# Enderby Island Cattle:

# A Breeding Strategy for Genetic Conservation

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## I. Introduction

Cloning, embryo transfer, and conservation genetics are phrases that have come to symbolise the potential for reproduction in endangered species and breeds today. Modern technology supplies unprecedented tools for influencing the way that animals reproduce, and it is now possible to control even minute details in the production of both domestic and wild species. In terms of endangered wild and domestic populations, these technologies seem to very nearly give humans a chance to take back their mistakes: when there's been too much inbreeding, when the habitat has been destroyed, when only a few individuals are left, these advances become the absolution by which humanity can restore a population.

In reality, the technologies of the future are simultaneously a source of successes and frustrations today. The trials associated with reviving the Enderby Island Cattle breed are symbolic of the progress that reproductive science has made, but also highlight the limitations and the degree to which luck still plays a role in conservation breeding. When, in 1998, the last surviving Enderby Island cow, Lady, was cloned using adult somatic cell nuclear transfer, it was a resounding success in the scientific community: Dolly the sheep had been cloned the same way just a year before, and Lady was the first animal ever cloned to save an endangered population.

It was also a thread of hope for the Enderby Island Cattle Project as the evidence that the breed could be saved had been dwindling. A single cow resisting reproduction is not conducive to restructuring a population. Eight years later, however, it is still not clear whether embryo transfer and *in vitro* fertilisation will be sufficient to continue the breed now that the maximum benefits of cloning have been reached for the breed. This report proposes a breeding scheme to be carried out starting with the genetics that are available, designed for highest chance of success in conservation of the Enderby Island breed.

## II. Background and Breed Description

The Enderby Island Cattle story begins in the 1890s, when settlers began attempting to farm Enderby Island, the northernmost island of the Auckland Islands group, 320 km south of New Zealand (A Rare Breed of New Zealand Origin, www.rarebreeds.co.nz/enderby.html). Nine cattle were introduced initially, but in 1910 farming was given up due to the inhospitable climate, and the cattle were left behind (Bunn 1998). Their breed origins are unknown. They were described in the ship's log as shorthorns, but they look very little like modern shorthorns and that may have just been a term used as a physical descriptor because they had short horns (A Rare Breed of New Zealand Origin, www.rarebreeds.co.nz/enderby.html).

In 1991, New Zealand's Department of Conservation (DoC) determined that the cattle, along with rabbits as another introduced species, should be eliminated as they were interfering with native plant and animal populations. An expedition was mounted to attempt eradication. During the expedition, 47 of the estimated 53 cattle on the island were killed. Oocytes were collected from 15 of the 25 females, and epididymal sperm was collected from 11 of the 22 males killed.

*In vitro* fertilisation was attempted using the sperm and oocytes during the expedition, but failed. Even when the ovaries could be removed from cows promptly, the oocytes died, in part due to the inefficient field laboratory system being used (Catt 1991, unpublished report). Although epididymal sperm is commonly used and generally considered mature, the Enderby Island semen collected showed morphological irregularities, thus lessening the efficacy of the sperm as well. The breed was, for all intents and purposes, considered extinct.

The marginal sperm was sent to be stored at DSIR, Palmerston North, as what was believed to be the last remaining viable Enderby Island genetic material. However, in 1992 it was discovered that two cows remained alive on the island: a cow that would be named Lady and her heifer calf. With the failure to regenerate the breed using collected sperm and oocytes, members of the Rare Breeds Conservation Society of New Zealand captured Lady and the heifer. They were then shipped to New Zealand for further attempts at genetic resuscitation of the breed. However, the calf died at Massey University, and Lady was taken to AgResearch, Hamilton, as the last hope for the breed.

At Ruakura, Lady resisted all attempts to reproduce. Although she had produced the calf while still on the island, under research conditions 35 attempts at *in vitro* fertilisation (IVF) were required before the bull calf Derby was born using the poor-quality semen collected from the bulls shot on the island. Because of the difficulties and limited success with IVF, researchers turned to cloning. Adult somatic cell transfer was used to clone Lady. Mural granulosa cells were collected from Lady's ovaries, cultured, and fused with enucleated donor egg cells. The fused cells were chemically activated and cultured. The embryos were analysed for quality of development, and cows were each implanted with two embryos showing good development. They were then permitted to develop as normal IVF embryos (Wells et al.1998).

Elective caesareans were performed on the cows that had retained normally developing embryos when they were close to term. Out of the 74 embryos implanted in 37 cows, five clones were born. One died shortly after birth, and two died later, in the years following transfer from Ruakura to Christchurch. Cloning is notoriously inefficient. Even under ideal circumstances, efficiency remains under 20%; most embryos continue to die before coming to term, possibly due to epigenetic complications in reprogramming the somatic donor cell (Gordon 2004). Clones tend to be plagued by metabolic irregularities as well, which may be caused by abnormal placental function (Wells et al.1998). In other experiments, cloned calves are often born excessively large with slow and/or weak behaviour patterns (Garry et al.1996). The cloning experiment, however, had succeeded in providing the females required to continue the Enderby breed.

Two clones – one of which is still alive, and one that died – produced two heifer calves each when bred to Lady's only son Derby. The need for cloning as an emergency measure had passed with the successful production of Lady's clones, so more conventional breeding methods could be used. However, although four calves survived, two were born dead; breeding Derby to the clones was equivalent genetically to breeding to Derby to Lady. Inbreeding, a classic hurdle in conservation and regeneration of small populations, had taken its first toll in the effort to rescue the remaining genes.

In May 2006, the population count remains at a total of seven Enderby Island Cattle. Derby died in 2004 of unknown causes, but a large quantity of high quality semen was collected and has been stored for future use. Fertilized embryos transplanted to recipient cows means that there are six calves expected next season; they have been bred from two of the clones' offspring and one clone, and fertilised by the semen collected from bulls killed during the eradication. The outcome of this year's breeding will play a substantial role in determining whether the breed can be continued further.

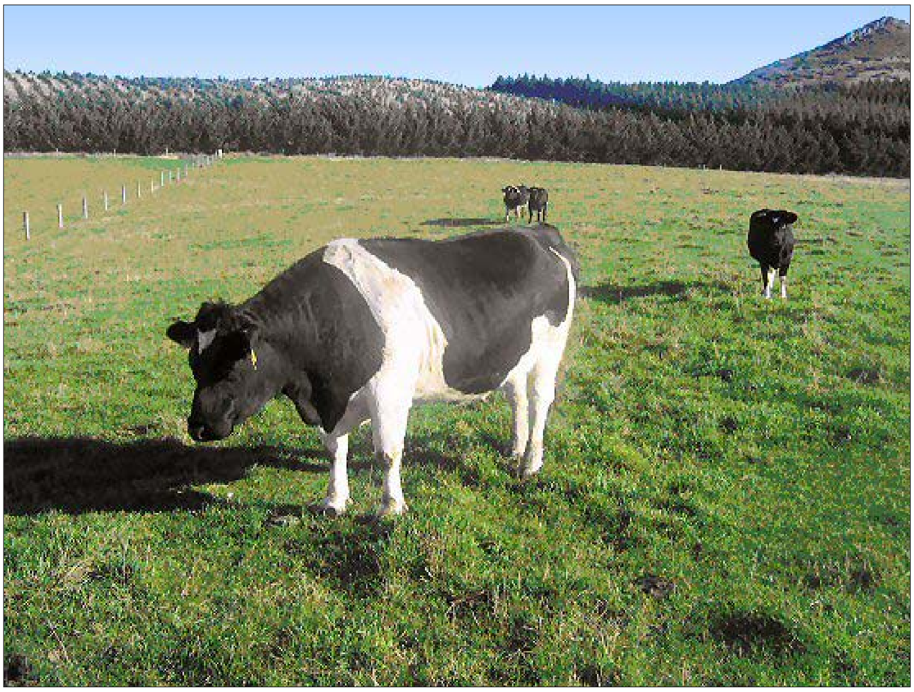

*Figure 1. Four of the Enderby Island cows*

The Enderby Island Cattle have an appearance that renders them closer to the Shetland Island cattle than the modern Shorthorn breed. They are typically black and white, although out of the 47 killed in 1991, three were red and white (Catt 1991). The implication is that they were not originally a pure breed, but most probably a mix of breeds resulting in cattle with short horns. The living specimens have long bodies with short legs, giving them a distinctive appearance [Figure 1].

Inspection of the culled animals on Enderby Island indicated that the cattle were nearly all in good physical condition when they were killed, and only two out of the 47 inspected had overgrown hooves. There was no evidence of parasitism. The cattle survived in Enderby's harsh climate eating seaweed, scrub, and Southern rata (A New Zealand Rare Breed Society Rescue Project, www.rarebreeds.co.nz/endcattlepro.html) for nearly 100 years, and apparently evolved to cope well with the environment. The rumen conditions of the cattle inspected post-mortem on the island showed that the rumen contents of the cattle very dry with a minimal fluid component compared to typical cattle (Catt 1991), the significance of which is not clear.

Other unique qualities of the breed have yet to be identified. When it is feasible, milk and meat analysis will be carried out to determine whether the Enderby Cattle have economically valuable characteristics that could be incorporated into the dairy and beef industries (Matheson 2006, personal communication). Before the breed can be adequately assessed, however, the population must be larger.

### III. Breeding Objectives

The objective for the Enderby Island Cattle breed currently is fundamentally simple. The goal is to create a population that is sufficiently genetically diverse and large enough to be self-sustaining; at this point, cloning and extensive embryo transfer are far from efficient enough to be responsible for carrying on the genetics of the entire breed, as shown by research by Wells et al. and Garry et al., as well as monetary costs. It has been recognised by researchers that continual cloning cannot maintain a population, and that the goal of cloning is to create a population of viable individuals to reproduce independently (Woolliams and Wilmut 1999). Once the population is large enough, further studies can be carried out to find out more about the unique qualities of the Enderby Island cattle.

### IV. Breeding Strategy

Given the limited number of individuals and genetic material available, methods for breeding a population are also limited. There are two basic strategies that can be used: one based on cross breeding and one based on interbreeding exclusively between Enderby cattle. The latter method is the one being employed currently, but ideally both would be used to provide maximum security for the genes and characteristics of the breed.

The interbreeding system being used currently involves breeding only the Enderby cows to the stored semen of the bulls killed on Enderby and Derby. The specifics of how this method will proceed depend in large part on the results of next season's calves, in terms of survival and sex ratios. Ideally, all calves will survive and three will be bull calves (Dave Matheson 2006, personal communication). If three bull calves are not born, breeding must continue until three are obtained, preferably each from a different cow and bull combination. Full genetic analysis will then be carried out on each of the new offspring. The bull calves will be used to each sire a lineage, beginning with matings that would result in the most genetically diverse offspring. The female calves of that cross would then be mated to Derby and the stored semen; their offspring could then be mated back to the less-related bulls.

The females born in this next generation would ideally be bred to Derby and different bulls stored semen as soon as possible; their offspring would then be bred with the bulls produced through other lines. Through this system, individuals and lineages will, over time, become genetically distinct from the others, allowing interbreeding between the lineages later.

A separate, complementary crossbreeding programme would ideally be performed along with the pure breeding scheme. Cross breeding was attempted on a small scale early in the process. Prior to the discovery of Lady, the stored sperm from the original bulls was used to inseminate non-Enderby cows, but the result was six bull calves (Bunn 1998), an unhelpful result for genetic conservation as any offspring from those bulls could only be 1/4 Enderby. A proposed scheme by Hugh Blair [Table 1] for cross breeding begins with insemination 60 unrelated cows of another breed with the stored sperm in the first year of a breeding programme. This is followed by breeding to the half-Enderby heifers using stored sperm two years later, then continuing to breed within the cross-bred cattle. This results in a theoretical total of 78 crossbreeds after eleven years of the scheme, with five offspring being 31/32 Enderby (Blair 1991, 2006).

**Cow Age**

| Year | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| | $N^c N^H$ | $N^c N^H$ | $N^c N^H$ | $N^c N^H$ | $N^c N^H$ | $N^c N^H$ | $N^c N^H$ | $N^c N^H$ |
| 1 | 0 | $15^0 6^{1/2}$ | $15^0 6^{1/2}$ | $15^0 6^{1/2}$ | $15^0 6^{1/2}$ | | | |
| 2 | 0 | | | | | | | |
| 3 | $23^{1/2} 9^{3/4}$ | | | | | | | |
| 4 | 0 | $22^{1/2} 9^{3/4}$ | | | | | | |
| 5 | $8^{3/4} 3^{7/8}$ | 0 | $21^{1/2} 8^{3/4}$ | | | | | |
| 6 | $8^{3/4} 3^{7/8}$ | $8^{3/4} 3^{7/8}$ | 0 | $20^{1/2} 8^{3/4}$ | | | | |
| 7 | $8^{3/4} 3^{7/8}$<br>$3^{7/8} 1^{15/16}$ | $8^{3/4} 7^{7/8}$ | $7^{3/4} 3^{7/8}$ | 0 | $19^{1/2} 8^{3/4}$ | | | |
| 8 | $8^{3/4} 3^{7/8}$<br>$6^{7/8} 2^{15/16}$ | $7^{3/4} 3^{7/8}$<br>$2^{7/8} 1^{15/16}$ | $7^{3/4} 3^{7/8}$ | $7^{3/4} 3^{7/8}$ | 0 | $18^{1/2} 7^{3/4}$ | | |
| 9 | $8^{3/4} 3^{7/8}$<br>$12^{7/8} 5^{15/16}$<br>$1^{15/16} 0$ | $7^{3/4} 3^{7/8}$<br>$5^{7/8} 2^{15/16}$ | $7^{3/4} 3^{7/8}$<br>$2^{7/8} 1^{15/16}$ | $7^{3/4} 3^{7/8}$ | $6^{3/4} 2^{7/8}$ | 0 | $17^{1/2} 7^{3/4}$ | |
| 10 | $7^{3/4} 3^{7/8}$<br>$11^{7/8} 4^{15/16}$<br>$3^{15/16} 1^{31/32}$ | $7^{3/4} 3^{7/8}$<br>$11^{7/8} 4^{15/16}$<br>$1^{15/16} 1^{31/32}$ | $7^{3/4} 3^{7/8}$<br>$5^{7/8} 2^{15/16}$ | $6^{3/4} 2^{7/8}$<br>$2^{7/8} 1^{15/16}$ | $6^{3/4} 2^{7/8}$ | $6^{3/4} 2^{7/8}$ | 0 | $16^{1/2} 6^{3/4}$ |

Table 1. Generation of Graded-up Enderby Cattle Herd by Continued Backcrossing to Enderby Cattle Semen. Table courtesy Hugh Blair, 2006. Notes: Cow age is at calving; year is year of mating; first mated at 14 months; $N^C(N^H)$ is number of cows mated (heifers born); superscripts are the proportion of Enderby cattle genes; 5% losses between years; 80% calves born to 2 cycles.

The semen collected from Derby would be used to supplement the sperm stored from the 1991 bulls in this process, as would any bulls born into the pure-breeding programme in the future.

After seven to ten years of operating both the purebred and cross-breed systems, an analysis of both programmes would take place to assess the status of the Enderby Island cattle population. If the purebred scheme was still faltering, the introduction of a few of the crossbreeds carrying 15/16 or 31/32 Enderby genes would be considered. If it appeared that the purebred scheme had been successful and was sufficiently stable so that the crossbred cattle would not be essential for conservation, the cross breeding program could then be discontinued or continued under some other auspices.

## V. Discussion of the Proposed Breeding Strategy

Neither crossbreeding nor pure-line breeding using just three related bulls and six related cows is an ideal way to construct a population. However, given the limitations, there are few other options available, and using both of these methods would ensure that the Enderby Island breed would move away from the ongoing brink of extinction and attempt to maintain fidelity to genetic heritage simultaneously.

Breeding exclusively within the Enderby breed would be ideal if enough genetic material (either through quality stored sperm/oocytes or living individuals) was available to breed a population that was not restricted by statistical limitations. If more live animals had been recovered from the island, or the oocytes collected had survived to create embryos through IVF, breeding the Enderby Island cattle could follow a more conventional programme. As the population stands currently, however, breeding must be performed carefully to protect from the negative impacts of inbreeding, and regardless of how controlled the process is in terms of genetics, inbreeding will undoubtedly have an effect.

Kristensen and Sørensen (2005) point out that according to several researchers, nearly all populations that have undergone a severe bottleneck will have lowered fitness, and that this reduction in fitness will lead to reduced genetic variation in the long term. They claim that the few reports from the field of conservation biology on populations which have been through severe bottlenecks but nevertheless prosper currently are not likely to be representative observations, a consideration that must be taken into account for the Enderby Island cattle.

Inevitably, variation is lost when individuals who are closely related are bred (Woolliams and Wilmut 1999), so it is undeniable that loss will continue to some degree for this population. Even when the sperm collected from the 11 males is taken into account, the effective population size is far below what is laid out by Meuwissen and Woolliams (1994) as the minimum for preventing a fitness decline. According to their model, an effective population size of 25 is required as an absolute minimum to maintain fitness in any population, with a minimum number of alleles being measured. Including the stored semen, there are 19 Enderby cattle, with low variability in the females given the cloning and artificial reproduction techniques used. The effects of inbreeding depression are recorded as causing breeding problems such as stillbirths (as seen at the extreme level in breeding Derby to his dams clones) as well as limitations in production and growth (Adamec et al. 2006).

The risks in breeding Enderby Cattle exclusively within the breed, therefore, are distinct. However, there are other considerations as well. Given that the cattle come from a population of about 50 that has been genetically isolated for a long period of time, it is likely that inbreeding has already occurred to a large extent, yet the breed has managed to persevere. Alleles with negative effects may have already been eliminated, through purging: intense natural selection can act on small inbred populations to filter out recessive deleterious alleles, ultimately resulting in a higher fitness level (Kristensen and Sørensen 2005).

Similarly, it has been observed that in some cases, it is possible for genetic variance to increase after severe depressions in population size (Hall 2004, p. 155). This also makes the success of the purebreeding programme seem more likely. Genetic analysis to pursue the level of homozygosity in the animals' genomes would provide more information about the potential for eliminating alleles through future inbreeding, and an estimate of the inbreeding that has already occurred. Given the evidence that inbreeding is not always as lethal for a population as is commonly thought, breeding exclusively within the Enderby population is a viable, if not guaranteed, solution.

Once three bull offspring are produced – whether this coming calving season or in subsequent years – the breeding of lineages can begin to control the effects of inbreeding. By using the bulls born, and stored semen from Derby and the other bulls, the lineages would become different enough that a large number of genetically variable individuals could be produced. This pattern would have to be continued for several generations and the rate of progress depends in part on the sex ratios produced, however. Enough bull calves would need to be produced to perpetuate genetic variability, but sufficient females would also be required to increase population size as well as providing material for diversity. This again brings chance into the equation; in such a small population, disproportionate representation of either sex is not unlikely, so several breeding seasons may be required to obtain the stock needed to begin breeding lineages.

The principal advantage for continuing breeding purebred Enderby Island stock is to maintain the breed genetically as it has survived for 100 years, rather than just salvaging individual genes. According to the Committee on Managing Global Genetic Resources, the preservationist regards existing breeds as unique genetic entities representing genes and gene combinations that have evolved over considerable periods of time to its unique environmental and production conditions (1993, p. 44). From the human perspective, this is the paradigm that most accurately reflects the reasoning behind maintaining the Enderby cattle without outcrossing. By breeding only within the breed, the unique combinations of genes that the cattle developed over the last century will be maintained, giving value to the breed as a whole rather than just single genes.

This principle is particularly recognised when related to unique adaptations in livestock: according to Stephen J. G. Hall, evidence is accumulating that genes at different loci can interact in relatively complicated ways to determine phenotype and crossbreeding can disrupt these interactions (2004, p. 72).

Regardless of how ideal it would be to continue breeding exclusively a pure line of Enderby Island Cattle, however, at this point the population is still too small to guarantee continued success, and outcrossing would be one way to ensure that even if the pure-breeding scheme fails, genetic material will not have been lost completely. After several generations, it would be possible to create a population that was in fact mostly Enderby Island genetics and hopefully similar in character to the original population. After 10 years it would be possible to have a population that was primarily 7/8 or 15/16 Enderby Island genes, using the scheme developed by Blair (1991, 2006). If the cattle were genotyped and carefully compared to existing cattle breeds, it would be possible to find a closely-matching breed to use for the crosses to attempt to preserve the unique aspects of the Enderby breed.

Cross-breeding does pose a risk to inheritance of complete genotypes, which may be part of what makes the Enderby cattle so unique. However, all the genes from the Enderby breed would still exist in the population resulting from outcrossing, and the genetic material, and would potentially be lost through selection or random chance through breeding the pure population if precautions were not taken anyway (Committee on Managing Global Genetic Resources: Agricultural Imperatives 1993, p. 66).

Cross-breeding also means that within the next decade, there would be some cattle carrying almost exclusively Enderby Island genes, and if at that point the purebred plan has not been successful, it would be possible to breed the pure-breds with the cattle that were almost completely Enderby Island genetics. In this way, carrying out both programmes is ideal because it allows the optimum outcome to develop a purebred Enderby population while also providing a genetic safety net should that scheme fail.

Carrying out both crossbreeding and purebreeding schemes, although the safest way to ensure that Enderby Island genes will continue, carries risks and negative aspects as well. Both proposals, in particular the cross-breeding system, require use of the semen stored from the bulls killed on Enderby in 1991. This is not an unlimited resource, nor is it particularly reliable, given the irregularities associated with using it in the past. It would have to be used judiciously, and it is possible that there simply would not be sufficient quantities to inseminate the required number of cows, even with the incorporation of Derby's semen.

In addition, conducting two independent breeding programmes means twice the amount of resources would be required. It would require twice the amount of human effort, and at least twice the cost. When applied to a population that has no particular known economic value, these are very relevant considerations. There is a point at which the investment becomes too great for any likely output, a factor that will have to be weighed by the decision-making entities in the Enderby Island Cattle Project.

## VI. Summary

Ideally, there would be no need for compensation for human mistakes in breeding. Breeds would not become so threatened that expensive last-chance efforts would need to be employed to preserve genetic qualities; an effective population size would always be maintained. Cloning would not be needed to save the last living individual of any breed or species. Ongoing embryo transfer would not be necessary just to build a base population.

The Enderby Island Cattle are a story of both numerous failures – seemingly, anything that could go wrong has gone wrong – but also impressive scientific successes and commitment by people determined to save the breed. Cloning was used for the first time to successfully preserve an endangered breed, setting a precedent for future conservation projects. Embryo transfer has been used to create a population, albeit a small one, out of a single cow and an assortment of sperm. From finding a cow of a breed everyone thought was extinct, to one bull being produced after 35 breeding attempts, to the first ever endangered clone being born, the options for the Enderby cattle have been minimal: either use all the science and money it takes to continue, or go extinct.

At this point, there are finally options for how to continue building the population. After so many resources have been sacrificed to develop the current population of seven, it is logical to do all that is possible to keep the breed from ever reaching the dire straits it has seen in the last 15 years. This means preserving both the genetic identities of the pure breed, and the genes the cattle carry through crossbreeding. If organisations or individuals were willing to commit the resources required, this method proposed here would be the ideal, genetically safest way to continue breeding.

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