2005 WESTIE EDUCATION SEMINAR

Genetics, Pediatrics, Fading Puppy Syndrome - Part 1 of 2

Editor's note: This is the handout that accompanied the very informative lecture given by Margret Casal at the WFA dinner and seminar during the WHWTCA Specialty weekend. This newsletter features the Genetics portion of the lecture. Pediatrics and Fading Puppy Syndrome will be covered in the winter edition of the WFA NEWS.

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A. GENETICS MODES OF INHERITANCE

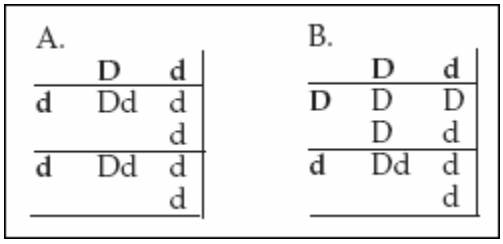
Keywords

Gene: Basic element of heredity that determines traits. A gene is transmitted from parents to offspring.
Allele: Alternative version of a given gene
Locus: Location on a chromosome where a gene with a specific function resides.
Homozygote: Pairs of alleles of a given gene are the same.
Heterozygote: Pairs of alleles of a given gene are different.
Genotype: Genetic make-up (blue print).
Phenotype: Observable properties as determined by the genotype (i.e. what the animal actually looks like).
In a simple analogy, the genotype can be described as the architectural blueprint and the phenotype as the building that is built from the blueprint In the following handout you will find information on modes of inheritance, an overview of genetic tests, and a list of the most common or well described genetic diseases in the West Highland White Terrier.

Mendelian Genetics
(Simple genetic traits)

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 (not sex determining chromosome) the risk to males and females is equal. Affected individuals are usually heterozygotes (Dd). At least on parent is affected, unless the condition is the result of a new mutation.

A.) *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).

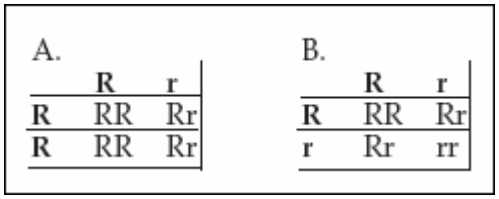


B.) *Affected x affected* matings (rare) produce 75% affected offspring. Only 25% of the offspring are normal (dd), but 75% are phenotypically affected but genotypically different; 50% are heterozygote (Dd) und 25% are homozygotes (DD). Double doses of dominant traits often lead to a more severe phenotype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

In autosomal dominant disorders that are either severely deleterious or that 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.

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; Rr). The normal individual has the “RR” genotype.

A.) *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.



B.) *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).

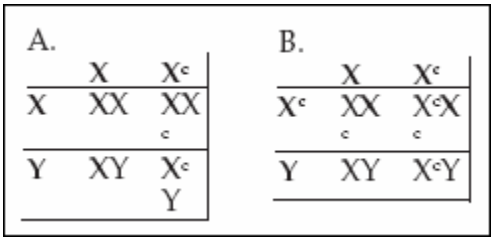
Autosomal recessive inherited diseases are by far the most common class of single gene disorders in domestic animals. In affected families, most affected animals are born to clinically normal parents that are carriers of a mutant allele that has been inherited from an ancestor that is common to the sire and the dam (some degree of inbreeding is present).

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.

A.) *Carrier female x normal male* matings result in 50% of the males being affected (X^cY). 100% of the females and 50% of the males are of a normal phenotype but half of the females are carriers(XX^c).



B.) *Carrier female x affected male* matings (rare) produce 50% affected offspring. Half of all males are affected (X^cY) and the other half normal (XY); whereas half of all females are carriers (XX^c) and the other half affected (X^cX^c).

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 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.

Complex Modes of Inheritance
(e.g. Polygenic traits)
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. 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. Examples include hip dysplasia, elbow dysplasia, heart disease and epilepsy.

GENETIC TESTS
Test Mating - If, as is the case with many genetic disorders, there is no available laboratory test for the carrier state, the only method by (Continued from page 3) which carriers can be detected is test mating. The animal to be tested is hypothesized to be a carrier and is usually mated to an animal known to be a carrier or affected. If any affected offspring are produced, we know that the tested animal is a carrier and the hypothesis is shown to be correct. If, however enough normal offspring and no affecteds are produced, we can reject the carrier hypothesis with some degree of statistical assurance.

Biochemical testing - For some genetic diseases, carriers are identifiable by biochemical testing (e.g., enzyme defects, clotting factor defects). These assays can be very useful and have been all that has been available for a number of years. However, compared with the possibility of DNA-based tests (see below), they have a number of disadvantages, including:

- Normal and carrier levels of the enzyme/substrate often overlap.
- A biochemical test may not be possible for a specific disease because the in vitro assay (laboratory test) results do not reflect the in vivo (real-life) conditions.
- A test may be possible but is not available because of expense.
- The enzyme or substrate may be unstable and would not survive shipment.
- Age matched controls are necessary.
- There may be lab to lab variation in the assay.
- The tissue needed for the assay may be difficult to obtain.

Molecular Genetic (DNA-Based) Tests for Affected and Carrier Animals -
DNA-based genetic tests identify differences in DNA sequences and are of two different varieties. One type of test, referred to as a mutation- based test, recognizes disease- causing mutations while a second type of test, the linkedpolymorphism test, recognizes DNA differences that are near the disease-causing gene and are used to track normal and mutant alleles of that gene through pedigrees. While there are significant differences between how these two types of tests are developed and how they are used, they both involve the same basic techniques.

Essentially all DNA-based genetic tests are based on the polymerase chain reaction, and consequently can be performed using a very small amount of DNA from the animal of interest. DNA-based genetic tests have the advantage (over biochemical assays) that DNA is very easy to obtain by fairly non-invasive techniques and is very stable. Common sources of DNA include: blood, hair follicles, cheek swabs, semen, and skin biopsies.

USES OF GENETIC TESTING
The more accurate the test, the quicker a disease can be eliminated from the breeding stock. The parents and relatives can be tested and their use as a breeder established if they are not carriers for the disease. Alternatively, if we know that a champion dog is a carrier of a specific disease but the dog has all the best qualities for its breed, then we are able to not only test the bitch he is to be bred to ensuring that she is not a carrier, but we can also test the offspring and retain only those for future breeding that are not carriers. Thus, we do not have to loose the desired traits in the champion dog.

The practicality of a **genetic screening program** depends on the following requisites: Disease must occur in a defined population (family, herd, breed) with sufficient frequency to be of economic or social importance. The test for the heterozygote is accurate and affordable. Removal of heterozygotes (carriers) does not deplete key genetic resources. Test and control program should be acceptable to breeders (precede by educational and public relations programs). Genetic counseling is available to breeders. Breed society has rules to insure control is based on test results (registries).

GENETIC COUNSELING: IS IT A GENETIC DISEASE?

This is probably the most common question posed to the veterinarian by the conscientious breeder when confronted with a puppy with an unusual illness. What are the chances of it happening again? What can be done about it? First, it is most important to make an accurate diagnosis. Second, one needs to know if the same disease has been seen in related animals, in the same breed or is known to be a genetic disease in other species. If any of these statements are true, then one is most likely dealing with a genetic disease. Or, to quote the "father" of small animal genetics, Dr. Donald Patterson, "Everything is inherited until proven otherwise!" Alternatively, if the same disease has never been seen in the breed and is not known to be inherited in other species, then one may be dealing with a developmental disorder that may have occurred during pregnancy as a result from toxins, malnutrition, medications, and such.

In summary, a disease has been seen in the breed before or occurs as a genetic disease in another species, it is likely to be genetic in the animal presented to the veterinarian. If one needs to make an educated guess as to the mode of inheritance, then it is helps to have an idea of the biochemical cause of disease. Most enzyme deficiencies are autosomal recessive and most structural defects are dominant. These are just rules of thumb, there are exceptions!

Table 1. List of the well-known genetic diseases in the West Highland White Terrier

Genetic Disease	Mode of Inheritance
Allergic Contact Dermatitis	Unknown
Atopy	Unknown
Cataract	Unknown
Conotruncal Heart Defects	Polygenic
Craniomandibular Osteopathy	Autosomal recessive
Cutaneous Histiocytoma	Unknown
Cystinuria Type I	Autosomal recessive
Ectopic Ureter	Polygenic
Epidermal Dysplasia	Autosomal recessive*
Familial Chronic Hepatitis	Autosomal recessive*
Glaucoma	Unknown
Globoid Cell Leukodystrophy	Autosomal recessive
Hypoadrenocorticism	Unknown
Ichthyosis	Autosomal recessive*
IgA deficiency	Unknown (autosomal recessive*)
Insulin-Dependent Diabetes Mellitus (IDDM)	Unknown
Ischemic Necrosis of the Femoral Head	Autosomal recessive*
Keratoconjunctivitis sicca (Dry eye)	Unknown
Malassezia (probably allergic)	Unknown
Microphthalmia and Multiple Congenital Ocular Defects	Unknown
Myotonia Congenita	Autosomal recessive
Persistent papillary membrane	Unknown
Pilomatrixoma	Unknown (Autosomal dominant*)
Pulmonic stenosis	Polygenic
Pyruvate kinase deficiency	Autosomal recessive
Retinal dysplasia	Autosomal recessive*
Seborrhea	Autosomal recessive*
Shaker dog syndrome	Unknown

*Suspected mode of inheritance

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Winter 2006, *NEWS*

B. PEDIATRICS IN A NUTSHELL

Pediatrics is defined as the study and treatment of children in health and disease. Childhood is defined as the period of life between infancy and puberty. In humans, there are volumes of books with tables, graphs, and guidelines such as the ¡°Denver scores¡± which one can refer to for assessment of growth and development in a pediatric patient. Unfortunately, we can only rely on very few tables, our own experience and the breeders¡¯ observations and thus may not be completely aware of the diversity present in the many different breeds of dogs and cats. This chapter deals with outward, physical characteristics that are common within the cat or dog population and that can be used either to simply determine the age of stray animal or to assess if a developmental delay is present due to congenital or acquired diseases.

History

Because of the unusual or nonspecific clinical signs, it is of great importance to obtain a comprehensive history not only of the patient himself, but also of the littermates, parents and other relatives. The history should include number of ill animals, the method by which they were raised, their normal environment, behavior of each puppy within the litter, body weight curves, duration and type of clinical signs, and medications given. The queen¡¯s or the bitch¡¯s history should include vaccination dates, estrous cycle (intervals and duration), breeding practice, medications or supplements given during pregnancy, and problems during pregnancy or birth. Has the disorder with which the patient is being presented, been seen in previous litters or in any of the relatives? In certain cases, clients are advised to bring the whole litter or at least one healthy littermate including the mother, so that the patient can be compared with its littermate(s). If the patient or its littermates have not been vaccinated yet, it may be better to have them come in the back door, to avoid exposure to all the infectious diseases that may linger in the waiting room.

Physical Examination

The physical examination of the neonate and the juvenile patient can be challenging. Owners as well as littermates that were brought in for comparison can be quite distracting. Usually, one cannot expect cooperation from the youngest of our patients, especially those that are already aware of their surroundings. They are so distracted by the new environment that something as simple as a menace reflex is difficult to elicit. While everyone knows how to perform a physical examination, the following two paragraphs focus on some of the differences to adults and are illustrated with some examples. The list is not complete, but comparison with the some of the developmental landmarks described below may be helpful in determining other abnormalities.

For the physical examination of a **neonate**, a pediatric stethoscope with a 2 cm bell is helpful. A digital thermometer allows rapid measurement of the body temperature, without causing great discomfort. Because the neonate can have a body temperature lower than 94°F, a digital thermometer that measures down to 92°F is practical. Neonates cannot regulate their body temperature during the first 2 weeks of life. Therefore, they should be examined on a warm, clean surface rather than the cold metal table. Checking the oral mucous membranes (MM) assesses hydration in the neonate, as their skin turgor is not developed as in adults. Moist MM are present in an adequate state of hydration, but tacky to dry MM indicate 5-7% dehydration. At 10% dehydration, the MM are very dry and there is a noticeable decrease in skin elasticity. The neonate is born with hair that covers most of the body except the ventral abdominal skin. Lack of hair or a sparse hair coat may indicate either a genetic abnormality of the skin or premature birth. The neonate normally has non-haired, dark-pink, ventral abdominal skin. Bluish or dark red discolorations are indicative of a neonate in distress (cyanosis or sepsis, respectively). Other than urine and feces, discharge from any orifice is abnormal in the neonate. The neonate¡¯s head, body, limbs, and tail are examined for symmetry and normal conformation. The head is specifically examined for open fontanels, cleft palates, bulging from behind closed eyelids, and formation of the nose and external ears. The presence of flattening or malformations of the chest are noted (e.g. swimmer syndrome, pectus excavatum), as are bulges in the neck area (e.g. gas in the esophagus, ectopic heart, goiter). Neonatal puppies are mildly pudgy and should never be bloated, which would be a sign of distress. The abdomen and urachus are especially examined for defects of the abdominal wall and ventral urine scalding (e.g. cannibalism due to an overzealous mother, ventral closure defects, persistent urachus). The genitals and the anus are checked for patency by stimulating urination and defecation using a moistened cotton ball. The presence of hair coat abnormalities over the dorsum may indicate the presence of a spina bifida. The tail is examined for muscle tone, length, curliness and kinks. Abnormalities in tone may be indicators for associated defects or problems (e.g. abnormal innervation of the distal pelvis).

The physical examination of the **older pediatric patient** follows the general guidelines as in the adult. However, many metabolic diseases with a genetic basis begin becoming apparent between 3 - 5 months of age. They are usually of a progressive, degenerative nature characterized by failure to thrive and clinical signs specific to the disease. Comparison to littermates is extremely useful in these cases, but not always possible. As above, the whole patient is examined for symmetry, conformation, body weight, and stature. The eyes deserve special attention, as many disorders especially infectious and hereditary diseases can be picked up at a very early age by a careful fundic examination. Ventro-lateral strabismus (down and out cross-eyed) may indicate the presence of a hydrocephalus, while nystagmus, either horizontal or vertical, may point towards inner ear infections, polyps in cats, trauma or other CNS disorders (hereditary or infectious). The neck again is examined for bulges in the esophagus (e.g. gas indicating a possible megesophagus or vascular ring anomaly; solid swellings indicating foreign bodies) and soft tissue lumps in the thyroid area (e.g. goiter). The limbs are examined for malformations and especially pain (e.g. hypertrophic osteodystrophy, panosteitis, elbow and hip dysplasia; vaccine reactions; trauma). Abnormal conformation of the genitals may indicate hermaphroditism, intersexes, cryptorchidism, or future problems (e.g. skin fold pyoderma in bitches with a small, tucked-in vulva). Urine scalding may be present in animals with chronic urinary tract infections and/or incontinence (e.g. immune deficiencies, bladder diverticulum, ectopic ureters, hour glass bladders, kidney malformations). Vaginal discharge or the presence of hair stuck together at the

ventral commisure of the vulva in bitches before their first heat is a common finding with puppy vaginitis. Fecal staining may indicate diarrhea, fecal incontinence, or the patient's inability to groom itself due to a more severe disorder.

Normal Development

During the **first week of life** newborn puppies sleep throughout most of the day (80%), and nurse vigorously for a short period of time every 2-4 hours. As the brain is not completely developed at birth, neuromuscular reflexes are missing and the only motor skills present are crawling, suckling, rooting, righting, and distress vocalization. The neonates only respond to stimuli such as odor, touch, and pain. The bitch initiates urination and defecation by licking the urogenital area. At three days of age, puppies should be able to lift their head, and by one week crawl in a coordinated manner. Puppies are unable to maintain their body temperature during the first few days of life. At this time, heat is produced by brown fat metabolism, which is under the control of the sympathetic nervous system (nonshivering thermogenesis), and the shiver reflex does not develop until after the first week of life. Therefore, their body temperature at birth (94.5-97.3°F) is lower than in adults and rises to 93.7-100.1 F during the first week of life. Heart and respiratory rates may be irregular at birth (P=160-200/min, R=10-20/min) and there is no abdominal component to their breathing. During the first week, the neonates begin to adjust to the new, extra-uterine physiology (200-220/ min, R=16-35/min). The umbilical cord dries out during the first day of life and should have fallen off by day 3-4. The flexor tone present at birth switches over to extensor tone after the 4^{th} day of life.

During the **second week of life**, puppies begin to crawl and their body temperature slowly rises towards normal adult levels. Puppies will have doubled their birth weight by 10 – 12 days and they begin to open their eyes at 10 – 12 days of age. Remnants of hyaloid artery attached to posterior lens capsule may be seen for a few days after the eyes open. The external ear canals open at 14 – 16 days of age. The iris is not very well pigmented, has a blue-gray color, and the cornea is slightly cloudy due to increased water content. By the end of three weeks of age puppies should be able to stand and have good postural reflexes.

At the end of the third, beginning of the fourth week of life, the body temperature has reached 99.3° - 101.5°F. At **two to three weeks of age** deciduous incisors and at 3 weeks deciduous canines begin erupting in puppies, respectively (Table 2). During this time puppies attempt to walk, and urination and defecation become voluntary. They begin to take an interest in their environment as their auditory, visual, and motor functions are developing. However, their sight is still poor at this time. By the end of three weeks of age, the puppies may be encouraged to eat solid food.

By the end of **4 – 6 weeks of age**, the iris color changes into the adult color and the mild corneal clouding disappears leading to greatly improved vision. By **6 weeks** of age the testicles should be descended in the dog. Puppies should be able to eat solid food without encouragement. The bitch will start weaning the puppies at 6-10 weeks of age. The optic disk may still appear smaller than in adults and has a different color due to incomplete myelination but the retinal vessels look similar to adult vessels. By the end of 8 weeks, all of the deciduous teeth have erupted in both

puppies. Because of extreme breed differences in dogs, puppies should be compared to litter mates to assess growth rates and weight gain.

Examination of the eyes reveals a blue-gray tapetum until reaching adult colors at about **4 –7 months** of age. By **four months** of age most breeds of puppies will have reached 50% of their expected adult weight. At this age, they lose their deciduous teeth and begin replacing them with permanent teeth, which is completed by **6 – 7 months of age**. With a few exceptions (large breed dogs, Basenjis, sight hounds) this coincides with **puberty**.

Table 2. Eruption of deciduous and permanent teeth in dogs.

DOGS			
Deciduous Teeth			Permanent Teeth
Weeks of Age	**Tooth**	**Weeks of Age**	**Tooth**
3-4	C	16-18	I^1, I^2, I^3, P^1, M^1
4-5	I^1, I^2	20-24	C, P^2, P^3, P^4, M^2
4-6	P^2, P^3	24-28	M^3
5-6	I^3		
6-8	P^4		

C: Canine; I: Incisor; P: Premolar; M: Molar

C. THE FADING PUPPY SYNDROME

The fading puppy syndrome is characterized by a pattern of increasing weakness, failure to nurse, weight loss, hypothermia, and death prior to weaning, usually in the first two weeks of life. It is actually not a single disease entity but rather common clinical signs caused by different underlying diseases processes. There are no obvious clinical signs or pathological findings, such as poor mothering, mastitis, neonatal canine herpes infections, or cleft palate, etc. Fading puppies usually have a low birth weight to begin with, are restless, and cry often beginning shortly after birth. In studies of purebred kennels from the 1950's and 60's, it was shown that 20-30% of puppies died before reaching weaning age. However, these studies were performed at a time where vaccines were not as readily available and diagnostics were less detailed. Thus, it is possible that some of the losses may be attributed to infectious diseases or non-recognized genetic defects. Studies performed in the 80's revealed that of 500 puppies about 30% had died before weaning (stillborn puppies were excluded). In 55% of these puppies, no cause of death was found even after careful histopathological and microbiological examination. This is consistent with our own findings in which we lose about 16% of pups before weaning age. These are dogs that are housed in a controlled, specific-pathogenfree environment.

There is a report of surfactant deficiency in lungs of fading puppies, but it was not clear if this was the defect leading to death or if this was secondary to the dying state. While the fading puppy syndrome is not a single disease entity, there is evidence that a large proportion of the neonatal mortality is due to homozygosity for deleterious genes. One study examined differences in mortality between purebred populations and outbred populations; that is, offspring of dogs of one breed being bred to dogs of another, unrelated breed. Another study looked at a colony of purebred beagles and found that there was a 26% mortality rate among puppies with a lower (0.26) coefficient of inbreeding to 75% mortality among puppies with a high (0.67) coefficient of inbreeding. The most obvious difference between kitten and puppy mortality is the biphasic

nature of deaths in kittens; while most of the fading puppies die at the time of birth, kittens die at birth and at the time of weaning. Despite these differences and in light of the findings in both puppies and kittens, every attempt should be made to obtain a diagnosis. Unfortunately, infectious diseases develop too rapidly in the neonate to obtain results quick enough to be of value to the affected individual but may be useful for litter mates. Urine metabolic screening and post mortem examinations should be performed on “fading” neonatal and juvenile animals. Although most cases will not, at our present level of knowledge, yield a positive diagnosis even with the use of microbial cultures and histopathological examination of fixed tissue, the performance of the post mortem examination is essential to diagnose those conditions which are recognizable. Also, because of the evidence that close inbreeding increases the rate of fading puppies, matings, which consistently produce a high percentage of neonatal deaths, should be avoided.