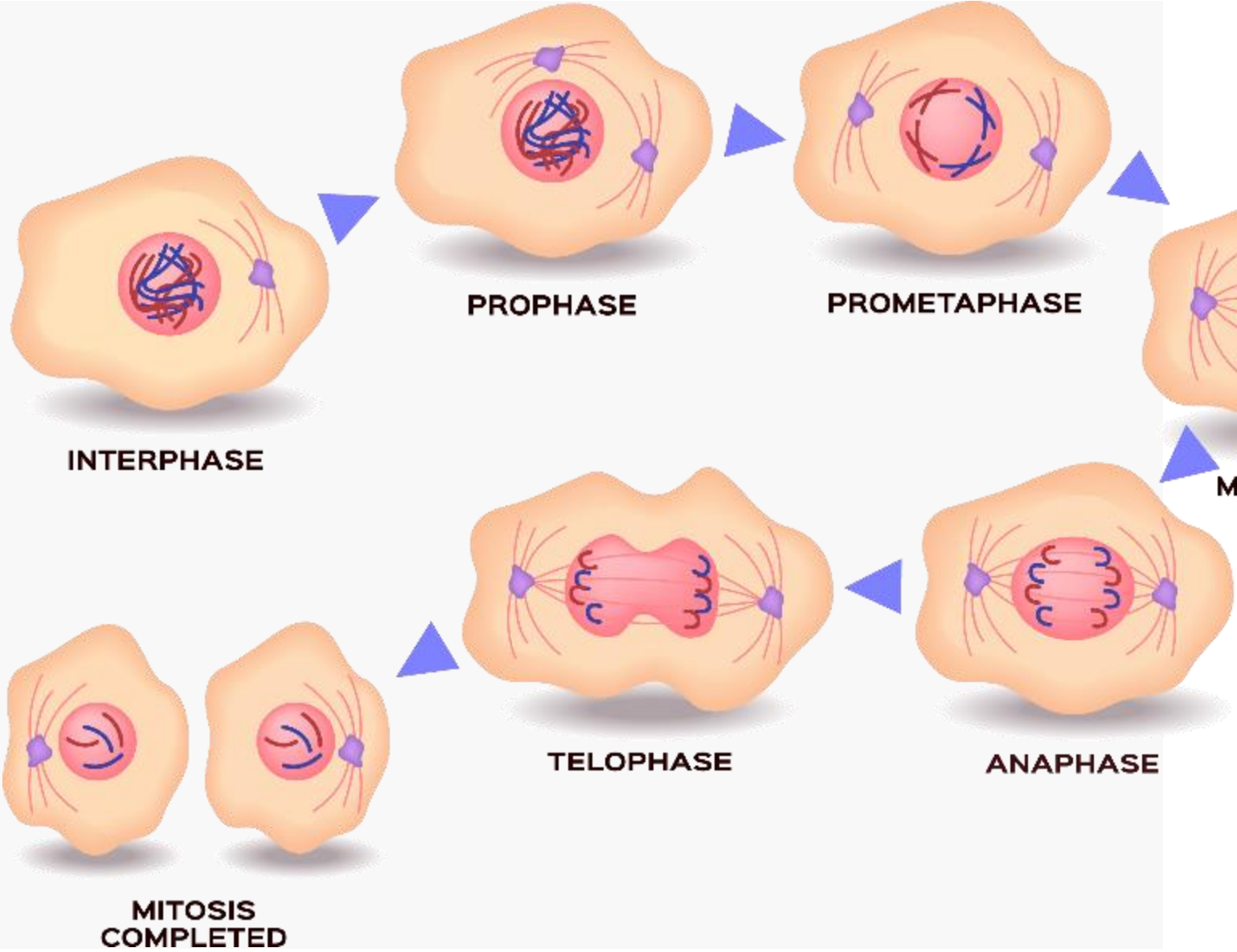
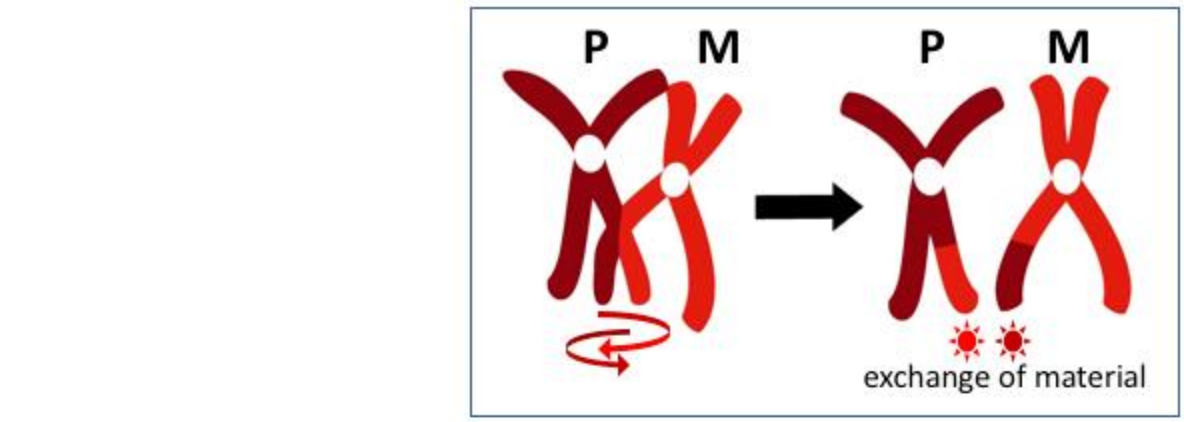
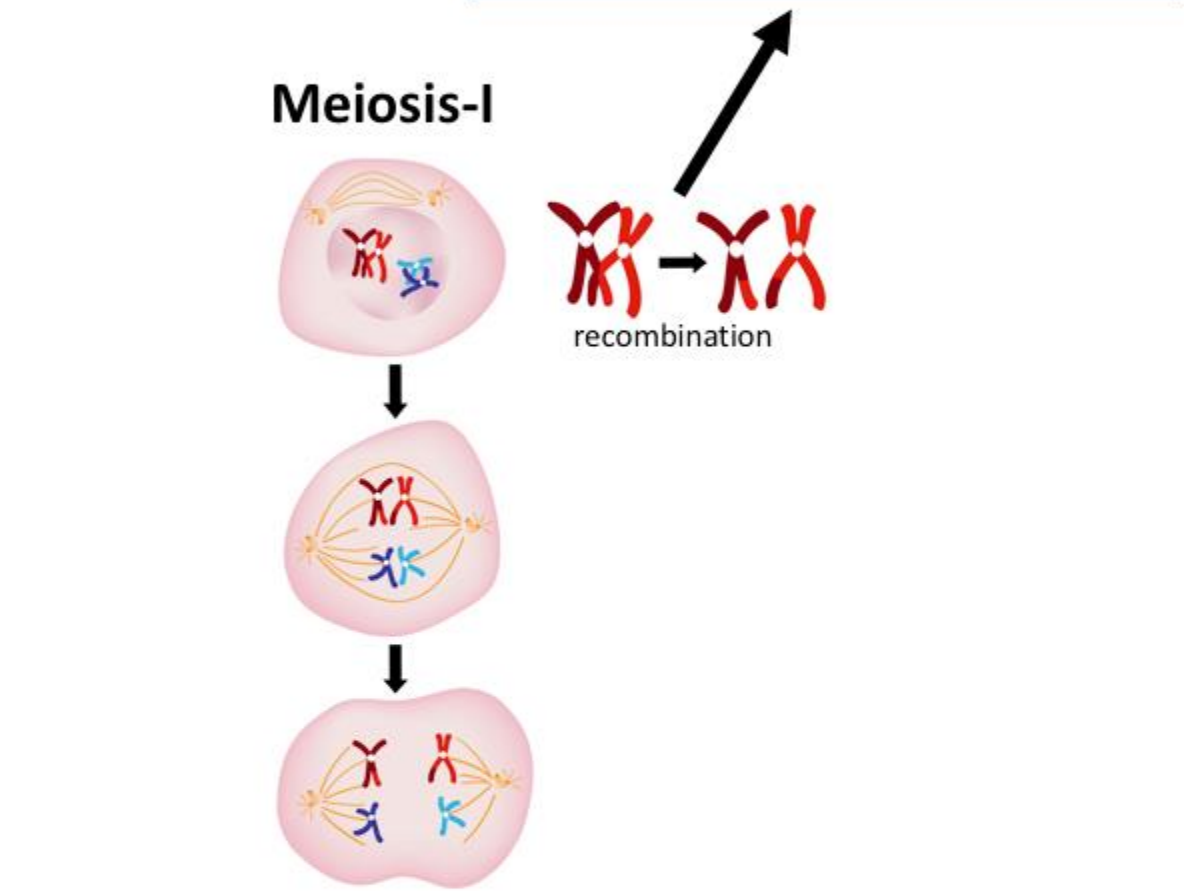


Figure 2. Mitosis. When a cell leaves interphase and enters the five phases of mitosis (prophase to telophase), the visible structural changes may be observed through a microscope. Once completed the cell splits into the two daughter cells, each with a full complement of genetic materia

Meiosis is the process of cell division that results in the gametes (egg and sperm). A major role of meiosis is to provide a mechanism, called genetic recombination, that facilitates the introduction of genetic variation to be passed to the next generation. The result of meiosis, the gametes, have only half the number of chromosome (a haploid set) of a somatic cell, and so there needs to be a fusion with another gamete of the same species to generate a new individual with a full complement of the genetic material for that species.

Meiosis is a process that occurs over two cycles, meiosis-I and meiosis-II (Figure 3). Before entering meiosis, the cell behaves much the same as in mitosis, duplicating its DNA content and gathering what it needs to divide. It is during meiosis-I that the process of genetic recombination occurs, providing opportunities for DNA from the two chromosomes of each pair to exchange information.


P
M
P
M
exchange of material


Meiosis-I
recombination

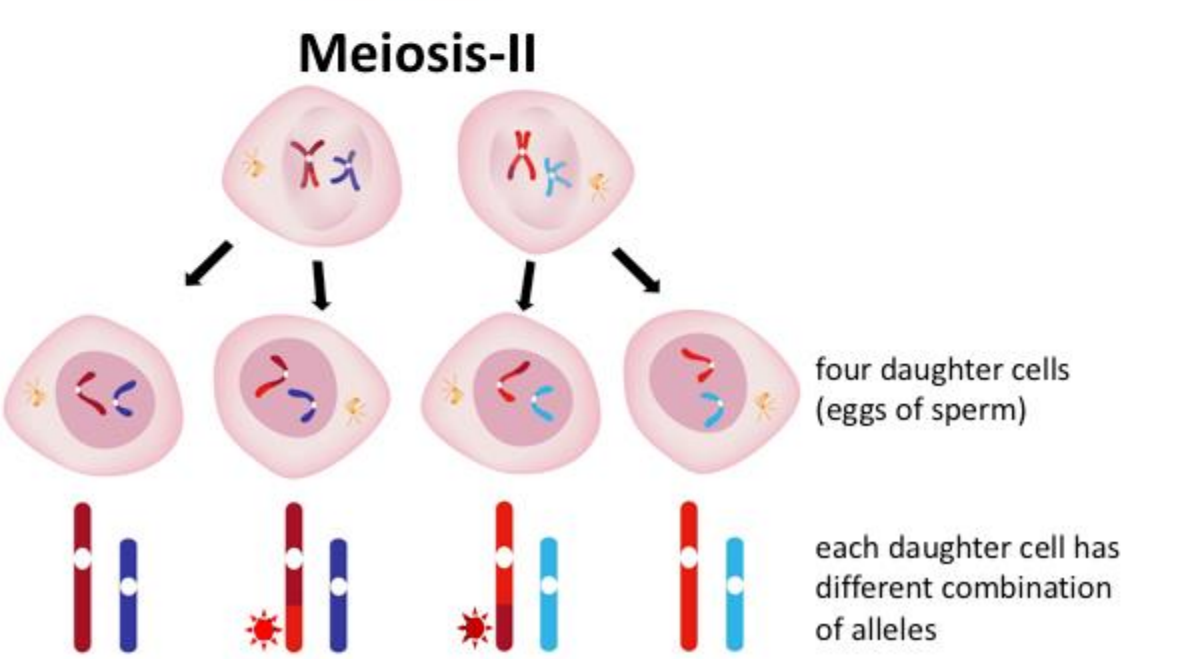

Meiosis-II
four daughter cells
(eggs of sperm)
each daughter cell has
different combination
of alleles

Figure 3. Meiosis. In meiosis-I the paternal and maternal chromosome pairs align and can exchange genetic information. The process effectively shuffles the alleles for each gene to create new genotype combinations. Once this has occurred, the cell divides into two and then meiosis-II proceeds very much like mitosis, with the exception that this second round of division produces four daughter cells, each with a haploid set of chromosomes. The genetic material is randomly distributed to the daughter cells, and it is the shuffling of genetic material in meiosis that results in genetic variation across a population.

This process creates a new set of alleles for the genes along the length of each chromosome. Meiosis-II proceeds very much like mitosis. Since this happens in the development of both gametes, the number of possible combinations emerging from a fusion of sperm and egg is enormous and explains the vast breadth of phenotypes. Puppies from a litter may look and behave very much like each other initially, but as they grow and develop, we notice differences among them that allows us to recognize them for the unique genetic entities they are.

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Canine Genetics for Dog Breeders: Part 3

By Dr. Matthew Breen
Dec 10, 2018 | 5 Minutes

Genetics is a fundamental field of biology that covers the passage of genetic information to successive generations (inheritance). More broadly, genetics covers the study of genes, how they are organized in cells, how they are regulated, and how they interact with each other and the environment. Genetics is a key component of genomics, a much broader area of the life sciences that is highly interdisciplinary and considers the structure, function, mapping and both natural and induced alterations of genomes.

Over the course of a series of short articles, we will cover the basics of what DNA is and how it is organized in cells, how cells divide and pass genetic information to the next generation, and how genetic variation (inherited and sporadic) impacts disease, and how purebred dogs can help advance genetic research.

How Are Genes Inherited?

Understanding how genes are passed from parent to offspring (inherited) requires a basic knowledge of some key terms.

- *Allele* – one or more alternative form of a gene.
- *Homozygous* – the two copies of the gene indicated are represented by the same allele.
- *Heterozygous* – the two copies of the gene indicated are represented by different alleles.
- *Genotype* – the set of genes that an individual possesses, inherited from the parents.
- *Trait* – a physical, behavioral, biochemical, or physiological characteristic, e.g. eye color, coat texture, etc.
- *Phenotype* – the appearance or manifestation of a trait (this may be physical, behavioral, biochemical, or physiological. This is what we can see or measure, e.g. blue eyes, straight coat.)

After mating, when fertilization occurs, the *haploid* set of chromosomes carried by each of the *gametes* of the male and female (sperm and egg) fuse to form a zygote. At the exact time of fertilization, the zygote thus acquires a *diploid* set of chromosomes; one haploid set from the mother and one from the father. A puppy, therefore, inherits a copy of the genome from each parent, with half the genes in each cell being derived from each parent. As such, for each autosomal gene, as well the sex chromosomes of a female puppy, there is a paternally and a maternally derived (inherited) copy. In males, the X and Y chromosomes carry different gene sets and so these are present only as one copy.

Every gene has one or more variant forms, called *alleles*. Since each individual has only two copies of each gene, an individual can only have two alleles even if there are many more across the population. If the allele of a gene inherited from both parents is the same, the gene is said to be *homozygous*. If the offspring inherits a different allele of a gene from each parent, the offspring is referred to as *heterozygous* for that gene. There are approximately 20,000 genes distributed across the canine

genome, each with a specific function. When we consider the alleles of multiple genes we refer to the combination possessed by an individual as the *genotype*. It is the enormous number of possible combinations of genes that lead to genetic variation and explains why no two individuals (except identical twins[1]) share the same *genotype* and hence *phenotype* for each *trait*.

Is it All in The Genes?

Most traits are influenced by numerous genes and impacted by the environment. For these traits, deciphering the pattern of inheritance is very complex and beyond the scope of this introductory piece. A small number of traits are primarily controlled by a single gene and so have a more predictable pattern of inheritance.

Most textbooks will highlight the major forms of inheritance as being either dominant or recessive. We will briefly explain the basics of these two simple inheritance patterns, but be aware that these terms can be misleading.

As we stated above, while each gene may be represented by one or more alleles, an individual can only have two alleles (a paternally and a maternally derived allele). The alleles may result in different phenotypes. Hypothetically, for fun, let's assume we are looking at a single dog gene that we will call *WOOF*, which only has two alleles, 'A' and 'a'. Allele 'A' results in loud barking and allele 'a' results in quiet barking.

When we look across the population we notice that dogs either bark loudly or quietly, but we do not observe dogs barking at a mid-volume. We also notice that many more dogs bark loudly than quietly. This type of scenario could be explained by the 'A' allele (loud barking) being fully dominant to the 'a' allele (quiet barking), which is referred to as 'recessive'. For this reason, notation dictates that the dominant allele is represented in an uppercase letter (in this case 'A') and the recessive allele is represented by a lowercase letter (in this case 'a').

With only these two alleles there are only three possible genotypes, AA, Aa, and aa. Since there are only two phenotypes, LOUD and QUIET, the presence of the A allele 'vetoes' the presence of the 'a' allele. We thus have the following summary:

Gene = *WOOF*

Alleles = A (loud barking); a (quiet barking)

GENOTYPE	**PHENOTYPE**
AA	loud barking

Aa	loud barking
aa	quiet barking

[1] There are exceptions to this general rule that relate to differential gene expression and environmental influences.

Without assessing the genotype by genetic testing, the dogs that exhibit loud barking would all appear the same and there is no indication as to whether their genotype is ‘AA’ or ‘Aa’. However, while the dogs with homozygous ‘AA’ or ‘aa’ will pass a copy of the ‘A’ or ‘a’ allele, respectively, to all their offspring, those with the heterozygous genotype ‘Aa’, will pass a copy of the ‘A’ allele to half of their offspring, and a copy of the ‘a’ allele to half their offspring.

Since it is fully dominant, the barking volume phenotype of all offspring that inherit the ‘A’ allele will be ‘loud, but the phenotype of offspring that inherit the ‘a’ allele will be determined by which allele is inherited from the other parent. If it is an ‘A’, the offspring will have the ‘loud’ phenotype. If it is another ‘a’ allele, the offspring will have the ‘quiet’ phenotype.

With just two alleles ‘A’ and ‘a’, we can display all possible combinations of genotypes and phenotypes (in parentheses) in the form of a Punnett square (Figure 1), where both parents are heterozygous ‘Aa’. This is termed a monohybrid cross, with the alleles, contributed from the sire across the top and those of the dam on the left.

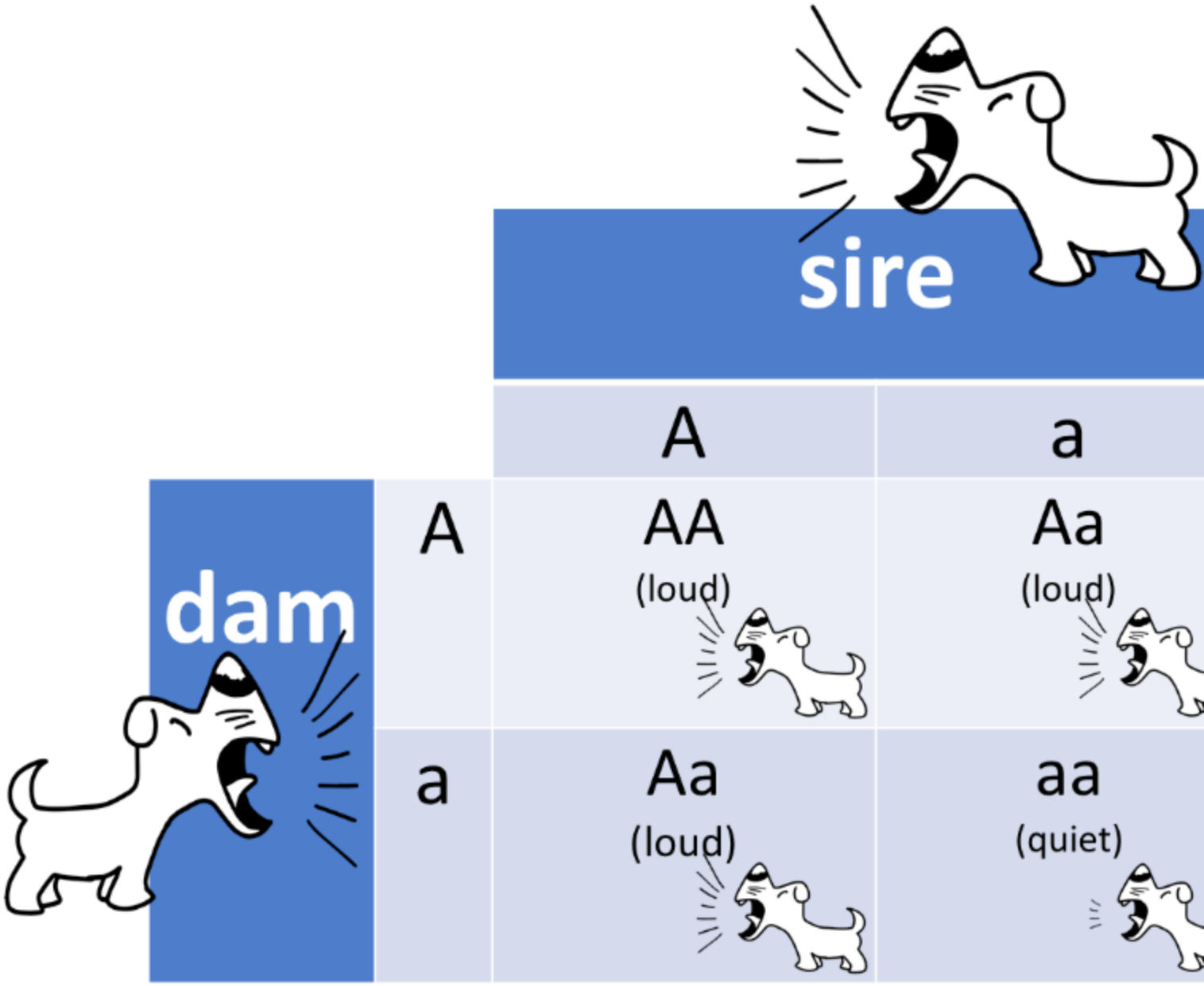

***Figure 1. Punnett square**. Simplified example showing possible combinations of genotypes and phenotypes when the parents are both heterozygous and one allele has complete dominance over the other.*

In this example, the fact that 'A' is dominant to 'a' means that whenever 'A' is present, the barking volume phenotype is 'loud'. This results in 75% of all offspring of this simplified mating scenario will be loud barkers. The only 'quiet' barkers are those homozygous recessive (aa) dogs, where the 'quiet' phenotype is observed because of the absence of an 'A' allele. It is important to note that those heterozygous dogs (AB), while all sharing the 'loud' phenotype, are carriers of the 'quiet' allele.

What Does This Tell Us?

This is very much an oversimplification to explain how genes pass from parent to offspring. In reality, very few traits are controlled by single genes and several other factors play a role in determining the final phenotype. Due to the way in which gametes are generated, the distribution of alleles to the

gametes is random. This example demonstrates a hypothetical case of a single gene and shows the autosomal dominant and autosomal recessive modes of inheritance.

Additional modes of inheritance, including X-linked dominant, X-linked recessive, Y-linked, codominance, multifactorial. Once a gene has been passed to offspring, there are also numerous interactions that occur to influence the final phenotype. While the genetic material provides the recipe for life, the way in which the ingredients are mixed and then interact with each other is controlled by a highly complex set of factors.

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