Cytoplasmic and mitochondrial genetic effects on economic traits in dairy cattle

Michael Mathias Schutz

Iowa State University

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Cytoplasmic and mitochondrial genetic effects on economic traits in dairy cattle

Schutz, Michael Mathias, Ph.D.

Iowa State University, 1991
Cytoplasmic and mitochondrial genetic effects on economic traits in dairy cattle

by

Michael Mathias Schutz

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

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For the Graduate College

Iowa State University
Ames, Iowa

1991
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EXPLANATION OF DISSERTATION FORMAT

This dissertation is presented in an alternative format, as described in the Iowa State University Graduate College Thesis Manual. Following an introduction and review of literature are three sections that report the author's work. Each of the three sections are in paper format and intended for publication in scientific journals. The organization of the second section is different than the first and third, because it is written for publication in a different scientific journal. A general summary intended to list major conclusions and implications and to suggest further areas of study follows the third article.
INTRODUCTION

Dairy cattle breeders have long talked about the importance of good cow families in breeding programs. Animal geneticists, generally adhering to belief in the singular importance of nuclear additive genetic effects, dismissed description of cow family effects as conjecture and hearsay. Over the past few years scientists have uncovered evidence that may, at least in part, account for observed cow family differences.

Maternal lineage effects have been shown to significantly account for differences in milk, fat, fat percentage, fat corrected milk, milk net return, days open, and days to first breeding in dairy cattle. Maternal lineages were defined by tracing backward through maternal pedigrees as many generations as possible, often to the beginning of a herd book registry. Because mtDNA in mammals is only known to be transmitted from female parents to offspring, maternal lineages are frequently considered indicative of cytoplasmic inheritance. Mitochondrial DNA (mtDNA) is transmitted by along with maternal cytosol and, being the only known extra nuclear genetic component in mammals, is a logical explanation for cytoplasmic inheritance.

Many polymorphisms in the mtDNA genome of dairy cattle have been found. Initial studies on the sequence of bovine mtDNA concentrated on the displacement-loop, because it is not known to code for specific gene products and may have a higher rate of sequence polymorphisms. Large numbers of base pair substitutions have been observed in bovine mtDNA displacement-loops and several restriction endonuclease cleavage sites
have been identified in gene coding regions of mtDNA. Combined with phenotypic differences, the occurrence of mtDNA sequence polymorphisms strongly suggests that sequence differences in the mtDNA genome may be directly associated with production or health differences.

Several shortcomings have been noted in previous analyses of the effects of cytoplasmic lineages. First, failure to account for relationships, or genetic covariance, among relatives does not preclude establishment of differences among maternal lineages by random drift of nuclear genes. Second, maternal nuclear genetic effects were not taken into account previously. Additive maternal genetic effects cause genetic differences among dams but are exhibited as strictly environmental influences with regard to offspring. Such effects do occur in beef cattle, but would be expected to be small in dairy cattle, where calves are removed from dams shortly after birth. A third problem deals with definition of maternal lineages. Even when traced to the beginning of a herd book registry, "distinct" lineages may branch from a single cow one or several generations before identification data was recorded.

The objectives of research reported in this dissertation are threefold. The first objective was to determine the importance of maternal lineage differences in traits of economic importance in dairy cattle from a source not previously investigated. Complete relationship information was considered and additive genetic maternal effects were explored to improve upon previously reported analyses. The second objective was to identify specific mtDNA markers by associating mtDNA sequence data from the same maternal lineages of cattle with production
or health traits of economic importance. A final objective was to use mtDNA sequence data to better define "cytoplasmic lineages" of cattle and evaluate effects of these lineages.
Mitochondria

Mitochondria are oval-shaped organelles about half a micrometer in diameter and from two to five micrometers long (Grivell, 1983). They are composed of a limiting outer membrane and a highly invaginated inner membrane (Tzagoloff, 1982). The inner membrane encloses a fluid matrix and the intermembrane space lies between inner and outer membranes. Typical mammalian cells contain several hundred mitochondria, each with several closed circular DNA genomes (Clayton, 1984). While mitochondria contain several hundred species of polypeptides, only a few are coded by the mitochondrial genome, and the remainder are encoded by nuclear genes and enter the mitochondria from the cytosol (Schatz, 1987). Ernster and Schatz (1981) and Tzagoloff (1988) extensively reviewed previous studies about mitochondrial structure and biogenesis.

Frequently, mitochondria are referred to as the "powerhouses" of cells. This analogy is apt. Thirty-four of the thirty-six molecules of adenosine triphosphate (ATP), the carrier of free energy in living systems, are generated inside mitochondria from complete oxidation of one molecule of glucose. ATP is generated through phosphorylation of adenosine diphosphate (ADP). Two molecules of ATP are generated in the cytosol of cells during glycolysis. In mitochondria, two molecules of ATP are generated in the citric acid, or Kreb's, cycle; and thirty-two
molecules are formed through the process of oxidative phosphorylation. Oxidative phosphorylation involves the transfer of electrons from NADH or FADH\textsubscript{2} to oxygen by a series of electron carriers and is closely associated with the inner membrane of the mitochondrion (Stryer, 1988).

**Review of mitochondrial genetics**

Among organelles in mammalian cells, mitochondria are remarkable in that they have an independent genetic system necessary for respiration. Ephrussi (1953) determined in 1952 that respiration deficiencies in yeast, *Saccharomyces cerevisiae*, were caused by non-Mendelian cytoplasmic genes. Furthermore, the properties of these deficient organisms suggested that such cytoplasmic genes might be associated with mitochondria. A decade later, in 1963, Nass and Nass (1963) provided electron micrographs showing that mitochondria possessed thread-like structures that could be digested by DNase, not by RNase. This was considered evidence that deoxyribonucleic acid (DNA) was present in chick embryo mitochondria. The past two decades have seen rapid progress in understanding the genetic capabilities of mtDNA.

Plant and yeast mtDNA also codes for functional gene products, but differs from animal mtDNA in important ways. Yeast mtDNA has five times as many nucleotides as animal mtDNA, and plant mtDNA is five times longer than that (Grivell, 1983). Plant mtDNA also has introns and may be either circular or linear (Wallace, 1982). In plants, chloroplasts also have some of their own genetic material (cpDNA).

Complete sequences of the 16,569-base pair human mitochondrial
genome were presented by Anderson et al. (1981). Although not exactly the same length, the 16,295-base pair mouse mitochondrial genome was highly homologous in gene organization to human mtDNA (Bibb et al., 1981). Anderson et al. (1982) presented the 16,338-base pair sequence of bovine mtDNA, and showed bovine mtDNA to be 63 to 79 percent homologous to human mtDNA with regard to genes encoding proteins. DNA sequence homology was only slight, however, in the displacement-loop region, which will be described in greater detail later. *Xenopus laevis* (frog) mtDNA (Roe et al., 1985) was 17,553-base pairs, which is longer than mammalian mtDNA. The displacement-loop was significantly longer, but there were several sites of sequence homology. Amphibian and mammalian genomes have similar gene order and compact organization. The mechanisms of replication, transcription, processing, and translation of mitochondrial genes are greatly conserved among higher vertebrates (Roe et al., 1985).

Replication and expression of the mitochondrial genome are limited to the organelle. Necessary ribosomal and transfer RNAs for translation of mitochondrial genes are encoded by mtDNA (Clayton, 1984). As shown in Figure 1, the circular double stranded DNA of mammalian mitochondria code for two ribosomal RNA subunits, 22 transfer RNAs, and 13 polypeptides involved in respiration (Clayton, 1984; Grivell, 1981). Polypeptides coded by the mitochondrial genome include seven subunits of NADH-Q reductase (also called NADH dehydrogenase), a proton pumping complex situated on the inner membrane. One unit of cytochrome reductase, three
Figure 1. Gene map of bovine mitochondrial DNA. Clockwise from the D-loop region (top center): Origin of heavy strand replication, Proline*, Threonine, Cytochrome reductase, Glutamic acid*, NADH-Q reductase (6)*, NADH-Q reductase (5), Histidine, Serine, Leucine, NADH-Q reductase (4), NADH-Q reductase (4L), Arginine, NADH-Q reductase (3), Glycine, Cytochrome Oxidase (3), ATPase (6), ATPase (8), Lysine, Cytochrome oxidase (2), Aspartic acid, Serine*, Cytochrome oxidase (1), Cysteine*, Tyrosine*, Origin of light strand replication, Tryptophan, Alanine*, Asparagine*, NADH-Q reductase (2), Isoleucine, Glutamine*, Methionine, NADH-Q reductase (1), Leucine, 16s ribosomal RNA, Valine, 12s ribosomal subunit, Phenylalanine (italicized genes are transfer RNAs, asterisk denotes coding on light strand).
subunits of cytochrome oxidase, and two subunits of ATP synthase are also
coded by mtDNA (Stryer, 1988).

Both ribosomal RNA subunits, 14 transfer RNAs, and 12 of the protein
subunits are coded on the heavy strand of mtDNA, and eight transfer RNAs
and one polypeptide are encoded on the light strand (Clayton, 1984). The
mammalian mitochondrial genome is extremely efficient. Only two signifi-
cant portions do not encode functional RNA. These are the displacement-
loop and the 32-base pair origin of light strand replication (Bibb et
al., 1981). The displacement-loop has promoters for both heavy and light
strand transcription (Chang et al., 1987).

Most gene coding regions in the mitochondrial genome are separated
by regions coding for the 22 transfer RNAs. In most genetic systems, the
transcription of genes is individually controlled. The scarcity of
noncoding regions in mammalian mtDNA suggests novel regulation of
transcription. Grivell (1983) indicated that one promoter site for RNA
synthesis on each strand of mtDNA meant transcription resulted in full
length RNA transcription of each strand. The primary RNA transcripts are
then cleaved to yield ribosomal RNAs, transfer RNAs, and messenger RNAs.
Apparently, primary transcripts fold in such a way that distinctive
shapes of transfer RNAs provide signal sites for RNA processing enzymes.
Cleaving occurs simultaneously with transcription.

Translation of messenger RNAs into polypeptide gene products is also
unique in mitochondria. Compared to 61 transfer RNAs in cytosol of
mammals, there are only 22 transfer RNAs in mitochondria (Anderson,
1981). Several differences between mammalian mitochondrial and nuclear
genetic codes are documented. Mammalian mitochondria read AGA and AGG as stop signals rather than as codons for arginine, AUA as methionine rather than isoleucine, and AUA and AUU can serve as start signals instead of AUG, which codes for methionine in nuclear DNA transcription. Also, tryptophan is specified by UGA as well as UGG (Grivell, 1983).

Barrell et al. (1980) proposed a mechanism which would allow 22 transfer RNAs to code for amino acids. They suggested that eight genetic code boxes with four codons for one amino acid had a single transfer RNA gene with T in the first, or wobble, position of the anticodon. Further, those transfer RNAs with U in the wobble position can recognize all four codons in genetic code boxes by "two out of three" base pair recognition or by U in the wobble position recognizing any other nucleotide.

Grivell (1983) updated a probable mechanism for translation by 22 transfer RNAs.

Each of the 22 transfer RNAs in mammalian mitochondria appears to be able to read a "family" of either two or four synonymous codons. The transfer RNAs for two-codon families seem to have conventional G:U wobble anticodons, that is, anticodons beginning with a G that is able to pair with U as well as with C or beginning with a U that can pair with G as well as with A. The transfer RNAs for four-codon families . . . apparently read all four synonymous anticodons. Their anticodons have a U in the 5' position. Either that U somehow pairs with all four of the 3' codon bases or the anticodons read only the first two bases and ignore the last one.

Grivell (1983) also pointed out differences in signalling and stopping translation of mtDNA. The signal for initiation of translation is the codon for methionine. In the cytosol, translation begins with methionine, but only following a recognition site for ribosomal binding. How
ribosomes in mitochondria recognize methionine start signals is not clear. Translation termination is complex. Stop codons are not present. However, cleavage of transfer RNAs leaves most gene transcripts with a UA end. This end is polyadenylated, like most eukaryotic messenger RNAs from the nucleus, leaving a UAA termination site.

The displacement-loop (D-loop) of mtDNA is not known to code for any functional gene transcript. Promoters for both heavy and light strand transcription are known to map in the D-loop, as does the origin of heavy strand replication. According to Chang et al. (1987) the promoter for light strand transcription is located upstream from the origin of mtDNA heavy strand replication. Transcription start sites for the light and heavy strands are separated by about 150-base pairs. Each has an associated analogous mitochondrial transcription factor binding site 12 to 40 base pairs upstream. Mitochondrial transcription factor and mitochondrial RNA polymerase, both originating from the cytosol, serve as the major protein effectors of RNA transcription in mitochondria. The mammalian D-loop has several sequences of high homology among species, but is apparently the least conserved segment of mtDNA (Anderson et al., 1982; Hauswirth et al., 1984; Upholt and Dawid, 1977).

Mitochondrial transcription factor and mitochondrial RNA polymerase are only two of the proteins transported into mitochondria from the cytosol. Hundreds of polypeptide species may be found in mitochondria, but only 13 are encoded by mammalian mtDNA and synthesized in mitochondria (Schatz, 1987). In all, there are about 60 respiratory chain proteins located on the inner membrane of mitochondria, with about 47
coded by nuclear genes (Capaldi, 1988). Other imported proteins are located not only on the inner membrane, but also on the outer membrane, or in the matrix or intermembrane space. Each protein must have information for recognition by mitochondria, as well as information for guiding it to its targeted location (Schatz, 1987). Respiratory subunits from cytosol are made as precursors on cytoplasmic ribosomes and are directed to mitochondria by leader sequences on N terminal extensions. Precursors bind to outer membrane receptors and are actively translocated to destined sites through a process involving energization of the mitochondrial inner membrane (Capaldi, 1988). Obviously, there exists intimate interaction between products of mitochondrial and nuclear genomes.

**Maternal inheritance**

In a well-known experiment, Hutchinson et al. (1974) compared mtDNA of the horse and donkey and their sterile offspring. Hybrids are defined as mules if born to a horse and sired by a donkey, or hinnies if born to a donkey and sired by a horse. Site-specific restriction endonuclease fragment patterns of horse and donkey were clearly distinguishable, and only mtDNA of the maternal species was detected in mules or hinnies. No mtDNA fragments specific for the paternal species was present. This finding was taken as evidence that mtDNA is maternally inherited in animals.

Hayashi et al. (1978) demonstrated that only mtDNA of the female parent was passed to offspring in Sprague-Dawley rats, known to have two types of mtDNA. Individual rats were homozygous with respect to EcoRI
cleavage patterns, when using a system able to detect as little as .02 mg mtDNA. They concluded that maternal inheritance of mtDNA is strict. This conclusion was also reached by Gyllensten et al. (1985). They examined reciprocal crosses of two widely divergent species of mice. The study spanned eight generations of backcrossing and showed that mtDNA inheritance was at least 99.9 percent maternal. Though the two species differed by an estimated 2000-base pair polymorphisms, each mtDNA was viable and fertile on the nuclear background of either species.

Inheritance of mitochondrial DNA has not yet been shown to involve a paternal contribution in mammals. Biparental inheritance of mtDNA has been detected in mussels (Hoeh et al., 1991). Eighty-five of 150 individuals examined were shown to be heteroplasmic. Exact reasons for seemingly strict maternal inheritance of mtDNA in mammals is not clear. Gyllensten et al. (1985) discuss the minuscule contribution of paternal mtDNA relative to the population of maternal mtDNA present in the egg immediately after fertilization. They estimated that 99.9 percent of mtDNA in a newly fertilized egg is maternal in origin. Simply by chance, it would be rare that paternal mtDNA could propagate to a detectable level.

Another potential explanation for maternal inheritance is that paternal mtDNA is physiologically incapable of replication in the fertilized egg. As long ago as 1871, Bütschli (1871) described a specialized mitochondria in the mitochondrial syncytium in the midpiece of sperm. Perhaps this "specialized" mitochondria is incapacitated in some way. Hecht et al. (1984), however, showed that paternal mtDNA is present in
the fertilized egg of mice, and is not grossly altered. Potential for paternal contribution of mtDNA in mammals appears to be present, but no such contribution has yet been discerned.

Maternal Lineages

Maternal effects

Traditionally, animal breeders have given most attention to additive direct nuclear effects, or additive nuclear effects genetically transmitted to offspring in a Mendelian fashion from each parent. Maternal effects have also been considered. Falconer (1989) described maternal effects as environmental influences, either prenatal or postnatal, of the mother on her offspring. Under this broad definition, three kinds of maternal effects have been suggested. First, in a 1972 symposium, Stormont discussed passive immunity as an environmental maternal influence. Second, Wagner (1972) at the same symposium summarized the possibility that mitochondria are transmitted maternally and may explain cytoplasmic inheritance in mammals. The third, and most traditionally recognized maternal effect is an additive maternal genetic effect.

Additive maternal genetic effects are strictly environmental with regard to the offspring on which they are measured. Phenotypic differences among dams for maternal effects are expressed only in the phenotypic values of their offspring. Maternal genetic differences are inherited by dams in a Mendelian fashion. Dams contribute not only a sample half of their genes to offspring, but also a maternal genetic influence. An example of such an effect is in preweaning growth in beef
cattle, where an offspring's performance is influenced by its own genes and the developmental environment provided by its dam (Willham, 1972).

Dickerson (1947) was among the first to describe genetic maternal effects. He looked primarily at the composition of the covariances among relatives. Willham (1963), in an often cited report, took a biometric approach to describing maternal effects in a linear genetic model. Eisen (1967) presented a method to partition maternal genetic effects from direct effects using covariances among relatives from three common mating designs. Willham (1980) pointed out problems in estimation of maternal effects, including confounding of dam's maternal effect and her genetic contribution to the offspring, as well as the possibility of a negative correlation between the direct and maternal effect. Van Vleck (1970) developed selection index equations for several traits with influences from direct and maternal genetic components. Newman et al. (1989) presented a method to differentiate between prenatal and postnatal maternal effects among crossfostered full sibs.

Maternal genetic effects in beef cattle are important for birth weight, preweaning gain, and weaning weight (Woldehawariat et al., 1977). Maternal genetic contributions to preweaning growth traits have been extensively reviewed by Koch (1972) and Woldehawariat et al. (1977). Cundiff (1972) suggested that maternal genetic influences are important for early postnatal growth of offspring nursing their dams, but have diminishing importance later in life.

As early as 1960, Brumby (1960) found that maternal effects explained 8 to 14 percent of total variation in milk and butterfat yields.
of dairy cattle. He, however, did admit the difficulty of separating additive genetic maternal differences from cytoplasmic differences. By using cow, dam, and grandam trios, Van Vleck and Bradford (1965) suggested that maternal genetic effects may account for 20 percent of within herd variance of milk yield. They later looked at up to three records of Holstein cows and found maternal effects had a large influence in first lactation, but decreasing importance in second or third lactation (Van Vleck and Bradford, 1966). On the other hand, Van Vleck and Hart (1966) looked at milk records of Holsteins expressed as deviations from herdmate averages, in part, to exclude confounding of sires and year-season averages. They interpreted results to show only additive direct effects were important for this trait.

Biologically, maternal genetic effects on offspring milk yield should be quite small in dairy cattle. Unlike beef cattle, dairy calves usually are not allowed to nurse their dams. Also, dairy cattle production records are initiated at about two years of age. If maternal genetic effects decrease with age (Cundiff, 1972), they would be quite small by the time the animal reaches productive age. Small effects could be related to neonatal environment or to immunity transferred to offspring through first milking colostrum immediately after calving.

Another potential kind of maternal genetic influence has been brought to light recently. Sapienza (1990) discussed parental imprinting of genes, or genomic imprinting. Nuclear genes inherited from the father or mother are temporarily and erasably marked in different ways, so that genes may be expressed only when inherited from a parent of one sex.
Evidence for genomic imprinting has been found in mice (Cattanach and Kirk, 1985). The mechanism of genomic imprinting is not understood, nor is it known what number and kind of genes are affected.

Gibson et al. (1988) presented gametic models to estimate genetic effects influenced by genomic imprinting. Maternally inherited genes would mimic maternal genetic effects, since the influence would be that of dam on offspring. Such imprinting, however, would not establish maternal lineages. This is precluded because, when individuals produce sperm or eggs, old imprints are erased and new ones specific to the sex of the individual are imposed (Sapienza, 1990).

**Deductive evidence for mitochondrial inheritance**

Bell et al. (1985) examined traditional evidence that cytoplasmic effects on production traits of dairy cattle are important. It must be noted that individual explanations are possible to explain these results, but they do not preclude cytoplasmic inheritance as an explanation for all of these situations. Three kinds of evidence were cited (Bell et al., 1985).

First, it is well known that daughter-dam regression usually results in higher estimates of heritability than paternal half-sib correlation in dairy cattle. Van Vleck and Bradford (1965, 1966) suggested maternal effects as an explanation for higher estimates from daughter-dam regression. Seykora and McDaniel (1983) found heritabilities of .27 and .29 from paternal half-sibs and .35 and .33 from regression of daughter on dam for milk and fat yield, respectively. Alternatively, such dif-
ferences may arise from the similar environments dam-daughter pairs would be subject to. This would be true only to a lesser degree for paternal half-sibs.

Another piece of traditional evidence is that dams' records are able to predict daughter performance, but relatively unable too predict son's genetic ability (Powell et al., 1981). This could be explained if maternal inheritance of mtDNA is strict. Powell et al. (1981) suggested that the environmental correlation between cow and daughter may be responsible for part of the difference. Preferential management of bull dams or daughters of certain bulls also may contribute to such a difference.

Perhaps the most widely noted evidence for existence of cytoplasmic effects is the differences between reciprocal crosses of dairy cattle. Bereskin and Touchberry (1966) found consistent differences between Guernsey and Holstein crosses when Guernsey was the breed of dam versus when Holstein was breed of dam. Similar results were also reported by McAllister (1986) in a Canadian crossbreeding study. That study involved reciprocal crosses of Holsteins and a synthetic Ayrshire breed. These results could be related to variability of mtDNA or its function in different breeds. Alternatively, differences could arise if breeds are providing different intrauterine environments. For example, Holsteins may have more intrauterine space than either Guernseys or Ayrshires. In fact, Rincon et al. (1982) detected a large effect on several production traits when Holstein was the dam breed in reciprocal crosses among Holsteins, Ayrshires, and Brown Swiss.
Phenotypic evidence for mitochondrial inheritance

Bell et al. (1985) examined records of 4461 cows in five herds to determine if cytoplasmic (maternal) lineage significantly accounted for variation in dairy cattle production traits and to find the relative importance of cytoplasmic and nuclear inheritance. Maternal lineages were defined by tracing female pedigrees to the first cow to enter the herd. There were 102 maternal lineages with at least 5 members. Number of generations from the maternal lineage foundation cow was at least 10 for most cows studied. This number was used in linear models in an attempt to account for ongoing mutation of mtDNA, but it was not a significant factor in any model and was ignored. The maximum of 21 generations was probably too short of a time frame to allow generation number to accurately account for mutation rate.

Additive genetic effects of sires and maternal grandsires were accounted in all models to remove cow's nuclear genetic ability to the extent possible. After adjustment for sire, as well as nuisance variables year and month of calving and age at calving, maternal lineages explained 2.0, 1.8, 1.8, and 3.5 percent of total variation of milk yield, milk fat yield, 3.7 percent fat-corrected milk yield, and milk fat percentage, respectively, during first lactation (Bell et al., 1985). Estimation of maternal lineage variance was by analysis of variance methods.

A further model, which included regression on the dam's first lactation production trait to account for more of her nuclear genetic contribution, still resulted in cytoplasmic effects being significant
for milk, fat corrected milk, and fat percentage and approaching significance \((P<.10)\) for milk fat yield. Ranges of maternal lineage estimates were 3353 kg milk, 98 kg fat, and .87% fat. Correlations among independent data sets agreed with expectations as an additional test for cytoplasmic lineage effect (Bell et al., 1985).

Bell et al. (1985) concluded that "maternally transmitted cytoplasmic components influence production traits in dairy cattle." They suggested maternal inheritance of mitochondria was a probable explanation of their findings, but admitted other maternally transmitted components could be involved. The authors also interpreted the greater effect of cytoplasmic lineage on fat percentage to be related to the role of mitochondria in fatty acid synthesis.

Huizinga et al. (1985) looked at records of 290 first lactation cows from a Dutch experimental herd. Cows were assigned to 74 maternal lineages by tracing maternal pedigrees backward to the beginning of the herd. Foundation cows were purchased as calves from 240 farms. Only one calf was purchased from any farm, so each foundation cow was considered as a separate cytoplasmic source. Records of cows calving between 1976 and 1982 were studied if two or more members of the cytoplasmic lineage were represented.

Cytoplasmic lineage was a significant source of variation in fat plus protein yield and milk returns (lactational net income less feed costs) (Huizinga et al., 1986). Cytoplasmic lineage accounted for 6, 5, 6, 10, and 13 percent of phenotypic variation in milk yield, fat percentage, protein percentage, fat plus protein yield, and milk returns,
respectively. The model accounted for the district from which the cytoplasmic source was purchased, sire's breed, year and season of calving, age at calving, and breeding values of sire and maternal grandsire. Although accounting for more variation in milk and fat percentage than in the study by Bell et al. (1985), cytoplasmic lineage was not significant for milk yield or fat percentage. This result may be related to the smaller number of degrees of freedom for F statistics in the study by Huizinga et al. (1986).

Ron (1989, personal communication) found that 3 percent of the variation in milk and fat yield from field data of dairy cattle in Israel was attributable to cytoplasmic effects. The range of maternal lineage effects was 2523 kg milk in 36 lineages from 28 herds. Faust et al. (1990) found a range in maternal lineage constants of 1447 to 1846 kg of 3.7% fat-corrected milk in data from 3413 cows in six North Carolina herds. All lineages had at least 5 members.

Tess et al. (1987) looked at 418 and 522 beef cows in two herds with 20 and 13 cytoplasmic lineages, respectively. Cytoplasmic effects were found to be important (P<.01) in the first herd and approached statistical significance (P<.10) in the second for milk yield predicted from a calf sucking technique. Cytoplasm accounted for 4 and 1 percent of phenotypic variance in the 2 herds, respectively. Furthermore, cytoplasm accounted for 5 percent of variation in average daily gain (ADG) and for 5 percent of variation in weight at 205 days (WT205) on the first farm and 2 percent of variation in ADG and 2 percent of variation in WT205 on the second farm. Correlations of ADG or WT205 with predicted milk yield
were high, giving evidence that "cytoplasmic effects were mediated through milk production," (Tess et al., 1987).

In her Ph.D. dissertation, Northcutt (1990) found significant effects of cytoplasmic lineage on preweaning growth traits by using least squares techniques to estimate variance. Cattle were from three synthetic crossbred lines of beef cattle differing in mature size. Cytoplasmic lineage variance for weaning weight ranged from 2.6 to 10.9 percent of total phenotypic variance. Failure to account for additive genetic covariances among individuals in relatively small cytoplasmic lineages, it was pointed out, may have inflated the cytoplasmic component of variance. In fact, when all genetic covariances were accounted, cytoplasmic influences were much smaller. No direct measure of milk yield was related to cytoplasmic influences.

Some phenotypic evidence suggests that cytoplasmic effects are not important. Considering only additive effects, Kennedy (1986) simulated a closed population similar in size to that of Bell et al. (1985). By using models considering cytoplasmic effects and finding cytoplasmic lineage accounted for 1.4 and 3.2 percent of phenotypic variance in milk yield and fat percentage [compared with 2 and 3.5 percent, respectively, shown by Bell et al. (1985)], he concluded that additive genetic effects in his model may have produced spurious maternal lineage effects. He suggested such spurious maternal lineage effects may arise from genetic drift if additive genetic covariances among individuals are not considered.

It should be pointed out that Kennedy (1986) simulated a closed
population, while Bell et al. (1985) studied cows from herds that were open to the extent that semen from bulls outside the herd was purchased. Falconer (1989) pointed out that before phenomena can be attributed to random genetic drift, it must be known that population size is small, sub-populations are isolated, and genes are subject to little selection. These conditions are more likely to hold in the simulated data of Kennedy (1986) in which the population was closed and culling was at random. Nevertheless, Kennedy (1986) made the very important point that additive genetic covariances among individuals should be considered. Faust et al. (1990), however, found no differences in cytoplasmic lineage constants when animal relationships were included or excluded from analyses of fat-corrected milk yield.

By using regression analysis of daughter-dam and granddaughter-grandam records, Reed and Van Vleck (1987) concluded that cytoplasmic effects accounted for no variation in either milk and fat yields or fat percentage. Correction for environmental effects, however, was made on all records for only the environment influencing daughter records. Thus, dam and grandam records were assumed subject to the same environmental effects as daughter records, an assumption not likely to be valid in field data.

Several studies involving crossbred dairy cattle have turned up no evidence for cytoplasmic inheritance. Ahlborn-Breier et al. (1988) examined records of 42,836 first lactation Friesians, Jerseys, and Friesian-Jersey crosses from 10 years. Breed source of heifer's cytoplasm was not important for any production trait studied. Matthes et al.
found negligible maternal effects on milk, fat, and protein yields, or fat and protein percentage of 31,400 first lactation heifers. These cattle were from stock available for developing the German Black Pied dairy breed. In both of these studies, cytoplasm was defined by breed source rather than distinct maternal or cytoplasmic lineages defined by pedigrees.

**Modeling cytoplasmic inheritance**

In his description of methodology to partition maternal and additive direct effects using covariances among relatives from 3 mating designs, Eisen (1967) mentioned but did not develop any way of estimating cytoplasmic contributions to variation. Rothschild and Ollivier (1987) developed expectations of causal components of variance, including mitochondrial material, from the 3 mating designs as well as a cross fostering design. They also compared relative statistical efficiencies of the designs and discussed consequences of adding a mitochondrial component to the original models. The underlying model was similar to that of Eisen (1967) except that it contained a component for mitochondrial variance. No nuclear by cytoplasmic interaction component was involved. Limitations of the proposed designs for application to dairy cattle included the difficulty of obtaining full-sibs (except through embryo transfer as proposed) and that milk production is a sex-limited trait.

Beavis et al. (1987) described a model for quantitative traits influenced by cytoplasmic interaction. Consideration was primarily given
to plant species. Cytoplasmic factors were assumed to be contributed only by the female parent. This, the authors claimed, makes it biologically impossible to "separate cytoplasmic effects from additive nuclear by cytoplasmic interactions because both are inherited as a unit in species that exhibit maternal inheritance of cytoplasm" (Beavis et al., 1987). Perhaps such limitations could be overcome by use of nuclear transfer and embryo cloning techniques in animal species.

Wilson et al. (1988) described mixed-model equations to consider additive genetic, maternal genetic, and cytoplasmic effects. A model was proposed to estimate the contribution of sire ($0.5 u_d$) and maternal grandsire ($0.25 u_d$) additive genetic contributions ($d$) to the dam compared to the contribution of maternal grandam ($0.25 u_{d+c}$) additive ($d$) plus cytoplasmic ($c$) effects. The differences between contributions ($u_{d+c} - u_d$) would measure cytoplasmic effects. Similar comparisons were made for maternal genetic effects comparing maternal grandsire ($0.5 u_m$) and maternal grandam ($0.5 u_{m+c}$) contributions. Any environmental covariance between daughter-dam-grandam trios would bias estimates of cytoplasmic effects upward. The authors point out that because mixed model techniques are used, differences would be regressed for unequal information, and care must be used in interpreting results.

A different model considering additive, maternal genetic, and cytoplasmic effects, as well as nuclear by cytoplasmic interaction, was put forth by Kirkpatrick and Dentine (1988). They arrived at an alternative conclusion to that of Reed and Van Vleck (1987) with regard to estimation of cytoplasmic effects from daughter-dam-grandam trios.
Kirkpatrick and Dentine (1988) concluded that observations were consistent with the existence of a positive maternal effect, cytoplasmic inheritance, and additive nuclear genetic effects. But they point out that definitive answers to questions about existence and magnitude of cytoplasmic influences will come only from planned experiments, likely employing nuclear transfer techniques.

The ability of animal models, employing use of complete relationship information among relatives, to separate maternal influences into their cytoplasmic and genetic components has been demonstrated (Southwood et al., 1989). By using simulated data along with true or incorrect models containing additive direct, additive maternal, cytoplasmic, and error variances, Southwood et al. (1989) concluded that certain animal models can partition variance caused by these components. The authors did not make recommendations to help decide which models were correct.

**Impact of cytoplasmic inheritance**

Mitochondrial genetic effects are expected not to be as large as nuclear effects, because of the small size of the mitochondrial genome. O'Neill and Van Vleck (1988) pointed out implications of cytoplasmic effects that may exist. Heritabilities from daughter-on-dam regression would be overestimated if important cytoplasmic effects were not accounted. Thus, genetic evaluation of dams of sires would be less accurate, but selection differentials would be only slightly less than optimum. Also, ignoring cytoplasmic effects would only decrease genetic gain slightly, because selection for cytoplasmic effects must be ac-
accomplished only in the dam to cow path of selection where intensity is least. Furthermore, overestimates of heritability have little effect on genetic selection differentials. But accuracy of selection would be lower and expected genetic progress predicted from such overestimates would be greater than realized gain. For the artificial insemination industry in the United States, small incremental gains are useful.

Van Vleck (1988) derived selection index equations considering cytoplasmic inheritance. He pointed out that overestimation of heritability by not considering cytoplasmic contribution would lead to overestimation of additive genetic gain from selection, because the accuracy of the selection index and the variance of indices will be overestimated. He went on to consider generation intervals for selection on cytoplasmic contribution and stated that cytoplasmic selection, relative to additive genetic selection, can be important only if the dam to cow generation interval can be shortened. On the other hand, Van Hendel (1989, personal communication), by using simulated data, showed that progress per year increased when cytoplasmic effects were considered in selection. Small or negative correlations between nuclear and cytoplasmic gene effects led to additional genetic progress over not considering cytoplasmic effects. Long-term selection was indicated to give decreased gains from selection on cytoplasmic effects, for cytoplasmic variation will decrease as a result of selection.
Mitochondrial Effects on Health Traits

**Health and fertility of dairy cattle**

Although milk yield and components of milk, especially fat and protein, are the traits most economically important in dairy cattle, some attention has been given to health differences. Measurement of genetic contribution to specific diseases in dairy cattle can be difficult because low incidences of disease make estimation on a population phenotype basis difficult. Phillipsson (1985) found incidences of veterinary treatments of Swedish cattle to be 7.5, 5.6, 4.4, 18.9, 2.7, and 3.4 percent for milk fever, retained placenta, ketosis, mastitis, teat problems, and feet problems, respectively. Lyons et al. (1991) looked at 22 separate health traits. Highest incidences were found for mastitis (.49), uterus infection (.15), udder injury (.11), milk fever (.09), and trimmed feet (.09). Heritabilities of these traits were .14, .06, .07, .40, and .08 for mastitis, uterus infection, udder injury, milk fever, and trimmed feet, respectively. Heritabilities around .11 were found for other feet and leg problems.

Young (1970) proposed a method for measuring health cost data rather than incidences. The method involved categorizing health related events into body system groups. Functional categories were digestive, mammary, locomotive, reproductive, respiratory, and others. Shanks et al. (1982) found heritabilities of health costs to be .03, .11, .01, .07, and .03 in first parity and .20, .18, .01, .13, and .45 in fourth and later parity for total cost, mammary cost, reproductive cost, locomotive cost, and
other health costs, respectively. Most heritabilities were intermediate from second or third parity. Lyons et al. (1991) grouped incidences of health traits and obtained heritability estimates for reproductive (.02), mammary (.07), digestive (.17), locomotive (.08), and respiratory (.01) systems.

The area of fertility and reproduction has been given more attention by geneticists. Freeman (1984) reviewed the genetics of fertility, as well as relationships between fertility and production. Most estimates of heritability for fertility measures were small ($h^2 < .10$). Despite small heritabilities, Freeman (1984) also pointed to evidence for a seemingly antagonistic relationship between fertility and production in cows, but a complementary relationship between heifer reproduction and production in first parity.

Hansen (1983) examined measures of fertility including days to first breeding, service period, days between services, number of services, conception lag, and days open. Heritabilities of these measures were from 0 to .03. Genetic antagonism between fertility traits was observed, but suggested to be of little consequence because estimates of genetic variance of fertility were small.

**Mitochondrial genetics of health traits**

Studies to relate health or reproductive traits to mitochondrial effects in dairy cattle are scant. Differences in mtDNA have been implicated in a wide range of human diseases. Examples of human diseases that are maternally inherited include mitochondrial myopathies, Leber's
hereditary optic neuropathy (LHON), progressive myoclonic epilepsy, cardiomyopathy, and possibly, chloramphenicol-induced blood dyscrasia (Merrill and Harrington, 1985). Grivell (1989) also mentions Kearns-Sayres syndrome (KSS) and chronic progressive external ophthalmoplegia (CPEO) as diseases associated with mtDNA.

Capaldi (1988) indicated that the most common forms of mitochondrial myopathy and encephalomyopathy are caused by respiratory chain defects. He went on to review results of such mitochondrial function abnormalities. Wallace (1989) further suggested that mtDNA mutations can be divided into 3 categories. LHON is an example of a mild missense mutation that is maternal and homoplasmic. LHON is correlated with a guanine-adenine transition that converts an arginine to a histidine in NADH dehydrogenase subunit 4 gene of mtDNA.

The second mutation category is deleterious point mutations that are maternal and heteroplasmic. An example is infantile bilateral striatal necrosis (IBSN) associated with LHON, but distinct from it (Wallace, 1989). It likely arises from a heteroplasmic deleterious point mutation segregating along a maternal lineage inflicted by LHON. Myoclonic epilepsy and ragged red muscle fiber disease (MERRF) is an even more compelling example of a heteroplasmic point mutation of mtDNA. With LHON and MERRF, the severity of clinical symptoms was directly proportional to the extent of defects of oxidative phosphorylation, and the order of tissues affected was consistent with their relative reliance on mitochondrial energy production. In the instance of LHON, deficiencies are
manifested in the death of the optic nerve and damage to cardiac muscle, both of which have a very high energy demand (Wallace, 1989).

The third category of mitochondrial mutations related to human disease is deletion mutations that are seemingly spontaneous (not inherited) and heteroplasmic. Deletions as large as 5.9 kilobases have been reported (Wallace, 1989). For example, patients with KSS had mtDNA with deletions of 2.0 to 7.0 kilobases, but this mutated mtDNA was at 45 to 75 percent of the total number of molecules.

The issue of tissue specificity of the previously mentioned mtDNA diseases may relate to the reliance of these tissues on oxidative phosphorylation energy (Wallace, 1989). Tissues differ for number of mitochondria and mtDNAs per cell. Expression of nuclear oxidative phosphorylation genes are also tissue specific. Given these facts, diseases caused by differences in mtDNA may be detectable in cattle under the stress of lactation, which is energy intensive and dependent on mitochondrial function.

Most kinds of mtDNA mitigated diseases are quite rare. Influence of mtDNA in some more common diseases may also occur. Palca (1990) proposed that Parkinson’s disease may be caused by mitochondrial abnormalities that may, at least in part, stem from mtDNA differences. Various cardiomyopathies may arise from mtDNA sources, and Huntington’s disease may be influenced by mitochondrial-nuclear interaction. It has been suggested that even aging in humans may result from an accumulation of mtDNA mutations during a person’s lifetime (Palca, 1990). Capaldi (1988) also pointed out a condition involving mitochondrial myopathy, encephalo-
myopathy, lactic acidosis, and stroke-like episodes (MELAS).

Effects of mtDNA mutations have not been shown in dairy cattle for health or reproductive traits. Possible relationships between reproductive performance and mitochondrial influences may be deduced. Bell et al. (1985) found smaller maternal lineage effects on production traits when they were first adjusted for days open (days from calving to subsequent conception), a measure of overall reproductive performance. The effect of cytoplasm on days open was also significant in that study, and the range of constants of maternal lineages was 14 days.

Faust et al. (1989) also looked at several measures of reproduction or fertility. Ranges of cytoplasmic lineage constants were 30 days for days from calving to first service, 65 percent for first service conception rate, and 1.7 for number of services. Standard deviations of cytoplasmic lineage constants were 1, 1, and .5 additive genetic standard deviations, respectively, for the three traits. While results were not significant in their study, Huizinga et al. (1986) found that cytoplasmic components accounted for as much as 10 and 13 percent of variation in number of inseminations and age at first calving. Freeman (1984) pointed out that age at first calving may be a measure more of maturity than fertility. Nibler et al. (1989) reported ranges of maternal lineages of 16.7 days between successive calvings in Friesians and 45.7 days in Flekvieh in a German study.

Bell et al. (1985) concluded that a small effect of cytoplasm on reproductive performance was present. They hypothesized mitochondria may influence reproduction because three steps in the biosynthesis of
steroids from cholesterol are related to mitochondria. Differences in mitochondrial function may influence reproductive performance. This in turn may influence lactational milk production because different portions of the production record would not be burdened by the additional energy demands of the developing fetus.

Harrison et al. (1990) looked at 10 high and 10 average producing Holstein cows. Days to first visual estrus and number of ovulations to first visual estrus, both of which would increase days open, were greater for high producing cows. No differences were detected between high and average producing cows for the interval from parturition to uterine involution or days to first ovulation. Moreover, the high producing cows had most negative energy balance (net energy in feed minus net energy of maintenance and milk produced) early in lactation. Energy yielding metabolites in blood were also most different between groups at that time.

Results of Harrison et al. (1990) meant that high and average producing cows resumed normal reproductive physiological activity at nearly the same time; however, expression of estrus was markedly different. High producing cows are more energy stressed in early lactation. The authors speculated that greater loss of body weight in high producing cows may represent greater mobilization of adipose and muscle tissues to support milk production. This weight loss and mobilization of tissues as well as differences in circulating energy metabolites may be important in the delay of expression of estrus in the high group. Such differences in energy demand and mobilization may relate to mitochondrial function.
Mitochondrial differences may influence reproductive performance through its association with milk production.

Previous results indicate two possible mechanisms for effects of mitochondrial genetics on production and reproduction. Mitochondrial differences may affect reproduction, thereby yielding milk production differences; or they may affect milk production, thereby altering reproductive performance. Both situations, also may be happening simultaneously, but results are not able to distinguish between the two mechanisms as yet.

**Differences in Mitochondrial Function and DNA**

**Mitochondrial function differences**

Lindberg et al. (1989) studied the relationship of mitochondrial proliferation and ATP production with milk production, as measured by crossfostered litter weight gain from day 1 to day 12 postpartum, in two selection lines of mice genetically divergent for milk yield. High milk selected mice had greater mammary weight, mammary total DNA, and RNA to DNA ratio. Mammary DNA per gram of tissue and protein per gram of tissue were similar between the two lines. Mitochondrial mass per gland was also higher for the milk selection line.

Actual differences in mitochondrial function also were studied by Lindberg et al. (1989). Rates of succinate-supported ATP production and ADP to oxygen ratio of isolated mitochondria differed. Authors concluded that between line differences in mitochondrial mass and efficiency of use of succinate to produce ATP were probably consequences of genetic
selection. Because function was measured on isolated mitochondria, effects of nuclear by cytoplasmic interaction could not be observed. Lindberg et al. (1989) proposed 3 mechanisms to explain differences in ATP production by mitochondria which could be related to mitochondrial replication, stimulation of mitochondria to produce ATP faster, and increased efficiency of substrate use.

Brown et al. (1988) examined mitochondrial function in dairy cattle. Correlations of mitochondrial respiratory activity and genetic ability of sires for milk or milk fat production ranged from -.35 to .15. Correlations of cow genetic indices or actual yields of milk with acceptor stimulated mitochondrial respiration, oxidative phosphorylation efficiency, or ATP synthesis rate, however, were much larger, ranging from .25 to .48. If correlations between mitochondrial function and milk production are influenced by mtDNA, they should be higher for cow indices or actual yield, because there would be no paternal contribution.

**mtDNA sequence differences**

Bovine mtDNA is heterogeneous in the population of dairy cattle, with many differences being point mutations in the D-loop (Hauswirth and Laipis, 1982; Laipis et al., 1988). This conclusion was drawn from analyses of restriction fragment length polymorphisms (RFLPs) in mtDNA. Comparison of fragment lengths to the published nucleotide sequence of the bovine mitochondrial genome (Anderson et al., 1982) allowed assignment of point mutations to the D-loop region. Such mutations in the D-loop may affect the abundance of mtDNA transcripts subserving ATP
synthetic capacity or the size of the mtDNA population in the cell.

Lindberg (1989) sequenced the entire D-loop (910 base pairs) light strand of mtDNA of cattle from 38 maternal lineages. Fifty-one sites of sequence variation were identified in this region of replicational and transcriptional control. Nucleotide substitutions at 48 sites, one base pair deletion, and 2 variable length poly G/poly C regions of 6 and 12 bases were found. Transversions (substitution of a pyrimidine for a purine or a purine for a pyrimidine) accounted for 10 of the 48 nucleotide substitutions observed. This number of transversions is much higher than would be predicted from knowledge of nuclear DNA.

Most nucleotide substitutions were found in the central GC-rich region of the D-loop rather than at the 5' end or the 3' end, which contains light and heavy strand transcriptional promoters and the origin of heavy strand replication (Lindberg, 1989). Overall, his data pointed to the presence of regions high in nucleotide sequence diversity, while other regions of the D-loop were quite strictly conserved. Phylogenetic trees were constructed and indicated two major divisions of mtDNA genomes in Holsteins.

Koehler (1989) used various restriction enzymes to conduct an RFLP analysis of the entire mtDNA genome of 38 maternal lineages. Twelve RFLPs were detected. In addition, one polymorphic site (detected with HpaII at nucleotide 363 in a poly G/poly C region and confirmed by nucleotide sequencing) varied in 29 percent of the maternal lineages. This site was considered hypervariable.
Johnston et al. (1991) investigated nucleotide sequence variability in the D-loop region from cattle in five Ayrshire, four Brown Swiss, seven Jersey, four Guernsey, and two Milking Shorthorn lineages. Ten nucleotide substitutions were observed, and the nucleotide diversity was calculated to be $1.8 \times 10^{-3}$, but their search was not extensive, limiting the usefulness of this calculation. No markers to identify any breed were found. In fact, nucleotide diversity seemed to be greater within than across breeds. Watanabe et al. (1985) also found within-breed differences at 3 restriction sites in cattle from 3 breeds in Japan. None of the differences were exclusive to a single breed. A similar result in sheep was reported by Hiendleder et al. (1991).

The extent of sequence variability for gene coding regions of mtDNA is not well documented for dairy cattle. Hiendleder et al. (1991) considered mtDNA of sheep to be similar to that of cattle. They found a total of 3 polymorphic base pair restriction sites at positions within NADH dehydrogenase subunit 5 and cytochrome b genes. Watanabe et al. (1989) found 7 polymorphic restriction sites in gene and transfer RNA coding regions of mtDNA from nine head of native Philippine cattle. Johnston (1991) observed 11 different sequences in ribosomal RNA subunits of mtDNA in 38 lineages of Holsteins.

**Association of mtDNA polymorphism with phenotypic traits**

Little work has been done to associate genome differences in mitochondrial DNA with differences in traits of economic importance in dairy cattle. Several associations between production traits and nuclear
genes or physiological markers have been shown. Hoeschele and Meinert (1990) found a chromosome segment with major effect on yield is linked to the chromosomal position of the recessive polymorphic allele for weaver (progressive degenerative myeloencephalopathy) in Brown Swiss. The gene region coding for prolactin may be closely related to a gene with a major effect on milk yield (M. R. Dentine, 1991, personal communication).

Also, Weigel et al. (1990) used a gene substitution model to associate various alleles of the bovine lymphocyte antigen complex A locus with production and health traits. For example, allele *w11* was related to udder health costs and decreased fat yield and percentage, while allele *w31* was associated with decreased fat percentage.

Concerning mtDNA markers, Brown et al. (1989) assumed mtDNA within maternal lineages was homogeneous and compared 283 records of cows from lineages polymorphic at a Hpa II site relative to a standard sequence (Anderson, et al., 1982) versus 833 lactations of cows from nonpolymorphic lineages. Milk yield was higher, though not significantly so, for polymorphic lineages. Fat percentage was significantly (*P*<.001) lower for those polymorphic lineages. It should be pointed out that this Hpa II site was later shown to be hypervariable and heterogenic with respect to maternal lineages (Koehler, 1989). That site should not be associated with production traits under the assumption that it is uniformly polymorphic or nonpolymorphic within a maternal lineage.

Brown et al. (1989) provided a method to examine associations between mtDNA sequence differences and traits of economic importance. Unlike with nuclear DNA markers, methods concerning mtDNA are not
encumbered by independent segregation and random assortment. The mtDNA genome is transmitted to the offspring as a whole unit. With few exceptions, it seems mtDNA is faithfully passed from mother to offspring, thereby forming homogeneous mitochondrial lineages. This assumption, with the exception of a Hpa II site previously mentioned, should be valid in modeling association of mtDNA sequence polymorphisms with traits of economic importance in dairy cattle.
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SECTION I. THE IMPORTANCE OF MATERNAL LINEAGE ON MILK PRODUCTION OF DAIRY CATTLE
ABSTRACT

Maternal lineage effects on milk production traits, considered indicative of cytoplasmic inheritance, were evaluated with animal models. Cattle were from a selection experiment begun in 1968. Maternal pedigrees were traced to the first female member in the Holstein-Friesian Herd Book, and purchased cows entering the herd, considered foundation females, were assigned to maternal lineage groups. All models accounted for year-season of calving, parity, and selection lines. Maternal lineage effects were included in a repeated records model with cow effects and preadjustment for sire and maternal grandsire transmitting abilities. Maternal lineage accounted for 5.2, 4.1, and 10.5% of phenotypic variation of preadjusted records of milk yield, fat yield, and fat percentage, respectively. Maternal lineage was evaluated as a fixed effect in an animal model including random animal and permanent environmental effects. Ranges of maternal lineage estimates were 2934 kg milk, 154 kg fat, and .907% fat. Tests of significance for large animal models were developed. Maternal lineage significantly affected fat percentage but not milk yield. Maternal genetic (nuclear) effects and their covariance with additive animal effects did not significantly account for additional variation nor did they influence maternal lineage estimates. Maternal lineage affected calculated net energy of milk, but was not important for solids-not-fat yield or concentration. Evidence exists that maternal lineage influences fat percentage, energy concentration, and to a lesser extent, fat yield in milk of dairy cattle.
Recent studies have demonstrated the existence of maternal lineage effects on production and reproduction of dairy cattle which may be indicative of cytoplasmic inheritance. Because mitochondria are transmitted only from female parents to ensuing offspring (Gyllensten et al., 1985), mitochondrial DNA (mtDNA) is a probable source of such cytoplasmic inheritance.

Mitochondrial DNA sequence is known to differ among dairy cattle (Hauswirth et al., 1982). Koehler (1989) used restriction enzymes to detect 11 polymorphisms among maternal lineages, and two additional polymorphisms occurred within lineages. Lindberg (1989) sequenced the entire D-loop region of mtDNA and identified 48 sites of nucleotide substitution plus one deletion and two variable-length regions among lineages.

Evidence of maternal lineage effects comes from two recent studies (Bell et al., 1985; Huizinga et al., 1986). In a study of 4461 cows representing 102 cytoplasmic lineages, Bell et al. (1985) showed that 2.0, 1.8, 1.8, and 3.5% of variation in milk yield, fat yield, 3.7% fat corrected milk yield (FCM), and fat percentage, respectively, was explained by cytoplasmic lineage. The authors concluded that cytoplasmic origin was a significant source of variation in production traits of dairy cattle. Huizinga et al. (1986) attributed 10 percent of variation in milk, fat, and protein production, and 13 percent of variation in milk economic returns to cytoplasmic components. By using field data from 36 lineages in 28 herds,
Ron (1989, personal communication) attributed 3 percent of variation in milk and fat yields to cytoplasmic effects.

Some evidence suggests that cytoplasmic effects may not be important. Considering only additive effects, Kennedy (1986) simulated a closed population, similar in size to that of Bell et al. (1985). Kennedy’s work showed that analyses which ignore covariances between observations, as was done by Bell et al. (1985), can lead to spurious F-test results. By using regression analysis of daughter-dam and granddaughter-grandam records, Reed and Van Vleck (1987) concluded that cytoplasmic effects accounted for no variation in either milk and fat yields or fat percentage. Correction for environmental effects, however, was made only for daughter records. Thus, dam and grandam records were assumed subject to the same environmental effects as daughter records, an assumption not likely to be valid in field data. Kirkpatrick and Dentine (1988) proposed a different model which gave an alternative explanation to Reed and Van Vleck’s (1987) conclusion. They concluded that observations were consistent with the existence of a positive maternal effect, cytoplasmic inheritance, and additive nuclear genetic effects.

Additive maternal effects cause genetic differences among dams, exhibited as strictly environmental influences with regard to offspring performance (Willham, 1963). It is unclear whether additive maternal effects influence production traits in dairy cattle. Maternal genetic effects are known to occur in beef cattle, in which genetic mothering ability influences preweaning growth of calves (Woldehawariat et al., 1977). In dairy cattle, calves generally do not nurse their dams, so additive maternal effects would be caused by intrauterine environment. In
1960, Brumby (1960) reported maternal genetic differences of 8 to 14% of total variance in milk yield, but admitted the difficulty of separating the effects of additive genetic maternal differences from the effects of cytoplasmic differences.

The ability to separate maternal influences into their cytoplasmic and additive genetic components by animal models has been demonstrated (Southwood et al., 1989). By using simulated data and true or incorrect models containing additive direct, additive maternal, cytoplasmic, and error variances, Southwood et al. (1989) concluded that certain animal models can be used to partition variation caused by these components.

The objective of the present study was to determine the extent of maternal lineage effects, which are indicative of cytoplasmic inheritance, on milk production traits in a herd of dairy cattle with known molecular variation in mtDNA (Koehler, 1989; Lindberg, 1989).
MATERIALS AND METHODS

Cattle studied were part of a selection experiment begun at Iowa State University's Breeding Research Herd in 1968. Foundation females were mated to Holstein AI sires with high or average transmitting abilities for milk to form two divergent genetic lines. A description of the design of this breeding experiment is presented by Bertrand et al. (1985). Records initiated through 1986 were included. At that time, the herd consisted of 150 milking cows, which differed by 1304 kg milk per lactation between high and average lines.

The genetic backgrounds of the original members of the herd were diverse. The 158 foundation females were purchased from 38 Holstein breeders throughout Iowa. But because maternal heritage was verified by tracing maternal lineage to the first female member recorded in the Holstein-Friesian Herd Book (Wales, 1885), the 133 registered females were assigned to 81 separate maternal lineages. Only records with unusual circumstances, such as those initiated by abortion or those with serious mastitis, were excluded. Lines with only one member with usable information were excluded also; thus, 53 maternal lineages from a total of 105 foundation cows were studied. Of these, 19 had members only in the high production line, 15 only in the average production line, and 19 in both lines. Foundation females were, on average, 19 generations removed from their matriarchs first recorded in the herd book. Inbreeding was negligible in this herd.

Mature equivalent (2X-305-ME) milk (MEMILK) and fat (MEFAT) yields and fat percentage were the production traits considered. A sire predicted
difference (PD) plus one-half maternal grandsire PD model, similar to model 3 of Bell et al. (1985), was used to analyze production traits for maternal lineage effects:

\[ Y_{imn} = \mu + YS_i + b_1(\text{age}) + b_2(\text{age}^2) + b_3(\text{TV}) + b_4(\text{GTO}) + ML_m + e_{imn} \]  

where \( Y_{imn} \) is MEMILK, MEFAT, or fat percentage record of cow \( n \) in maternal lineage \( m \) calving in year-season of calving \( i \) (seasons were October to April and May to September and some early years were combined because of too few records); \( b_1 \) and \( b_2 \) are linear and quadratic regressions on age at calving to account for specific herd effects because mature equivalent adjustments are on a regional basis; \( b_3 \) is regression on estimated transmitting value (TV = 1982 PD value of the sire plus one-half 1982 PD value of the maternal grandsire of the \( n \)th cow); \( b_4 \) is the regression on the number of generations to the maternal lineage origin (GTO); \( ML_m \) is the effect of maternal lineage \( m \); and \( e_{imn} \) is the residual. All effects except maternal lineage and residual were considered fixed. Variance of maternal lineages was estimated using the RANDOM option of the general linear models procedure of the Statistical Analysis System [(SAS Institute Inc., 1985) SAS PROC GLM]. Estimates are according to Henderson's method 3 (Henderson, 1984). Because foundation females were purchased from other herds and because sire and maternal grandsire PD values could not always be obtained, records of foundation females were excluded from this part of the study. Only cows from subsequent generations were used. Separate analyses were conducted for first and second parity with Model [1].
Also, a repeated records model was used to estimate variance components based on as many as seven records per cow. Records were preadjusted for sire plus one-half maternal grandsire 1982 PD values to account for a portion of additive nuclear contributions.

The repeated records model was

$$Y_{ijklm} = \mu + YS_i + P_j + b_1(\text{age}) + b_2(\text{age}^2) + \text{ML}_2 + C(\text{ML})_{1:k} + e_{ijklm}$$

where $Y_{ijklm}$ is the record $m$ in year-season $i$ and parity $j$ of cow $l$ in maternal lineage $k$. Effects are as in Model [1]; and $C(\text{ML})_{1:k}$ is the effect of cow $l$ nested in maternal lineage $k$. In this model, maternal lineage, cow, and residual were treated as random effects. Expectations of maternal lineage, cow, and error variance were zero, and variance among maternal lineages was $\text{var}(\text{ML}) = I\sigma_M^2/\sigma_e^2$ and among cows was $\text{var}(C) = I\sigma_C^2/\sigma_e^2$, where $\sigma_e^2$ is error variance and $I$ is an identity matrix.

Restricted maximum likelihood (REML) estimates of variances of maternal lineage and error and solutions for maternal lineages used an expectation-maximization algorithm (Meyer, 1987). Convergence was declared when change in all estimates expressed as a percentage was less than $1 \times 10^{-4}$ (Meyer, 1987). Inclusion of cow effects, in addition to preadjustment for sire plus one-half maternal grandsire 1982 PD values, accounted for a portion of additive nuclear differences.

Detailed animal models including all known additive genetic covariances among related individuals have been proposed, however (Henderson, 1984). Recent computing methods have made animal model analyses feasible (Berger
et al., 1989; Meyer, 1989; Misztal and Gianola, 1988). Records of all cows in the herd including foundation females were analyzed according to this animal model:

\[ Y_{ijklmno} = \mu + Y_{S_1} + P_j + ML_k + L_1 + G(L)_{m:1} + PE_n + a_n + e_{ijklmno}. \]  

[3]

Effects in the model are as previously defined, except that \( L_1 \) is the "high" or "average" sire selection line; \( G(L)_{m:1} \) is sire birthyear group \( m \) nested in selection line 1; \( PE_n \) is the permanent environmental effect of animal \( n \) with a record; and \( a_n \) represents the additive genetic effect of the animal \( n \). Permanent environment, animal, and residual effects were considered random and independently distributed with zero expectations. Variance among permanent environments was \( \text{var}(PE) = I\sigma_{PE}^2/\sigma_e^2 \), where \( \sigma_e^2 \) is error variance and \( I \) is an identity matrix. Variance among animals was \( \text{var}(a) = A\sigma_a^2/\sigma_e^2 \), where \( A \) is the numerator relationship matrix. \( A \) included sires and dams of all cows back to foundation cows and relationships among sires and paternal grandsires of artificial insemination bulls represented by daughters with records were included.

Based on Model [3], a derivative-free REML procedure (Meyer, 1989) was used to estimate variance components for permanent environmental and animal effects. The procedure uses a simplex or polytope method to evaluate explicitly the maximum log-likelihood. Used in this way, Model [3] corresponds to Model 2 of Meyer (1989). For purposes of testing the hypothesis that maternal lineage effects differ, maternal lineages were considered fixed.
Because animal models are usually of large order, conventional tests of significance, requiring elements of variance-covariance matrices and, hence, direct inversion, are often unfeasible. Thus, an alternative test based on mixed model conjugate normal equations (Harville, 1979) was developed to determine the significance of maternal lineage effects (See Appendix 1). This procedure results in an approximate test, which is exact if estimated variance ratios are true. Variance ratios for random effects in the model used variance component estimates from analyses with Model [3]. Because of concern about increased bias when using variance ratios estimated from the same data (Henderson, 1984), variance ratios based on heritabilities of .2, .2, and .5 and repeatabilities of .5, .5, and .7 (G. Wiggans, 1990, Personal Communication) for MEMILK, MEFAT, and fat percentage, respectively, were also used.

For both sets of variance ratios, genetic groups were fixed sire birth year groups. Both sets of variance ratios were also used in testing maternal lineage effects in models that differed from Model [3] in definition of genetic groups. Genetic groups were defined according to Westell et al. (1988) and unknown parent groups accounted for additional genetic trend not explained by known additive genetic relationships. Thus, four tests of significance were conducted.

Both sets of variance ratios were also used for testing maternal lineage effects in models that differed from Model [3] only in definition of genetic groups. In these models, genetic groups were defined according to Westell et al. (1988) to account for additional genetic selection not explained by known genetic relationships.

Variance component estimation with Model [3] was expanded to include
variance caused by additive maternal genetic effects and covariance between additive animal and maternal genetic effects. The model used was

$$Y_{ijklmnop} = \mu + Y_{i} + P_{j} + M_{k} + L_{l} + G(L)_{m,l} + PE_{n} + a_{n} + M_{o} + e_{ijklmnop}$$  \[4\]

where effects and assumptions are as in Model [3], except $M_{o}$ is the effect of dam $o$. Maternal genetic effects also had expectation of zero. Variance among maternal genetic effects was $\text{var}(M) = \frac{A\sigma_{M}^{2}}{\sigma_{e}^{2}}$ and covariance between animal and maternal genetic effects was $\text{cov}(a,M) = A\sigma_{a,M}/\sigma_{e}^{2}$, where $A$ and $\sigma_{e}^{2}$ are as previously defined. Model [4b] differed from model [4] by considering additive animal and maternal genetic effects to be uncorrelated $[\text{cov}(a,M) = 0]$. 
RESULTS AND DISCUSSION

Number of cows and overall means for the three production traits for first and second parity are in Table 1. Numbers of records in subsequent parities decreased quickly and results from later parities will not be reported. Means increased from first to second parity for MEMILK and MEFAT, but remained nearly constant for fat percentage. Increases may have resulted from mature equivalent age factors not being exact for a single herd. Culling of cows in first parity also may have contributed to increased means in second parity. After culling for involuntary reasons, voluntary culling was based on transmitting ability for milk. Any culling was without regard to maternal lineage. Standard deviations of traits were nearly identical in both parities.

The results in Table 2 are based on Model [1]. Year-season of calving and regression on sire plus one-half maternal grandsire 1982 PD values were highly significant ($P < .01$) for all traits in both parities. Linear or quadratic regressions on age were not significant for any trait. Because analysis was separate for parities, this finding may not be unusual. As expected, however, $F$ values for regressions on age were smaller for MEMILK and MEFAT, because they are already age-adjusted. Regressions on generations to origin were not significant in either parity. Bell et al. (1985) obtained similar results. Hence, generations to origin were not considered in subsequent analyses. The effect of cytoplasmic lineage (Table 1) was highly significant ($P < .01$) for fat percentage in both parities and MEFAT in first parity and was significant ($P < .05$) for MEFAT in second parity and MEMILK in first parity.
Table 1. Means and effects of maternal lineage on production traits from Model [1]

<table>
<thead>
<tr>
<th>Trait</th>
<th>No. Cows</th>
<th>Overall Mean</th>
<th>SD</th>
<th>M.L. a</th>
<th>( \sigma^2_{ML}/\sigma^2_e )</th>
<th>Range b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEMILK c, kg</td>
<td>664</td>
<td>7643</td>
<td>1721</td>
<td>.020</td>
<td>.041</td>
<td>5493</td>
</tr>
<tr>
<td>MEFAT c, kg</td>
<td>661</td>
<td>271</td>
<td>60</td>
<td>.002</td>
<td>.058</td>
<td>195</td>
</tr>
<tr>
<td>FAT, %</td>
<td>662</td>
<td>3.62</td>
<td>.41</td>
<td>.001</td>
<td>.084</td>
<td>1.24</td>
</tr>
<tr>
<td>Parity 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEMILK d, kg</td>
<td>409</td>
<td>8360</td>
<td>1718</td>
<td>.170</td>
<td>.028</td>
<td>3258</td>
</tr>
<tr>
<td>MEFAT d, kg</td>
<td>407</td>
<td>299</td>
<td>57</td>
<td>.017</td>
<td>.072</td>
<td>148</td>
</tr>
<tr>
<td>FAT, %</td>
<td>409</td>
<td>3.64</td>
<td>.42</td>
<td>.001</td>
<td>.140</td>
<td>1.12</td>
</tr>
</tbody>
</table>

a M.L. is Maternal Lineage.

Range of maternal lineage solutions.

c MEMILK = mature equivalent milk yield (2x-305-ME).

d MEFAT = mature equivalent fat yield (2x-305-ME).

Ratios of estimates of maternal lineage variance to error variance are presented in Table 1. Ratios for MEMILK and fat percentage were greater than those previously reported from similar models using actual (Bell et al., 1985) or simulated (Kennedy, 1987) records. This finding suggests that maternal lineage may account for an appreciable portion of residual variance in models not considering its influence. Ranges of maternal lineage least squares means from Model [1] are also presented in Table 1. Ranges for all traits were much greater than one phenotypic standard deviation. Moreover, ranges for all three traits were greater than those reported in previous work (Bell et al., 1985).
Table 2. F statistics and residual mean squares for Model [1]

<table>
<thead>
<tr>
<th>Traits</th>
<th>Parity 1</th>
<th>Parity 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>MEMILK&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Year-season</td>
<td>27</td>
<td>2.43**</td>
</tr>
<tr>
<td>Age at calving</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>1</td>
<td>.01</td>
</tr>
<tr>
<td>Quadratic</td>
<td>1</td>
<td>.01</td>
</tr>
<tr>
<td>Transmitting value</td>
<td>1</td>
<td>30.63**</td>
</tr>
<tr>
<td>Generations to origin</td>
<td>1</td>
<td>.12</td>
</tr>
<tr>
<td>Maternal lineage</td>
<td>52</td>
<td>1.47*</td>
</tr>
<tr>
<td>Residual mean square</td>
<td>580</td>
<td>2,236,085</td>
</tr>
</tbody>
</table>

<sup>a</sup>MEMILK = mature equivalent milk yield (2x-305-ME).
<sup>b</sup>MEFAT = mature equivalent fat yield (2x-305-ME).
<sup>c</sup>Units for residual mean squares are in parentheses.

**P > F < 0.01.
*P > F < 0.05.
†P > F < 0.10.
Table 3 has numbers of records, cows, and lineages used in the repeated records Model [2] with preadjustment for sire plus one-half maternal grandsire 1982 PD values for each of the three traits. There was an average of 2.4 records per cow and 12.6 cows per cytoplasmic lineage. Resulting variance components for cytoplasmic lineage, cows within lineage, and residuals also appear in Table 3. Ideally, 1982 PD values might have been regressed to account for herd level and herd variance when preadjusting. Such regression coefficients, however, could not be determined accurately for later parities, thus 1982 PD values were used as additive adjustments.

Ratios of variance components from repeated records analysis are in Table 4. Variance caused by cytoplasmic lineage accounted for 4 to 10 % of phenotypic variance after removal of a portion additive nuclear effects. Phenotypic variance was defined as the sum of maternal lineage, cow, and residual variances, as listed in Table 3. The ratio of cytoplasmic to residual variance ranged from 12 to 38 %. This ratio was much greater than that from the analysis using Model [1] or from previous reports (Bell et al., 1985; Kennedy, 1987). One explanation is that inclusion of cow effects in the model with repeated measures decreased residual variance, and inflated the ratio of maternal lineage to residual variance. Perhaps ratios of maternal lineage to phenotypic (5.2 to 10.5 %) or to cow variance (6.6 to 17.2 %) are more stable measures of the importance of maternal lineage than are ratios to residual variance.

Ranges of REML procedure solutions for maternal lineages are also presented in Table 4. Ranges of solutions, which are regressed because
Table 3. Distribution of records in repeated records Model [2] and variances for maternal lineage, cows within lineage, and error

<table>
<thead>
<tr>
<th>Trait</th>
<th>No. Records</th>
<th>No. Cows</th>
<th>No. Lineages</th>
<th>$\sigma^2_{\text{ML}}$</th>
<th>$\sigma^2_{\text{C}}$</th>
<th>$\sigma^2_{\text{e}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEMILK$^a$, kg</td>
<td>1595</td>
<td>669</td>
<td>53</td>
<td>126,385</td>
<td>1,258,207</td>
<td>1,039,727</td>
</tr>
<tr>
<td>MEFAT$^b$, kg</td>
<td>1595</td>
<td>669</td>
<td>53</td>
<td>161</td>
<td>2,442</td>
<td>1,300</td>
</tr>
<tr>
<td>FAT, %</td>
<td>1593</td>
<td>667</td>
<td>53</td>
<td>.0146</td>
<td>.0556</td>
<td>.0388</td>
</tr>
</tbody>
</table>

$^a$MEMILK = mature equivalent milk yield (2x-305-ME).
$^b$MEFAT = mature equivalent fat yield (2x-305-ME).
Table 4. Variance ratios and ranges of REML solutions for maternal lineages from Model [2]a

<table>
<thead>
<tr>
<th>Trait</th>
<th>$\hat{\sigma}^2_{ML}/\hat{\sigma}^2_P$</th>
<th>$\hat{\sigma}^2_{ML}/\hat{\sigma}^2_e$</th>
<th>$\hat{\sigma}^2_{ML}/\hat{\sigma}^2_C$</th>
<th>$\hat{\sigma}^2_C/\hat{\sigma}^2_P$</th>
<th>Rangeb</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEMILKc, kg</td>
<td>.052</td>
<td>.121</td>
<td>.100</td>
<td>.519</td>
<td>1000</td>
</tr>
<tr>
<td>MEFATd, kg</td>
<td>.041</td>
<td>.124</td>
<td>.066</td>
<td>.626</td>
<td>34</td>
</tr>
<tr>
<td>FAT, %</td>
<td>.105</td>
<td>.376</td>
<td>.172</td>
<td>.614</td>
<td>.37</td>
</tr>
</tbody>
</table>

\[ \frac{\hat{\sigma}^2_P}{\hat{\sigma}^2_C} = \frac{\hat{\sigma}^2_{ML}}{\hat{\sigma}^2_{ML}} + \frac{\hat{\sigma}^2_C}{\hat{\sigma}^2_e}. \]

a. Range of maternal lineage solutions.
b. Range of maternal lineage solutions.
c. MEMILK = mature equivalent milk yield (2x-305-ME).
d. MEFAT = mature equivalent fat yield (2x-305-ME).

maternal lineages were treated as random for variance estimation, were approximately one phenotypic standard deviation for fat percentage and slightly less for MEMILK and MEFAT yields. In all instances, ranges of solutions for lineages were at least twice the greatest prediction error variance of these regressed solutions. This is evidence that, after inclusion of cow effects, appreciable differences exist among maternal lineages, especially for fat percentage.

The use of animal models (Henderson, 1984) allows partitioning of cow effects into permanent environmental and animal effects. Table 5 has prior variance ratios for derivative-free REML estimation of variance components with Model [3]. Variances are expressed as a ratio to phenotypic variance for animal ($h^2$) and permanent environment ($c^2$).
Table 5. Prior variance ratios for random effects for DFREML estimation with Model [3]

<table>
<thead>
<tr>
<th>Trait</th>
<th>Animal ((h^2))</th>
<th>Perm. Env. ((c^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEMILK (^a)</td>
<td>.33</td>
<td>.30</td>
</tr>
<tr>
<td>MEFAT (^b)</td>
<td>.25</td>
<td>.35</td>
</tr>
<tr>
<td>FAT (^c)</td>
<td>.40</td>
<td>.50</td>
</tr>
</tbody>
</table>

\(^a\)MEMILK = mature equivalent milk yield (2x-305-ME).
\(^b\)MEFAT = mature equivalent fat yield (2x-305-ME).

Priors for Model [4] used variance estimates resulting from analysis with Model [3] for animal and permanent environmental components. Maternal genetic variance \((m^2)\) and covariance between animal and maternal genetic component ratios were set near zero. For both models, convergence was declared when variance of function values in the simplex was less than 1.0 \(x 10^{-5}\) (Meyer, 1989).

Estimates of variance components from Model [3], including animal and permanent environment, are in Table 6. Phenotypic variance is the sum of animal, permanent environmental, and error variance for Model [3]. Animal variances as a ratio to total phenotypic variances (heritabilities) from Model [3] are in Table 7. Heritabilities for milk and fat yields fall into the ranges of recent estimates for Holsteins (Moore et al., 1991;
Table 6. Variance estimates with or without maternal genetic effects

<table>
<thead>
<tr>
<th>Trait</th>
<th>Model</th>
<th>Animal (a)</th>
<th>Permanent Environment (PE)</th>
<th>Maternal Genetic (M)</th>
<th>Covariance (Cov)</th>
<th>Phenotype (P)</th>
<th>Error (e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEMILK, kg²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model [4b]d</td>
<td>583,040</td>
<td>734,214</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model [3]b</td>
<td>1,130</td>
<td>1,009</td>
<td></td>
<td></td>
<td>3,434</td>
<td>1,295</td>
<td></td>
</tr>
<tr>
<td>Model [4]c</td>
<td>1,179</td>
<td>989</td>
<td>1</td>
<td>-14</td>
<td>3,448</td>
<td>1,293</td>
<td></td>
</tr>
<tr>
<td>Model [4b]d</td>
<td>1,130</td>
<td>992</td>
<td>1</td>
<td></td>
<td>3,423</td>
<td>1,299</td>
<td></td>
</tr>
<tr>
<td>FAT, %²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model [3]b</td>
<td>.0944</td>
<td>.0242</td>
<td></td>
<td></td>
<td>.1554</td>
<td>.0368</td>
<td></td>
</tr>
<tr>
<td>Model [4]c</td>
<td>.0863</td>
<td>.0223</td>
<td>.0101</td>
<td>-.0004</td>
<td>.1551</td>
<td>.0369</td>
<td></td>
</tr>
<tr>
<td>Model [4b]d</td>
<td>.0846</td>
<td>.0234</td>
<td>.0098</td>
<td></td>
<td>.1546</td>
<td>.0368</td>
<td></td>
</tr>
</tbody>
</table>

MEMILK - mature equivalent milk yield (2x-305-ME).
Model [3] includes a and PE.
Model [4] includes a, PE, M, and Cov(a,M).
Model [4b] includes a, PE, and M.
MEFAT - mature equivalent fat yield (2x-305-ME).
Table 7. Parameter estimates with or without maternal genetic effects

<table>
<thead>
<tr>
<th>Trait</th>
<th>Model</th>
<th>Animal $h^2$</th>
<th>Permanent Environment $c^2$</th>
<th>Maternal Genetic $m^2$</th>
<th>Gov.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model [4b]</td>
<td>25.39</td>
<td>31.97</td>
<td>.00</td>
<td></td>
</tr>
<tr>
<td>MEFAT®</td>
<td>Model [3]</td>
<td>32.90</td>
<td>29.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Model [4]</td>
<td>34.20</td>
<td>28.68</td>
<td>.04</td>
<td>-.41</td>
</tr>
<tr>
<td></td>
<td>Model [4b]</td>
<td>33.01</td>
<td>28.99</td>
<td>.04</td>
<td></td>
</tr>
<tr>
<td>FAT, %</td>
<td>Model [3]</td>
<td>60.80</td>
<td>15.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Model [4b]</td>
<td>54.70</td>
<td>15.16</td>
<td>6.36</td>
<td></td>
</tr>
</tbody>
</table>

MEMILK = mature equivalent milk yield (2x-305-ME).
Model [3] includes a and PE.
Model [4] includes a, PE, M, and Cov(a,M).
Model [4b] includes a, PE, and M.
MEFAT = mature equivalent fat yield (2x-305-ME).

Schutz et al., 1990). Heritability of fat percentage was slightly higher than recent reports (Schutz et al., 1990), but similar to the estimate by deJager and Kennedy (1987). Ratios of permanent environmental variance to phenotypic variance are also in Table 7.

Variance estimates from Model [4], which includes maternal genetic effects and covariance between additive animal and maternal genetic effects, are also in Table 6. Ratios of maternal genetic variance ($m^2$) and
covariance (Cov) to phenotypic variance from this model are also in Table 7. Maternal genetic ratio was small for milk and fat yield, but was .065 for fat percentage. Inclusion of maternal genetic and covariance terms in Model [4] decreased the portion of variance previously partitioned to additive genetic effects (Model [3]) for milk and fat percentage. Total phenotypic variance explained by each model was nearly identical. The covariance ratio of .0599 was not readily explained. It may, however be a result of sampling error. Confounding between additive and maternal effects could produce covariance among errors of estimates.

Model [4] was reanalyzed, this time assuming no covariance between additive direct and maternal genetic components. Results of this Model [4b] are also in Tables 6 and 7. Use of likelihood ratio tests (Meyer et al., 1990) showed that covariance terms were not significantly different from zero at a .05 significance level. Log-likelihoods for models including a maternal genetic component were actually smaller than those for the model with only animal and permanent environmental components. Maternal genetic effects and covariances were not important in this study.

Because mtDNA is passed from female to offspring with no segregation, inclusion of maternal lineage effects, indicative of mtDNA, as fixed effects in a mixed model is arguably appropriate. Mitochondrial DNA is exactly duplicated in offspring of the same lineage. Southwood et al. (1989) included cytoplasmic effects as random when reporting the ability of animal models to partition them from maternal genetic effects. Small maternal genetic variance ratios from Model [4] help clarify the question of "whether reported values are true estimates of cytoplasmic variance or
due to random fluctuations of other maternal genetic effects," posed by Southwood et al. (1989). Fixed maternal lineage estimates were very nearly identical under Models [3] or [4] for MEMILK, MEFAT, and fat percentage and further supported the conclusion that maternal lineage effects are not caused by unaccounted nuclear maternal genetic differences, because their inclusion did not change differences among maternal lineage estimates.

Ranges of maternal lineages estimates from model [3] and Model [4] are in Table 8. Ranges for fixed estimates were 1.9, 2.6, and 2.3 phenotypic standard deviations for MEMILK, MEFAT, and fat percentage.

Table 8. Ranges of estimates or solutions of maternal lineage effects from animal model analysis

<table>
<thead>
<tr>
<th>Maternal Lineage Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>MEMILK, kg</td>
</tr>
<tr>
<td>MEFAT, kg</td>
</tr>
<tr>
<td>FAT, %</td>
</tr>
</tbody>
</table>

$\hat{\sigma}_p = (\hat{\sigma}_a^2 + \hat{\sigma}_{PE}^2 + \hat{\sigma}_e^2)^{1/2}$.

$^a$Maternal lineages used the inverse of $\hat{\sigma}_{PE}/\hat{\sigma}_e$ from Table 4.

$^b$Model [3] includes a and PE.

$^c$Model [4] includes a, PE, M, and Cov(a,M).

$^d$MEMILK = mature equivalent milk yield (2x-305-ME).

$^e$MEFAT = mature equivalent fat yield (2x-305-ME).
respectively. Ranges were slightly smaller for MEMILK and fat percentage, but higher for MEFAT than those reported by Bell et al. (1985). When maternal lineages were considered random, ratios of error to maternal lineage variance were the inverse of ratios in Table 4. Random maternal lineage solutions had ranges of .44, .43, and .77 for MEMILK, MEFAT, and fat percentage, respectively.

Tests of significance of maternal lineage effects are in Table 9. For Model [3], with variance ratios estimated from these data, F values were 1.04, 1.08, and 1.38 for MEMILK, MEFAT, and fat percentage, respectively, but significant only for fat percentage. Because all sires were from outside this herd, effects of maternal lineages on production traits also were tested in Model [3], but with variance ratios more typical of values used on a national basis (G. Wiggans, 1990, Personal communication). The F values (Table 9) were somewhat greater, and associated probability values were much smaller. Small changes in F values affect probability values greatly with many degrees of freedom. The F values were influenced appreciably by the use of different variance ratios. Significant maternal lineage effects were observed for MEFAT and fat percentage.

Effects of maternal lineages also were tested according to Westell grouping strategies to better account for genetic similarities among base cows and selected AI sires (Westell et al., 1988) in conjunction with both sets of variance ratios described. Probability levels associated with F values did not differ greatly under either grouping scheme for the same variance ratios. In all instances, maternal lineage effects on fat
Table 9. Tests of significance of maternal lineage effects

<table>
<thead>
<tr>
<th>Variance Ratio Source</th>
<th>DFREML</th>
<th></th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trait</td>
<td>F</td>
<td>P &gt; F</td>
<td>MSE</td>
</tr>
<tr>
<td>Fixed sire groups</td>
<td>MEMILK, kg</td>
<td>1.01</td>
<td>.447</td>
</tr>
<tr>
<td></td>
<td>MEFAT, kg</td>
<td>1.08</td>
<td>.325</td>
</tr>
<tr>
<td></td>
<td>FAT, %</td>
<td>1.38</td>
<td>.039</td>
</tr>
<tr>
<td>Westell groups</td>
<td>MEMILK, kg</td>
<td>.94</td>
<td>.597</td>
</tr>
<tr>
<td></td>
<td>MEFAT, kg</td>
<td>1.03</td>
<td>.416</td>
</tr>
<tr>
<td></td>
<td>FAT, %</td>
<td>1.48</td>
<td>.015</td>
</tr>
</tbody>
</table>

aDegrees of freedom for maternal lineages = 52.
bMSE - Mean squared error.
cResidual degrees of freedom = 1829.
dResidual degrees of freedom = 1845.
eMEMILK - mature equivalent milk yield (2x-305-ME).
fMEFAT - mature equivalent fat yield (2x-305-ME).
percentage were significant. When variance ratios like those from national evaluations, were used MEFAT also was influenced significantly by maternal lineages.

Because fat is the component containing the most energy in milk and was significantly influenced by maternal lineages, that are considered indicative of mtDNA, perhaps effects of maternal lineage are exhibited through differences in efficiencies of conversion of precursors to milk fat by the cow. Lactose and protein are also energy containing components in milk, but only information for solids-not-fat (SNF) was complete for this study. Milk net energy, as reported in Table 10, was calculated according to Tyrrell and Reid (1965) as follows:

\[
\text{Net Energy} = 41.84(\text{Fat \%}) + 22.29(\text{SNF \%}) - 25.58.
\]

Lactation net energy was calculated by multiplying net energy by MEMILK yield.

Variance components of random effects in Model [3] for SNF, SNF percentage, milk energy, and lactation energy are in Table 10. Heritability of SNF was smaller than MEFAT, but heritability of SNF percentage was nearly the same as for fat percentage. The ratio of permanent environmental to phenotypic variance was much smaller for SNF percentage than for fat percentage. Variance ratios for net energy in milk were nearly identical to those for fat percentage, perhaps because fat percentage receives the highest weight in calculation of milk net energy.
Table 10. Variance estimates of solids-not-fat and calculated energy in milk.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Animal (a)</th>
<th>Permanent Environment (PE)</th>
<th>Phenotype (P)</th>
<th>Error (e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNF, kg^2</td>
<td>4390 (23.09)</td>
<td>6425 (33.79)</td>
<td>19,011</td>
<td>8196</td>
</tr>
<tr>
<td>SNF, %</td>
<td>.0618 (58.51)</td>
<td>.0037 (3.53)</td>
<td>.1056</td>
<td>.0401</td>
</tr>
<tr>
<td>Energy, (kcal/kg)^2</td>
<td>1308 (60.82)</td>
<td>347 (16.12)</td>
<td>2,150</td>
<td>496</td>
</tr>
<tr>
<td>Lactation energy, (Mcal x 10)^2</td>
<td>3307 (26.75)</td>
<td>3948 (31.94)</td>
<td>12,361</td>
<td>5105</td>
</tr>
</tbody>
</table>

^Model [3] includes a and PE.
^Ratios to phenotypic variance in parentheses.

Possibly for a similar reason, variance ratios for lactation net energy were similar to those for MEMILK.

Table 11 shows ranges of maternal lineage estimates. These ranges were 2.1, 1.6, 2.6, and 2.5 phenotypic standard deviations for SNF, SNF percentage, net energy, and lactation net energy of milk, respectively. The F statistics and associated probability levels are also in Table 11. Maternal lineages significantly affected energy concentration in milk from this herd of dairy cattle.
Table 11. Ranges of maternal lineage estimates and tests of significance for effects on solids-not-fat and calculated energy in milk from Model [3]a,b,c

<table>
<thead>
<tr>
<th>Trait</th>
<th>Ranged</th>
<th>$\sigma_P$</th>
<th>$F$</th>
<th>$P &gt; F$</th>
<th>MSEe</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNF, kg</td>
<td>290</td>
<td>138</td>
<td>1.00</td>
<td>.475</td>
<td>8,190</td>
</tr>
<tr>
<td>SNF, %</td>
<td>.524</td>
<td>.325</td>
<td>1.02</td>
<td>.444</td>
<td>.040</td>
</tr>
<tr>
<td>Energy, (kcal/kg)</td>
<td>119</td>
<td>46</td>
<td>1.42</td>
<td>.028</td>
<td>501</td>
</tr>
<tr>
<td>Lactation energy, (Mcal x 10)</td>
<td>273</td>
<td>111</td>
<td>1.03</td>
<td>.423</td>
<td>5,091</td>
</tr>
</tbody>
</table>

a. $\sigma_P = (\sigma_a^2 + \sigma_{PE}^2 + \sigma_e^2)^{1/2}$.

b. Model [3] includes a and PE.

c. 52 degrees of freedom for maternal lineages, and 1845 degrees of freedom for residual.

d. Range of maternal lineage estimates.

e. Mean squared error.
CONCLUSIONS

Maternal lineage effects, considered indicative of cytoplasmic inheritance that is likely related to mtDNA, were significant for fat percentage and net energy of milk, and to a lesser extent, for MEFAT yield. Ranges of maternal lineage estimates were 2.3, 2.6, and 2.6 phenotypic standard deviations for fat percentage, MEFAT yield, and calculated net energy of milk, respectively. Maternal lineages did not significantly affect MEMILK, SNF or SNF percentage, or lactational energy of milk. Because these traits are composites of lactose, proteins, and minerals, future work to examine these milk constituents separately is warranted.

Unaccounted maternal genetic effects or their covariance with additive animal effects did not seem important as an explanation for maternal lineage effects. Variance components for maternal genetic and covariance terms were not significantly different from zero. Animal models with variance components only for animal and permanent environment were most appropriate for this analysis. Models with complete additive relationships should eliminate most concerns that maternal lineage effects could be caused by spurious additive genetic effects.

Several possible consequences of maternal lineage effects on traits of economic importance are foreseen. Because mitochondria are transferred to offspring via mother only, there has been no exploitation of potential gains from selection of more efficient genotypes. First, maternal lineage differences could be employed in embryo transfer programs to choose donor and recipient females to produce replacement heifers. Second, adjustment for maternal lineage when selecting potential bull mothers could increase
the accuracy of predicting a son's breeding value. Finally, current cloning techniques in dairy cattle involve transfer of cells to enucleated ova, without regard to cytoplasmic content. Potential exists for increasing performance by evaluating ova from nuclear-genetically inferior females from superior maternal lineages.
ACKNOWLEDGMENTS

Appreciation is expressed to National Association of Animal Breeders and 21st Century Genetics Cooperative for partial financial support. K. Meyer is acknowledged for kindly providing computer programs. Helpful comments from D. A. Harville and B. W. Kennedy are greatly appreciated.
REFERENCES


When considering the importance of an effect in a linear mixed model, one often wishes to test a null hypothesis such as

\[ H_0: \beta_{M1} - \beta_{M2} - \beta_{M3} - \cdots - \beta_{Mn}, \]

where \( \beta_i \)'s correspond to \( m \) levels of \( M \), or the effect of interest. An example of mixed model equations in this form could be:

\[
\begin{bmatrix}
X'X & X'M & X'Z \\
X'M' & X'M' & X'M'Z \\
Z'X & Z'M & Z'Z + G
\end{bmatrix}
\begin{bmatrix}
\hat{\beta} \\
\hat{\beta}_M \\
\hat{u}
\end{bmatrix}
= \begin{bmatrix}
X'Y \\
X'M'Y \\
Z'Y
\end{bmatrix},
\]

where \( X_M \) is a known incidence matrix of the fixed effect of interest, \( X \) is a known incidence matrix of other fixed effects, \( Z \) is a known incidence matrix of random effects, \( Y \) is a known vector of dependent variables, \( \hat{\beta}_M \) is an unknown vector of estimates of levels of the effect of interest, \( \hat{\beta} \) is an unknown vector of estimates of other fixed effects, \( \hat{u} \) is a vector of unknown solutions of random effects, and \( G \) is the variance of \( u \) and is assumed known.

When equations are few enough to permit obtaining an inverse or generalized inverse of the coefficient matrix, conducting a statistical test of significance is straightforward. Define a matrix of all independent linear pairwise comparisons, each an estimable function of the
effect of interest. An example is:

\[ H = \begin{bmatrix}
1 & 1 & 1 & \cdots & 1 \\
-1 & 0 & 0 & \cdots & 0 \\
0 & -1 & 0 & \cdots & 0 \\
0 & 0 & -1 & \cdots & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
0 & 0 & 0 & \cdots & -1
\end{bmatrix} \]

\( H \) is an \( m \times (m-1) \) matrix for a fixed effect of interest having \( m \) levels. Graybill (1976) defined the test criterion for this hypothesis test as

\[
W = \frac{(H'\hat{\beta}_M)' [H'G_MH]^{-1} (H'\hat{\beta})}{N - r(X)} \cdot \frac{\text{m - 1}}{
(Y'Y - \hat{\beta}_X'Y - \hat{\beta}_M'Y - \hat{\beta}_Z'Y)
}
\]

where \( W \) has an \( F \) distribution with \( m - 1 \) and \( N - r(x) \) degrees of freedom, \( G_M \) is the portion of the inverse (or generalized inverse) of the coefficient matrix corresponding to \( M \), the effect of interest, \( N \) is the number of observations, \( r(X) \) is the rank of the fixed effects portion of the coefficient matrix, and \( m \) is the number of levels of \( M \).

As discussed by Takahashi (1989), obtaining the generalized inverse of a coefficient matrix for large order mixed model equations, such as animal models with several random effects, is often computationally
impossible. Frequently, these large sets of equations are solved iteratively, and, hence, no generalized inverse is obtained. Henderson (1974) proposed a method to obtain the variance of an estimable function iteratively, using the same coefficient matrix as do the mixed model equations, but with the coefficient vector of a linear estimable function as the right-hand side. Harville (1979) termed these equations the mixed model conjugate normal equations. These may be depicted as

\[
\begin{bmatrix}
X'X & X'X_n & X'Z \\
X_n'X & X_n'X_n & X_n'Z \\
Z'X & Z'X_n & Z'Z + G
\end{bmatrix}
\begin{bmatrix}
\hat{\Phi} \\
\hat{\Phi}_n \\
\hat{\Phi}
\end{bmatrix}
= \begin{bmatrix}
0 \\
H \\
0
\end{bmatrix}.
\]

The coefficient matrix is as previously defined. \(\hat{\Phi}_n\) is the \(m \times (m-1)\) portion of solutions to the mixed model conjugate normal equations pertaining to the effect of interest, \(\hat{\Phi}\) represents the remainder of solutions, \(H\) is as previously defined for the stated null hypothesis and is a series of \(m-1\) independent linear estimable functions.

When an inverse or a generalized inverse of the coefficient matrix is computationally feasible, \(\text{var}(H'\hat{\Phi}_M)\) is estimated by \([H'G_MH]^{-1}\sigma^2_e\). Harville (1979) demonstrated, however, that \(\text{var}(H'\hat{\Phi}_M)\) can be estimated by \(\hat{\Phi}_M'H\sigma^2_e\) from iterative solutions to mixed model conjugate normal equations. Hence,
\[ W^* = \frac{(H'\hat{\beta}_M)'(\hat{\phi}_M'H)^{-1}(H'\hat{\beta})}{N - r(X)} \]

\[ (Y'Y - \hat{\beta}X'Y - \hat{\beta}_M'X'M'Y - \hat{\mu}Z'Y) \]

where \( W^* \) has an \( F \) distribution with \( m - 1 \) and \( N - r(X) \) degrees of freedom. Provided that the number of levels, \( m \), in the effect of interest, \( M \), is not too large, \( (\hat{\phi}_M'H)^{-1} \) can be easily obtained after the mixed model conjugate normal equations are solved iteratively.
SECTION II. ASSOCIATION OF BOVINE MITOCHONDRIAL DNA WITH TRAITS OF ECONOMIC IMPORTANCE
Maternal lineage effects, likely indicative of mitochondrial DNA (mtDNA) inheritance, have been previously shown for production and reproduction in dairy cattle (Bos taurus). Sequence variation of mtDNA is known to exist in the bovine. Displacement-loop sequence polymorphisms of bovine mtDNA were associated with milk production, reproduction, and health costs incurred by dairy cattle. One base-pair (bp) substitution was associated with additional production of 842 kg milk and 37 kg milk fat per cow per lactation. Another bp substitution was associated with a decrease of 36 days and one breeding between successive calvings. Effects of this size are economically important and have broad implications in genetic selection of dairy cattle.
Dairy cattle breeders have made remarkable and consistent progress improving milk production. The current estimate of annual genetic gain is 123 kg milk per cow per lactation (Powell, 1991). Such gains have been accomplished by using Mendelian principals and statistical methodology to estimate breeding values of superior individuals. The genetic increase in production has resulted almost entirely from use of additive genetic variation of nuclear origin.

Several studies have demonstrated the existence of cytoplasmic lineage influences on measures of production and reproduction in dairy cattle. Inheritance of mitochondrial DNA (mtDNA) is indicated by these results, because mitochondria are transmitted only from female parents to ensuing offspring (Gyllensten et al., 1985). Limited biparental inheritance has been reported in Mytilus (mussels), but not in mammals (Hoeh, 1991). Analyses of 4,461 cows representing 102 maternal lineages revealed that 2.0, 1.8, and 3.5% of phenotypic variation in milk yield, milk fat yield, and percentage of fat in milk, respectively, was explained by cytoplasmic inheritance (Bell et al., 1985). Other studies have shown even higher percentages of phenotypic variation in milk and milk component production to be attributable to maternal lineages (Huizinga et al., 1986; Schutz et al., 1991).

Milk volume and percentage of milk fat and milk protein (two constituents of milk, along with lactose and minerals) are economically the most important traits of dairy cattle. Reproduction and health of
cattle, however, merit attention as well, because the interval between successive calvings and health costs also determine profitability of dairy cows. Effects of cytoplasmic inheritance on reproductive measures have been shown for number of days open (days from calving to next conception), days from calving to first detected estrus, first service conception rate, and number of services (Bell et al., 1985; Faust et al., 1990; Huizinga et al., 1986).

Mitochondrial lineage influences on health differences of cattle have not been examined. Much work associating human diseases to mitochondrial DNA (mtDNA) sequence differences has been reported (Merrill and Harrington, 1985). Kearns-Sayres Syndrome (KSS) and Leber’s hereditary optic neuropathy (LHON) are examples of such diseases (Grivell, 1989; Wallace, 1989). In fact, LHON has been shown to be correlated with a single guanine-adenine transition which converts an arginine to a histidine in NADH dehydrogenase subunit 4 gene of mtDNA (Wallace, 1989). Such mtDNA sequence substitutions may affect health in cattle.

Molecular variation in bovine mtDNA has been demonstrated through RFLP analysis (Brown et al., 1989; Koehler et al., 1991; Watanabe, 1985) and comparison of nucleotide sequences (Olivo et al., 1983). Displacement loop (D-loop) sequences of mtDNA from 36 distinct registered maternal lineages available for this study were previously compared (Lindberg, 1989). Fifty-one sequence differences were located, including 48 single base pair (bp) substitutions, one 9 bp deletion, and two variable length poly G-C runs. Where possible, D-loops from two or more animals of the same maternal lineage were sequenced to verify accuracy of
mtDNA isolation and nucleotide sequencing and to confirm constancy of mtDNA within maternal lineages (Lindberg, 1989).

The D-loop region of mtDNA does not code for any known gene products, hence sequence polymorphisms there would not alter specific metabolic chain subunits. Promoters for transcription of both heavy and light strands of mtDNA as well as the origin of heavy strand replication, however, lie within the D-loop. Thus, sequence differences in the mtDNA D-loop may alter transcription or replication rates. Moreover such D-loop polymorphisms may mark differences elsewhere on the mtDNA genome in coding regions that are associated with phenotypic expression of traits.

Cows in this study were from a selection experiment founded at Iowa State University in 1968. Heifers for this herd were purchased from 38 Holstein breeders throughout Iowa to keep the herd as genetically broad-based as possible. Cows were bred artificially to sires from commercial artificial insemination organizations, allowing a continuous influx of nuclear genes. Frequencies of bovine lymphocyte antigen phenotypes were similar to frequencies in the U.S. Holstein population, meaning that these nuclear genes are likely representative of the entire U.S. Holstein population (Weigel et al., 1990). Females were assigned to groups and artificially mated to bulls with either high or average estimated additive genetic transmitting ability for milk yield. Females born in each group were mated to new bulls chosen for that group, thus forming divergent selection lines that differed by 1308 kg of milk per cow per lactation when these data were analyzed.
Ancestral pedigrees of registered foundation females in the herd were tracked backward through the Holstein-Friesian Herd Book (Wales, 1885). Eighty-one distinct maternal lineages were defined by convergence of maternal pedigrees after 1885. It is possible that these lineages would have been found to converge to fewer lineages had registration records been kept prior to importation of these cows from Europe in about 1885.

Thirty-six maternal lineages had surviving members in the herd when samples for nucleotide sequencing were collected. These lineages had from one to six purchased foundation females in the herd. Nucleotide sequence polymorphism data were obtained and all cows within the same maternal lineage that were ever in the herd were assumed to have identical mtDNA.

Table 1 has location, type, and frequency of the 17 most common sequence polymorphisms of the mtDNA D-loop in this herd of Holstein dairy cattle. Only those polymorphisms occurring in at least 4 percent of cows in the herd are listed, since information on markers occurring in a very small number of cows would not be statistically informative. Transitions at bp 169 and 216 occurred in 80 and 84 percent of cattle, respectively. The probable explanation is that the cow originally sequenced (Anderson et al., 1982) had the rarer genotype at those two sites. From 30 to 608 cows were polymorphic at individual bp sites of the least and most frequent mtDNA D-loop sequence difference, respectively.

Production records of all cows in the 36 maternal lineages with known mtDNA D-loop sequences were considered. Milk and fat yield
Table 1. Location, type, and frequency of seventeen most common sequence polymorphisms of mtDNA D-loops in a herd of dairy cattle

<table>
<thead>
<tr>
<th>Location in D-loop</th>
<th>Polymorphic event</th>
<th>Frequency</th>
<th>Location in D-loop</th>
<th>Polymorphic event</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>G-A</td>
<td>.07</td>
<td>16058</td>
<td>C-T</td>
<td>.12</td>
</tr>
<tr>
<td>106</td>
<td>T-C</td>
<td>.14</td>
<td>16074</td>
<td>T-C</td>
<td>.07</td>
</tr>
<tr>
<td>169</td>
<td>A-G</td>
<td>.80</td>
<td>16085</td>
<td>T-C</td>
<td>.05</td>
</tr>
<tr>
<td>216</td>
<td>Var. length</td>
<td>.84</td>
<td>16111</td>
<td>A-C*</td>
<td>.04</td>
</tr>
<tr>
<td></td>
<td>G-C run</td>
<td></td>
<td>16113</td>
<td>T-C</td>
<td>.11</td>
</tr>
<tr>
<td>363</td>
<td>C-G*</td>
<td>.46</td>
<td>16141</td>
<td>T-C</td>
<td>.11</td>
</tr>
<tr>
<td>16022</td>
<td>G-A</td>
<td>.14</td>
<td>16230</td>
<td>C-T</td>
<td>.06</td>
</tr>
<tr>
<td>16049</td>
<td>C-T</td>
<td>.08</td>
<td>16231</td>
<td>C-T</td>
<td>.12</td>
</tr>
<tr>
<td>16057</td>
<td>G-A</td>
<td>.12</td>
<td>16247</td>
<td>C-T</td>
<td>.13</td>
</tr>
</tbody>
</table>

*Location is defined by the first published mtDNA sequence (Anderson et al., 1982). Polymorphic event and frequency is also with regard to that sequence.

*Base pair substitution is a transversion.

records adjusted to a uniform age and lactation length, or mature equivalent (ME), basis were obtained. Percentages in milk of fat and solids-not-fat (SNF) which is total solids in milk less fat in milk, were known for each record of each cow. Up to seven production records were used for individual cows.
Because mitochondria play an extensive role in energy metabolism, mtDNA polymorphism may alter energy content in milk. Fat, protein, and lactose are the carriers of energy in milk; however, information was complete since 1968 only for fat and SNF, which combines protein, lactose, and minerals. Net energy concentration in milk, which is based on fat and SNF (Tyrrell and Reid, 1965), was calculated in terms of kilocalories per kilogram and multiplied by lactation milk yield to approximate lactation energy production in terms of megacalories.

To evaluate the effect of mtDNA D-loop sequence polymorphism, each cow was assigned a value of 1 if polymorphic or 0 if not polymorphic with respect to the first published mtDNA sequence (Anderson et al., 1982) at each of the 17 locations considered. Each production trait was analyzed individually with the following animal model (Henderson, 1984; Westell et al., 1989):

\[ Y_{ijkn} = \mu + YS_i + P_j + X_k + \beta_1 + ... + \beta_{17} + PE_n + a_n + e_p \]

where \( Y_{ijkn} \) is the milk, fat, fat percentage, SNF, SNF percentage, energy concentration, or lactation energy record; \( \mu \) is an overall mean; \( YS_i \) is the effect common to all cows calving in year-season \( i \); \( P_j \) is the effect common to all cows in parity \( j \); \( X_k \) is the effect common to cows in either the high or average selection line; \( \beta_1 \) to \( \beta_{17} \) are the binomial regressions of production record on mtDNA D-loop sequence polymorphisms; \( PE_n \) is permanent environmental effect common to all records of cow \( n \); \( a_n \) is the effect of animal \( n \) and is composed of the additive genetic
contribution of sire and dam breeding values and a Mendelian sampling effect; and $e$ is a random residual.

Regression of production traits on mtDNA D-loop polymorphism was of primary interest. Effects of the overall mean, year-season of calving, parity, and selection line were treated as fixed effects in the mixed model to account for explainable environmental background. Additive genetic covariances among related individuals were incorporated in this model (Henderson, 1984; Westell et al., 1988). Permanent environment and additive genetic effects were treated as random and have properties of Best Linear Unbiased Prediction (BLUP) (Henderson, 1984). Random effects were assumed to be normally and independently distributed with mean expectations of zero. Variance among permanent environments was assumed to be $\text{var}(\text{PE}) = I\sigma_{PE}^2/\sigma_e^2$, where $I$ is an identity matrix and $\sigma_e^2$ is error variance. Variance among animals was assumed to be $\text{var}(a) = A\sigma_a^2/\sigma_e^2$, where $A$ is the numerator relationship matrix. A included sires and dams of all cows back to foundation cows and included information for sires and paternal grandsires of bulls with daughters in the herd. Regression coefficients for sequence polymorphism are Best Linear Unbiased Estimates and were obtained by iterative methods described elsewhere (Schutz et al., 1991).

A total of 1800 records of 728 cows were used in this study. Along with effects of 17 mtDNA locations, there were 33 year-season; 7 parity; 2 selection line, 728 permanent environment, and 950 animal effects. Animal, or additive genetic, effects were for 728 cows with records, 197 relatives without records, and 25 phantom parent groups (Westell et al.,...
Table 2 has overall means of production traits along with their regressions on nucleotide sequence differences. The polymorphism at base pair (bp) 363 has previously been associated with milk and fat yield and fat percentage (Brown et al., 1989). This site has since been shown to be highly heterogeneous within maternal lineages (Koehler, et al., 1991), and is therefore unstable for use as a marker. Regressions of production on the polymorphism at bp 363 will not be reported because the sequence difference can not be assumed uniform within maternal lineages.

Eleven nucleotide sequence polymorphisms significantly influenced at least one production trait and traits were influenced in both positive and negative directions. A single Adenine to Guanine transition at bp 169 related to increased production of 482 kg of milk, 24 kg of fat, 51 kg of SNF, and 456 megacalories of energy per cow per lactation. On a purely evolutionary basis, site 169 previously has been found to demarcate two distinct mitochondrial families of cattle in the Holstein population (Lindberg, 1989).

Polymorphism at site 16074 had a large positive effect on milk, fat, and SNF yield and lactation energy, while polymorphism at site 16231 had a negative effect on the same traits. Fat percentage in milk and energy concentration of milk were significantly affected in cows with polymorphism at sites 16058, 16085, 16230, and 16247. Effects of polymorphism on SNF percentage were small. Possibly, larger effects would have been
Table 2. Regression of production on sequence polymorphisms in the mtDNA D-loop and overall production means in a herd of dairy cattle

<table>
<thead>
<tr>
<th>Location in D-loop</th>
<th>Milk (kg)</th>
<th>Fat (kg)</th>
<th>SNF (kg)</th>
<th>Lactation Energy (Mcal)</th>
<th>Fat (%)</th>
<th>SNF (%)</th>
<th>Energy Concentration (Kcal/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>235</td>
<td>1</td>
<td>19</td>
<td>85</td>
<td>-.21</td>
<td>-.04</td>
<td>-21</td>
</tr>
<tr>
<td>106</td>
<td>464</td>
<td>16</td>
<td>47</td>
<td>343</td>
<td>-.03</td>
<td>.08</td>
<td>2</td>
</tr>
<tr>
<td>169</td>
<td>482*</td>
<td>24**</td>
<td>51*</td>
<td>456*</td>
<td>.05</td>
<td>.05</td>
<td>6</td>
</tr>
<tr>
<td>216</td>
<td>-157</td>
<td>1</td>
<td>-7</td>
<td>-10</td>
<td>.07</td>
<td>.09†</td>
<td>10</td>
</tr>
<tr>
<td>16022</td>
<td>-113</td>
<td>-6</td>
<td>-26</td>
<td>-162</td>
<td>.04</td>
<td>.01</td>
<td>3</td>
</tr>
<tr>
<td>16049</td>
<td>989†</td>
<td>29</td>
<td>102†</td>
<td>756</td>
<td>.07</td>
<td>.17</td>
<td>1</td>
</tr>
<tr>
<td>16057</td>
<td>-577†</td>
<td>-21</td>
<td>-48</td>
<td>-395</td>
<td>.01</td>
<td>.11</td>
<td>7</td>
</tr>
<tr>
<td>16058</td>
<td>39</td>
<td>20</td>
<td>9</td>
<td>225</td>
<td>.30*</td>
<td>.06</td>
<td>31†</td>
</tr>
<tr>
<td>16074</td>
<td>842*</td>
<td>37**</td>
<td>85*</td>
<td>749**</td>
<td>.14</td>
<td>.05</td>
<td>16</td>
</tr>
<tr>
<td>16085</td>
<td>-197</td>
<td>-20</td>
<td>-36</td>
<td>-343</td>
<td>-.21†</td>
<td>-.19†</td>
<td>-29†</td>
</tr>
<tr>
<td>16111</td>
<td>107</td>
<td>-5</td>
<td>15</td>
<td>52</td>
<td>-.05</td>
<td>.05</td>
<td>-3</td>
</tr>
<tr>
<td>16113</td>
<td>32</td>
<td>3</td>
<td>-10</td>
<td>3</td>
<td>.09</td>
<td>-.21†</td>
<td>-3</td>
</tr>
<tr>
<td>16141</td>
<td>-336</td>
<td>-8</td>
<td>-35</td>
<td>-198</td>
<td>.10</td>
<td>-.05</td>
<td>6</td>
</tr>
<tr>
<td>16230</td>
<td>383</td>
<td>33</td>
<td>39</td>
<td>490</td>
<td>.39**</td>
<td>.06</td>
<td>39*</td>
</tr>
<tr>
<td>16231</td>
<td>-650†</td>
<td>-28</td>
<td>-61†</td>
<td>-522†</td>
<td>-.12</td>
<td>-.01</td>
<td>-12</td>
</tr>
<tr>
<td>16247</td>
<td>351</td>
<td>21</td>
<td>32</td>
<td>346</td>
<td>.17*</td>
<td>-.01</td>
<td>16†</td>
</tr>
<tr>
<td>Overall Mean†</td>
<td>8085</td>
<td>288</td>
<td>745</td>
<td>5888</td>
<td>3.63</td>
<td>9.21</td>
<td>732</td>
</tr>
</tbody>
</table>

*aOverall standard deviations are in parentheses.
**P > t ≤ .01.
*P > t ≤ .05.
†P > t ≤ .10.
observed for lactose and protein if data for those components were available. Effects as large as those found are certainly economically important.

An overall test of significance was used to determine whether effects of D-loop polymorphisms were specific for individual locations. That the effect of a D-loop sequence polymorphism being present differs among bp sites was tested versus the null hypothesis that the effect of the presence of a polymorphism at one site equals the effect at any other site. Effects of presence of D-loop polymorphism at different sites were significant for milk fat yield ($P > F \leq .09$) and fat percentage ($P > F \leq .06$). Smallest significance levels of individual regressions of production on polymorphisms (Table 2) were also for milk fat yield and fat percentage.

Reproductive and health traits also have economic importance in dairy cattle improvement programs. Table 3 has regressions of reproduction and health costs on sequence polymorphisms in the mtDNA D-loop. Number of days open and number of breedings (artificial inseminations), along with reproductive costs, were the measures of reproduction considered. Reproductive costs included costs of insemination and semen, as well as costs of reproductive exams and treatments for post-calving disorders such as metritis and retained placenta.

In addition to reproductive costs, health differences were measured by mammary costs and total health costs. Using costs of health disorders was necessary because incidences of specific events or diseases occur too infrequently to be of use in analysis of data from a single herd. Health
Table 3. Regression of reproduction and health costs on sequence polymorphisms in the mtDNA D-loop and overall means of reproduction and health cost means in a herd of dairy cattle

<table>
<thead>
<tr>
<th>Location in D-loop</th>
<th>Days open (d)</th>
<th>Number of Breedings (n)</th>
<th>Reproduction Costs ($)</th>
<th>Mammary Costs ($)</th>
<th>Total Health Costs ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>-31.7</td>
<td>-.73</td>
<td>-6.12</td>
<td>24.79†</td>
<td>10.22</td>
</tr>
<tr>
<td>106</td>
<td>0.5</td>
<td>.13</td>
<td>-2.66</td>
<td>16.24†</td>
<td>21.28†</td>
</tr>
<tr>
<td>169</td>
<td>14.6†</td>
<td>.34</td>
<td>6.05†</td>
<td>.84</td>
<td>11.47</td>
</tr>
<tr>
<td>216</td>
<td>-0.5</td>
<td>.12</td>
<td>.07</td>
<td>-9.70†</td>
<td>-14.54†</td>
</tr>
<tr>
<td>16022</td>
<td>-13.4</td>
<td>-.12</td>
<td>-3.89</td>
<td>9.77</td>
<td>1.83</td>
</tr>
<tr>
<td>16049</td>
<td>16.1</td>
<td>.28</td>
<td>.88</td>
<td>4.58</td>
<td>17.94</td>
</tr>
<tr>
<td>16057</td>
<td>-9.7</td>
<td>.14</td>
<td>-2.90</td>
<td>-9.31</td>
<td>-15.93</td>
</tr>
<tr>
<td>16058</td>
<td>28.3†</td>
<td>.68</td>
<td>2.44</td>
<td>2.94</td>
<td>18.44</td>
</tr>
<tr>
<td>16074</td>
<td>3.7</td>
<td>.34</td>
<td>6.28</td>
<td>13.56</td>
<td>25.93†</td>
</tr>
<tr>
<td>16085</td>
<td>-36.3*</td>
<td>-.99*</td>
<td>-12.82†</td>
<td>-2.31</td>
<td>-19.70</td>
</tr>
<tr>
<td>16111</td>
<td>-14.1</td>
<td>-.64</td>
<td>-4.06</td>
<td>-8.94</td>
<td>-11.19</td>
</tr>
<tr>
<td>16113</td>
<td>-5.5</td>
<td>-.63</td>
<td>-1.64</td>
<td>1.18</td>
<td>-2.24</td>
</tr>
<tr>
<td>16141</td>
<td>-11.7</td>
<td>-.36</td>
<td>-3.01</td>
<td>-15.67†</td>
<td>-19.58</td>
</tr>
<tr>
<td>16230</td>
<td>-4.4</td>
<td>-.02</td>
<td>-2.04</td>
<td>13.10</td>
<td>12.12</td>
</tr>
<tr>
<td>16231</td>
<td>-16.0</td>
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<td>-3.53</td>
<td>-4.81</td>
<td>-11.30</td>
</tr>
<tr>
<td>16247</td>
<td>-8.2</td>
<td>.02</td>
<td>2.47</td>
<td>3.19</td>
<td>7.71</td>
</tr>
<tr>
<td>Overall mean</td>
<td>135</td>
<td>2.61</td>
<td>38.73</td>
<td>19.07</td>
<td>77.67</td>
</tr>
</tbody>
</table>

Overall standard deviations are in parentheses.

*P > t ≤ .05.
†P > t ≤ .10.
costs were grouped by body systems and total health cost is the sum of reproductive, digestive, mammary, respiratory, and skin and skeletal costs. Mammary costs reflected costs of treatment and medications required for cows with mastitis or injured teats, but discarded milk value was not included.

Polymorphism at bp 169 was associated with days open, and reproductive costs and days open were increased in animals polymorphic at bp 16058. There was a very large favorable impact on the reproductive complex in cows with mtDNA D-loop sequence difference at bp 16085. This single T to C transition was related to a decrease of 36 days open, one insemination, and $12.82 in reproductive costs. Thirty-six days open is nearly 2 reproductive cycles.

Mammary costs were altered in cows with mtDNA D-loop sequence polymorphisms at four locations. None were locations significantly affecting milk or milk fat yield or fat percentage. Total health costs were increased when polymorphisms at bp 106 and 16074 occurred, and decreased when polymorphisms at bp 216 occurred. Significant effects were not observed for costs of digestive, respiratory, and skin skeletal cost categories. Incidences of health disorders in these systems were sparse.

Current dairy cattle breeding programs are centered around selection of bulls used for artificial insemination. Two stage selection is practiced for bulls entering artificial insemination organizations. The first stage is pedigree selection, based on sire and dam information. Bulls are finally chosen for extensive use based on a progeny test. The
largest selection differentials are for the sire to bull pathway (VanTassell and Van Vleck, 1991) where mtDNA polymorphism is not important if the mitochondrial genome is transmitted only from female parents. The dam to bull pathway is equally important, but the accuracy of selection is less than at the sire to bull pathway. A bull’s estimated transmitting ability based on pedigree may be biased if the contribution from his dam is not adjusted for mitochondrial influence on her records. While the bull would acquire mtDNA from the dam, it would not be transmitted to his offspring. Adjustment of bull dam’s records for mtDNA influences would allow more accurate prediction of expected genetic contribution of a bull to his daughters.

The dam to cow pathway has traditionally been selected least intensely (VanTassell and Van Vleck, 1991). However, new developments in reproductive technology and embryo manipulation seem poised to make this pathway of selection more viable. Differences in mtDNA could be incorporated into embryo transfer breeding programs to better choose donor and recipient females to produce replacement heifers. Current cloning techniques require nuclear transplantation into an enucleated ovum without regard to cytoplasmic content. Potential exists for using mtDNA sequence polymorphism to identify ova of females with inferior nuclear genetics in superior mtDNA background as candidates for enucleation and subsequent introduction of nuclei with greater genetic potential.
ACKNOWLEDGMENTS

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REFERENCES


SECTION III. GROUPING OF MATERNAL LINEAGES BY MITOCHONDRIAL DNA GENOTYPES
Maternal lineage groups defined by several methods of classification using mitochondrial DNA sequence characteristics were evaluated with animal models. Cattle were from a selection experiment begun in 1968. Maternal pedigrees were traced to the first female member in the Holstein-Friesian Herd Book, and foundation females were assigned to maternal lineages. Mitochondrial DNA displacement-loop sequence data was available for 36 lineages, and all cows within lineages were considered identical for useful DNA sequence polymorphisms. Base pair substitution (Adenine to Guanine) at nucleotide 169 defined two maternal lineage groups and significantly accounted for increased milk fat and estimated milk energy production. Clustering the 36 maternal lineages using 16 mitochondrial DNA displacement-loop sequence differences produced groups with significant influence on fat percentage and energy concentration. Reducing the number of clustered groups from 24 to 14 produced larger $F$ statistics, but significant outcome was for the same traits. Genotype groups defined by sequence and restriction fragment length differences as reported by Lindberg et al. (1991) did not significantly affect production by the cows in the 29 maternal lineages divided in that way.
INTRODUCTION

Several recent reports have suggested the existence of maternal lineage effects on production (Bell et al., 1985; Faust et al., 1990; Huizinga et al., 1986; Schutz et al., 1991) and reproduction (Bell et al., 1985; Faust et al., 1989; Nibler et al., 1989; Schutz et al., 1991b). Authors concluded that results are likely indicative of cytoplasmic inheritance. Mitochondrial DNA (mtDNA) is a probable source of such cytoplasmic inheritance, because in mammals mitochondria are only known to be transmitted from female parents to ensuing offspring (Gyllensten et al., 1985), although partial biparental inheritance has been established in mussels (Hoeh et al., 1991).

Some reports have discredited the existence of cytoplasmic inheritance using simulated data (Kennedy et al., 1986) or analysis of daughter-dam-grandam trios (Reed and Van Vleck, 1987). Nevertheless, the ability to partition maternal influences into cytoplasmic and additive genetic components by use of well-defined animal models has been demonstrated (Southwood et al., 1989). Southwood et al. (1989) used simulated data and true or incorrect models containing additive direct, additive maternal, cytoplasmic, and error variances, and concluded that certain animal models correctly partition these components of variance. Schutz et al. (1991) found additive maternal variation of nuclear origin to be negligible for production traits of dairy cattle.

Mitochondrial DNA sequence is known to differ among dairy cattle (Laipis et al., 1988). Koehler (1989) used restriction enzymes to detect
11 polymorphisms among maternal lineages, and two additional polymorphisms occurred within lineages. One polymorphism within a single lineage appeared to result from a single mutational event, but the other occurred within several lineages and is considered heteroplasmic with regard to lineages. No other such sites of heteroplasmy have been documented (Koehler, 1991). Johnston et al. (1991) found ten nucleotide substitutions by restriction analysis of five breeds of dairy cattle. Lindberg et al. (1991) sequenced entire mtDNA displacement-loops (D-loops) and identified 48 sites of nucleotide substitution plus one deletion, and two variable-length regions among 36 maternal lineages. Johnston (1991) observed 11 different sequences in ribosomal RNA subunits of mtDNA in 38 lineages of Holsteins.

Studies associating maternal lineages with production traits have defined maternal lineage sources as foundation cows in the herd studied (Bell et al., 1985; Huizinga et al., 1986; Nibler et al., 1989) or as maternal lineage matriarchs found by tracing maternal pedigrees to the beginning of a herd book (Faust et al., 1990; Schutz et al., 1991). Defining maternal lineages this way has several disadvantages. In either definition of maternal lineages, females in multiple lineages may have a single common maternal ancestor prior to earliest recorded pedigree information. Also, misidentification of maternal ancestor or erroneously recorded registration numbers leads to incorrect assignment to lineages. Most commonly, branches of single maternal pedigrees are inadvertently defined as separate maternal lineages.
Lindberg et al. (1991) demonstrated the potential for 27 restriction endonuclease target sites in the bovine mtDNA D-loop region to be used to describe Holstein cytoplasmic genotypes. Single base pair substitutions in bovine mtDNA D-loops have been associated with production differences in Holsteins (Brown et al., 1989; Schutz et al., 1991). The objective of this study was to define maternal lineages according to mtDNA molecular characterization and to associate maternal lineages, so defined, with production traits in a herd of Holsteins.
MATERIALS AND METHODS

Cattle studied were part of a selection experiment begun at Iowa State University's Breeding Research Herd in 1968. Foundation females were mated to Holstein AI sires with high or average transmitting abilities for milk to form two divergent genetic lines. Records initiated through 1988 were included in analyses.

Genetic backgrounds of original foundation females in the herd were quite diverse; however, frequencies of Bovine Lymphocyte Antigen genotypes were similar to those in the U.S. Holstein population (Weigel et al., 1990), strongly suggesting that nuclear genes are representative of that population. Cows were bred artificially to sires from commercial artificial insemination organizations, allowing continuous introgression of nuclear genes.

The herd was assembled through purchases of 158 foundation females acquired from 38 Holstein breeders located throughout Iowa. But because maternal heritage was determined by tracing maternal lineage to the first female member recorded in the Holstein-Friesian Herd Book (Wales, 1885), the 133 registered females were assigned to 81 separate maternal lineages. Thirty-six registered lineages had members remaining in the herd for restriction and sequence analysis of the mtDNA genome. There were 71 foundation females in these 36 lineages and they were, on average, 19 generations removed from their matriarchs first registered in the Herd Book. Lineages were represented by from 1 to 6 foundation females in the herd for lineages with the fewest or most foundation females, respectively.
Mature equivalent (2X-305-ME), milk (MEMILK), and fat (MEFAT) and fat percentage, as well as SNF yield and SNF percentage, were production traits available for analysis. Milk net energy has been shown to be affected by maternal lineages (Schutz et al., 1991). Milk net energy concentration, in terms of Kcal/kg, was calculated using lactation average of test day fat and SNF percentages according to Tyrrell and Reid (1965) as follows:

\[ \text{Net Energy} = 41.84 \times (\text{Fat} \%) + 22.29 \times (\text{SNF} \%) - 25.58. \]

Lactation net energy was calculated by multiplying net energy concentration by MEMILK yield. Lactation information for protein and lactose was not complete. Production records were assigned to year-season of calving subclasses. Seasons were from October to April and May to September to account for winter and summer feeding and management differences. Up to seven records per cow were included for analyses.

Base pair 169

Lindberg (1989) used mtDNA D-loop nucleotide sequence data from these 28 maternal lineages to construct a phylogenetic tree by parsimony analysis. His analysis generated a two-part evolutionary tree based on division of the population at ntl69. Nucleotide information for this study was available for cows from 36 maternal lineages. All members of a single maternal lineage were assumed to have identical mtDNA genotype with respect to ntl69. Lineages were assigned values of 0 or 1 if they
were identical or polymorphic, respectively, at ntl69 compared to the first published mtDNA sequence (Anderson et al., 1982). Effect of this binomial data on each production trait was individually analyzed using the following mixed animal model:

\[ Y_{ijknp} = \mu + YS_i + P_j + X_k + \beta_1(ntl69) + PE_n + a_n + e_p, \]  

where \( Y_{ijknp} \) are 1800 milk, fat, fat percentage, SNF, SNF percentage, energy concentration, or lactation energy records; \( \mu \) is an overall mean; \( YS_i \) is the effect common to all cows calving in year-season \( i \) (\( i = 1 \) to 33); \( P_j \) is the effect common to all cows in parity \( j \) (\( j = 1 \) to 7); \( X_k \) is the effect common to cows in either the high or average selection line; \( \beta_1 \) is the binomial regression of production record on mtDNA D-loop sequence polymorphism at ntl69; \( PE_n \) is the permanent environmental effect common to all records of cow \( n \) (\( n = 1 \) to 728); \( a_n \) is the additive genetic value of cow \( n \) and is composed of sire and dam breeding values and a Mendelian sampling effect; and \( e_p \) is residual error.

Overall mean, year-season, parity, selection line, and binomial regression on polymorphism at ntl69 were considered fixed effects. Permanent environment, animal additive genetic, and residual effects were considered random, and independently distributed with expectations equal to zero. Variance among permanent environments was \( \text{var}(PE) = I\sigma_{PE}^2 / \sigma_e^2 \), where \( \sigma_e^2 \) is the residual variance and \( I \) is an identity matrix. Variance
among animals was \( \text{var}(a) = A \sigma_a^2 / \sigma_e^2 \), where \( A \) is the numerator relationship matrix. \( A \) included sires and dams of all cows with records in the herd and sire and paternal grandsire relationships among artificial insemination bulls represented by daughters with records. \( A \) also included unknown parent groups, giving 950 animal equations. Estimates of variances were obtained from Schutz et al. (1991).

Regression of production on nucleotide substitution at nt169 was of primary interest. Significance of this substitution effect was tested by solution of mixed model conjugate normal equations (Harville, 1979, Henderson, 1974). Such tests of significance are exact if estimates of variances are assumed to be true.

**Maternal Lineage Clusters**

The same 36 maternal lineages were grouped by clustering techniques using mtDNA D-loop sequence information. The 17 most frequent sequence substitution sites in maternal lineages in this herd were described by Schutz et al. (1991). One of those sites, nt363, was found to be hypervariable and, therefore, not useful for clustering of lineages. For each of the remaining sixteen nucleotide positions, maternal lineages are given a value of 0 or 1, respectively, if identical or polymorphic with respect to the first published bovine mtDNA sequence (Anderson et al., 1982). Each lineage had a total of 16 values (0 or 1) and lineages with the same polymorphisms were identical in their combination of 0s and 1s.
A matrix of distances between maternal lineages based on binomial sequence data was computed as \([1 - \text{Jaccard coefficient}]\) for each pair of lineages. Jaccard coefficients are measures of similarity, calculated by:

\[
\text{Jaccard coefficient}(i,j) = \frac{n_1}{n_1 + n_2 + n_3},
\]

where

- \(n_1\) = number of nucleotide sites polymorphic for maternal lineages i and j,
- \(n_2\) = number of nucleotide sites polymorphic only for maternal lineage i,
- \(n_3\) = number of nucleotide sites polymorphic only for maternal lineage j (Jacquard, 1974).

Number of nucleotide sites not polymorphic in either maternal lineage does not enter the equation because this occurrence would be far more common, and thus inclusion would decrease the impact of polymorphic sites in determining similarities of distances between lineages. Thus, a matrix of distances was created that was of the order of the number of maternal lineages. The matrix was symmetric with zeros on diagonals and \([1 - \text{Jaccard coefficients}]\) on the off diagonals. Off diagonals for two maternal lineages with identical polymorphisms were also zero.

Based on the matrix of pairwise distances, maternal lineages were organized into most homogeneous groups possible by average-linked cluster analysis (Sneath and Sokal, 1973). Average linkage clustering of groups
is based on average distance between pairs of observations, one in each
group and comparing all possible comparisons of each member of one group
with each member of the group being compared. This clustering method
tends to form clusters with small variances and avoids extreme results
inherent to other methods which consider only the nearest or farthest
members from each group compared.

Two lineages were non-polymorphic at all sixteen nucleotide sites.
These lineages were joined to form a single cluster, but were not so
assigned by average linkage analysis, which only joined clusters based on
polymorphic nucleotide sites. The 36 lineages were grouped into maternal
lineage clusters of either 24 groups (Clustering 1) or 14 groups (Clus-
tering 2) for separate analyses. The term "Clustering" is used instead
of cluster to avoid confusion with the individual clustered groups. With
cluster analysis any number of clusters may be chosen. Clustering into
24 groups was the first for which the normalized distance between groups
joined was greater than zero. Clustering into 14 groups was somewhat
arbitrarily chosen as the smallest number for which the joined groups had
normalized distances of less than .6.

Clusterings of maternal lineages were analyzed with the following
animal model:

\[ Y_{ijkmnp} = \mu + Y_{i} + P_{j} + X_{k} + MLC_{m} + PE_{n} + a_{n} + e_{np} \]  [2]

where all effects are as previously defined, except MLC_{m} is the fixed
effect of maternal lineage cluster group m (m = 1 to 24 for Clustering 1
and \( m = 1 \) to \( 14 \) for Clustering 2). Assumptions, and expectations and variances of random effects are as previously defined. Residuals obtained with either Clustering 1 or Clustering 2 in this model were examined with a model consisting of actual maternal lineages to compare the ability of the two clusterings to account for maternal lineage differences.

Sequence genotypes

Lindberg et al. (1991) reported the ability of RFLP and sequence data accurately described the mitochondrial genotypes of bovine cytoplasmic lineages. In Table 2 of their report (Lindberg et al., 1991), 29 lineages are assigned to 21 genotypes according to restriction enzyme and sequence analyses. Sequence information was obtained on 2 or more members of each lineage, and sequence differences were confirmed, where detectable by commercially available restriction enzymes. Of the 29 lineages, 28 were registered and included in the ntl69 and clustering analyses in this study. One lineage was not registered and maternal ancestry could not be verified through registry information; however, cows in this lineage were included in this portion of this study because genotype as defined by mtDNA D-loop sequence was known (Lindberg et al., 1991).

The model employed to examine effects of mtDNA genotypes on production traits was:

\[
Y_{ijkmnp} = \mu + YS_i + P_j + X_k + G_m + PE_n + a_n + e_p, \tag{3}
\]
where all effects, again, are as previously defined, except $G_m$ is the fixed effect of mtDNA genotype $m$ ($m = 1$ to 21). Numbers of fixed effect classes remained the same, yet because the number of lineages was fewer, there were 1407 records of 572 cows ($PE_n, n = 572$) and 787 animal equations.
RESULTS AND DISCUSSION

Base pair 169

Overall means and standard deviations of records of all cows in the available 36 maternal lineages are in Table 1. Of the 728 cows in these lineages, 583, or 80.1 percent, of the cows were polymorphic at mtDNA D-loop ntl69 with respect to the first published sequence (Anderson et al., 1982). Probably, that first cow had the sequence which was less common compared to the entire Holstein population. These 583 cows had 1468 records versus 332 records for 145 cows identical to the reference sequence.

Table 1. Means and standard deviations of production traits for records by cows in 36 maternal lineages

<table>
<thead>
<tr>
<th>Production trait</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEMILK, kg</td>
<td>8085</td>
<td>1771</td>
</tr>
<tr>
<td>MEFAT, kg</td>
<td>288</td>
<td>60</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.63</td>
<td>0.44</td>
</tr>
<tr>
<td>Solids-Not-Fat, kg</td>
<td>745</td>
<td>165</td>
</tr>
<tr>
<td>Solids-Not-Fat, %</td>
<td>9.21</td>
<td>0.40</td>
</tr>
<tr>
<td>Energy Concentration, Kcal/kg</td>
<td>732</td>
<td>50</td>
</tr>
<tr>
<td>Lactation Energy, Mcal</td>
<td>5888</td>
<td>1225</td>
</tr>
</tbody>
</table>
Table 2 has regressions of production traits on binomial ntl69 sequence data. Highly significant effects of having the Adenine to Guanine transition on heavy strand mtDNA at ntl69 on MEFAT, fat percentage, and energy concentration were detected ($P > t < .01$). The effect on lactation energy concentration was also significant ($P > t < .05$).

Previous reports, based on cytoplasmic lineages, have suggested a larger impact on fat and energy than on milk (Bell et al., 1985; Schutz et al., 1991). Schutz et al. (1991b) also reported a more significant impact on fat yield than on milk yield when the effect of an Adenine to Guanine transition at ntl69 was analyzed concurrently with other mtDNA D-loop sequence differences.

### Table 2. Regression of production traits on mtDNA

<table>
<thead>
<tr>
<th>Production trait</th>
<th>Binomial Regression Coefficient</th>
<th>$t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEMILK, kg</td>
<td>349</td>
<td>0.96</td>
</tr>
<tr>
<td>MEFAT, kg</td>
<td>39</td>
<td>2.77**</td>
</tr>
<tr>
<td>Fat, %</td>
<td>0.16</td>
<td>3.30**</td>
</tr>
<tr>
<td>Solids-Not-Fat, kg</td>
<td>49</td>
<td>1.48</td>
</tr>
<tr>
<td>Solids-Not-Fat, %</td>
<td>0.06</td>
<td>1.56</td>
</tr>
<tr>
<td>Energy Concentration, kcal/kg</td>
<td>8</td>
<td>3.05**</td>
</tr>
<tr>
<td>Lactation Energy, Mcal</td>
<td>277</td>
<td>2.30*</td>
</tr>
</tbody>
</table>

*$P > t \leq .05$.

**$P > t \leq .001$.
Lindberg et al. (1989) determined that sequence polymorphism at nt169 marked an evolutionarily important bifurcation in phylogenetic trees based on mtDNA sequence information. Such distinct lineages may have evolved while geographically separated. Further, separation may have been accompanied by differences in artificial selection potentially leading to significant effects on production like those observed.

No known gene products are coded by the D-loop of mtDNA. However, the D-loop is a site of important transcriptional and replicational control. Differences in production associated with sequence polymorphism in that region of mtDNA may relate to control of mtDNA function, or may serve as markers for important sequence variation elsewhere in the genome, which is inherited in its entirety. Therefore, mtDNA D-loop polymorphism may become established in artificially selected populations if affected traits depend on control of transcription and replication of mtDNA. Alternatively, sequence variants may, by chance alone, become fixed in subpopulations with differences in mtDNA gene coding regions.

Maternal Lineage Clusters

Data for analysis of maternal lineage clusters was the same as for the previous analysis (Table 1). Assignment of lineages to cluster groups are in Table 3 along with number of cows in each cluster group. Lineages in a cluster group are separated by double spaces. With 24 cluster groups only a single cluster (maternal lineages 14 and 18) had members with differing genotypes. All other clusters were exactly identical with respect to these 16 nucleotides.
<table>
<thead>
<tr>
<th>Maternal Lineage</th>
<th>Binomial Sequence Codes</th>
<th>Maternal Lineage</th>
<th>Binomial Sequence Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 (47)</td>
<td>0001000000000000</td>
<td>20 (47)</td>
<td>0001000000000000</td>
</tr>
<tr>
<td>29</td>
<td>0001000000000000</td>
<td>29</td>
<td>0001000000000000</td>
</tr>
<tr>
<td>3 (133)</td>
<td>0011000000000000</td>
<td>2 (203)</td>
<td>001100000000000001</td>
</tr>
<tr>
<td>32</td>
<td>0011000000000000</td>
<td>3</td>
<td>001100000000000000</td>
</tr>
<tr>
<td>33</td>
<td>0011000000000000</td>
<td>32</td>
<td>001100000000000000</td>
</tr>
<tr>
<td>41</td>
<td>0011000000000000</td>
<td>33</td>
<td>001100000000000000</td>
</tr>
<tr>
<td>51</td>
<td>0011000000000000</td>
<td>37</td>
<td>00110000000000000011</td>
</tr>
<tr>
<td>61</td>
<td>0011000000000000</td>
<td>40</td>
<td>00110000000000000001</td>
</tr>
<tr>
<td>77</td>
<td>0011000000000000</td>
<td>41</td>
<td>001100000000000000</td>
</tr>
<tr>
<td>2 (45)</td>
<td>001100000000000001</td>
<td>51</td>
<td>001100000000000000</td>
</tr>
<tr>
<td>40</td>
<td>0011000000000000</td>
<td>58</td>
<td>00110000000000000011</td>
</tr>
<tr>
<td>17 (42)</td>
<td>0010000000000000</td>
<td>61</td>
<td>001100000000000000</td>
</tr>
<tr>
<td>52</td>
<td>0010000000000000</td>
<td>77</td>
<td>001100000000000000</td>
</tr>
<tr>
<td>37 (25)</td>
<td>00110000000000000011</td>
<td>17 (42)</td>
<td>001000000000000000</td>
</tr>
<tr>
<td>58</td>
<td>00110000000000000011</td>
<td>52</td>
<td>001000000000000000</td>
</tr>
<tr>
<td>14 (43)</td>
<td>0111001000001000</td>
<td>13 (99)</td>
<td>011100000000000000</td>
</tr>
<tr>
<td>18</td>
<td>0111000000001000</td>
<td>14 (53)</td>
<td>01110000000000001000</td>
</tr>
<tr>
<td>10 (30)</td>
<td>1011000100001000</td>
<td>18 (53)</td>
<td>01110000000000001000</td>
</tr>
<tr>
<td>55 (23)</td>
<td>1011000100000000</td>
<td>66</td>
<td>01110000000000001000</td>
</tr>
<tr>
<td>13 (9)</td>
<td>0111000000000000</td>
<td>10 (53)</td>
<td>1011000100001000</td>
</tr>
</tbody>
</table>

aDouble spaces divide cluster groups within a clustering.

bNumber of cows in cluster groups is in parentheses.

cBinomial sequence codes are for nucleotides 8, 106, 169, 216, 16022, 16049, 16057, 16058, 16074, 167085, 16111, 16113, 16141, 16230, 16231, and 16247, respectively, and Is correspond to polymorphic sites.
Table 3. Continued

<table>
<thead>
<tr>
<th>Clustering 1</th>
<th>Clustering 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Lineage</td>
<td>Binomial Sequence Codes</td>
</tr>
<tr>
<td>66 (47)</td>
<td>0111000000001000</td>
</tr>
<tr>
<td>8 (15)</td>
<td>0011100000000000</td>
</tr>
<tr>
<td>67 (30)</td>
<td>0011100000100000</td>
</tr>
<tr>
<td>5 (12)</td>
<td>0000110000000000</td>
</tr>
<tr>
<td>57 (9)</td>
<td>0001110000000000</td>
</tr>
<tr>
<td>39 (19)</td>
<td>0001000010000010</td>
</tr>
<tr>
<td>74 (7)</td>
<td>0001000010000000</td>
</tr>
<tr>
<td>45 (9)</td>
<td>0011001100000000</td>
</tr>
<tr>
<td>60 (22)</td>
<td>0011000101000000</td>
</tr>
<tr>
<td>69 (15)</td>
<td>0001000011000000</td>
</tr>
<tr>
<td>4 (7)</td>
<td>0010000010000000</td>
</tr>
<tr>
<td>75 (45)</td>
<td>0011000000000110</td>
</tr>
<tr>
<td>71 (35)</td>
<td>0011111000010000</td>
</tr>
<tr>
<td>22 (23)</td>
<td>0010000100000001</td>
</tr>
<tr>
<td>35 (36)</td>
<td>0000000000000000</td>
</tr>
<tr>
<td>46</td>
<td>0000000000000000</td>
</tr>
</tbody>
</table>
With 14 cluster groups, members obviously had more diverse genotypes, but maternal lineages within a single cluster never differed at more than 3 base pair sites and that occurred in only one cluster (maternal lineages 13, 14, 18, and 66). Clustering proved successful in grouping maternal lineages with similar genotypes.

Twenty-four groups in Clustering 1 had from 17 records of 7 cows to 311 records of 133 cows in the smallest and largest groups, respectively. Clustering 1 averaged 30.3 cows and 75 records per group, and Clustering 2 averaged 52 cows and 128.6 records per group. Clustering 2, with 14 groups, had from 19 records of 7 cows to 509 records of 203 cows. Significance tests of the effect of cluster groups on production traits and ranges of cluster group estimates are in Table 4. Clustering 1 had a significant \( \left( P > F \leq .05 \right) \) effect on fat percentage and energy concentration. An effect of further clustering is to increase the among-group variability. Combining maternal lineages into 14 groups increased \( F \) statistics for all traits, although Clustering 2 had statistically significant effects on the same traits. Ranges of estimates were larger for Clustering 1. The \( F \) statistics and probability levels were in line with those previously reported for maternal lineage effects (Schutz et al., 1991). Not surprisingly, however, Clustering 1 was in somewhat better agreement to maternal lineage results because combining fewer lineages than with Clustering 2 was obviously more analogous to that analysis.

Coefficients of determination \( (R^2) \) were from .806 and .802, respectively, for fat percentage and energy concentration for model [2] with
Table 4. Tests of significance of maternal lineage clusters on production traits and ranges of estimates

<table>
<thead>
<tr>
<th>Production trait</th>
<th>Clustering 1</th>
<th></th>
<th>Clustering 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P &gt; F</td>
<td>Range</td>
<td>F</td>
</tr>
<tr>
<td>MEMILK, kg</td>
<td>.93</td>
<td>.549</td>
<td>1402</td>
<td>1.03</td>
</tr>
<tr>
<td>MEFAT, kg</td>
<td>1.15</td>
<td>.282</td>
<td>75</td>
<td>1.33</td>
</tr>
<tr>
<td>FAT, %</td>
<td>1.68</td>
<td>.023</td>
<td>.64</td>
<td>2.44</td>
</tr>
<tr>
<td>Solids-Not-Fat, kg</td>
<td>.98</td>
<td>.490</td>
<td>135</td>
<td>1.08</td>
</tr>
<tr>
<td>Solids-Not-Fat, %</td>
<td>1.23</td>
<td>.208</td>
<td>.46</td>
<td>1.00</td>
</tr>
<tr>
<td>Energy Concentration, Kcal/kg</td>
<td>1.58</td>
<td>.041</td>
<td>81</td>
<td>2.19</td>
</tr>
<tr>
<td>Lactation Energy, Mcal</td>
<td>1.07</td>
<td>.375</td>
<td>1245</td>
<td>1.21</td>
</tr>
</tbody>
</table>

a36 maternal lineages were clustered into 24 groups for clustering 1 and 14 groups for clustering 2.

bRange of maternal lineages.

Clustering 1. $R^2$ was only slightly lower for each trait with Clustering 2 than with Clustering 1, being .805 for fat percentage and .801 for net energy concentration. Analysis of residuals from the model with either Clustering 1 or Clustering 2 with a model including only maternal lineage demonstrated the ability of clustered lineages to account for the underlying maternal lineage effects. Maternal lineage effects on residuals could still have been appreciable if not accounted for by clusterings. Even with clustering of 36 lineages into 14 groups, statistical effects of maternal lineages on residuals were negligible ($P > F \leq 1.00$). Maternal lineages did, however, account for about twice as much residual variation with Clustering 2 as with Clustering 1. Again,
this result is expected since Clustering 1 is more analogous to maternal lineages as originally defined.

Sequence Genotypes

The purpose of this analysis was to determine if economic traits can be associated with lineages assigned to homogeneous groups by sequence and restriction enzyme analysis. Overall means and standard deviations of records by cows in the 29 maternal lineages assigned to 21 genotypes by Lindberg et al. (1991) are in Table 5. Means and standard deviations are similar to those of the 36 lineages from the prior analyses. The 21 genotypes had from 17 records of 7 cows to 197 records of 70 cows for the smallest and largest genotype groups, respectively. Genotypes on average had 67 records and 27.2 cows.

Table 5. Overall means and standard deviations of production traits for records by cows of 21 mtDNA genotypes

<table>
<thead>
<tr>
<th>Production trait</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEMILK, kg</td>
<td>7975</td>
<td>1760</td>
</tr>
<tr>
<td>MEFAT, kg</td>
<td>286</td>
<td>59</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.65</td>
<td>0.44</td>
</tr>
<tr>
<td>Solids-Not-Fat, kg</td>
<td>736</td>
<td>164</td>
</tr>
<tr>
<td>Solids-Not-Fat, %</td>
<td>9.23</td>
<td>0.39</td>
</tr>
<tr>
<td>Energy Concentration, Kcal/kg</td>
<td>734</td>
<td>49</td>
</tr>
<tr>
<td>Lactation Energy, Mcal</td>
<td>5830</td>
<td>1215</td>
</tr>
</tbody>
</table>
Table 6 has tests of significance of mtDNA genotypes on production traits. Genotype was significant ($P > F < .085$) for energy concentration, but was not significant at $P > F < .10$ for any other trait. The $F$ statistics were as high or nearly as high as those from testing maternal lineages in a previous report by Schutz et al. (1991). But associated probability levels were much higher because of fewer numerator and denominator degrees of freedom. Ranges of estimates tended to be smaller than for cluster analysis. For model [3], $R^2$ values were .816 for fat percentage and .807 for net energy concentration. These values were somewhat higher than for either clustering under model [2]. Comparisons must be taken with some caution since data were not identical.

Table 6. Tests of significance of mtDNA genotypes on production traits and ranges of estimates

<table>
<thead>
<tr>
<th>Production trait</th>
<th>$F$</th>
<th>$P &gt; F$</th>
<th>Range$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEMILK, kg</td>
<td>.99</td>
<td>.465</td>
<td>1334</td>
</tr>
<tr>
<td>MEFAT, kg</td>
<td>.87</td>
<td>.628</td>
<td>51</td>
</tr>
<tr>
<td>Fat, %</td>
<td>1.42</td>
<td>.105</td>
<td>.38</td>
</tr>
<tr>
<td>Solids-Not-Fat, kg</td>
<td>.95</td>
<td>.528</td>
<td>125</td>
</tr>
<tr>
<td>Solids-Not-Fat, %</td>
<td>1.01</td>
<td>.453</td>
<td>.36</td>
</tr>
<tr>
<td>Energy Concentration, Kcal/kg</td>
<td>1.46</td>
<td>.085</td>
<td>76</td>
</tr>
<tr>
<td>Lactation Energy, Mcal</td>
<td>.90</td>
<td>.584</td>
<td>1016</td>
</tr>
</tbody>
</table>

$^a$Range of genotype group estimates.
CONCLUSIONS

The intent of this work was not so much to determine optimal methods of grouping cows based on mtDNA differences but to examine the effects on production of previously proposed grouping methods. The significance of A to G transition at ntl69 in the mtDNA D-loop on fat and energy produced by dairy cattle may have evolutionary implications as proposed by Lindberg et al. (1991). It also indicates the benefit of mixed models in associating potentially important mtDNA sequence polymorphisms. Effects of groups used to more accurately define maternal lineages by clustering on D-loop sequence polymorphism or by mtDNA genotype identification were most important for fat percentage and energy concentration. The magnitude of these effects was similar to that of maternal lineage effects previously reported (Schutz et al., 1991). Optimal definition of mitochondrial lineages will also incorporate sequence variation in gene coding regions of mtDNA, where mitochondrial effects more likely originate. Further research may determine mechanisms by which mtDNA sequence variation affects precise assignment of cows into maternal groups with similar mitochondrial genetic value.
ACKNOWLEDGMENTS

Appreciation is expressed to National Association of Animal Breeders and Eastern Artificial Insemination Cooperative for partial financial support. Funding was also provided by United States-Israel Binational Agricultural Research and Development grant number US-1519-88R.
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GENERAL SUMMARY

Maternal lineage effects, considered to represent mitochondrial DNA (mtDNA) differences, were significant for fat percentage and net energy of milk, and to a lesser extent, for MEFAT yield. Maternal lineages did not significantly affect solids-not-fat yield or percentage. These findings were from models that accounted for additive genetic relationships, reducing the likelihood that random drift of nuclear genes could account for significant maternal lineage effects. Additive nuclear maternal effects were not important as an alternative explanation for differences between maternal lineages.

Several base-pair substitutions in the bovine mtDNA displacement-loop region were significantly associated with increases and decreases in production of milk and its components. One base-pair transition marked a very large favorable impact on reproductive measures of days open, number of breedings, and reproductive costs. Mitochondrial DNA sequence data also were used in several ways to define groups of maternal lineages which had important effects of fat percentage and net energy content of milk.

Important implications of this work are foreseen. Because mitochondria are transmitted to offspring by only their mother, there has been little intentional exploitation of possible gains from selection of more efficient mtDNA genotypes. This has been especially true in the dairy industry in which genetic gain has been predominantly through sire selection. If part of a bull dam's genetic superiority is from mito-
chondrial differences, the bull's predicted genetic value would be overestimated because mtDNA would not be transmitted to his offspring. The opposite would hold if the dam was genetically inferior. Identification of differences in mitochondrial lineages would allow more accurate pedigree prediction of the additive genetic value of bulls, especially for fat percentage.

The advance of reproductive technologies opens further avenues for advantageous use of mtDNA differences. Maternal lineage differences could be employed in embryo transfer programs to choose donor and recipient females to produce replacement heifers. Donors would be from lineages whose mtDNA favorably influences traits of economic importance. Also, cloning techniques in dairy cattle involve transfer of cells to enucleated ova, currently without regard to cytoplasmic content. Possibility exists for increasing performance of dairy cattle by enucleating ova from nuclear-genetically inferior females from superior maternal lineages and introducing nuclei from the best additive genetic sources. Identification of maternal lineages through pedigree information would be extremely difficult on a population basis, because pedigrees usually are not on electronic media beyond a few generations. But more exact characterization of cows into lineages may come from DNA fingerprinting or sequence analysis of the mitochondrial genome.

The field of mitochondrial genetics in cattle is relatively young and many questions remain. To this point, research has involved primarily institutional and research herds. Findings need to be corroborated in other herds and using field data if possible. Effects of
mitochondrial genotypes on growth and conformation traits may be revealing, because growth, like milk and milk fat production, is very energy intensive.

All records of a single cow are influenced by her additive genetics, permanent environment, maternal lineage and non-additive genetic effects (additive by additive, dominance, and epistasis). Simultaneous evaluation of more than two variance components at one time is computationally demanding. In the near future, advances in computation and methodology will allow estimation of the genetic component of variance from maternal lineages simultaneously with other random components.

Work is also needed to gain further knowledge about the mode of action of mtDNA sequence differences. Detection of base pair polymorphism has concentrated on the mtDNA displacement-loop. Of even more interest now would be detection of sequence variants in the gene coding regions. Because only 13 out of hundreds of polypeptides involved in mitochondrial processes are coded by mtDNA, interaction between mitochondrial and nuclear DNA is likely to be of great importance. Reproductive technologies like cloning and nuclear transplantation may allow evaluation of effects of different mtDNA genotypes in identical nuclear backgrounds. Specific mtDNA genetic effects on traits of economic importance may soon be useful in selection of dairy cattle.
ACKNOWLEDGMENTS

The author wishes to express his deepest thanks to his advisor, Dr. A. E. Freeman, whose advice, leadership, encouragement, support, challenges, and friendship can not go unnoticed. Having a renowned advisor, who shares his sense of humor and cultivates relationships with graduate students on a professional and personal basis must be a unique opportunity.

The author recognizes Dr. Beitz, Dr. Harville, Dr. Rothschild, and Dr. Willham for serving on his graduate committee. They each shared something that can not be bought even with tuition fees--they shared their own areas of expertise.

The author could not begin to list the many graduate students who have toiled, laughed, complained, joked, played, studied, worked, or in some other way survived the ISU experience with him. Some, however, can not escape mention. Keith B.--what can I say (besides 'Hey Willie, HAA!')? Very few have contributed more to my education. Thanks for your friendship and teaching. Curt V., Mark B., Sally N. and Chris S., your support and friendship have often gone without thanks, but never without appreciation or notice. Bevin H. thanks for putting up with me as an officemate. Don’t worry, Americans aren’t all like this. Bob and Dee congrats on "land"ing the job you set your "mark" on.

The author must recognize Dave Kelley, whose role as animal breeding group and office historian (237 HA!) was critical in allowing education to be acquired from those predecessors who previously walked the second
floor hallway. Dave, thanks for always being available to answer questions, to give advice and encouragement, or even to visit. What was Chaucer's first name again?

The author also thanks his many friends outside the animal breeding group. Family back in Minnesota, the teachers at St. Cecilia, the softball and bowling teams, the friends in Nut. Phys., the ISU dairy club and judging teams, and the rest of the cast of thousands have made these four years speed by.

Lastly, the author thanks his parents, Nick and Regina Schutz, for their unconditional love, support, and encouragement. From you I learned the importance of education and I acquired an interest in agriculture, and dairy cattle in particular. This Ph.D. dissertation is dedicated to both of you.
APPENDIX A
<table>
<thead>
<tr>
<th>Maternal Lineage</th>
<th>Lineage Matriarch</th>
<th>Foundation females in IOSTATE herd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6008 HHB</td>
<td>Akkrummer</td>
</tr>
<tr>
<td>2</td>
<td>7294 HHB</td>
<td>Alpona</td>
</tr>
<tr>
<td>3</td>
<td>959DFHB</td>
<td>Kingsma’s Antje 12th</td>
</tr>
<tr>
<td>4</td>
<td>968DFHB</td>
<td>Aukje Wartena</td>
</tr>
<tr>
<td>5</td>
<td>911WHFA</td>
<td>Plum Creek Beauty</td>
</tr>
<tr>
<td>6</td>
<td>1913DFHB</td>
<td>Marianna Beets 6th</td>
</tr>
<tr>
<td>7</td>
<td>854DFHB</td>
<td>Bekker 3d</td>
</tr>
<tr>
<td>8</td>
<td>465WFHB</td>
<td>Bertha Mercedes</td>
</tr>
<tr>
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APPENDIX B
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Table B1. (continued)

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<th>Line Code</th>
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<th>MEMILK (lbs.)</th>
<th>MEFAT (lbs.)</th>
<th>Fat Pct. (%)</th>
<th>SNF (lbs.)</th>
<th>SNF Energy Pct. (%)</th>
<th>SNF Conc. (Kcal/lb.)</th>
<th>Lactation Energy (Mcal)</th>
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Following are a series of programs to test significance of fixed
effects in large mixed models, and specifically intended for use with
animal models including a numerator relationship matrix. As mentioned in
the internal documentation, the programs use a grouping strategy based on
proxy parents, but could be used with defined sire groups treated as fixed
and the conventional relationship matrix. The first program (AINV) is to
create the relationship matrix from an input pedigree file. The next three
(COEF, ITPACK, and FTEST) create the coefficient matrix for a specified
model, solve by iteration, and use mixed model conjugate normal techniques
to conduct a test of significance for a classes fixed effect. The fifth
program (RSQ) calculates the coefficient of determination \( R^2 \) for the
model. The last three programs (COEFCOV, ITPACK, and TTEST) are analogous
to the previously mention programs, but are for testing a covariable. More
details as well as description of input and output data files are contained
in the internal documentation.
// AINV JOB
// /* JOBPARM DUPLEX=NO,FLASH=NONE,KEEP=YES
// /* STEPO EXEC SCRUNC
// /* SYSIN DD *
// A3$MMS.RELX
// /* S1 EXEC FORTUCLG
// /* FORT.SYSIN DD *

//***************************************************************************
// THIS IS A PROGRAM TO CALCULATE A HALF STORED RELATIONSHIP*
// MATRIX INVERSE BASED ON WESTELL GROUPING AND PROXY *
// OR PHANTOM PARENTS *
// UNIT 10 IS A FILE WITH ANIMAL, SIRE, DAM, AND TYPE. *
// TYPE 1 = BOTH PARENTS KNOWN *
// 2 = ONE PARENT KNOWN *
// 3 = NO PARENTS KNOWN *
// ZEROS ARE NOT EXPECTED FOR SIRE OR DAM IN ANY *
// RECORD *
// ONLY CHANGE IS NA-NUMBER OF ANIMALS AND PHANTOM PARENTS *
// WHICH IS DEFINED IN THE PARAMETER STATEMENT. *
// INPUT *
// UNIT 10 RELATIONSHIP FILE *
// OUTPUT *
// UNIT 90 INVERSE OF RELATIONSHIP FOR *
// COEF PRG. *
// NOTE THAT CONVERSION OF THE PROGRAM TO CALCULATE *
// HENDERSON'S RELATIONSHIP INVERSE INVOLVES ONLY *
// CHANGING THE VALUES ADDED TO DIFFERENT PARTS OF *
// THE MATRIX WHEN PARENTS ARE UNKNOWN. *

IMPLICIT DOUBLE PRECISION (A)

PARAMETER(NA=1001,NAEF=NA*(NA+1)/2)
INTEGER TYPE, TOTAL
REAL*8 AINV
DIMENSION AINV(NAEF)

CALL MNULL(AINV,NAEF,1)
KOUNT1=0
KOUNT2=0
KOUNT3=0

* USE OF THESE COEFFICIENTS ARE BASED ON USING WESTELL GROUPING.
* TO EXCLUDE PHANTOM GROUPS CERTAIN COEFFICIENTS ARE IGNORED,
* DEPENDING ON THE NUMBER OF KNOWN PARENTS, THAT IS O'S ARE
* ADDED TO THE RELATIONSHIP IN CERTAIN PLACES AS IN HENDERSON.

A1=2.DO
A2=-1.DO
A3=1.DO/2.DO
A4=4.DO/3.DO
A5=-2.DO/3.DO
A6=1.DO/3.DO
A7=1.DO
A8=-A3
A9=1.DO/4.DO

N=NA
100 CONTINUE

C READ RELATIONSHIP FILE
READ(10,*,END=199) I, J, K, TYPE
WRITE(6,*) I, J, K, TYPE
IF(TYPE .EQ. 1) THEN
  KOUNT1=KOUNT1+1
  AINV(IHMSSF(I,I,N))=AINV(IHMSSF(I,I,N))+A1
  AINV(IHMSSF(J,J,N))=AINV(IHMSSF(J,J,N))+A3
  AINV(IHMSSF(K,K,N))=AINV(IHMSSF(K,K,N))+A3
  IF(I .LT. J) THEN
    AINV(IHMSSF(I,J,N))=AINV(IHMSSF(I,J,N))+A2
  ELSE
    AINV(IHMSSF(J,I,N))=AINV(IHMSSF(J,I,N))+A2
  END IF
  AINV(IHMSSF(I,K,N))=AINV(IHMSSF(I,K,N))+A2
  AINV(IHMSSF(J,K,N))=AINV(IHMSSF(J,K,N))+A3
ELSE IF(TYPE .EQ. 2) THEN
  KOUNT2=KOUNT2+1
  AINV(IHMSSF(I,I,N))=AINV(IHMSSF(I,I,N))+A4
  AINV(IHMSSF(J,J,N))=AINV(IHMSSF(J,J,N))+A6
  AINV(IHMSSF(K,K,N))=AINV(IHMSSF(K,K,N))+A6
  IF(I .LT. J) THEN
    AINV(IHMSSF(I,J,N))=AINV(IHMSSF(I,J,N))+A5
  ELSE
    AINV(IHMSSF(J,I,N))=AINV(IHMSSF(J,I,N))+A5
  END IF
  AINV(IHMSSF(I,K,N))=AINV(IHMSSF(I,K,N))+A5
  AINV(IHMSSF(J,K,N))=AINV(IHMSSF(J,K,N))+A6
ELSE
KOUNT3-KOUNT3+1
AINV(IHMSSF(I,I,N))=AINV(IHMSSF(I,I,N))+A7
AINV(IHMSSF(J,J,N))=AINV(IHMSSF(J,J,N))+A9
AINV(IHMSSF(K,K,N))=AINV(IHMSSF(K,K,N))+A9

IF(I .LT. J) THEN
   AINV(IHMSSF(I,J,N))=AINV(IHMSSF(I,J,N))+A8
ELSE
   AINV(IHMSSF(J,I,N))=AINV(IHMSSF(J,I,N))+A8
END IF

AINV(IHMSSF(I,K,N))=AINV(IHMSSF(I,K,N))+A8
AINV(IHMSSF(J,K,N))=AINV(IHMSSF(J,K,N))+A9

* WHEN NO PARENTS ARE KNOWN AN EXTRA .25 IS ADDED TO THE DIAGONAL
* TO MAKE IT 1.00 WHEN BOTH PHANTOM PARENTS ARE THE SAME(SIRE=DAM).
* THIS WAS INDEPENDENTLY NOTED BY QUASS AND WESTELL.

IF(J .EQ. K) THEN
   AINV(IHMSSF(K,J,N))=AINV(IHMSSF(K,J,N))+A9
END IF

END IF
GO TO 100
199 CONTINUE

TOTAL-KOUNT1+KOUNT2+KOUNT3
DO 300 I=1,NAEF

C WRITE OUT THE HALF STORED RELATIONSHIP INVERSE
WRITE(90) AINV(I)
C WRITE(6,*) AINV(I)

300 CONTINUE

WRITE(6,*) 'TOTAL ANIMALS EXCLUDING PHANTOM GROUPS = ', TOTAL
WRITE(6,*) KOUNT1, ' WITH BOTH PARENTS KNOWN'
WRITE(6,*) KOUNT2, ' WITH ONE PARENT KNOWN'
WRITE(6,*) KOUNT3, ' WITH NO PARENTS KNOWN'
END

C INTEGER FUNCTION IHMSSF(I,J,N)
C FUNCTION TO WORK OUT ADDRESS IN A HALFSTORED SYMMETRIC MATRIX OF
C ORDER N; CONSIDER THE UPPER TRIANGLE. I-ROW, J-COLUMN
C WRITTEN BY K. MEYER.
C
IF (I .LE. J) THEN
C

SUBROUTINE MNULL(A,N,M)
C ROUTINE TO FILL VECTORS/MATRICES WITH ZEROS
C
C SET ALL ELEMENTS OF MATRIX A, DIMENSION N*M TO ZERO
DIMENSION A(N,M),D(M,N)
DOUBLE PRECISION A
DO 2 I-1,N
DO 2 J-1,M
2 A(I,J)=0.DO
RETURN

ENTRY RMNULL(D,M,N)
C
C SET A REAL *4 MATRIX TO ZERO
DO 6 J-1,N
DO 6 I-1,M
6 D(I,J)=0.
RETURN
END

C ____________________________________________________________
//GO.FT10FO01 DD UNIT=DISK,DSN=A3$MMS.RELFILE,DISP=(OLD,KEEP)
//GO.FT90FO01 DD UNIT=DISK,DSN=A3$MMS.RELX,DISP=(NEW,CATLG),
// SPACE=(6233,(700,100),RLSE),DCB=(RECFM=FB,LRECL=8,BLKSIZEx=6232)
//COEF JOB
//*JOBPARM DUPLEX-NO,FLASH-NONE,KEEP-YES
//STEPO EXEC SCRUNC
//SYSIN DD *
A3$MMS.RHNDS
A3$MMS.YPY
A3$MMS.ITDAT
//S1 EXEC FORTVCLG
//FORT.SYSIN DD *

//*****************************************************************
//*****************************************************************
//* N1-N6 CORRESPOND TO LEVELS FOR EFFECTS 1 TO 6. *
//* NT, THE NUMBER OF MODEL EFFECTS, MUST BE SPECIFIED. *
//* THE NUMBER OF EFFECTS MAY BE CHANGED BY ADDING OR REMOVING *
//* COPIES OF APPROPRIATE LINES OF CODE. *
//* NANIM IS THE EFFECT IN THE MODEL WHICH WILL HAVE THE RELATION- *
//* SHIP MATRIX FROM THE AINV PROGRAM ADDED TO IT. THE REL- *
//* ATIONSHIP INPUT MATRIX IS UNIT 11. *
//* NA MUST BE CHANGED TO ADD THE NUMBER OF LEVELS OF ALL INCLUDED*
//* EFFECTS. *
//* VARIANCE RATIOS MUST BE CHANGED TO CORRESPOND TO TRAIT BEING *
//* ANALYZED. *

INPUT
* 
* 
* UNIT 10 PRODUCTION FILE *
* 
* UNIT 11 RELATIONSHIP INVERSE *
* 
*

OUTPUT
* 
* UNIT 90 COEF MATRIX FOR ITPACK PRG *
* 
* UNIT 91 Y'Y FOR F-TEST PRG *
* 
* UNIT 92 RIGHT-HAND SIDES FOR ITPACK*

//*****************************************************************
//*****************************************************************

PARAMETER(N1-29
* ,N2=7
* ,N3=2
* ,N4=53
* ,N5=764
* ,N6=1001
C NUMBER OF TRAITS
* ,NT=6
* ,NANIM=N6
C

* ,NA-N1+N2+N3+N4+N5+N6, NE=NA*(NA+1)/2)
INTEGER*4 COEF(NE), F(NT), EX(2)
REAL*8 DY(NA), DYPY, DAUG, AINV, DVARAN, Y, YDUM(2)
COMMON /BIG/ COEF, DY
DIMENSION MISS(NT)
DYPY=0
KOUNT=0
C ***************************************************************
C PRIORS FOR VARIANCE RATIOS ARE ADDED HERE. **
C ***************************************************************

DVARPE=1.331059170
DVARAN=1.70177411

C ***************************************************************
C ***************************************************************

KNTPE=O
KNTAINV=O
KNONZER=O

C NUMBER OF K'S MUST BE ONE LESS THAN THE NUMBER OF EFFECTS

K0=0
K1=N1
K2=K1+N2
K3=K2+N3
K4=K3+N4
K5=K4+N5

C SET VECTORS TO ZERO

DO 7 I=1,NT
    MISS(I)=0
7 CONTINUE

DO 9 I=1,NA
    DY(I)=0.DO
9 CONTINUE

DO 11 I=1,NE
    COEF(I)=0
11 CONTINUE

C ***************************************************************
C ***************************************************************
C CHANGE THE READ STATEMENT TO READ THE APPROPRIATE PRODUCTION *
C DATA. THE ORDER OF EFFECTS MAY BE CHANGE BY SWITCHIN F( )'S.*
C A FORMATTED READ COULD EASILY BE USED.*
C
C F(I) CORRESPONDS TO THE Ith EFFECT IN THE MODEL. AND Y IS *
C TRAIT OF INTEREST. DUMMY VARIABLES CAN BE INSERTED TO READ *
C DATA ON THE PRODUCTION FILE THAT IS NOT TO BE INCLUDED IN *
C THE MODEL OR FOR ADDITIONAL DEPENDENT VARIABLES. *
C EXAMPLES ARE EX( ) AND YDUM( ). *
12 READ(10,*,END=199) F(6), F(1), F(2), F(3), F(4), F(5), EX(1)
   *  Y, YDUM(1)

C CALCULATE Y PRIME Y
   DYPY=DYPY+Y*Y
   KOUNT=KOUNT+1

C CHECK FOR MISSING VALUES.
   DO 75 J=1,NT
      IF (F(J) .LE. 0) THEN
         MISS(J)=MISS(J)+1
         GO TO 198
      END IF
   75 CONTINUE

M1=F(1)
M2=F(2)+K1
M3=F(3)+K2
M4=F(4)+K3
M5=F(5)+K4
M6=F(6)+K5

COEF(IHMSSF(M1,M1,NA))=COEF(IHMSSF(M1,M1,NA))+1
COEF(IHMSSF(M1,M2,NA))=COEF(IHMSSF(M1,M2,NA))+1
COEF(IHMSSF(M1,M3,NA))=COEF(IHMSSF(M1,M3,NA))+1
COEF(IHMSSF(M1,M4,NA))=COEF(IHMSSF(M1,M4,NA))+1
COEF(IHMSSF(M1,M5,NA))=COEF(IHMSSF(M1,M5,NA))+1
COEF(IHMSSF(M1,M6,NA))=COEF(IHMSSF(M1,M6,NA))+1

COEF(IHMSSF(M2,M2,NA))=COEF(IHMSSF(M2,M2,NA))+1
COEF(IHMSSF(M2,M3,NA))=COEF(IHMSSF(M2,M3,NA))+1
COEF(IHMSSF(M2,M4,NA))=COEF(IHMSSF(M2,M4,NA))+1
COEF(IHMSSF(M2,M5,NA))=COEF(IHMSSF(M2,M5,NA))+1
COEF(IHMSSF(M2,M6,NA))=COEF(IHMSSF(M2,M6,NA))+1

COEF(IHMSSF(M3,M3,NA))=COEF(IHMSSF(M3,M3,NA))+1
COEF(IHMSSF(M3,M4,NA))=COEF(IHMSSF(M3,M4,NA))+1
COEF(IHMSSF(M3,M5,NA))=COEF(IHMSSF(M3,M5,NA))+1
COEF(IHMSSF(M3,M6,NA))=COEF(IHMSSF(M3,M6,NA))+1

COEF(IHMSSF(M4,M4,NA))=COEF(IHMSSF(M4,M4,NA))+1
COEF(IHMSSF(M4,M5,NA))=COEF(IHMSSF(M4,M5,NA))+1
COEF(IHMSSF(M4,M6,NA))=COEF(IHMSSF(M4,M6,NA))+1

COEF(IHMSSF(M5,M5,NA))=COEF(IHMSSF(M5,M5,NA))+1
\[ \text{COEF(IHMSSF(M5,M6,NA))} - \text{COEF(IHMSSF(M5,M6,NA))} + 1 \]
\[ \text{COEF(IHMSSF(M6,M6,NA))} - \text{COEF(IHMSSF(M6,M6,NA))} + 1 \]

C BUILD RIGHT-HAND SIDES
\[ \text{DY(M1)} - \text{DY(M1)} + Y \]
\[ \text{DY(M2)} - \text{DY(M2)} + Y \]
\[ \text{DY(M3)} - \text{DY(M3)} + Y \]
\[ \text{DY(M4)} - \text{DY(M4)} + Y \]
\[ \text{DY(M5)} - \text{DY(M5)} + Y \]
\[ \text{DY(M6)} - \text{DY(M6)} + Y \]

198 GO TO 12
199 CONTINUE

C *****************************************************************
C *****************************************************************
C TO ELIMINATE LINEAR DEPENDENCIES, (FIXED EFFECTS OTHER THAN *
C THE MEAN OR THE EFFECT CONTAINING IT) THIS PART OF THE *
C PROGRAM AUGMENTS FIXED EFFECTS WITH BLOCKS OF ONES. *
C *
C TO BLOCK OVER EFFECT \( T \), CHANGE DO'S TO \( K(T-1) \) AND \( K(T) \), *
C WHERE EFFECT \( T \) MUST BE CONSTRAINED. NESTED EFFECTS CAN BE *
C HANDLED, BUT THE BLOCK MUST COVER ONLY THOSE LEVELS NESTED *
C IN EACH LEVEL OF THE MAIN EFFECT. FOR EXAMPLE FROM *
C \( K(3-1) \) TO \( K(3.5) \) AND \( K((3.5)-1) \) TO \( K(4) \). \( K3.5 \) WOULD HAVE TO *
C BE SPECIFIED. *
C *
C COMMENT OUT ANY CONSTRAINTS NOT NEEDED. *
C *****************************************************************
C *****************************************************************

DO 202 I=K1+1,K2
   DO 201 J=I,K2
      COEF(IHMSSF(I,J,NA)) = COEF(IHMSSF(I,J,NA)) + 1
   201 CONTINUE
202 CONTINUE

DO 212 I=K2+1,K3
   DO 211 J=I,K3
      COEF(IHMSSF(I,J,NA)) = COEF(IHMSSF(I,J,NA)) + 1
   211 CONTINUE
212 CONTINUE

DO 222 I=K3+1,K4
   DO 221 J=I,K4
      COEF(IHMSSF(I,J,NA)) = COEF(IHMSSF(I,J,NA)) + 1
   221 CONTINUE
222 CONTINUE
C OUTPUT SECTION OF THIS PROGRAM

DO 250 II-1,NA
DO 249 JJ-II,NA

C ***********************************************************
C ***********************************************************
C IF PE IS TRAIT X, THEN SET THE FOLLOWING AS K(X-1) AND KX.*
C PE CAN BE ANY ADDITIONAL RANDOM EFFECT, SO LONG AS ITS *
C VARIANCE STRUCTURE IS AN IDENTITY MATRIX AND ITS ERROR TO *
C PE VARIANCE RATIO IS SPECIFIED.
C ***********************************************************

IF (II .GT. K4 .AND. II .EQ. JJ .AND. II .LE. K5) THEN
   DAUG = COEF(IHMSSF(II,JJ,NA))+DVARPE
   KNTPE = KNTPE + 1
   GO TO 214
END IF

C ADD ON RELATIONSHIP INVERSE.
IF (II .GT. (NA-NANIM)) THEN
   C READ RELATIONSHIP MATRIX
   READ(11,END-251) AINV
   KNTAINV=KNTAINV+1
   DAUG=COEF(IHMSSF(II,JJ,NA))+(AINV*DVARAN)
   GO TO 214
END IF

DAUG=COEF(IHMSSF(II,JJ,NA))

214 CONTINUE
IF (DAUG .NE. O.DO) THEN
C WRITE OUT ROW COL AND ELEMENT OF COEF MATRIX.
   WRITE(90) II,JJ,DAUG
   WRITE(6,*), II,JJ,DAUG
   KNONZER=KNONZER+1
END IF

249 CONTINUE
250 CONTINUE
251 CONTINUE

C WRITE OUT Y PRIME Y

WRITE(91) DYPY
WRITE(6,*), '------------------------------------------'
WRITE(6,*), 'RIGHT-HAND SIDES'
WRITE(6,*), '------------------------------------------'
DO 300 I=1,NA
C WRITE OUT RIGHT-HAND SIDES
 WRITE(92) DY(I)
 C WRITE(6,*), DY(I)
 300 CONTINUE
WRITE(6,*), '----------------------------------------------------------'
WRITE(6,*), 'Y PRIME Y =', DYPY
WRITE(6,*), 'NONZERO ELEMENTS WERE WRITTEN TO ITDAT'
WRITE(6,*), 'RECORDS READ =', KOUNT
WRITE(6,*), 'NUMBER OF PERMANENT ENVIRONMENTS =', KNTPE
WRITE(6,*), 'NUMBER OF ANIMALS AND PHAN GRPS =', KNTAINV
DO 310 I=1,NT
 WRITE(6,*) MISS(I), 'HAVE TRAIT ', I, ' MISSING'
 310 CONTINUE
END
C
INTEGER FUNCTION IHMSSF(I,J,N)
C
FUNCTION TO WORK OUT ADDRESS IN A HALF STORED SYMMETRIC MATRIX OF
C ORDER N; CONSIDER THE UPPER TRIANGLE. I=ROW, J=COLUMN
C WRITTEN BY KAREN MEYER

IF (I .LE. J) THEN
Il=I-1
IHMSSF=N*Il-I*Il/2+J
ELSE
Jl=J-1
IHMSSF=N*Jl-J*Jl/2+I
END IF
RETURN
END
C
//GO.FT10F001 DD UNIT=DISK, DSN=A3$MMS.PROFILE, DISP=(OLD,KEEP)
//GO.FT11F001 DD UNIT=DISK, DSN=A3$MMS.RELX, DISP=(OLD,KEEP)
//GO.FT90F001 DD UNIT=DISK, DSN=A3$MMS.ITDAT, DISP=(NEW,CATLG),
// SPACE=(TRK,(5,5),RLSE), DCB=(RECFM-FB, LRECL-16, BLKSIZE-19056)
//GO.FT91F001 DD UNIT=DISK, DSN=A3$MMS.YPY, DISP=(NEW,CATLG),
// SPACE=(TRK,(1,1),RLSE), DCB=(RECFM-FB, LRECL-8, BLKSIZE-19064)
//GO.FT92F001 DD UNIT=DISK, DSN=A3$MMS.RHNDSD, DISP=(NEW,CATLG),
// SPACE=(TRK,(5,5),RLSE), DCB=(RECFM-FB, LRECL-8, BLKSIZE-19064)
//ITPACK JOB
/*JOBPARM DUPLEX-NO,FLASH-NONE,KEEP-YES,LINES-35
//STEP0 EXEC SCRUNC
//SYSIN DD *
A3$MMS.CONJISOL
A3$MMS.CONJRHS
A3$MMS.ITLSOL
//S1 EXEC FORTVCLG,
// REGION.LKED-1028K,REGION.GO-8M,TIME.GO-25,
// PARM.GO='NOOCTSTATUS'
*****************************************************************************
****
**** THIS IS A FORTRAN VERSION OF THE DRIVER PROGRAM USED TO SET
**** UP THE VECTORS THAT ARE USED IN THE ITPACK ROUTINES.
****
**** NOTE! MORDER AND MELEM MUST BE GIVEN VALUES BEFORE THE
**** PROGRAM IS RUN. ALSO THE MAXIMUM NUMBER OF ITERATIONS
**** ALLOWED CAN BE CHANGED(ITMAX) AS WELL AS OTHER PARAMETERS
**** IN THE "SETTING PARAMETERS" SECTION.
****
**** RIGHT-HAND SIDES FOR THE CONJUGATE NORMAL EQUATIONS ARE
**** DETERMINED AND OUTPUT AS WELL.
****
**** INPUT
****
**** UNIT 11 ITDAT FROM COEF PRG
**** UNIT 12 RIGHT-HAND SIDES FROM COEF PRG
****
**** OUTPUT
****
**** UNIT 91 SOLUTION OF MIXED MODEL EQUATIONS
**** UNIT 92 SOLUTION OF MIXED MODEL CONJUGATE NORMAL EQUATIONS
**** UNIT 93 RIGHT-HAND SIDES FROM CONJUGATE NORMAL EQUATIONS
****
**** NOTE THAT THIS PROGRAM IS DESIGNED TO OUTPUT DATA TO BE
**** USED WITH AN F-TEST. CONJUGATE NORMAL RIGHT-HAND SIDES
**** ARE CONTRASTS AMONG LEVELS OF THE EFFECT. THESE CONTRASTS
**** INDIVIDUALLY COULD BE TESTED WITH A STUDENT'S T-TEST.
****
**** ONE CAN USE A T STATISTIC TO TEST FOR COVARIABLES. INPUT
**** MUST BE FROM THE COEFCOV PROGRAM. CONJUGATE NORMAL RIGHT-
**** HAND SIDES MUST BE CHANGED SO THAT THEY ARE SIMPLY A 1
**** CORRESPONDING TO THE COVARIABLE TO BE TESTED, INSTEAD OF 1
**** -1 CONTRASTS.
****
*****************************************************************************
**** MORDER = NUMBER OF EQUATIONS TO BE SOLVED
**** MELEM = NUMBER OF ELEMENTS IN THE COEFFICIENT MATRIX
**** MENTRY = MELEM IF RHS ARE READ SEPARATELY, ELSE MELEM+MORDER
/** NF = NUMBER OF FACTORS INCLUDED */
/** NTEST = ORDERED NUMBER OF THE FACTOR TO BE TESTED */
****************************************************************
//FORT,SYSIN DD *
PARAMETER (MORDER=1856
* ,MELEM=18912
* ,MENTRY=MELEM
* ,NF=6
* ,NTEST=4
* ,ITMAX=1500
* ,NW=4*MORDER+(2*ITMAX))
REAL*8 COEF COEFY
INTEGER IA(MORDER+1),JA(MELEM),IPARM(12),IWKSP(3*MORDER),
* ELECNT, ELEMENT, K(NF)
REAL*8 A(MELEM),RHS(MORDER),U(MORDER),WKSP(NW),
* RPARM(12)
DATA LEVEL/2/,IDGTS/1/
IRND=1
N=MORDER
C*****************************************************************************
C*****************************************************************************
C INPUT NUMBER OF FACTORS. THIS NUMBER MAY BE EXPANDED BUT MUST *
C MATCH THE NUMBER AND LEVELS IN THE COEF PROGRAM. *
*****************************************************************************
N1=29
N2=7
N3=2
N4=53
N5=764
N6=1001
C*****************************************************************************
C*****************************************************************************
C K'S MAY ALSO BE CHANGED. UNLIKE IN PRC COEF K'S EQUAL THE NUMBER C EFFECTS IN THE MODEL.
C*****************************************************************************
K(1)=N1
K(2)=K(1)+N2
K(3)=K(2)+N3
K(4)=K(3)+N4
K(5)=K(4)+N5
K(6)=K(5)+N6
C**** THIS VERSION SOLVES IN CORE
C**** READING AND STORING MATRIX AND RHS
ELECNT=1
C SOLVE MIXED MODEL EQUATIONS
DO 10 ELEMENT=1,MENTRY
   READ(11,END=35) IR,IC,COEF
   A(ELECNT)=COEF
JA(ELECNT) = IC
IF(IR .EQ. IC) IA(IC) = ELECNT
ELECNT = ELECNT + 1

10 CONTINUE
35 CONTINUE
DO 40 ELEMENT = 1, MORDER
   READ(12, END = 55) COEFY
   RHS(ELEMENT) = COEFY
40 CONTINUE
55 CONTINUE
IA(MORDER + 1) = ELECNT

C**** SETTING PARAMETERS
CALL ERRSET(208, 0, -1, 1.0)
CALL DFALUT(IPARM, RPARAM)
IPARM(1) = ITMAX
IPARM(8) = (NW * NW)
IPARM(2) = LEVEL
IPARM(12) = IDGTS

C**** SETTING SOLUTION VECTOR TO FIRST GUESS
CALL VFILL(N, U, O, DO)
CALL JCG(N, IA, JA, A, RHS, U, IWKSP, NW, WKSP, IPARM, RPARAM, IER)

C**** PRINT SOLUTIONS
DO 75 I = 1, MORDER
   WRITE(91) U(I)
   WRITE(6, *) I, U(I), RHS(I)
75 CONTINUE

C***** THIS IS THE LOOP FOR THE CONJUGATE NORMAL EQUATIONS

WRITE(6, *) ' THESE ARE THE SOLUTIONS TO THE CNE.'

C*****************************************************************
C THE K NUMBERS IN THE LOOP WOULD HAVE TO BE ADJUSTED TO
C ACCOMODATE TESTING A NESTED EFFECT.
C NKEEP WOULD BE CHANGE WHEN PROCEEDING TO THE FIRST LEVEL NESTED
C IN THE NEXT MAIN EFFECT.
C*****************************************************************

NSTART = K(NTEST - 1) + 2
NKEEP = K(NTEST - 1) + 1
MCOL = 0
DO 80 LL = 1, MORDER
RHS(LLL)=0.DO
80 CONTINUE

C THE CONJ RHS HAVE A 1 CORRESPONDING TO THE FIRST LEVEL.

RHS(NKEEP)=1.DO
DO 150 ELEMENT=NSTART,K(NTEST)
MCOL=MCOL+1

C THE FIRST CONJ RHS HAS A -1 CORRESPONDING TO LEVEL 2
C SUBSEQUENT CONJ RHS'S HAVE A -1 CORRESPONDING TO LEVEL 3
C THEN 4 THEN 5 ETC.

RHS(ELEMENT)=-1.DO

CALL ERRSET(208,0,-1,1.0)
CALL DFAULT(IPARM,RPARM)
IPARM(1)=ITMAX
IPARM(2)=LEVEL
IPARM(8)=(NW*NW)
IPARM(12)=IDGTS

WRITE(6,*) 'COLUMN ROW SOLUTION RHS'
DO 110 I=NKEEP,K(NTEST)

C DO 110 I-1,MORDER
WRITE(92) U(I)
WRITE(93) RHS(I)
WRITE(6,*) MCOL, I, U(I), RHS(I)
110 CONTINUE
RHS(ELEMENT)=0.DO
150 CONTINUE
STOP
END
//GO.FT92F001 DD UNIT=DISK,DSN=A3$MMS.CNJSL,DISP=(NEW,CATLG),
// SPACE=(TRK,(5,5),RLSE),DCB=(RECFM=FB,LRECL=8,BLKSIZ=19064)
//GO.FT93F001 DD UNIT=DISK,DSN=A3$MMS.CNJRH,DISP=(NEW,CATLG),
// SPACE=(TRK,(5,5),RLSE),DCB=(RECFM=FB,LRECL=8,BLKSIZ=19064)
FTEST JOB
/*JOBPARM DUPLEX-NO,FLASH-NONE,KEEP-YES
S1 EXEC FORTVCLG,
REGION.LKED=1028K,REGION.GO=612K,TIME.GO=05
FORT.SYSIN DD *
*******************************************************************************/
/* PROGRAM TO TEST THE SIGNIFICANCE OF A FIXED EFFECT IN *
/* A MIXED MODEL USING SOLUTIONS OF THE EFFECTS AS WELL *
/* AS SOLUTIONS FROM MIXED MODEL CONJUGATE NORMAL *
/* EQUATIONS. THIS PROGRAM IS RUN AFTER THE ITPACK *
/* PROGRAM TO SOLVE THE MIXED MODELS. *
*******************************************************************************/
INPUT
UNIT 11 CONJUGATE NORMAL SOLUTIONS *
UNIT 12 CONJUGATE NORMAL RHS *
UNIT 13 SOLUTIONS TO MME'S *
UNIT 14 MME RIGHT-HAND SIDES *
UNIT 15 Y PRIME Y *
NOTE UNITS 11-13 ARE FROM THE ITPACK PROGRAM AND UNITS*
14 AND 15 ARE FROM THE COEF PROGRAM. *
*******************************************************************************/
/* THE NUMBER OF LEVELS OF EFFECTS IN THE MODEL MUST *
/* MATCH PREVIOUS PROGRAMS, COEF AND ITPACK. *
*******************************************************************************/
NRANK IS THE RANK OF THE FIXED EFFECTS PORTION OF THE *
EQUATIONS OF CONCERN. *
NNNN IS THE NUMBER OF RECORDS FROM COEF JOB OUTPUT *
*******************************************************************************/
PARAMETER (NRANK=88 *
,NNNN=1933
* ,N1=29 *
* ,N2=7 *
* ,N3=2 *
* ,N4=53 *
* ,N5=764 *
* ,N6=1001 *
* ,NA=N1+N2+N3+N4+N5+N6
*******************************************************************************/
C DEFINE THE TRAIT YOU WISH TO TEST!
C NUSE= THE TRAIT YOU WISH TO TEST (SAY N1 OR N2)
C NEUSE=SIZE OF HALF STORED MATRIX (NEED NOT BE CHANGED)
C KUSE= NUMBER OF EQUATIONS AHEAD OF THE TRAIT OF INTEREST
*******************************************************************************/
* ,NUSE=N4 *
* ,NEUSE=NUSE*(NUSE+1)/2 *
* ,KUSE=N1+N2+N3
*******************************************************************************/
* ,NUSEM1=NUSE-1)
C****************************************************************
C SET PARAMETERS ACCORDING TO THE NUMBER OF LEVELS OF THE FACTOR*
C FOR WHICH THE TEST IS DESIRED. *
C****************************************************************

REAL*8 PHI(NSU,NUSEM1), CONJRHS(NSU,NUSEM1), NUM,
  *H(NSU,NUSEM1), WKSP(NUSEM1), T(NUSEM1),
  *HALF(NSU), CROSS, ITSOL(NA), UHAT(NSU), NUM2, NUMSS,
  *YPY,
  *DENSS, SUBTR, F, RHNDS(NA)
INTEGER*4 IFLAG(NUSEM1)

K1=1
K2=K1+2
K3=K2+3
K4=K3+4
K5=K4+5
K6=K5+6

C**** SECTION TO READ THE SOLUTION MATRIX OF THE CONJUGATE NORMAL
C**** EQUATIONS AND ALSO THE MATRIX OF THE n(NSU)-1 LINEAR
C**** CONTRASTS USED AS RIGHT-HAND SIDES.

DO 11 I-1,NUSEM1
  DO 10 J-1,NSU
    READ(ll) PHI(J,I)
  READ(12) CONJRHS(J,I)
10 CONTINUE
11 CONTINUE

C**** THIS SECTION OF THE PROGRAM HALF STORES H.

DO 33 K-1,NUSEM1
  DO 32 L-K,NUSEM1
    HALF(IHMSSF(L,K,NUSEM1))=H(L,K)
 32 CONTINUE
33 CONTINUE

C**** SECTION TO BUILD THE H-MATRIX. REALLY PHI'*LAMBDA, WHERE
C**** PHI IS THE SOLUTIONS TO CONJUGATE NORMALS AND LAMBDA IS THE
C**** CONTRAST MATRIX.

DO 22 K-1,NUSEM1
  DO 21 L-1,NUSEM1
    NUM=0.DO
    DO 20 M-1,NSU
      NUM=NUM+(PHI(M,L)*CONJRHS(M,K))
20 CONTINUE
21 CONTINUE
22 CONTINUE
CALL KAREN MYER SUBROUTINE TO INVERT HALF-STORED SYMMETRIC MATRIX. HALF IS RETURNED AS THE INVERSE OF HPH.

CALL DMSVHF(HALF,WKSP,IFLAG,NUSEM1)
WRITE(6,*)'HALF INVERSE'
DO 37 M=1,NEUSE
     WRITE(6,*) HALF(M)
37 CONTINUE

READ ITERATED SOLUTIONS AND THE SECTION OF THEM FOR THE EFFECT OF INTEREST.

DO 41 I=1,NA
     READ(13) ITSOL(I)
     WRITE(6,*) ITSOL(I)
41 CONTINUE

WRITE OUT SOLUTIONS FOR EFFECT OF INTEREST
WRITE(6,*) 'uuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuuu
DO 42 I=1,NUSE
     UHAT(I)=ITSOL(KUSE+I)
     WRITE(6,*) UHAT(I)
42 CONTINUE

THE T MATRIX IS SIMPLY LAMBDA'*UHAT, WHERE LAMBDA IS THE MATRIX OF CONTRASTS AND UHAT THE SECTION OF ITERATED SOLUTIONS PERTAINING TO THE EFFECT OF INTEREST.

DO 55 I=1,NUSEM1
    NUM2=0.D0
    DO 54 M=1,NUSE
        NUM2=NUM2+(UHAT(M)*CONJRHS(M,I))
    54 CONTINUE
    T(I)=NUM2
55 CONTINUE

CALL QUAFRM (A SUBROUTINE BY KAREN MEYER) TO MULTIPLY T'*HPhinv*T TO GET NUMERATOR SUM OF SQUARES.

CALL QUAFRM(HALF,T,NUMSS,NUSEM1)
WRITE(6,*) 'NUMERATOR SS =',NUMSS

OBTAIN DENOMINATOR SS AND THEN THE F-TEST

DO 65 I=1,NA
     READ(14) RHNSD(I)
65 CONTINUE
     SUBTR=0.D0
     DO 67 I=1,NA
\begin{verbatim}
SUBTR = SUBTR + (ITSOL(I) * RHNDSD(I))

CONTINUE
WRITE(6,*) 'XXXIT-', SUBTR
READ(15) YPY

DENSS = YPY - SUBTR
WRITE(6,*) 'DENOMINATOR SS = ', DENSS

F = (NUMSS / NUSEM1) / (DENSS / (NHHN - NRRANK))
WRITE(6,*) 'F = ', F

C*** ADD COMMENT FOR THE TEST YOU ARE CURRENTLY DOING. ************
C*******************************************************************
WRITE(6,*) 'TEST FOR MEMILK WITH WESTELL GROUPS. DFREML
*VARS.'
END

INTEGER FUNCTION IHMSSF(I, J, N)
C
C FUNCTION TO WORK OUT ADDRESS IN A HALFSTORED SYMMETRIC MATRIX
C CONSIDER THE UPPER TRIANGLE

IF(I .LE. J) THEN
  I1 = I - 1
  IHMSSF = N * I1 - I * I1 / 2 + J
ELSE
  J1 = J - 1
  IHMSSF = N * J1 - J * J1 / 2 + I
END IF
RETURN
END

SUBROUTINE DKMVHF(A, V, IFLAG, N)
C
C * * * ROUTINE TO INVERT A HALFSTORED SYMMETRIC MATRIX * * *

C IF THE MATRIX IS NOT OF FULL RANK THE GENERALISED INVERSE
C IS RETURNED, SETTING N-RANK(A) ROWS/COLUMNS TO ZERO AND
C OBTAINING THE REGULAR INVERSE OF THE FULL RANK SUBMATRIX
C THIS IS A REWRITE OF HENDERSON'S MATRIX INVERTOR "DJNVHF",
C USING HIS ALGORITHM BUT AVOIDING TO REARRANGE ROWS AND COL.S

PARAMETERS :
C A : DOUBLE PRECISION VECTOR OF LENGTH N*(N+1)/2, CONTAINING

\end{verbatim}
THE MATRIX TO BE INVERTED HALFSTORED ON ENTRY AND THE
INVERSE ON EXIT
V : DOUBLE PRECISION VECTOR OF LENGTH N, USED AS WORKSPACE
IFLAG : INTEGER VECTOR OF LENGTH N, CONTAINING THE ORDER IN
WHICH ROWS/COLS WERE PROCESSED ON EXIT, EXCEPT FOR THE N-TH
ELEMENT WHICH GIVES THE RANK OF THE MATRIX
N : ORDER OF THE MATRIX

KARIN MEYER
NOVEMBER 1983

DOUBLE PRECISION A(1),V(1),XX,DMAX,AMAX,BMAX,ZERO,DIMAX
INTEGER IFLAG(1)

IF(N.EQ.1)THEN
XX=A(1)
IF(DABS(XX).GT.ZERO)THEN
A(1)=1.D0/XX
IFLAG(1)=1
ELSE
A(1)=0.D0
IFLAG(1)=0
END IF
RETURN
END IF

N1=N+1
NN=N*N1/2
DO 1 I=1,N
1 IFLAG(I)=0

SET MINIMUM ABSOLUTE VALUE OF DIAGONAL ELEMENTS FOR
NON-SINGULARITY (MACHINE SPECIFIC )
ZERO=1.D-20

START LOOP OVER ROWS/COLS

DO 8 I=1,N

... FIND DIAGONAL ELEMENT WITH BIGGEST ABSOLUTE VALUE
DMAX=0.D0
AMAX=0.D0
KK=N
DO 2 I=1,N

... CHECK THAT THIS ROW/COL HAS NOT BEEN PROCESSED
IF(IFLAG(I).NE.0)THEN
KK=KK+N1-I
8 CONTINUE
 ELSE  
   KK=KK+N1  
   BMAX=DABS(A(KK))  
   IF(BMAX.GT.AMAX)THEN  
     DMAX=A(KK)  
     AMAX=BMAX  
     IMAX=I  
   END IF  
   KK=KK-I  
 END IF  
 2 CONTINUE  
 C ... CHECK FOR SINGULARITY  
 IF(AMAX.LE.ZERO)GO TO 11  
 C ... ALL ELEMENTS SCANNED,SET FLAG  
 IFLAG(IMAX)=II  
 C ... INVERT DIAGONAL  
 DIMAX=1.DO/DMAX  
 C ... DEVIDE ELEMENTS IN ROW/COL PERTAINING TO THE BIGGEST  
 C DIAGONAL ELEMENT BY DMAX  
   IL=IMAX-N  
   DO 3 I=1,IMAX-1  
     IL=IL+N1-I  
     XX=A(IL)  
     IF(XX.NE.0)A(IL)=XX*DIMAX  
 3 V(I)=XX  
 C ... NEW DIAGONAL ELEMENT  
   IL=IL+N1-IMAX  
   A(IL)=-DIMAX  
   DO 4 I=IMAX+1,N  
     IL=IL+1  
     XX=A(IL)  
     IF(XX.NE.0)A(IL)=XX*DIMAX  
 4 V(I)=XX  
 C ... ADJUST THE OTHER ROWS/COLS :  
 C A(I,J)=A(I,J)-A(I,IMAX)*A(J,IMAX)/A(IMAX,IMAX)  
   IJ=0  
   DO 7 I=1,N  
     IF(I.EQ.IMAX)THEN  
       IJ=IJ+N1-I  
     ELSE  
       XX=V(I)  
       IF(XX.NE.0.DO)THEN  
         XX=XX*DIMAX  
         DO 5 J=I,N  
           IJ=IJ+1  
           IF(J.NE.IMAX)A(IJ)=A(IJ)-XX*V(J)  
 5 CONTINUE  
 ELSE  
 6 IJ=IJ+N1-I
END IF
END IF
7 CONTINUE

C ... REPEAT UNTIL ALL ROWS/COLS ARE PROCESSED
8 CONTINUE

C-----------------------------------------------
C END LOOP OVER ROWS/COLS
C-----------------------------------------------
C ... REVERSE SIGN
   DO 9 I=1,NN
   9 A(I)=-A(I)
C ... AND THAT'S IT
C PRINT 10,N
10 FORMAT( 'FULL RANK MATRIX INVERTED, ORDER =',I5)
C RETURN RANK AS LAST ELEMENT OF FLAG VECTOR
   IFLAG(N)=N
RETURN

C-----------------------------------------------
C MATRIX NOT OF FULL RANK, RETURN GENERALISED INVERSE
C-----------------------------------------------

11 IRANK=II-1
   IJ=0
   DO 14 I=1,N
      IF(IFLAG(I).EQ.0) THEN
      C ... SET REMAINING N-II ROWS/COLS TO ZERO
         DO 12 J=I,N
            IJ=IJ+1
            A(IJ)=0.DO
         CONTINUE
      ELSE
         DO 13 J=I,N
            IJ=IJ+1
            IF(IFLAG(J).NE.0) THEN
            C ... REVERSE SIGN FOR II-1 ROWS/COLS PREVIOUSLY PROCESSED
            A(IJ)=-A(IJ)
            ELSE
            A(IJ)=0.DO
            END IF
         CONTINUE
      END IF
   CONTINUE
14 CONTINUE
   PRINT 15,N,IRANK
15 FORMAT( 'GENERALISED INVERSE OF MATRIX WITH ORDER =',I5,
            1 ' AND RANK =',I5)
SUBROUTINE QUAFRM(A,Y,QQ,N)

C PURPOSE : ROUTINE TO EVALUATE THE QUADRATIC FORM Y'AY FOR A
C BEING A HALFSTORED SYMMETRIC MATRIX

C PARAMETERS :
C A : DOUBLE PRECISION VECTOR OF LENGTH N(N+1)/2,
C CONTAINING THE UPPER TRIANGLE OF A STORED ROWWISE
C Y : DOUBLE PRECISION VECTOR OF LENGTH N,
C CONTAINING THE DATA POINTS
C QQ : DOUBLE PRECISION VARIABLE TO RETURN THE VALUE OF
C Y'AY
C N : ORDER OF A AND Y

C SUBROUTINES REQUIRED : NONE

IMPLICIT DOUBLE PRECISION (A-H,O-Z)

DIMENSION A(1),Y(1)

N1=N+1
QQ=0.DO
IJ=0
DO 1 I=1,N
YY=Y(I)
IF(YY.EQ.0.DO)THEN
IJ=IJ+N1-I
ELSE
IJ=IJ+1
QQ=QQ+YY*YY*A(IJ)
XX=0.DO
DO 2 J=I+1,N
IJ=IJ+1
2 XX=XX+Y(J)*A(IJ)
QQ=QQ+2.DO*XX*YY
END IF
1 CONTINUE

RETURN
END
//GO.FT11F001 DD UNIT=DISK, DSN=A3$MMS.CONJSOL,DISP=(OLD,KEEP),
// DCB=(RECFM=FB,LRECL=8,BLKSIZE=19064)
//GO.FT12F001 DD UNIT=DISK, DSN=A3$MMS.CONJRHS,DISP=(OLD,KEEP),
// DCB=(RECFM=FB,LRECL=8,BLKSIZE=19064)
//GO.FT13F001 DD UNIT=DISK, DSN=A3$MMS.ITSOL,DISP=(OLD,KEEP),
// DCB=(RECFM=FB,LRECL=12,BLKSIZE=19068)
//GO.FT14F001 DD UNIT=DISK, DSN=A3$MMS.RHNDSD,DISP=(OLD,KEEP),
// DCB=(RECFM=FB,LRECL=8,BLKSIZE=19064)
//GO.FT15F001 DD UNIT=DISK, DSN=A3$MMS.YPY,DISP=(OLD,KEEP),
// DCB=(RECFM=FB,LRECL=8,BLKSIZE=19064)
//RSQ JOB
//JOBPARM DUPLEX-NO,FLASH-NONE,KEEP-YES
//S1 EXEC FORTVCLG,FVPOPT=3
//FORT.SYSIN DD *

PROGRAM TO CALCULATE R SQUARE (COEFFICIENT OF VARIATION)
FOR THE SPECIFIED MODEL. N1-N6 CORRESPOND TO LEVELS FOR
EFFECTS 1 TO 6.

N1-N6 AND NT MUST BE SPECIFIED.

NC IS THE NUMBER OF COVARIABLES AND MUST BE SPECIFIED. THIS
PROGRAM CAN BE ALTERED TO ELIMINATE COVARIABLES BY IGNORING
ANY COVARIABLES IN THE READ STATEMENT AND COMMENTING OUT
THE SMALL DO LOOP INVOLVING THEM.

COMMENT OUT ANY EXTRA EFFECTS IF THERE ARE TOO MANY,
AND CHANGE THE NUMBERS ADDED FOR NA TO THE NUMBER
OF TRAITS USED. INCLUDE NC IF THERE ARE COVARIABLES

ALSO CHANGE THE READ STATEMENT TO MATCH
INPUT DATA.

UNIT 10 PRODUCTION FILE
(SAME AS FOR COEF OR COEFCOV)
UNIT 11 Y PRIME Y FROM COEF OR COEFCOV
UNIT 12 SOLUTION FILE FROM ITPACK

PARAMETER(N1=29
, N2=7
, N3=2
, N4=53
, N5=764
, N6=1001
C NC IS THE TOTAL NUMBER OF COVARIATES
, NC=17
C NUMBER OF TRAITS
, NT=6
C

* NA=N1+N2+N3+N4+N5+N6+NC
INTEGER 4 F(N1), FC(NC), EX(2), K(10)
REAL 8 YPY, Y, YDUM(7), BHAT(NA), THAT, SSE, MEAN, RMEAN,
* RSQ

KNTSOL=0
KNTREC=0
K(1)=0
K(2)=N1
K(3)=K(2)+N2
K(4)=K(3)+N3
K(5)=K(4)+N4
K(6)=K(5)+N5
K(7)=K(6)+N6

7 READ(11) YPY

DO 9 I=1,NA
   READ(12)BHAT(I)
   KNTSOL=KNTSOL+1
9 CONTINUE

NNN=NT+1

C **********************************************************************
C **********************************************************************
C CHANGE THE READ STATEMENT TO READ THE APPROPRIATE PRODUCTION DATA. THE ORDER OF EFFECTS MAY BE CHANGE BY SWITCHING F(I)'S.
C A FORMATTED READ COULD EASILY BE USED.
C
C F(I) CORRESPONDS TO THE Ith EFFECT IN THE MODEL. AND Y IS
C TRAIT OF INTEREST. DUMMY VARIABLES CAN BE INSERTED TO READ
C DATA ON THE PRODUCTION FILE THAT IS NOT TO BE INCLUDED IN
C THE MODEL OR FOR ADDITIONAL DEPENDENT VARIABLES.
C EXAMPLES ARE EX(I) AND YDUM(I).
C
C THE FC(L) READ CAN BE ELIMINATED WITH NO COVARIABLES
C **********************************************************************
C **********************************************************************

12 READ(10,*,END=199)F(6),F(1),F(2),F(3),F(4),F(5),EX(1),
   *(FC(L) L=1,NC),Y,YDUM(1)

KNTREC=KNTREC+1
YSUM=YSUM+Y
YHAT=0.0

DO 14 L=1,NT
   YHAT=YHAT+BHAT(K(L)+F(L))
14 CONTINUE

C **********************************************************************
C SECTION TO CONSIDER COVARIABLES.
C **********************************************************************

C DO 15 M=1,NC
C KKK=K(NT+1)+M
C YHAT=YHAT+(BHAT(KKK)*FC(M))
C 15 CONTINUE

SSE=(YHAT*Y)+SSE
GO TO 12

199 CONTINUE

MEAN=YSUM/KNTREC
RMEAN=KNTREC*(MEAN**2)
RSQ=(SSE-RMEAN)/(YPY-RMEAN)

WRITE(6,*) '-------------------------------------'
WRITE(6,*) ' COEFFICIENT OF DETERMINATION '
WRITE(6,*) '-------------------------------------'
WRITE(6,*)
WRITE(6,*) 'Y PRIME Y =',YPY
WRITE(6,*)
WRITE(6,*) KNTSOL, 'SOLUTIONS WERE READ FROM ITSOL FILE'
WRITE(6,*) 'RECORDS READ =', KNTREC
WRITE(6,*)
WRITE(6,*) 'THE MEAN =', MEAN
WRITE(6,*) 'REDUCTION DUE TO FITTING THE MEAN =', RMEAN
WRITE(6,*) 'REDUCTION DUE TO FITTING MODEL =', SSE
WRITE(6,*) 'COEFFICIENT OF DETERMINATION (Rsq)=', RSQ
WRITE(6,*) 'TRAIT IS MEMILK CLUSTER 1 '
END

//GO.FT10F001 DD UNIT=DISK,DSN=A3$MMS.PROFILE,DISP=SHR
//GO.FT11F001 DD UNIT=DISK,DSN=A3$MMS.YPY,DISP=SHR
//GO.FT12F001 DD UNIT=DISK,DSN=A3$MMS.ITSOL,DISP=SHR
/* JOBPARM DUPLEX-NO, FLASH-NONE, KEEP-YES */

// COEFCOV JOB
/* JOBPARM DUPLEX-NO, FLASH-NONE, KEEP-YES */

// STEPO EXEC SCRUNG
// SYSIN DD *
A3$MMS.RHNDSD
A3$MMS.YPY
A3$MMS.ITDAT

// S1 EXEC FORTVCLG, FVPOPT=3
// FORT.SYSIN DD *

PARAMETER(N1=29
  *  ,N2=7
  *  ,N3=2
  *  ,N4=53
  *  ,N5=764
  *  ,N6=1001
)

C NC IS THE TOTAL NUMBER OF COVARIATES
  *  ,NC=17

C NUMBER OF TRAITS
  *  ,NT=6
  *  ,NANIM=N6

C

    INTEGER*4 COEF(NE), F(NT), FC(NC), EX(2)
    REAL*8 DY(NA), DYPY, DAUG, AINV, DVARAN, Y, YDUM(6)
    DIMENSION MISS(NT)
COMMON /BIG/ COEF

DYPY=0
KOUNT=0

C PRIORS FOR VARIANCE RATIOS ARE ADDED HERE.

DVARPE=1.293
DVARAN=1.537

KNTPSE=0
KNTAINV=0
KNONZER=0

K0=0
K1=N1
K2=K1+N2
K3=K2+N3
K4=K3+N4
K5=K4+N5
K6=K5+N6

DO 7 I-1,NT
    MISS(I)=0
7 CONTINUE

DO 9 I-1,NA
    DY(I)=0.DO
9 CONTINUE

DO 11 I-1,NE
    COEF(I)=0
11 CONTINUE

C ************************************************************************
C ************************************************************************
C CHANGE THE READ STATEMENT TO READ THE APPROPRIATE PRODUCTION *
C DATA. THE ORDER OF EFFECTS MAY BE CHANGE BY SWITCHIN F( )'S.*
C A FORMATTED READ COULD EASILY BE USED. *
C *
C F(I) CORRESPONDS TO THE Ith EFFECT IN THE MODEL. AND Y IS *
C TRAIT OF INTEREST. DUMMY VARIABLES CAN BE INSERTED TO READ *
C DATA ON THE PRODUCTION FILE THAT IS NOT TO BE INCLUDED IN *
C THE MODEL OR FOR ADDITIONAL DEPENDENT VARIABLES. *
C EXAMPLES ARE EX( ) AND YDUM( ). *
C *
C FC(L) READS COVARIABLES.
C ************************************************************************
C ************************************************************************

12 READ(10,*,END-199)F(6),F(1),F(2),F(3),F(4),F(5),EX(1),
   * (FC(L),L-1,NC), Y, YDUM(1)
DYPY = DYPY + Y*Y
KOUNT = KOUNT + 1

C CHECK FOR MISSING VALUES.
DO 75 J = 1, NT
    IF (F(J) .LE. 0) THEN
        MISS(J) = MISS(J) + 1
        GO TO 198
    END IF
75 CONTINUE

M1 = F(1)
M2 = F(2) + K1
M3 = F(3) + K2
M4 = F(4) + K3
M5 = F(5) + K4
M6 = F(6) + K5

COEF(IHMSSF(M1, M1, NA)) = COEF(IHMSSF(M1, M1, NA)) + 1
COEF(IHMSSF(M1, M2, NA)) = COEF(IHMSSF(M1, M2, NA)) + 1
COEF(IHMSSF(M1, M3, NA)) = COEF(IHMSSF(M1, M3, NA)) + 1
COEF(IHMSSF(M1, M4, NA)) = COEF(IHMSSF(M1, M4, NA)) + 1
COEF(IHMSSF(M1, M5, NA)) = COEF(IHMSSF(M1, M5, NA)) + 1
COEF(IHMSSF(M1, M6, NA)) = COEF(IHMSSF(M1, M6, NA)) + 1

DO 81 L = 1, NC
    MC = L + K5
    COEF(IHMSSF(M1, MC, NA)) = COEF(IHMSSF(M1, MC, NA)) + FC(L)
81 CONTINUE

COEF(IHMSSF(M2, M2, NA)) = COEF(IHMSSF(M2, M2, NA)) + 1
COEF(IHMSSF(M2, M3, NA)) = COEF(IHMSSF(M2, M3, NA)) + 1
COEF(IHMSSF(M2, M4, NA)) = COEF(IHMSSF(M2, M4, NA)) + 1
COEF(IHMSSF(M2, M5, NA)) = COEF(IHMSSF(M2, M5, NA)) + 1
COEF(IHMSSF(M2, M6, NA)) = COEF(IHMSSF(M2, M6, NA)) + 1

DO 82 L = 1, NC
    MC = L + K5
    COEF(IHMSSF(M2, MC, NA)) = COEF(IHMSSF(M2, MC, NA)) + FC(L)
82 CONTINUE

COEF(IHMSSF(M3, M3, NA)) = COEF(IHMSSF(M3, M3, NA)) + 1
COEF(IHMSSF(M3, M4, NA)) = COEF(IHMSSF(M3, M4, NA)) + 1
COEF(IHMSSF(M3, M5, NA)) = COEF(IHMSSF(M3, M5, NA)) + 1
COEF(IHMSSF(M3, M6, NA)) = COEF(IHMSSF(M3, M6, NA)) + 1

DO 83 L = 1, NC
    MC = L + K5
    COEF(IHMSSF(M3, MC, NA)) = COEF(IHMSSF(M3, MC, NA)) + FC(L)
83 CONTINUE

COEF(IHMSSF(M4, M4, NA)) = COEF(IHMSSF(M4, M4, NA)) + 1
COEF(IHMSSF(M4, M5, NA)) = COEF(IHMSSF(M4, M5, NA)) + 1
COEF(IHMSSF(M4, M6, NA)) = COEF(IHMSSF(M4, M6, NA)) + 1
DO 84 L=1,NC  
   MC=L+K5  
   COEF(IHMSSF(M4,MC,NA))=COEF(IHMSSF(M4,MC,NA))+FC(L)  
84 CONTINUE

   COEF(IHMSSF(M5,M5,NA))=COEF(IHMSSF(M5,M5,NA))+1  
   COEF(IHMSSF(M5,M6,NA))=COEF(IHMSSF(M5,M6,NA))+1

DO 85 L=1,NC  
   MC=L+K5  
   COEF(IHMSSF(M5,MC,NA))=COEF(IHMSSF(M5,MC,NA))+FC(L)  
85 CONTINUE

   COEF(IHMSSF(M6,M6,NA))=COEF(IHMSSF(M6,M6,NA))+1  
   DO 86 L=1,NC  
      MC=L+K5  
      COEF(IHMSSF(M6,MC,NA))=COEF(IHMSSF(M6,MC,NA))+FC(L)  
86 CONTINUE

C CODE COEF OF COVARIABLES WITH THEM SELVES  
DO 92 K=1,NC  
   DO 91 L=K,NC  
      MCK=K+K5  
      MCL=L+K5  
      COEF(IHMSSF(MCK,MCL,NA))=COEF(IHMSSF(MCK,MCL,NA))+(FC(K)*FC(L))  
91 CONTINUE
92 CONTINUE

C BUILD RIGHT-HAND SIDES  
   DY(M1)=DY(M1)+Y  
   DY(M2)=DY(M2)+Y  
   DY(M3)=DY(M3)+Y  
   DY(M4)=DY(M4)+Y  
   DY(M5)=DY(M5)+Y  
   DY(M6)=DY(M6)+Y  
   DO 101 K=1,NC  
      MC=K+K5  
      DY(MC)=DY(MC)+(FC(K)*Y)  
101 CONTINUE

198 GO TO 12  
199 CONTINUE

C *************************************************************************  
C *************************************************************************  
C TO ELIMINATE LINEAR DEPENDENCIES,(FIXED EFFECTS OTHER THAN  
C THE MEAN OR THE EFFECT CONTAINING IT) THIS PART OF THE  
C PROGRAM AUGMENTS FIXED EFFECTS WITH BLOCKS OF ONES.  
C  
C TO BLOCK OVER EFFECT T, CHANGE DO'S TO K(T-1) AND K(T),  
C
WHERE EFFECT T MUST BE CONSTRAINED. NESTED EFFECTS CAN BE *
HANDLED, BUT THE BLOCK MUST COVER ONLY THOSE LEVELS NESTED *
IN EACH LEVEL OF THE MAIN EFFECT. FOR EXAMPLE FROM *
K(3-1) TO K(3.5) AND K((3.5)-1) TO K(4). K3.5 WOULD HAVE TO *
BE SPECIFIED. *
COMMENT OUT ANY CONSTRAINTS NOT NEEDED. *
*****************************************************************
DO 202 I=K1+1,K2
   DO 201 J=I,K2
      COEF(IHMSSF(I,J,NA))=COEF(IHMSSF(I,J,NA))+1
   201 CONTINUE
202 CONTINUE
DO 212 I=K2+1,K3
   DO 211 J=I,K3
      COEF(IHMSSF(I,J,NA))=COEF(IHMSSF(I,J,NA))+1
   211 CONTINUE
212 CONTINUE
DO 222 I=K3+1,K4
   DO 221 J=I,K4
      COEF(IHMSSF(I,J,NA))=COEF(IHMSSF(I,J,NA))+1
   221 CONTINUE
222 CONTINUE
C OUTPUT SECTION OF THIS CRAZY PROGRAM
DO 250 II=1,NA
   DO 249 JJ=II,NA
   C ***********************************************************
   C ***********************************************************
   C IF PE IS TRAIT X, THEN SET THE FOLLOWING AS K(X-1) AND KX.*
   C PE CAN BE ANY ADDITIONAL RANDOM EFFECT, SO LONG AS ITS *
   C VARIANCE STRUCTURE IS AN IDENTITY MATRIX AND ITS ERROR TO *
   C PE VARIANCE RATIO IS SPECIFIED. *
   C ***********************************************************
   IF (II .GT. K3 .AND. II .EQ. JJ .AND. II .LE. K4) THEN
      DAUG = COEF(IHMSSF(II,JJ,NA)) + DVARPE
      KNTPE = KNTPE + 1
      GO TO 214
   END IF
   C ADD ON RELATIONSHIP INVERSE.
   IF (II .GT. (NA-NANIM-NC) .AND. II .LE. (NA-NC)) THEN
      IF (JJ .LE. (NA-NC)) THEN

READ(11,END=251) AINV
KNTAINV=KNTAINV+1
DAUG=COEF(IHMSSF(II,JJ,NA))+(AINV*DVARAN)
GO TO 214
END IF
END IF

DAUG=COEF(IHMSSF(II,JJ,NA))

214 CONTINUE
IF (DAUG .NE. 0.DO) THEN
WRITE(90) II,JJ,DAUG
WRITE(6,*) II,JJ,DAUG
KNONZER=KNONZER+1
END IF

249 CONTINUE
250 CONTINUE
251 CONTINUE
WRITE(91) DYPY
WRITE(6,*) II,JJ,DAUG
WRITE(6,*) 'RIGHT-HAND SIDES'
DO 300 I=1,NA
WRITE(92) DY(I)
WRITE(6,*) I, DY(I)
300 CONTINUE
WRITE(6,*) 'Y PRIME Y =',DYPY
WRITE(6,*) KNONZER, 'NONZERO ELEMENTS WERE WRITTEN TO XPRIMEX'
WRITE(6,*) 'RECORDS READ =', KOUNT
WRITE(6,*) 'NUMBER OF PERMANENT ENVIRONMENTS =', KNTPE
WRITE(6,*) 'NUMBER OF ELEMENTS FROM AINV =', KNTAINV
DO 310 I=1,NT
WRITE(6,*) MISS(I), 'HAVE TRAIT ', I, 'MISSING'
310 CONTINUE
END

C-----------------------------------------------------------------------
INTEGER FUNCTION IHMSSF(I,J,N)
C FUNCTION TO WORK OUT ADDRESS IN A HALFSTORED SYMMETRIC MATRIX OF
C ORDER N; CONSIDER THE UPPER TRIANGLE. I=ROW, J=COLUMN
C-----------------------------------------------------------------------
IF (I .LE. J) THEN
Il=I-I-1
IHMSSF=N*Il-I*Il/2+J
ELSE
J1=J-1
IHMSF=N*J1-J*J1/2+I
END IF
RETURN
END

C --------------------------------------------------
GO.FT10F001 DD UNIT=DISK, DSN=A3$MMS.GARY17.PROFILE, DISP=(OLD,KEEP)
GO.FT11F001 DD UNIT=DISK, DSN=A3$MMS.GARY17.RELX, DISP=(OLD,KEEP)
GO.FT90F001 DD UNIT=DISK, DSN=A3$MMS.ITDAT,
   DISP=(NEW,CATLG),
   SPACE=(TRK,(5,5),RLSE), DCB=(RECFM=FB,LRECL=16, BLKSIZE=19056)
GO.FT91F001 DD UNIT=DISK, DSN=A3$MMS.YPY, DISP=(NEW, CATLG),
   SPACE=(TRK,(1,1),RLSE), DCB=(RECFM=FB,LRECL=8, BLKSIZE=19064)
GO.FT92F001 DD UNIT=DISK, DSN=A3$MMS.RHNDSD,
   DISP=(NEW,CATLG),
   SPACE=(TRK,(5,5),RLSE), DCB=(RECFM=FB,LRECL=8, BLKSIZE=19064)
// TTPACK JOB
/* JOBPARM DUPLEX-NO, FLASH-NONE, KEEP-YES, LINES-20
// STEPO EXEC SCRUNC
// SYSIN DD *
A$MMS.TSOL
A$MMS.ITSOL
// S1 EXEC FORTVCLG, FVPOPT-3,
// REGION.LKED=8M, TIME.GO=100, REGION.GO=8M,
// PARM.GO='NOOCTSTATUS'

********************************************************************************
// **
// ** THIS IS A FORTRAN VERSION OF THE DRIVER PROGRAM USED TO SET
// ** UP THE VECTORS THAT ARE USED IN THE ITPACK Routines.
// **
// ** NOTE! MORDER AND MELEM MUST BE GIVEN VALUES BEFORE THE
// ** PROGRAM IS RUN. ALSO THE MAXIMUM NUMBER OF ITERATIONS
// ** ALLOWED CAN BE CHANGED (ITMAX) AS WELL AS OTHER PARAMETERS
// ** IN THE "SETTING PARAMETERS" SECTION.
// **
// ** RIGHT-HAND SIDES FOR THE CONJUGATE NORMAL EQUATIONS ARE
// ** DETERMINED AND OUTPUT AS WELL.
// **
// **
// ** INPUT
// **
// **
// ** UNIT 11 ITDAT FROM COEF PRG
// ** UNIT 12 RIGHT-HAND SIDES FROM COEF PRG
// **
// **
// ** OUTPUT
// **
// **
// ** UNIT 91 SOLUTION OF MIXED MODEL EQUATIONS
// ** UNIT 92 SOLUTION OF MIXED MODEL CONJUGATE NORMAL EQUATIONS
// **
// **
// ** NOTE THAT THIS PROGRAM IS DESIGNED TO OUTPUT DATA TO BE
// ** USED WITH A T-TEST.
// **
// **
// ** ONE CAN USE A T STATISTIC TO TEST FOR COVARIABLES. INPUT
// ** MUST BE FROM THE COEFCOV PROGRAM. CONJUGATE NORMAL RIGHT-
// ** HAND SIDES MUST BE ENTERED SO THAT THEY ARE SIMPLY A 1
// ** CORRESPONDING TO THE COVARIABLE TO BE TESTED.
// **
// **
// ********************************************************************************
// ** MORDER - NUMBER OF EQUATIONS TO BE SOLVED
// ** MELEM - NUMBER OF ELEMENTS IN THE COEFFICIENT MATRIX
// ** MENTRY - MELEM IF RHS ARE READ SEPARATELY, ELSE MELEM+MORDER
// ** NF - NUMBER OF FACTORS INCLUDED (COV. 'S ARE THE LAST FACTOR
// ** NTEST - ORDERED NUMBER OF THE FACTOR TO BE TESTED
// **
// ** THE NUMBER OF COVARIATES IS THE LAST FACTOR
// ********************************************************************************
// FORT.SYSIN DD *
PARAMETER (MORDER=1873
*MELEM-*, MENTRY-MELEM*
*NF-7*
*NTEST-7*
*ITMAX-1500*
*NW-4*MORDER+(2*ITMAX))

REAL*8 COEF COEFY
INTEGER IA(MORDER+1), JA(MELEM), IPARM(12), IWKSP(3*MORDER),
* ELECNT, ELEMENT, K(NF)
REAL*8 A(MELEM), RHS(MORDER), U(MORDER), WKSP(NW),
* RPARM(12)
DATA LEVEL/2/, IDGTS/1/
IRND=1
N=MORDER

C**** INPUT NUMBER OF FACTORS****************************************************
N1=29
N2=7
N3=2
N4=53
N5=764
N6=1001
N7=17

C*******************************************************************************
K(1)=N1
K(2)=K(1)+N2
K(3)=K(2)+N3
K(4)=K(3)+N4
K(5)=K(4)+N5
K(6)=K(5)+N6
K(7)=K(6)+N7

C**** THIS VERSION SOLVES IN CORE
C**** READING AND STORING MATRIX AND RHS
ELECNT=1
DO 10 ELEMENT=1,MENTRY
   READ(11,END=35) IR, IC, COEF
   A(ELECNT)=COEF
   JA(ELECNT)=IC
   IF(IR.EQ. IC) IA(IC)=ELECNT
   ELECNT=ELECNT+1
10 CONTINUE
15 CONTINUE
DO 40 ELEMENT=1, MORDER
   READ(12,END=55) COEFY
   RHS(ELEMENT)=COEFY
40 CONTINUE
55 CONTINUE
IA(MORDER+1)=ELECNT

C**** SETTING PARAMETERS
CALL ERRSET(208,0,-1,1.0)
CALL DFAULT(IPARM,RPARM)
C**** SETTING SOLUTION VECTOR TO FIRST GUESS
CALL VFILL(N,U,0.D0)
CALL JCG(N,IA,JA,A,RHS,U,IWKSP,NW,WKSP,IPARM,RPARM,IER)

C**** PRINT SOLUTIONS
WRITE(6,*)'EQUATION','SOLUTION','RHS'
DO 75 I=1,MORDER
   WRITE(91) U(I)
   WRITE(6,*) I,U(I),RHS(I)
75 CONTINUE
WRITE(6,*)
C WRITE OUT RHS IF WANTED
C DO 76 I=1,MORDER
C WRITE(6,*) RHS(I)
C 76 CONTINUE

C***** THIS IS THE LOOP FOR THE CONJUGATE NORMAL EQUATIONS
WRITE(6,*) 'THESE ARE THE SOLUTIONS TO THE CNE.'
NSTART=K(NTEST-1)+1
MCOL=0
DO 80 LL=1,MORDER
   RHS(LL)=0.DO
80 CONTINUE
C SET RHS TO 1.0 FOR COVARIABLE BEING TESTED.
DO 150 ELEMENT=NSTART,K(NTEST)
   MCOL=MCOL+1
   RHS(ELEMENT)=1.DO
150 CONTINUE

C**** SETTING PARAMETERS FOR ITPACK
CALL ERRSET(208,0,-1,1.0)
CALL DFAULT(IPARM,RPARM)
IPARM(1)=ITMAX
IPARM(2)=LEVEL
IPARM(8)=(NW*NW)
IPARM(12)=IDGTS

C**** SETTING SOLUTION VECTOR TO FIRST GUESS
CALL VFILL(N,U,0.D0)
CALL JCG(N,IA,JA,A,RHS,U,IWKSP,NW,WKSP,IPARM,RPARM,IER)

C**** PRINT SOLUTIONS
WRITE(6,*) 'COVARIABLE SOLUTION RHS'
WRITE(92) U(ELEMENT)
WRITE(6,*) MCOL, U(ELEMENT), RHS(ELEMENT)
110 CONTINUE
   RHS(ELEMENT) = 0. DO
150 CONTINUE
STOP
END

//LKD.SYSLIB DD
//   DD
//   DD
//   DD
//   DD DSN=SYSU.LINPACK.VSUBLIB,UNIT=DISK,DISP=SHR
//   DD DSN=SYSU.ITPACK.SUBLIB,UNIT=DISK,DISP=SHR
//GO.FT11F001 DD UNIT=DISK,DSN=A3$MMS.ITDAT,
//   DISP=(OLD,KEEP),DCB=(RECFM=FB,LRECL=16,BLKSIZE=19056)
//GO.FT12F001 DD UNIT=DISK,DSN=A3$MMS.RHNDSD,
//   DISP=(OLD,KEEP),DCB=(RECFM=FB,LRECL=8,BLKSIZE=19064)
//GO.FT91F001 DD UNIT=DISK,DSN=A3$MMS.ITSL,
//   DISP=(NEW,CATLG),SPACE=(TRK,(1,1),RLSE),
//   DCB=(RECFM=FB,LRECL=12,BLKSIZE=19068)
//GO.FT92F001 DD UNIT=DISK,DSN=A3$MMS.TSOL,DISP=(NEW,CATLG),
//   SPACE=(TRK,(5,5),RLSE),DCB=(RECFM=FB,LRECL=8,BLKSIZE=19064)
TTEST JOB
/*JOBPARM DUPLEX-NO,FLASH-NONE,KEEP-YES
//S1 EXEC PORTVCLG,
// REGION.LKED=1028K,REGION.GO=612K,TIME.GO=05
//PORT.SYSIN DD *

//***********************************************
//** PROGRAM TO TEST THE SIGNIFICANCE OF A COVARIABLE IN A
//** MIXED MODEL USING SOLUTIONS TO MIXED MODEL CONJUGATE
//** NORMAL EQUATIONS. SEE HIROSHI TAKAHASHI M.S. THESIS,
//** IOWA STATE UNIVERSITY, UNPUBLISHED FOR DETAILS OF THE
//** TEST. THIS PROGRAM IS RUN AFTER TTPACK PROGRAM.
//**
//** INPUT
//**
//** UNIT 11 CONJUGATE NORMAL SOLUTIONS
//** UNIT 13 SOLUTIONS TO MME'S
//** UNIT 14 MME RIGHT-HAND SIDES
//** UNIT 15 Y PRIME Y
//**
//** NRANK IS THE RANK OF THE FIXED EFFECTS PORTION OF THE*
//** EQUATIONS OF CONCERN. *
//** NNNN IS THE NUMBER OF RECORDS FROM COEF JOB OUTPUT *
//**

PARAMETER (NRANK=108
* ,NNNN=1933
G**** INPUT NUMBER OF FACTORS*******************************************
*   ,N1=33
*   ,N2=7
*   ,N3=2
*   ,N4=53
*   ,N5=764
*   ,N6=1001
*   ,N7=17
*   ,NA=N1+N2+N3+N4+N5+N6+N7
C******************************************
C DEFINE THE TRAIT YOU WISH TO TEST!
C NUSE= THE POSITION OF THE COVARIATES
C NEUSE=SIZE OF HALF STORED MATRIX (NEED NOT BE CHANGED)
C KUSE= NUMBER OF EQUATIONS AHEAD OF THE TRAIT OF INTEREST
C******************************************
*   ,NUSE=N7
*   ,NEUSE=NUSE*(NUSE+1)/2
*   ,KUSE=N1+N2+N3+N4+N5
C******************************************
*   ,NUSEM1=NUSE-1)

C*********************************************************
C SET PARAMETERS ACCORDING TO THE NUMBER OF LEVELS OF THE FACTOR*
C FOR WHICH THE TEST IS DESIRED.

REAL*8 PHI(NUSE), BHat(NUSE),
*ITSOL(NA), YPY,
*SIGSE, SUBTR, RHNDSD(NA), T(NUSE)

K1=N1
K2=K1+N2
K3=K2+N3
K4=K3+N4
K5=K4+N5
K6=K5+N6

C**** SECTION TO READ THE SOLUTION MATRIX OF THE CONJUGATE NORMAL EQUATIONS.

DO 11 I-1,NUSE
    READ(11) PHI(I)
11 CONTINUE

C**** OBTAIN DENOMINATOR SS AND THEN THE T-TEST

WRITE(6,*)'EQUATION SOLUTION R-H-S'

DO 65 I=1,NA
    READ(14) RHNDSD(I)
    READ(13) ITSOL(I)
C WRITE(6,*) I, ITSOL(I), RHNDSD(I)
65 CONTINUE

SUBTR=0.DO
DO 67 I=1,NA
    SUBTR-SUBTR+(ITSOL(I)*RHNDSD(I))
67 CONTINUE

READ(15) YPY
WRITE(6,*)'SUBTR=',SUBTR
WRITE(6,*)'YPY=',YPY

CALCULATE ESTIMATE OF ERROR VARIANCE
SIGSE=(YPY-SUBTR)/(NNNN-NRANK)

WRITE(6,*)'LACTATIONAL ENERGY'
WRITE(6,*)'RANK OF FIXED EFFECTS',NRANK
WRITE(6,*)'NUMBER OF OBSERVATIONS',NNNN
WRITE(6,*)'ESTIMATE OF RESID. VARIANCE',SIGSE
WRITE(6,*)' COVARIABLE SOLUTION CONJ. SOL
* T value'

DO 68 I=1,NUSE
BHIAT(I)=ITSOL(K5+I)

C CALCULATE THE T STATISTIC
   T(I)=(BHIAT(I)/(DSQRT(SIGSE*PHI(I))))
WRITE(6,*)I,BHIAT(I),PHI(I),T(I)
68 CONTINUE

C FOR LARG DEGREES OF FREEDOM THESE ARE ASSOCIATED PROB. LEVELS
WRITE(6,*)'******************************************'
WRITE(6,*)'*** T VALUE P > T ***'
WRITE(6,*)'*** 0.674 0.5 ***
WRITE(6,*)'*** 0.842 0.4 ***
WRITE(6,*)'*** 1.036 0.3 ***
WRITE(6,*)'*** 1.282 0.2 ***
WRITE(6,*)'*** 1.645 0.1 ***
WRITE(6,*)'*** 1.960 0.05 ***
WRITE(6,*)'*** 2.326 0.02 ***
WRITE(6,*)'*** 2.576 0.01 ***
WRITE(6,*)'*** 3.291 0.001 ***
WRITE(6,*)'******************************************'

END

//GO.FT11F001 DD UNIT=DISK,DSN=A3$MMS.TSOL,DISP=(OLD,KEEP),
// DCB=(RECFM=FB,LRECL=8,BLKSIZE=19064)
//GO.FT13F001 DD UNIT=DISK,DSN=A3$MMS.ITSOL,DISP=(OLD,KEEP),
// DCB=(RECFM=FB,LRECL=12,BLKSIZE=19068)
//GO.FT14F001 DD UNIT=DISK,DSN=A3$MMS.RHNDSD,
// DISP=(OLD,KEEP),DCB=(RECFM=FB,LRECL=8,BLKSIZE=19064)
//GO.FT15F001 DD UNIT=DISK,DSN=A3$MMS.YPY,DISP=(OLD,KEEP),
// DCB=(RECFM=FB,LRECL=8,BLKSIZE=19064)