

# DNA PROFILING

## Multiple Sires

Edited by Kim Miles and Pam Thompson

A breeder once told me that even though there were two males in a pen with one of his females, he could distinguish which was the sire just by looking at the pups. If faced with the possibility of two sires, what would you do? Could you tell which one was the sire?



Well, this year, something similar happened to us. Our female came into heat early, and we hadn't yet removed her from the pen. We anticipated that she might be pregnant, but hoped that she wouldn't be. At least if she was pregnant, we were pretty

sure which male would be the father since we had seen Ash, the Alpha male, tied with Mia.

From what we had observed, Maverick, the beta male, had shown no interest in Mia, and he also seemed to be sufficiently cowed by the alpha. Although there was a chance that Maverick could have bred Mia, all indications pointed to Ash.

However, nothing about this litter was to be as expected. The estrus was early, which is why Mia was still in the pen with two males to begin with. The litter was unexpected; we had intended on breeding Mia, but with the birth of our daughter this year, we did not want to breed Mia until next year, at the earliest. But it was the pups, them-



selves, that presented us with the ultimate surprise.

In the past, I would roll my eyes whenever I heard someone say they had an "accidental" litter. I couldn't help but think, "You have a female in heat...it's in with a male. Ok, where's the 'accident'?" But my thoughts have become a little more "generous"



or "understanding" because of what happened to us this year.

Let me explain. Our animals normally breed during mid-February, so we separate the males and females during the last weekend of January. This has worked for many years with no mishaps. However, this year, on January 16, a commotion led me to the front kennel



where Mia and Ash were “tied.” Mia had exhibited no signs of swelling. In fact, there was nothing to indicate that she had come into heat, and she exhibited no indication or willingness to mate. When we saw them tied, though, we moved her immediately, calling our vet and asking him what our best options were.

He advised against any medical action concerning the potential pregnancy. So all we could do was simply hope that she was not pregnant—and face the consequences if she was. It was not long before we had our answer, however. Mia was definitely pregnant and we were definitely going to be the unexpected human parents of some “accidental” puppies.

But we were now faced with a big question: “Who’s the dad?” We just “knew” that Ash was the father. After all, we had seen Ash and Mia tied. But we couldn’t rule out Maverick, the second male in the pen, as a possible (or even a multiple) sire.

Even though we had not seen Mia and Maverick mate, we couldn’t be 100% sure that such a mating did not occur; and while multiple-sired litters (i.e., litters with more than one

father) are uncommon, they can and do happen. So we felt we only had one course of action: DNA profiling of the dam, possible sires and puppies.

#### **DNA PROFILING:**

The last five years has seen a sharp rise in DNA profiling for verifying paternity, and there are several labs to choose from, some of which are listed at the end of this article.

These labs send out swab test kits free of charge, along with detailed instructions. In the test kits, nylon bristle swabs for sample collection seem to be the most commonly used collecting tool, although foam swabs are also available.

The sample collection method is non-invasive (i.e. no blood or hair bulb tissue is needed). The nylon bristle swab or the foam swab is inserted into the animal’s mouth between the gum and the cheek, where it collects loose cheek cells. The swab is then packaged and mailed back to the lab.

DNA profiling is used on pets for identification and to verify ancestry. According to Genomic, a com-

pany specializing in DNA profiling, true parentage can be detected even within highly inbred lines to a reported accuracy of 99.8 %. Once the test is completed, a certificate is mailed to the owner, reporting the dog’s DNA profile in an alphanumeric code sequence and a color bar code.

Our veterinarian assisted us in our search for a competent lab that would perform DNA profiling on wolfdogs. He contacted one lab that immediately hung up on him after he mentioned the word “wolfdog.” However, I was a little more successful. The labs I called were very willing to accept wolfdogs, clarifying only that “testing cannot determine the breed.”

#### **THE PUPPIES**

Mia gave birth to five healthy puppies in March. One was black, like Maverick, and four were brown. Most wolf and high content wolfdog puppies are born dark—even the white arctic wolves—and lighten in color as they age. Brown puppies generally become agouti gray in color, which is the coloration of both

Mia and Ash. My husband was sure that the black pup (#2) would be Maverick's and that the remaining puppies in the litter would be Ash's. On the other hand, I thought two pups would be Maverick's (the black one and one other) and that the remaining puppies would be Ash's. To our complete surprise, none of the puppies

were sired by Ash—even though we had seen Mia and Ash coupled. All of the puppies were sired by Maverick.

This only reaffirms my personal thoughts on the breeder's comments posted at the beginning of this article; one can only question all of the pedigrees of puppies he produced if there were more than one male present during breeding season.

Registering animals and documenting lineage is worthwhile, but this, alone, should not be considered "verified." My situation has caused me to do a lot of thinking about taking advantage of the genetic testing services that are now available—both for self-assurance and for assuring my puppy buyers: with DNA profiling, they will truly be getting

Example of A Genemath Pedigree™

Sire	AA	BG	DK	BC	AT	SS	RY	CD	KM	AJ
Pup 1	AB	BP	DK	CL	NT	PS	AR	DP	MN	JQ
Dam	BZ	PS	KK	LS	MN	PD	AH	PO	MN	QR
Excluded Male	FZ	TS	AK	CL	MA	DS	YH	PO	NK	AR

Name of Lab	Contact information	Costs per Animal	Processing Time
<b>MMI Genomic</b>	1-800-362-3644 (press #5 for canine services)	\$55.00 per dog	10 days (will phone results upon request)
<b>Veterinary Genetics Lab University of California</b>	1-530-752-2211	\$40.00 per dog or \$300.00 for litter (includes sire, dam & pups)	3-4 weeks processing time
<b>PeagGen, Inc.</b> through AKC (DNA operations)	1-919-816-3508	\$35.00 per dog (need <u>all</u> involved) \$25.00 to "read DNA" and provide results on litter otherwise, will send instructions on how to do so.	6 weeks
<b>VetGen</b>	www.vetgen.com 1-800-483-8436	\$60.00 - Confirms Parentage of offspring (includes Profile) DNA required from offspring/dam/sire	Minimum of 3 weeks * Also does genetic testing for breed specific diseases and coat color in Labradors, etc.

Single Marker Example from Two pups,  
Sir, Dam, & Second Male



Sire = **FJ**

Dam = **HH**



Excluded Male **HH**

(Pups would have contained **HH** markers on profile if sired by this male.)



Grey Pup = **HJ**,    Black Pup = **FJ**