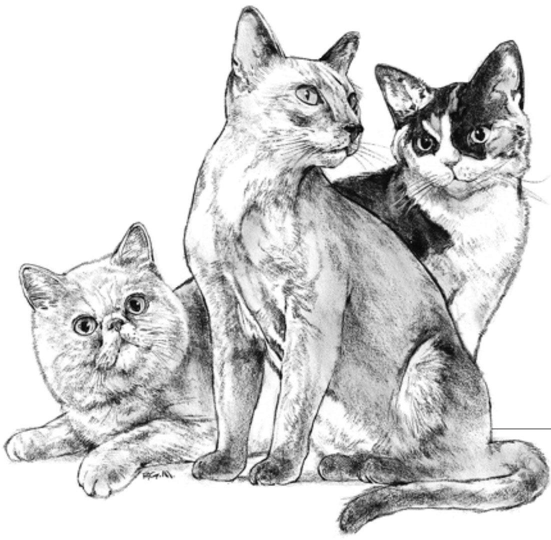

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Geneticists Work to Build the Feline Genetic Map

A highly detailed feline genetic map is being built. This map will show the architecture of the 19 pairs of feline chromosomes and identify markers anchored to genes. Ultimately, it will become a useful tool for researchers studying various feline genetic diseases and traits of interest. The more dense the map, the easier it will be for researchers to identify gene mutations.

Several hundred feline genetic diseases and traits of interest have been identified, yet only about 13 genetic mutations have been discovered. William Murphy, Ph.D., associate professor of genetics at Texas A&M College of Veterinary Medicine, and his research team are working with collaborators at the National Cancer Institute and the National Center for Biotechnology Information to build the feline genetic map. Their goal is to develop gene mapping tools to help advance studies of genetic diseases that will improve feline health through the development of tests for genetic diseases and other traits.

> "Our goal is to provide the tools to help advance understanding about feline genetic diseases. Once disease mutations are found, scientists can develop tests that will enable breeders to screen cats to determine carriers. Using judicious breeding, breeders will be able to reduce the incidence of genetic disease and help cats to live long, healthy lives."
>
> *William Murphy, Ph.D., associate professor of genetics at Texas A&M College of Veterinary Medicine*

Using the Sequenced Feline Genome

The completion of the sequencing of the feline genome last fall has helped the researchers to develop their feline genetic map. "We are taking advantage of the sequenced genome to help design markers," Murphy says. "It has helped us to find new markers and put them on the map. We are constantly refining our work, going back and rechecking. A lot of resources are used to confirm the building and rebuilding of the map."

Scientists at Agencourt Bioscience Corp., a biotechnology firm started by scientists originally involved in the Human Genome Project, did the sequencing. The $5.5 million project included more than 100 people and involved geneticists at the Broad Institute of MIT and Harvard and the National Cancer Institute. An Abyssinian cat named "Cinnamon" was chosen for the sequencing because she was highly inbred, meaning there was little variation in traits between her and her close relatives. To produce the best genome sequence, it was important to select a cat with little variation in traits and thus few polymorphisms.

The feline genome was sequenced two times using a whole genome strategy (WGS), a technique in which many millions of pieces representing the entire genome is sequenced at once. Compared to the canine genome, which was sequenced seven-and-a-half times, the sequenced feline genome has relatively low coverage, yet it still provides an independent source of confirmation about the location of genes.

Combining Mapping Tools

The first feline genetic tool was a linkage map based on recombination frequencies (chromosome exchange events that occur during meiosis) between pairs of genetic markers along chromosomes. Developed in 1999 by Marilyn Raymond of the National Cancer Institute, part of the National Institutes of Health, this map identified gene markers for disease genes that are close to chromosomes, but did not represent the true distance between markers.

"It's very hard to find genes associated with disease or coat color if you don't have an educated guess at what the genes might be to begin with," Murphy explains. "Markers help to navigate across strands of DNA to find genes. They have the best predictive value when they are close to the disease gene in question. Increasing map density improves the precision with which a trait can be mapped."

Working in the laboratory of Stephen J. O'Brien, chief of the Laboratory of Genetic Diversity at the National Cancer Institute, in 1997, Murphy began constructing the radiation hybrid (RH) panel and began a RH map to complement the linkage map. This map uses statistics to provide information about the physical location of genes. First published in 2000, the RH map made it easy to map several hundred genes and microsatellites at a time. What the RH map did not do is tell researchers whether pieces of DNA were in the wrong spot.

Cytogenetic Mapping

Building the chromosome map that Murphy is currently working on entails matching pieces of DNA to chromosomes using mulicolor fluorescence *in situ* hybridization (FISH), a process that reveals the precise chromosomal location of DNA fragments and/or genes. The researchers work with selected cones (large pieces of DNA) that were used to build the genome sequence and map them to chromosomes. The process anchors the sequencing information to the actual chromosomes and allows the researchers to be sure that all chromosomal regions are represented in the finished data.

Feline Genetic Map
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FISH mapping will provide an important tool for validating the existing gene maps and sequence assembly. "We are cytogenetically mapping over 100 markers from the current RH map to anchor the statistically generated RH maps by physical means to cat chromosomes," Murphy says. "This will add a final level of quality control to the RH maps and will be used to resolve discrepancies between the existing linkage and RH maps. Integration of these markers with the RH map will increase the quality of the cat-human genome comparative map to ensure the positional cloning efforts."

Comparative Mapping

The researchers have tapped into the human, mouse and dog genomes in developing the feline gene map. Ninety-five percent of the cat genome is shared with dogs and humans. Comparative genomics help to unlock information about the feline genome and find genetic mutations responsible for disease. Genome comparisons using many different mammalian genomes can reveal the unknown and even unsuspected biological functions by providing information about the records or evolutionary changes that have occurred over millions of years.

The first part of Murphy's work in building the feline gene map involved filling gaps in the feline-human comparative gene map. Using primers from a database of roughly 4,000 testis, heart and uterus expressed sequence tags (ESTs) and 80,000 traces from the feline sequence assembly, they compared the cat sequence to the human and dog genome sequences. ESTs are small pieces of genes that code for protein.

"For markers that gave high-scoring reciprocal hits in the same regions of the human and dog genomes, we designed PCR (polymerase chain reaction) primers that would amplify a fragment of 100 to 250 nucelotides in length," Murphy says. "We then selected 550 candidate primer pairs from the database that filled existing gaps in the feline-human comparative coverage on the latest feline hybrid map."

Advancing Feline Health

Eventually, an integrated cytogenetic/RH/genetic linkage map — the cat gene map that Murphy and his colleagues are developing — will be available online and accessible to researchers who are studying feline genetic disease or those interested in the cat as an animal model. This enhanced gene map will be a useful tool to help identify disease genes for both simple and complex inherited diseases.

"Our goal is to provide the tools to help advance understanding about feline genetic diseases," Murphy says. "Once disease mutations are found, scientists can develop tests that will enable breeders to screen cats to determine carriers. Using judicious breeding, breeders will be able to reduce the incidence of genetic disease and help cats to live long, healthy lives." ■

Winn Feline Foundation Helps to Fund Cat Genetic Map

The Winn Feline Foundation, a nonprofit organization that supports studies into medical problems affecting cats, has helped to fund the genetic mapping research under way at Texas A&M University College of Veterinary Medicine. Two Winn Feline grants have supported the work of William Murphy, Ph.D., associate professor of genetics. The first one allowed the researchers to fill gaps in the feline radiation hybrid (RH) map, and the second one supported the mulicolor fluorescence *in situ* hybridization (FISH) cytogenetic mapping that will lead to the final integrated map.

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