Pedigree analysis and inherited canine & feline reproductive diseases
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Abstract
As theriogenologists, breeders come to us not only for diagnosis and treatment but also with questions pertaining to the genetic nature of a specific disease. The recent advances in molecular genetics and the availability of genetic maps have made it possible to discover the basis of many genetic diseases in the dog and cat. Over 600 inherited diseases have been described in dogs and about 200 in cats, with more genetic diseases being added each year. This has led to the development and growing need for incorporation of clinical genetics into veterinary practice with the reproductive specialist playing an important role in the detection of potentially new genetic diseases and to provide genetic counselling. To provide assistance to the breeder, we as theriogenologists already appreciate the importance of accurately diagnosing a disease and now need to be able to decipher pedigrees along with historical information that will allow us to suggest a reasonable mode of inheritance for a given disease.

Keywords: Pedigree analysis, modes of inheritance, inherited reproductive diseases.

Introduction
The first step to understanding the mode of inheritance is precise phenotyping, i.e. the disease of interest must be accurately diagnosed. For example, two related bullmastiffs are both one year of age; are polyuric and polydipsic; have elevated serum renal values; have a urine specific gravity of 1.011; and a urinary protein to creatinine ratio of 5. However, one has renal dysplasia and the other an inherited glomerulonephropathy. Had the two been lumped together as one disease, the pedigree analysis would have been incorrect. The second step to understanding the mode of inheritance is analyzing the provided pedigrees. Figure 1 shows a typical pedigree of a dog registered with one of the European kennel clubs. Tracking genetic diseases through these types of pedigrees is not easy and it becomes clear that more than just a three-generation pedigree is needed. To visualize inheritance patterns, we typically convert written pedigrees into diagrammatic pedigrees as demonstrated below. Once converted, the inheritance patterns will present as one of the modes if inheritance as outlined. This information is essential in providing a thorough work up of any genetic disease.

Definitions and symbols
A gene is defined as the basic element of heredity that determines traits. A gene is transmitted from parents to progeny. An allele is an alternative version of a given gene. The chromosomal location at which a gene with a specific function resides is called a locus. If pairs of alleles of a given gene are the same, they are called homozygous (or homozygote when referring to the genotype in the individual). Heterozygote means that the pairs of alleles of a given gene are different.

The genotype is defined as the genetic constitution and consists of internally coded, heritable information. The genotype could essentially be viewed as the individual’s architectural blue print. Usually, only alleles of genes of interest listed, e.g. B for black, b for brown, e for yellow if Labrador hair color is of interest. The phenotype is the summary of observable properties of an organism, and the outward, physical manifestation of the genotype. In other words, the phenotype is the implementation of the blue print.

Any gene that is located on a non sex chromosome is considered autosomal. Genes on the X or Y chromosome are called X-linked and Y-linked, respectively. In dominant traits, the allele is expressed in the phenotype when present on only one chromosome (in heterozygotes). For incomplete dominant traits, the allele is expressed in heterozygotes, but homozygotes are more severely affected and in complete
dominance, homozygotes are indistinguishable from heterozygotes. In contrast, in recessive disorders, the allele can only be expressed when present on both chromosomes (in homozygotes).

Pedigrees are of great importance in recording the occurrence of a known or suspected genetic disease in families and in determining the mode of inheritance. The recommended method of drawing lines indicative of relationships between relatives in domestic animals differs somewhat from that used in humans. This is because in domestic animals, inbreeding is common and the generations are often overlapping, making the human pedigree format awkward. The basic principle used in drawing animal pedigrees is that a mating is indicated by vertical lines that come out of the bottom of the symbols of the two parents and are connected by a horizontal line on which the offspring are arrayed (Figure 2). To convert the written pedigree, animal names are coded by numbers, their sexes indicated by symbols (squares are males and circles are females), and their relationships indicated by lines (Figure 3). There are commercially available products that assist with drawing pedigrees (e.g. Pedigree/Draw, Southwest Foundation for Biomedical Research, San Antonio, TX) and that calculate coefficients of inbreeding if needed. However, in most cases drawing a quick five to 10 generation pedigree will allow for an educated guess as to the mode of inheritance of a given trait or disease.

**Modes of inheritance with examples**

**Autosomal dominant inheritance**

One allele of a given gene is enough to determine the phenotype (trait/disease allele D with d being normal) and since the gene is located on an autosome (non sex determining chromosome) the risk to males and females is equal. Affected individuals are usually heterozygotes (Dd). At least one parent is affected, unless the condition is the result of a new mutation.

*Affected x normal* matings produce 50% affected offspring. 50% of the animals are normal (phenotype) and are homozygous in the normal allele (dd) and 50 % of the animals are phenotypically affected and are heterozygous for a normal and a trait determining allele (Dd; Figures 4a1 and 4b).

*Affected x affected* matings (rare) produce 75% affected offspring. Only 25% of the offspring are normal (dd), but 75% are phenotypically affected but genotypically different; 50% are heterozygote (Dd) and 25% are homozygotes (DD; Figures 4a2 and 4b). Double doses of dominant traits often lead to a more severe phenotype that can lead to early morbidity and mortality.

In autosomal dominant disorders that are severely deleterious (have a fitness close to zero), or which would be selected against by breeders, most of the cases observed in a population will represent new mutations. These will occur as rare sporadic cases with no prior evidence of their occurrence in related animals. A number of such mutations affecting collagen metabolism in dogs and cats have been found in recent years. In these, fragility of the skin and joint laxity preclude survival under ordinary circumstances and proof of their dominant nature was obtained only by breeding studies in a protected laboratory environment.

The mating of two individuals with a dominant disorder will be rare, but in such cases the number of offspring may be reduced because the homozygous state is lethal in utero. Owing to the loss of homozygotes (one out of four embryos in matings between two heterozygotes), the proportion of affected offspring at birth will be 2/3 rather than 3/4. Examples include desired traits such as Chinese crested dogs, Mexican hairless dogs, and Scottish fold cats in which the trait is encoded by a single “defective” gene. When the animals with the desired traits are bred together, one quarter of the offspring will be homozygous for that trait, which is then lethal in the hairless dogs and the homozygous Scottish fold cats will have severe cartilage defects.

It should be fairly straightforward to eliminate traits inherited as simple autosomal dominant, if they are easily recognized and their onset occurs before the age of reproduction. In such cases, only the affected the animal has the mutation and it can be removed from the breeding pool before it reproduces. However, the autosomal dominant mode of inheritance is the more rare occurrence. It appears that most disorders, in dogs and cats at least, are inherited as recessive traits that are transmitted by either a single gene (autosomal recessive) or, more likely, multiple genes (complex inheritance). It is more difficult to reduce transmission of disorders that are inherited in this manner.
**Autosomal recessive inheritance**

An animal has to have two trait determining (disease) alleles to express the phenotype or be affected (rr). Again both female as well as male animals are equally affected. An animal that has one disease allele is phenotypically normal but is called a carrier (for the disease allele, r; carrier = Rr). The normal individual has the “RR” genotype.

*Carrier x normal* mating produces 50% carrier offspring. 100% of the animals are of a normal phenotype. However, 50% of these are homozygous in the normal allele (RR) and 50% of the animals are carriers (Rr) and thus heterozygous for a normal and a trait determining allele (Figures 5a1 and 5b).

*Carrier x Carrier* matings produce 75% phenotypically normal offspring. However, 2/3 of these are carriers (Rr, rR, RR). Without specific tests it is often impossible to distinguish the normals from the carriers; 25% of the offspring are affected (rr; Figures 5a2 and 5b).

Autosomal recessive inherited diseases are by far the most common class of single gene disorders in domestic animals. In affected families, most affected animals are born to clinically normal parents that are carriers of a mutant allele that has been inherited from an ancestor that is common to the sire and the dam (some degree of inbreeding is present). Often when a “new” disease shows up in a dog or cat, the breeders tend to discount autosomal recessive inheritance because this “new” disease has not been seen in over five generations. However, as evident in Figure 5c, the original mutation may have occurred many generations ago but it wasn’t until the sixth generation that two carriers came together to produce an affected animal.

As theriogenologists, we are most concerned with disorders that affect the reproductive tract, which brings additional problems to the analysis, such as sex-limited expression of a particular disorder. That is, the disorder causes an abnormal phenotype in one sex, not both, so sterility or infertility only occurs in one sex. Another example of sex-limited expression is cystinuria in Newfoundland dogs, which is present in both males and females but it is almost always the affected male dog that presents to the clinic because the cysteine stones lodge in the narrow male urethra and cause blockage.3,4

The other, unaffected, sex can continue to transmit the mutation. Since the genotype is not expressed in the phenotype of one, it makes pedigree analysis difficult even if the disorder is inherited as a simple Mendelian autosomal recessive trait: the parent that cannot express the phenotype, although it looks normal, can be carrying one or two copies of the mutation, or it could have two normal copies of the gene. To deduce the genotype of the parent that cannot express the trait, one would need to perform experimental matings with progeny testing. This is a good reason to support research to identify the causative mutation, create a DNA test, and genotype animals directly. Another problem is that a disorder can have a severe phenotype in some cases, and a less severe phenotype in others. A disorder that exemplifies both types of pedigree problems is persistent Müllerian duct syndrome in the miniature schnauzer.5 Females are unaffected by this mutation, as are male carriers. Males that are homozygous for the mutation have normal testes and internally have oviducts, a complete uterus, a cervix and cranial vagina. Only half the affected dogs have associated cryptorchidism, which often elicits clinical investigation, while the other half have scrotal testes, and thus appear to be normal males.

**X-chromosomal recessive inheritance**

The gene of interest is located on the X-chromosome: therefore two copies of the trait determining (disease) allele are necessary in females but only one copy is needed in males for the phenotype to be expressed.

Affected males are hemizygotes (they only have one X chromosome). Most affected offspring are males, born of matings between carrier females and normal males. In such matings, 50% of the sons are affected and 50% of the daughters are carriers. Affected females only occur as the result of matings between affected males and carrier or affected females. When the male is the only affected parent, male to male transmission of the condition is never observed.
Carrier female x normal male matings result in 50% of the males being affected (X<sup>c</sup>Y); 100% of
the females and 50% of the males are of a normal phenotype but half of the females are carriers
(XX<sup>c</sup>)(Figures 6a1 and 6b).

Carrier female x affected male matings (rare) produce 50% affected offspring. Half of all males
are affected (X<sup>c</sup>Y) and the other half normal (XY); whereas half of all females are carriers (XX<sup>c</sup>) and the
other half affected (X<sup>c</sup>X<sup>c</sup>)(Figures 6a2 and 6b).

In domestic animals, an important feature of X-linked recessive disorders is that in matings of
carrier females to normal males, one half of the male offspring will be affected, regardless of whether the
male (sire) is related to the female. Thus, inbreeding is not a prominent feature in X-linked recessive
disorders. This is in contrast to autosomal recessive inheritance, in which inbreeding is often present, the
parents of affected offspring having inherited the mutant gene from ancestors which they share.
Examples of X-linked recessive diseases include severe combined immunodeficiency in the Corgi and
Basset hound<sup>6,7</sup> and ectodermal dysplasia in various breeds (usually a new mutation).<sup>8</sup>

X-linked dominant inheritance

X-linked dominant traits are more commonly found in females than males (twice as common in
rare traits, since females have two chances to receive an X with the mutant allele, while males have only
one). If the mutant allele is lethal to hemizygous male embryos, the disorder will be found only in
females. The chief characteristic of X-linked dominant inheritance in families is that affected males
transmit the trait to all of their daughters and none of their sons. Affected females are usually
heterozygous and it may not be possible to distinguish between autosomal dominant and X-linked
dominant inheritance from their offspring. One half of the females and one half of the males will be
affected in both cases. However, this would be the case only when dominance is complete. That is, when
the effect in a heterozygous female is essentially the same in a hemizygous male. An example of X-
linked inheritance in which the mutation is "incompletely dominant" in heterozygous females is found in
a form of hereditary renal disease in Samoyed dogs. In this case, females with the mutation on one X
chromosome have a slowly progressive form of glomerular defect and usually survive for long periods.
Males, whose only X contains the mutant gene, have a much more severe disorder, rapidly progressing to
renal failure and death by 15 months of age. This disorder has been shown to be due to a mutation in the
X-chromosomal gene that encodes the alpha-5 chain of type IV collagen, a constituent of the glomerular
basement membrane.<sup>9</sup>

Complex mechanisms and complex modes of inheritance

Many diseases that are of great concern to both breeders and veterinarians are caused not by a
single gene but by the interactions of several genes (Figure 7). To make matters more difficult for the
breeder and the geneticist, the phenotype (or the appearance of the trait or disease) can often be modified
by environmental influences such as nutrition or exercise. In other words, complex or polygenic
disorders are caused by mutations or sequence variations in just a few or many genes with the interaction
of the environment and are thus more difficult to evaluate. Complex traits may occasionally have a
monogenic basis, in that a DNA repair gene suffers a mutation causing mutations in other genes. Other
examples of monogenic diseases mimicking complex traits include diseases with variable expression or
incomplete penetrance. The variable expression of these diseases with single gene disease-causing
genotype may be due to environmental influences as well as modification through other genes. In
oligogenic traits, only a few different genes are involved in expression of disease. Finally, the most
complic complex of all, some diseases are considered multifactorial, in that a particular disease is caused by many
genes, each of small effect but when added convey susceptibility. When exposed to a specific
environmental situation, the disease then becomes evident.

In cases of complex inheritance, it is not uncommon for the phenotype to vary in severity
between affected animals. Cryptorchidism may exemplify this type of complexly inherited disorder, as
the phenotypes include late testis descent, unilateral or bilateral cryptorchidism, and several locations for
the undescended testis. In addition, cryptorchidism could be an example of another problem: genetic
heterogeneity. This means that the same phenotype can be caused by mutations in different genes. In that example, we could find that unilateral cryptorchidism is caused by mutations in genes A and B in the Irish setter, while mutations in genes C and D cause the same phenotype in the miniature poodle. In that case, we would need to study both breeds to identify their specific mutations, and design DNA tests specific for each breed.\textsuperscript{10}

**Conclusion**

As theriogenologists, we can make an impact by seeking definitive diagnoses, but also developing diagnostic criteria that distinguish the degrees of severity in the affected phenotype. As expert phenotypers, we can also participate in research studies that require precisely diagnosed cases and controls to identify causative mutations. For example, in Genome Wide Association Studies (GWAS), several affected and control animals that are related to each other are needed to obtain a definitive result (Figure 8).\textsuperscript{11}

Finally, how can we approach other diseases that may have an inherited basis but that have an adult onset? For example, how could we approach an investigation into genetic susceptibility to pyometra? For now, that type of study appears out of reach. First it would likely require long term studies (multigenerational pedigrees) just to identify enough clearly defined normal and affected phenotypes within a group of related animals. Secondly, as it would likely be a complex trait, many more animals would be needed than for a trait inherited as a simple Mendelian trait. However, this should not discourage us from compiling information such as accurate diagnoses and pedigrees to lay the foundation for working up a genetic disease.

**References**

Figure 1. Typical pedigree of a registered dog. Note that the pair listed to the right of any given dog are the parents. Males will always be listed on top and females on the bottom of such pairs.

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<td><strong>Bay Breeze Horse Run</strong>&lt;br&gt;SHSB 537256 AKD PP 45465901&lt;br&gt;weiß, Gross 61,5, HD B, Int-Champion, VDH-Champion, L-Champion, I-Champion, A-Champion, CH-Champion 24.08.1998</td>
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<td><strong>Del Zarzoso Pensando-en-Gina</strong>&lt;br&gt;SHSB 544437 LOE 0743097&lt;br&gt;weiß, Gross 58, HD C, CH-Champion 03.11.1999</td>
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Figure 2. Pedigrees are typically converted for ease of scrutiny by eye. Squares are males, circles females, filled in symbols are affected animals, and empty symbols represent clinically normal animals. Animals that have died can be indicated by placing a cross underneath the individual or drawing a diagonal line across the individual’s symbol. Parents are connected by lines and the offspring are indicated on the connecting line.

The propositus (#7) is an affected male whose mother is also affected. The litter containing the propositus is the result of a mating between half siblings.
Figure 3b.
Figure 3c. Conversion of a standard pedigree into a graphical pedigree used for analysis. 3a) Standard pedigree. 3b) Names are converted to numbers to quickly identify multiple occurring parents. 3c) Converted pedigree showing only the most important, directly related individuals.
Figure 4a.

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Figure 4b.

AUTOSOMAL DOMINANT CONDITIONS

heterozygous affected X normal

heterozygous affected X heterozygous affected

Figure 4. Autosomal dominant inheritance. 4a1) Punnett square demonstrating possible outcomes when pairing affected to normal individuals. 4a2) Punnett square demonstrating possible outcomes when pairing affected to affected individuals. 4b) Pedigrees demonstrating the autosomal dominant mode of inheritance.
Figure 5. Autosomal recessive inheritance. 5a1) Punnett square demonstrating possible outcomes when pairing carrier to normal individuals. 5a2) Punnett square demonstrating possible outcomes when pairing carrier to carrier individuals. 5b) Pedigrees demonstrating the autosomal recessive mode of inheritance. 5c) Pedigree demonstrating that even if a disease has a straightforward autosomal recessive mode of inheritance, it may not manifest itself until many generations later.
Figure 6a.

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Figure 6b.

**X-LINKED RECESSIVE CONDITIONS**

- Normal male X carrier female
- Hemizygous affected male
- Homozygous affected female
- Normal male and female
- Heterozygous carrier female

Figure 6. Autosomal dominant inheritance. 4a1) Punnett square demonstrating possible outcomes when pairing a carrier female to a normal male. 4a2) Punnett square demonstrating possible outcomes when pairing an affected male to a carrier female. 4b) Pedigrees demonstrating the X-linked recessive mode of inheritance.
Figure 7. Typical pedigree of a complex genetic disorder in which there is variable expressivity in the offspring, despite the fact that two clearly affected dogs were bred together.
Figure 8. Pedigree developed by breeding affected female (arrow) to carrier father to produce 40 dogs for linkage analysis to discover the basis of centronuclear myopathy in the Labrador retriever.