The fourth article in a series

It perhaps marks the rapid development of the industry, and particularly the use of superior sires, that there have been so many enquiries about deer blood-typing during the past year. Typically, a test tube of blood will arrive at Invermay, well wrapped, with a question such as "Could you tell me the percentage of Wapiti in this stag?" While the researchers are glad to help when they can, and certainly don't want to throw a wet blanket on such interest, these requests often reflect a misunderstanding of what blood-typing entails, and what it can tell.

In this article, Peter Dratch and Peter Fennessy, Invermay Agricultural Research Centre, explain not only the use of blood-typing, but the basics of how it is done in domestic animals, and how far it has progressed in deer.

Peter Dratch

Peter Fennessy

Directions in deer breeding

Blood typing

Parent detection in the protein

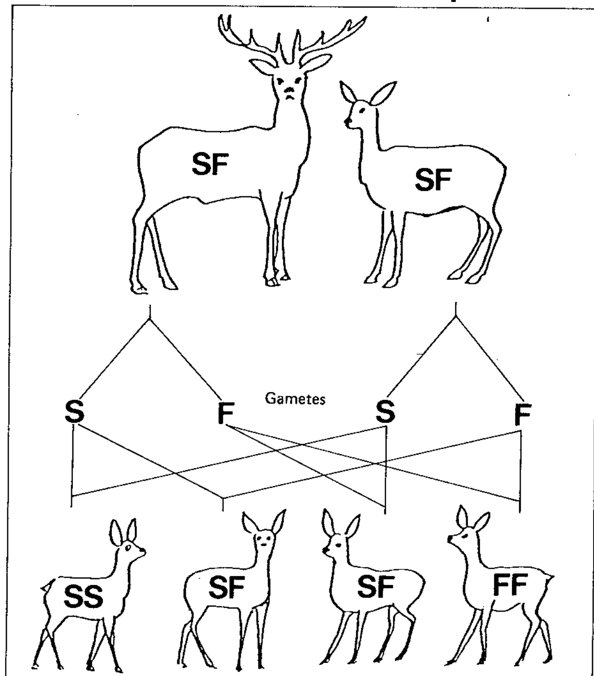

Figure 1. *When deer which are both heterozygous for transferrin (Tf SF), calves of three genotypes will be produced. But you are twice as likely to get calves of the same transferrin type as their parents as either of the homozygous types, Tf SS and Tf FF.*

JUST AS there are differences between individual deer in body size and coat colour, so there are differences in the blood which characterise individuals.

Because their primary use has been to "mark" individuals or groups of animals, these differences are commonly called blood markers. Obviously, the more differences that are found, the better the picture of the individual.

A big advantage of using blood markers rather than external characteristics is that blood markers are much harder to mask by environmental conditions. A stag might have a different weight and colour during the rut than during the summer, but his blood proteins will be the same.

Uses

The most obvious application of blood-typing is to the positive identification of individuals. These fingerprints in the blood (to use a morbid mixed metaphor) can make sure that the

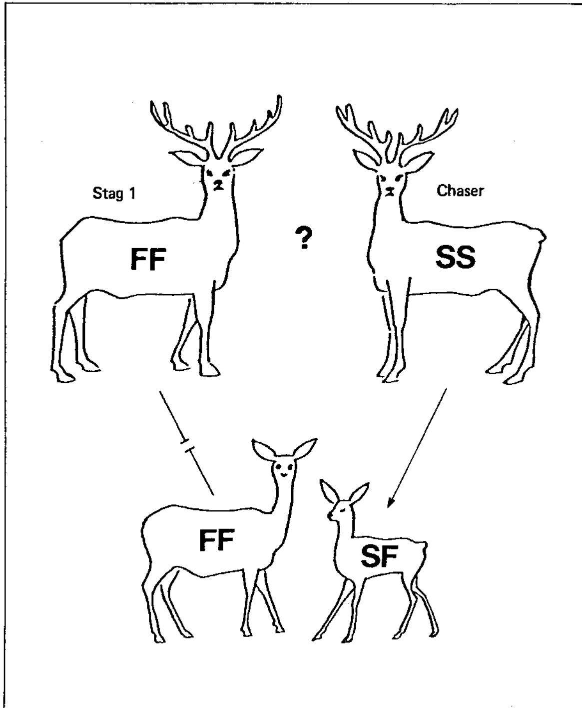

Figure 2. *If the FF stag 1 had bred with this FF hind then the calf would have to be Tf FF. Since the calf is Tf SF, the SS chaser must be the sire.*

animal which was purchased is the same one that is delivered. Likewise, by blood-typing the horses after a race, punters can be sure that the winner was the animal on the card and not a ring-in.

Because blood types are inherited, their most widespread use is in determining parentage. Resolving paternity cases is what gets blood-typing into the newspapers, especially when the cases involve horses or that most domestic of animals, humans. Though philandering fathers get the headlines, blood types in deer could equally be used to confirm suspected cases of mis-mothering.

As with any powerful tool, blood-typing is finding new application. It can be used by both conservationists and farmers to tell whether hybridisation is occurring between two groups of animals, or to estimate the amount of potential variation within a species. We will touch on all of these applications in explaining how blood-typing is done.

Techniques

Blood-typing is a brand of biochemical genetics, and it is the meeting ground between the lab scientist and the animal breeder. The techniques and recipes fill volumes of scholarly journals, and detailing them can be used by those in white coats to bore those in gumboots (getting their own back for decades of AgReport). But all of the techniques fall into two basic groups: Electrophoresis and serology. If you understand the principles which underly electrophoresis and serology, you are in a position to interpret the blood-typing results.

Electrophoresis measures differences in the electrical charge of proteins, and these differences can be revealed by subjecting samples to an electrical field. One early problem in separating proteins according to their charge was that when the power was turned off, those differences would disappear. This was overcome by inserting the samples into a gel made of starch (from potatoes) or agarose (from seaweed) or another neutral substance, and then subjecting the gel to the electrical field. When the juice is turned off the protein separations remain in the gel.

Another problem is that most proteins are colourless, and this difficulty is overcome by linking the protein reaction to a dye and then staining the gel. Haemoglobin, which is one of the proteins which is often tested, is already red so the gel doesn't need to be stained to see charge differences for it.

Since electrophoresis was discovered three decades ago, gel jockeys have applied the method to all of the common deer species. They have looked at proteins in both blood and tissues, and have found a surprising amount of variation. Not only do the proteins mark clear differences between species, but in some deer (such as Reds, Reindeer and White-tails) there is considerable variation between individuals. In other species, such as Pere David's and Fallow, individuals show little or no electrophoretic variation.

Serology measures the reaction which ▷

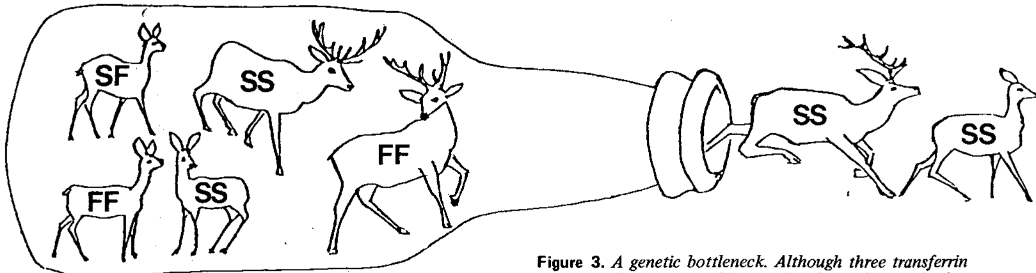

Figure 3. *A genetic bottleneck. Although three transferrin types may be represented in an original population, the few surviving individuals may by chance be all of the one transferrin type; so genetic variation has been lost.*

occurs on the surface of red blood cells when they come into contact with known reagents. The red cells are obtained from fresh blood samples, and the reagents are developed over time by immunising one deer with the blood of another.

In contrast to electrophoresis, very little serology has been done in deer, as animals in the wild are much less easily analysed by this method. Not only is a fresh blood sample required from the animals being tested (now no problem with deer on farms) but a herd of deer is also needed to develop the reagents to test those blood samples against.

Serology has been developed in horses, cattle and sheep because the economic value of the animals made it worthwhile.

The most widely known serological test is the ABO group in humans. If you have given blood, a nurse has pricked your thumb, added a drop of your blood to a few reagents, and in a moment you are classified A, B, AB or O. (If you have both factor A and B, you are AB; if you have neither, you're O). It is important to realise that this is but one blood group of the several hundreds now known in humans.

Getting a transfusion from someone with the same ABO group could still kill you, and a complete blood-typing takes many further tests. Returning to four-legged examples, about twenty blood groups are used in horse blood-typing at present, plus a dozen electrophoretic proteins.

Sex

As we alluded to earlier, the power of blood-typing lies in the fact that the electrophoretic variants and serological factors are inherited. As with any inherited trait, an individual – whether it is a deer or a human – has two copies, one from each parent. Your gametes (sperm in males and ova in females) have only one copy, as do the gametes of whomever you are copulating with. The gametes join to form the zygote of the offspring which again has two copies for each trait.

If the copies from each parent are the same, the offspring is homozygous for the trait; if they are different, the offspring is heterozygous. Figure 1 illustrates this for transferrin (abbreviated Tf) which shows variation in Red deer. As the name implies, the role of this protein is to carry iron in the blood.

Parentage determination

The next question is how can these homozygotes and heterozygotes be used to determine parentage? If we are trying to determine paternity, we blood sample the calf, the mother and the possible fathers. Say a calf is heterozygous for transferrin (Tf SF); it has both slow and fast bands on a starch gel stained for transferrin. The hind is homozygous FAST for transferrin (Tf FF). Of the two stags which were in the paddock at the rut, the first is homozygous FAST, like the hind and the chaser if homozygous SLOW (Tf SS). Which stag did the deed? The problem is illustrated in Figure 2.

Since the hind is homyzygous FAST, she must have given a fast transferrin band to her calf. Since the calf is heterozygous, it must have received a slow transferrin band from the sire. Since the first stag has no slow transferrin band (recall it was Tf FF) it could not be the sire. It is excluded. The chaser has a slow transferrin band to provide (in fact it has two, as it was Tf SS), so it is the probable sire.

If more stags got through the fence, they too would need to be blood typed. If transferrin did not exclude them, another protein might.

The previous example illustrates how variation in a single protein is used. Obviously, the more variation we can find by electrophoresis and serology, the better our chances of getting an exclusion. At present in Red deer we have electrophoretic variation for about 10 proteins, and most of those have been reported in the last five years. We are likely to find considerably more variation, especially when serology is developed in deer species.

Hybridisation

In the first article of this series, we introduced the idea of genetic distance. The greater the genetic distance between animals, the more they will differ in physical characteristics, including their blood proteins and red blood cells, and also the more difficult it will be to hybridise them. Thus it is not surprising that it is relatively easy to differentiate the blood of a Red deer from that of a Fallow. Because they cannot interbreed, their proteins have become progressively more different.

We also have found two proteins in the blood which readily distinguish Red deer and Pere David's deer (transferrin is one of them). So in this case the lab scientists are waiting for the breeders to provide a first generation of hybrids. Those hybrids should all be heterozygous for transferrin, with one Red deer band and one Pere David band.

At the NZDFA Annual Conference, we reported an electrophoretic difference in haemoglobin that sorts out Red deer and Wapiti. In this case we already have the controlled crosses ▷

grazing at Invermay, and as you would expect, the blood of those calves has bands from both parental types. The next question, of course, is how does the blood of Wapiti in Fiordland compare.

Electrophoresis has also been used to detect more subtle differences. In Scottish Red deer for example, variation was found in the enzyme Glucose phosphate isomerase (all enzymes are proteins; this one is called GPI for short). No GPI variation was found in Red deer from continental Europe, until some population geneticists from Stockholm found the same variant in one Swedish deer park. They were pretty excited, and they then found out that some of the deer in that park had been imported from Britain – decades before. Apparently they brought their rare GPI genes with them.

Conservation

As mentioned earlier, some deer species show much reduced levels of electrophoretic variation. Fallow deer and Pere David's are two such species, and a look at their breeding histories can give us clues as to why this loss of genetic variation might have occurred.

Pere David's deer were extinct in the wild for several centuries before they were found in a walled royal hunting park in China by the French priest. This bottleneck in numbers likely resulted in a loss of genetic variation. Moreoever, because only 16 breeding deer were brought to Woburn Abbey to start the present population, these founders may not have had all of the variation that remained in the Chinese hunting park. And yet another founder effect could occur in bringing the Pere David's deer to New Zealand. This is illustrated in Figure 3, again using the protein transferrin as an example.

Fallow deer also have shown little electrophoretic variation so far, but the reasons are less obvious. A founder effect when the animals were brought to Britain is one possibility. It is also possible that in using a small number of sires and strongly selecting for coat colour during the last century, other variation in Fallow was lost.

An important question we have just begun to address is whether these species also have low levels of variation in physiological characteristics which are important for selection by deer farmers. Geoff Asher's results on Fallow deer at Ruakura certainly suggest that this could be the case. Alternatively, such species might be used when a consistent product is of ultimate importance.

Conclusion

Using electrophoresis, lab scientists have already found considerable variation in deer, and this variation has several direct applications to the deer-farming industry. The more distantly related the animals, such as Reds and Wapiti, the more promising the results thus far. In deer serology, it is very early days yet. With such a variety of deer behind fences, New Zealand is in a good position to pioneer this field. That calls for further research commitment: What is needed is a small herd of deer to develop reagents, and a laboratory to do the tests which have become routine in other highly valued livestock.