AN ABSTRACT OF THE THESIS OF

Roger LeRoy Kray for the degree of Doctor of Philosophy in Genetics presented on 4 January 1977

Title: ASSOCIATION OF GENETICALLY DETERMINED POLYMORPHIC BLOOD COMPONENTS WITH PROLIFICACIES OF PUREBRED AND CROSSBRED PIGS.

Abstract approved: Ralph Bogart

Crossmatings were accomplished among three semi-closed lines within a population of pigs, for the purpose of investigating effects of crossbreeding on reproductive traits. Two of the lines were purebred (Berkshire and Yorkshire); the third line ("Composite") consisted of descendants from inter se matings among offspring of crosses of the Berkshire and Yorkshire lines. Other crossbred categories consisted of offspring sired by members of the closed lines and farrowed by daughters which had been produced from Berkshire-Yorkshire crossmatings. Reproductive performance was expressed as number of fetuses (dead or alive) per litter.

Demonstrations of genetic contrasts among the categories were accomplished by means of electrophoretic analyses of hemolysates and blood serum, and by serology of erythrocytes. Those methods were used to detect phenotypic variations in four serum proteins, two erythrocyte enzymes, and seven antigenic determinants located on the erythrocyte membrane. Among 275 pigs from which specimens were obtained for those analyses, there were dams which had produced a total of 480 litters. A sub-group among the subjects of those studies included 36 families consisting of a mated sow and boar, and one or more offspring of the mating. Inclusion of those families within the population of subjects provided opportunity for some verification of previously proposed modes of inheritance of the blood components.

Certain alleles were found to be heterogeneously distributed among the Berkshire, Yorkshire, and Composite lines. Distribution of phenotypes among those categories was such that crossmatings could result in

formation of heterozygous combinations of alleles which were found only in the homozygous state in the Berkshire and Composite lines. Distribution of alleles found at one locus was such that crosses of Yorkshires with either Berkshires or Composites could be expected to produce a phenotype which was not present in any of the Berkshire, Yorkshire, or Composite subjects. Those observations provided motivation for accomplishing blood component phenotyping on specimens from daughters produced from various types of crossmatings.

Performance data on sows of separate breed categories (crossbred and purebred) were analyzed separately, as seven different subsets of the total performance data. Blood component phenotypes were shown to be associated with variance in reproductive performance, by the method of least squares analysis. Non-genetic variables also considered in these analyses were the age of dam at date of farrowing, and the semester during which conception occurred. Series of least squares analyses were accomplished with data from each of seven different breed categories.

Some evidence was found to suggest an overdominant mode of gene expression in certain breed categories. That mode of gene expression was correlated with the transferrin phenotypes in the Yorkshire and Composite lines, and with the amylose-modifier phenotypes in daughters of the Berkshire-Composite crossmatings. Somewhat less persuasive evidence also suggested an overdominant mode of gene expression correlated with the phosphohexose isomerase phenotypes in another crossbred category. The additive mode of gene expression was associated with transferrin and phosphohexose isomerase phenotypes in daughters from Berkshire-Composite crossmatings.

Equally noteworthy results of the study were the consistencies in correlations of certain blood components with performance, without regard to whether gene expression appeared to be additive or non-additive. The evidence indicated high probabilities that transferrin phenotypes were real correlates of performance in three breed categories and that this was also true of amylose-modifier phenotypes in two of the breed categories. There was also fairly persuasive evidence that transferrin and amylose-modifier phenotypes were both real correlates of performance in two other breed categories as well.

Association of Genetically Determined Polymorphic Blood Components with Prolificacies of Purebred and Crossbred Pigs

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Commencement June 1977

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Date thesis is presented 4 January 1977

Typed by Barbara York for Roger LeRoy Kray

ACKNOWLEDGMENTS

Graduate Program Committee Members

Dr. Ralph Bogart (Major advisor)
Dr. Lyle Brown
Dr. Janet Cardenas
Dr. Warren Kronstad
Dr. Paul Roberts

Dr. D. C. England significantly aided the accomplishment of the studies reported here, by allowing total access to the extensive records on reproduction of the Oregon State University swine herd, and by offering suggestions about contents of portions of this thesis. Dr. England aided also by organizing adequate assistance at collection of blood samples.

Dr. W. D. Hohenboken provided valuable advice on the statistical analysis of data.

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ASSOCIATION OF GENETICALLY DETERMINED POLYMORPHIC BLOOD COMPONENTS WITH PROLIFICACIES OF PUREBRED AND CROSSBRED PIGS

INTRODUCTION

Fundamental techniques of biometrical analyses have been applied to rather large bodies of data, toward the goal of understanding the bases for variation of litter size in pigs (Cummings et al., 1947; Boylan et al., 1961; Urban et al., 1966; Revelle and Robison, 1973). Those analyses indicated that an individual gilt's reproductive performance is unlikely to be predicted with practical reliability by the criterion of performance of its dam. Because litter size is of obvious economic importance, there have been continuing efforts toward understanding the bases for the individual variation. Specifically designed experiments have yielded evidence that differences in litter size indeed have a genetic basis.

Hybrid vigor has been a typical result of crossing of unrelated populations of pigs (Winters et al., 1935; Chambers and Whatley, 1951; Squiers et al., 1952; Smith and King, 1964). Offspring of the crosses were superior in terms of early viability or fitness, in comparison to the pooled average of the parental groups.

The breeding program for the Oregon State University swine herd since 1960 has consisted, in part, of various crossmatings involving three semi-closed lines. Separate lines were derived from each of two stocks obtained from established purebred herds of the Berkshire and Yorkshire breeds. The third line was developed from a small group of first-generation offspring from crossmatings of the two purebred lines; this was designated as the Composite line. Crossmatings involving members of the latter line were begun in 1968. Crossmatings, by 1973, showed evidence of hybrid vigor in terms of average litter sizes in two of the crossbred categories. Daughters from matings between Berkshires and Yorkshires farrowed, at specific parities, litters which were larger than the averages of either of the two lines of which their

parents were members. A quite similar observation was obtained when the average size of litters born to daughters of crossmatings of Yorkshires and Composites was compared to averages of the Yorkshire and Composite lines.

The observation of hybrid vigor in the daughters from Berkshire-Yorkshire crossmatings is consistent with the results from crosses among other breeds (Squiers et al., 1952; Smith and King, 1964). However, the finding that a similar degree of hybrid vigor was obtained from crossing the Yorkshire and Composite lines was not anticipated on the basis of any previous findings. Due to the results of crossing the Yorkshire and Composite lines, interest was drawn toward the question of the degree to which the two lines were genetically distinct from each other. Some basis existed for supposing that a considerable degree of distinctiveness might exist between those lines. Parents of the Yorkshire-Composite crossbreds were separated by as many as six generations from ancestors common to the two lines. Furthermore, the lineages from two Yorkshire boars of early generations were not represented in the Yorkshires used in the crossmatings. It was felt that objective evidence about genetic differences between lines could aid understanding of the genetic basis for hybrid vigor.

This study of the genetics of litter size in the herd described above consisted of two separate phases. Those phases involved the testing of separate hypotheses. The goal of the initial phase was to verify the expectation that a chosen battery of serological and biochemical analyses could be effective in demonstrating genetic differences among the Berkshire, Yorkshire, and Composite lines. Results of that phase were the basis for extending the process of single-gene analyses into a second phase. Data were gathered during the latter phase for evaluating the extent to which variance in litter size could be attributed to additively and non-additively acting determinants present in specifically identified genetic linkage groups.

It was reasoned that the assignment of such genetic determinants to specific linkage groups could constitute important fundamental information that would be of value in designing future studies of this metric trait. Verification of any of the standing hypotheses about

the basis for hybrid vigor as it occurs in swine reproductive traits, could probably be made more likely, subsequent to identification of the linkage group locations of those genetic determinants.

BACKGROUND LITERATURE

Swine Prolificacy

Despite the use of Sus scrofa as research material in a wide variety of bio-medical disciplines (e.g., see the symposium proceedings compiled by Bustad and McClellan, 1966), the elucidation of a genetic basis for variation in prolificacy has seldom been attempted by any approach other than purely statistical evaluation of rates of reproduction. That approach has been useful for providing indications that the variation has some genetic basis. A stimulating aspect of the observations collected to date is the evidence that litter size in at least some populations may be partly dependent upon genetic factors which have a non-additive mode of action.

Studies of various swine populations have demonstrated the advantage of using the heritability statistic in attempting to evaluate the nature of determinants of individual variation of litter size. Methods for estimating the heritability, and the description of usefulness of that statistic, have been presented by Falconer (1960). A trait for which the heritability value is found to be 1.0, would be one for which differences among individuals are determined strictly by additively acting genetic factors.

Heritability of the number of live-born per litter, farrowed by gilts, was estimated by Cummings et al. (1947), using records of both inbred and crossbred lines. The pooled estimate for those several breed types was not significantly different from zero. A larger quantity of data from three inbred lines, also pertaining to litters born to gilts, was analyzed by Boylan et al. (1961). The estimates obtained for each of those three lines were not significantly different from zero. The data on 3119 litters born in nine purebred and two crossbred categories were analyzed by Urban et al. (1966), to estimate heritability of the number born dead or alive per litter. Data from litters subsequent to the first-born were not excluded. The resulting estimate of heritability, 0.09 ± 0.04, was significantly different

from zero. A more recent study involved both crossbred and purebred Yorkshire and Duroc populations (Revelle and Robison, 1973). Heritability of number of live-born per litter was estimated as 0.13 ± 0.06. Revelle and Robison have suggested possible reasons for the low values typically obtained as estimates of heritability of swine prolificacy. Those suggestions included the occurrence of negative correlations of separate components of the trait, and control of the trait by genes which are not additively expressed.

Studies of the reproductive physiology of litter-bearing species (Nalbandov and Bahr, 1974; Tachi and Tachi, 1974) make it apparent that the occurrence of negatively correlated individual components of the litter size trait is a suggestion which should be seriously regarded. However, there is also a considerable body of evidence in favor of the concept that important causes of variance in litter size may be genotypes consisting of genes which are not additively expressed.

Two- and three-breed crosses involving Poland China, Duroc Jersey, and Chester White purebred populations provided early evidence of the "crossbreeding effect" on the trait (Winters et al., 1935). That study showed that when daughters born from crosses of two breeds were mated to boars of a third breed, the average number of live-born per litter was distinctly greater than the average live-born per litter from females who were instead backcrossed to boars of a parental breed (9.33 vs. 8.13). No difference between numbers born dead per litter was found for the two types of matings.

A population of the Duroc breed was used by Chambers and Whatley (1951) in a program of crossmatings which yielded somewhat more substantial evidence about relative merits of differing patterns of crossmating. The average number of piglets per litter, in litters produced by matings between separate lines, was found to be larger than that of inbred litters, but the difference was not statistically significant. However, a highly significant difference was found when a comparison was made between the average for litters from inbred dams and the average for litters farrowed from matings between line-cross daughters and boars of a third line. The average size of litters

farrowed by the crossbred, crossmated gilts was 1.64 piglets greater than the average for the litters from inbred dams.

A crossbreeding effect was also apparent in a study by Squiers et al., (1952). Subjects of that study consisted of three inbred lines, one outbred population, and, derived from crossings of those groups, six different subgroups of crossbred offspring. Reproductive tracts were recovered from mated females slaughtered at 25 days after estrus. Counts were made of the number of corpora lutea and the apparently viable embryos contained in reproductive tracts. The average numbers of corpora lutea per gilt for each of the six crossbred categories were compared to the average of the two parental categories from which the crossbred population was derived. Four of the six crossbred populations exceeded the average of the respective parental groups by at least 1.3 corpora per gilt. The data for all crossbreds were pooled and compared to pooled data from the parental groups. The mean difference by which the crossbreds exceeded the purebreds (1.19 corpora per gilt) was highly significant ($P<0.01$). The data on ovulation were coupled with embryo counts for a study of early in utero mortality. Comparisons between each crossbred category and the respective parental populations showed that in five of the six crossbred types, in utero mortality to the 25th day was less than the average of the two respective parental lines. Pooling of the data for the crossbreds for comparison to pooled data from the purebred categories yielded the result that, to the 25th day, crossbred gilts lost 0.81 fewer embryos per gilt than did purebreds. Finally, a comparison was made between the purebred parental groups and the crossbreds for numbers of normal embryos present at the 25th day. Each of the six crossbred types exceeded the average for the respective parental categories by values ranging from 0.5 to 2.8 embryos per gilt. The difference between pooled crossbred and pooled purebred groups, 1.85 embryos per gilt, was highly significant.

Among the four parental populations from which crossbred populations were derived for the study by Squiers et al., three were inbred lines having average degrees of inbreeding amounting to 45, 30, and 25%. A later study by Johnson and Omtvedt (1975) used three

separate breeds in an experimental design having much similarity to that of Squiers et al. (1952). A contrast between those studies was the practically insignificant extent of inbreeding of the three purebred stocks used as parents of the crossbred subjects in the latter project (Johnson et al., 1973). Some contrasts are also found between the results of the two studies. Johnson and Omtvedt (1975) found that average numbers of corpora lutea per gilt in each of the three crossbred types was less than the average for respective parental categories (unlike the results found by Squiers et al. (1952)). Pooled data on all crossbreds and pooled data on the purebreds showed there were 0.45 fewer corpora lutea per crossbred gilt, compared to purebred gilts. However, comparison of the numbers of apparently viable embryos at 30 days yielded results which were consistent with those of Squiers et al. Number of embryos in each of the three classes of crossbreds was greater than the average in the respective parental group. The total crossbred average exceeded the purebred average by 0.77 embryos per gilt. Finally, the ratio of live embryos per corpus luteum in the crossbreds, as a pooled group, exceeded that of the purebreds by 9.52%, a highly significant difference.

Other evidence of non-additive gene action was provided from a larger quantity of data (Smith and King, 1964). The data were obtained from more than 500 farms through auspices of the Pig Industry Development Authority in Great Britain, and consisted of records on about 28,000 litters produced from females of either the Large White, Landrace, or Wessex breeds, or daughters born to crosses of any two of those breeds. Daughters born to crossmatings of Wessex and Landrace, or to crossmatings of Wessex and Large Whites, farrowed litters which were larger than those produced by females in the purebred parental lines. Production from the daughters born to crossmatings of the Landrace and Large White breeds seemed to have some dependency on the breed of the service boar. Daughters from the crossmatings of Landrace and Large Whites, when backcrossed to Large White boars, farrowed larger litters than did either Landrace or Large White females mated to Large White boars. Such superiority relative to both parental

breeds was not observed when daughters of the crossmating of Landrace and Large Whites were mated to Landrace boars.

Crosses of two semi-closed purebred lines (of the Berkshire and Yorkshire breeds) at Oregon State University were the basic stock from which a third closed line was derived. Spurr (1969) provided a description of the contrasts of average litter sizes among the three lines, along with results of some more detailed studies of the bases for variance in litter size. During a six-year period following crossing of the two purebred lines, the line from the hybrid base was developed by inter se matings among successive generations of offspring of those crossmatings. There were efforts to identify determinants of litter size by estimating effects of age of dam at time of farrowing and of the degrees of inbreeding of the dam and of the litter born. Mean values of the number of fetuses per litter were calculated, with adjustments for variation of those factors. The adjusted value obtained for number of fetuses (dead or alive) per litter farrowed by Berkshire dams was 8.46. Calculations of the adjusted averages for the Yorkshire and hybrid lines yielded identical values of 10.69 fetuses per litter.

A subsequent study of variation in performance among groups within that herd was made by utilizing data on 889 litters. In that study, Liu (1974) utilized a succinct means of distinguishing between the first-generation (F_1) offspring of Berkshire-Yorkshire reciprocal matings, and descendants of inter se matings of those F_1 offspring; the latter individuals were designated as the Composite line. Liu studied the results from mating females of each line to boars of the same line, as opposed to mating with boars of a different line. Mating of Yorkshire females to Berkshire boars yielded litters in which the average number of live-born was 9% higher than in litters produced by intra-line matings of Yorkshires. A similar result was obtained when the effect of mating Composite females to Yorkshire boars was compared to the intra-line mating of Composites. The advantage of mating Berkshire females to Yorkshire boars (number of live-born per litter was 15.8% greater than the average from matings of Berkshire females to Berkshire boars) was found to be highly significant.

Genetic Bases for Hybrid Vigor

Two concepts having some distinctiveness from each other were devised for the purpose of providing hypotheses about the bases for hybrid vigor. Neither of these concepts comprises any postulate of the actual degree of complexity of the array of genes controlling any specific trait. Rather, they provide concepts about the qualitative nature of those genes. Subsequently, others presented somewhat more elaborate concepts which served to emphasize that complex patterns of organization of genes affecting a particular trait may have evolved through natural selection.

Efforts by Crow (1948), at tracing the origins of the hypotheses about heterosis showed that, in 1910, Bruce, Keeble, and Pellew proposed that favorable alleles are dominant to the inferior alleles at several or many of the loci involved. Inferior alleles which diminish the phenotypic value of the individual are supposedly present at loci which are not the same in the two populations used for the crossmatings. Therefore, offspring of the crossmatings possess significantly fewer loci at which a deleterious allele is present in the homozygous state. Crossbred populations in which it is found that the vigor achieved by the initial cross cannot be successfully fixed within at least some selected descendants are not necessarily viewed as refutations of this concept. Such findings are considered to be explainable by the occurrence of repulsion linkage of the more favorable alleles.

An alternative concept, that of overdominance, apparently had its origins at about the same date. East (1936) was able to cite his own publication, in 1912, of an opinion that the hybrid vigor expressed in the first-generation progeny of a cross-fertilization is "roughly proportional to the genic distinctiveness" between the two parental stocks. Decades after this proposal, the concept was refined by means of the postulate that, with greater divergence in functional characteristics of alleles, the action of those alleles became more nearly additive in the heterozygote (East, 1936). The essential

feature of the concept remains the idea that the value of a particular heterozygous genotype is greater than the values of either of the homozygous types formed from the two alleles.

The particular relevance of the concept of overdominance to "fitness" was later emphasized. Lerner (1954) stressed that heterozygosity per se probably provides distinct advantages. Phenotypes which are the more extreme deviants from the normal have the poorest viability, except in very uncommon environmental conditions. During ontogeny, development of an extreme phenotype is less likely in the case of individuals possessing heterozygous genotypes, compared to individuals of homozygous genotypes. Thus, according to Lerner, the totality of the characteristics which determine fitness exhibit the quality of overdominance. It seems appropriate to note that the thesis of Lerner does not strictly insist on the importance of true heterozygosity. The proposed concept allows that evidence of overdominance may in fact be pseudo-overdominance. That is, the advantageous combinations may actually consist of particular genotypes located at separate, closely linked genes.

Mather (1955) stressed an alternative to the concept of the value of heterozygosity. Hybrid vigor might be due to the fact that the separate characters which determine superiority are controlled by several or perhaps many gene combinations in which there is an interrelatedness that is partly due to pleiotropic gene action, partly due to physical linkage, and perhaps partly because of interactions of the separate characters themselves. Mather contends that in outbreeding species, selection must have operated on the complexes present in the heterozygous configuration, since those complexes would have only seldom been present in the homozygous state. Thus, an important consideration might be that of the "balance" of gene combinations which have evolved by natural selection acting upon the many different combinations possible in a polygenic system.

Genetic Bases of Some "Simple" Traits

Development of a medium for electrophoresis of tissue fluids without the disadvantage of convection (Smithies, 1955) was a breakthrough which made possible a highly advantageous new approach to analysis of genetic variation among individuals. Use of the approach in studies of blood serum specimens provided suggestions of its considerable value.

Blood serum samples from a heterogeneous population of cattle were subjected to the starch gel electrophoresis method devised by Smithies (Ashton, 1958). Variations among individual sera were observed in components of the beta-globulin segment of the electropherogram. Ashton concluded that the variation was due to co-dominant alleles at a single locus. Later, auto-radiography of electropherograms served to identify the variant beta-globulin fractions as the iron-binding proteins of serum (Giblett et al., 1959). The name transferrin had been applied to the serum components which exhibited that characteristic, following purification and characterization of such a component from the beta-globulin fraction of swine serum (Laurell and Ingelman, 1947; Holmberg and Laurell, 1947).

Interest in heritable variants of blood serum components extended to pigs within a fairly short time after the demonstration of such a component in cattle. Kristjansson (1960) presented evidence of an individual variation with a genetic basis, in pigs, and subsequently demonstrated that the component was transferrin (Kristjansson and Cipera, 1963). Two alleles at the transferrin (Tf) locus, Tf^A and Tf^B, were described by those studies. Existence of two additional alleles was later suggested, and those have been designated as Tf^C (King, 1962) and Tf^D (Schroffel, 1966). Kristjansson used autoradiography to verify that those proposed additional alleles were Tf (Baker, 1968a). Following that early demonstration of simply determined variation of blood components, a series of studies indicated that several other components displayed such variation.

Pre-albumin is a protein which was named on the basis of its distinctive behavior during electrophoretic separation of serum components. Analysis of sera from 358 pigs provided evidence of variation of that fraction among individuals (Kristjansson, 1963). Family studies within that population indicated a single-locus mode of inheritance with two alleles present in the population.

Ceruloplasmin. Successful purification of a distinctive metalloprotein containing copper was accomplished in 1948 using swine serum as the source (Holmberg and Laurell). The name ceruloplasmin, proposed upon initial purification of the protein, has been retained. Electrophoretic methods available at that time indicated that the protein had the mobility of an alpha-globulin. It was found to possess oxidase activity toward para-phenylenediamine. That enzymic property was later utilized in conjunction with starch gel electrophoresis by Imlah (1964) to demonstrate that ceruloplasmin variants occurred in pigs. Family studies within one breed showed that the variation could be attributed to two alleles at a single locus.

Hemopexin. Separate workers independently exploited knowledge of the peroxidase activity of heme-containing proteins for purposes of locating such components of pig serum on electrophoresis gels (King, 1962; Imlah, 1965; Graetzer et al., 1965). Those efforts revealed the existence of genetically determined variants of the hematin-binding component. Variations were found to be due to presence of four alleles at a single locus. Existence of a serum globulin having such a function had been suggested earlier, at which time the name hemopexin was proposed (Grabar et al., 1960). Apparently, the genetic variation of that component in pigs had been observed in an earlier study. However, that study (Kristjansson, 1961) did not distinguish between hemopexin and the hemoglobin-binding protein, and apparently, the variant components were mistaken as hemoglobin-binding protein.

Slow alpha-2 macroglobulin. A component from human serum, having the mobility of the alpha-globulins in a filter-paper electrophoresis system, was found by Poulik and Smithies (1958) to behave differently in a starch gel electrophoresis system. That component migrated at a distinctly slower rate in the latter system than did the alpha- and

beta-globulins. Those investigators reasoned that the difference in relative mobilities in the two systems might be due to especially large molecular size, causing a slower migration in gel than on the paper medium. Descriptions of results from the different systems were facilitated by assigning to that component the name, slow alpha-2 macroglobulin (Sa). Schroffel (1965) observed a component of swine serum having a mobility distinctly less than that of beta-globulins, and chose to refer to it as the slow alpha-2 macroglobulin. Analyses of sera from a heterogeneous population of pigs led to the conclusion that individual variations of that component could be attributed to three alleles at a single locus.

Amylase. Coincident with electrophoretic observations of the para-phenylenediamine oxidase activity of swine serum, Graetzer et al. (1965) observed that one or two discrete bands having a different translucency occurred along the migration path of each specimen, after a period of incubation. Further attention was given to those bands as they occurred in specimens from 123 litters of pigs. Results were compatible with the concept of genetically determined variation of positions of those bands, due to three alleles at a single locus. Graetzer et al. (1965) did not report that a protein at the positions of those bands was evident after application of a protein stain. However, more detailed studies provided some indication that the components were proteins. Although those studies did not provide any more definite evidence about the specific nature of the components, it has been suggested that they might be serum amylase (Moustgaard and Hesselholt, 1965). That hypothesis is suggested by the apparent alteration of the structure of the starch gel. That hypothesis can be considered reasonable, in view of evidence from specific assays showing that amylase activity is present in swine serum (Ryan et al., 1975).

Specific enzymes are locatable on starch gels following electrophoresis of tissue fluids. Mixtures of the substances necessary for the functioning of a particular enzyme may be applied over the surface of the gel after electrophoresis. Location of the enzyme on the gel may be disclosed by including, in the applied mixture, a chromogenic compound which will undergo a color change as a result of the action

of the enzyme. (Most commonly, the chromogen is not a substrate for the enzyme; the color change simply occurs as a secondary consequence of the enzyme activity.) This technique for obtaining evidence about variant forms of a particular enzyme was devised by Markert and Hunter (1957), who proposed that the gels processed in this way should be called zymograms.

Erythrocyte enzymes. Prior to the innovation of gel electrophoresis and development of zymograms from gels, evidence began to accumulate to indicate that the circulating mammalian erythrocyte was probably the site of considerable enzyme activity (Bartlett and Marlow, 1953). Such evidence, along with the relative ease of procurement of adequate volumes of blood from a variety of mammalian species, facilitated pursuits of evidence of variant forms of some specific enzymes, besides those present in serum. Saison (1968) applied the hemolysates from 15 families of pigs to a starch gel electrophoresis system. Development of a zymogram from the gel demonstrated the existence of variation of electrophoretic mobility of 6-phosphogluconate dehydrogenase (Pgd). A single-gene basis for that variation seemed evident. Later, Saison and O'Reilly (1971) reported that variation of phosphohexose isomerase (Phi) in swine erythrocytes also had such a genetic basis, with two alleles apparent at that locus.

Erythrocyte membrane antigens. Other blood components display variation detectable by techniques which are substantially different from those which elucidate enzyme and serum protein variants. Certain structural features of the swine erythrocyte membrane are found to vary among individuals. As with the components described above, the variation has been found to be genetically based to a considerable extent (Saison and Ingram, 1962; Andresen, 1962). More specifically, much of the individual variation had been found to be attributable to allelic variations at several genetic loci. Those findings provided additional means by which to describe the degree of differences of genetic constitutions among individuals.

Demonstration of such differences of membrane composition have been possible due to the fact that those variant components have the property of immunogenicity. The essential consequence of that property

is that blood sera can be obtained, from specifically immunized individuals, which contain a class of proteins having an antibody function. The individual variations in the compositions of erythrocyte membranes are evidenced by contrasts in the results observed upon mixing the erythrocytes with the serum containing the specific antibody (antiserum). The essence of this phenomenon was first described in 1901 by Landsteiner, who later summarized its ramifications in a comprehensive treatise (Landsteiner, 1962).

Four of the separate genes known by 1962 as being determinants of variation of swine erythrocyte antigens were given the respective alphabetic designators G, H, I, and L. (Andresen, 1962, 1964). Two alleles which control the antigen structures were identified to each of those four loci, and were designated[1], respectively, as G^a, G^b, H^a, H^b, I^a, I^b, L^a, and L^b. Subsequently, identification of additional alleles at the H locus was reported (Hojny, 1973).

Another separate gene responsible for the antigenic variation was discovered independently by researchers in Europe and Canada, and designated as the N gene (Hojny et al., 1966). Two alleles, N^a and N^b, were found at that locus.

The variation which is described as the A-o blood group system differs from the above in some essential respects. The A^A allele at that locus apparently is expressed as a trait that is completely dominant to the a^o allele (Saison and Ingram, 1962). Furthermore, it has become apparent that variation of the A-o phenotypes is not simply due to a single locus. The initial evidence of a gene which exerts an epistatic effect upon the A-o gene was provided by Rasmusen (1964). More recent evidence indicated that this gene, which can suppress the A and o phenotypes, is closely linked to the H gene (Rasmusen, 1972; Hojny, 1974).

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[1] Rasmusen (1975a) has proposed nomenclature which makes possible a distinction between the antigens present on the cell membrane, and the genes which determine the various antigens. Antigenic determinants are specified by single alphabetic symbols with subscripts attached (the only exceptions are the symbols for phenotypes controlled by the A-o locus, viz., A, o, and "-"). The genes controlling the antigens are specified instead by alphabetic symbols with superscripts.

Additional genes have been identified as being determinants of the antigenic components of the swine erythrocyte. The loci designated as D (Rasmusen, 1967; Hradecky, 1967; Hradecky and Linhart, 1970) and F (Andresen, 1962; Hradecky and Hojny, 1970) each have two alleles which have been identified to date. At least two alleles exist at the K locus (Andresen, 1962). The antigens designated as the E system apparently are a rather complex group. Evidence is being sought as to whether or not that group of variants is due to only a single allelic series (Andresen, 1962; Saison and Ingram, 1962; Hojny and Hradecky, 1972, 1973).

Molecular Bases for Polymorphism

There is much uncertainty about the physiological significance of the occurrence of variant forms of individual components of tissues. Almost certainly, any actual significance could be made more apparent if the structural bases for the variations were known. There have been some beginnings at achieving this goal; however, very few of the components have been subjected to sufficient analyses so that explicit evidence about variance in structure has been obtained. Instead, for most components mentioned, it is possible to pose only tentative concepts about the basis for the variations. Mostly, the basis for those concepts is evidence about structural variation in homologous components of other species which have been more intensively studied.

Transferrin. Often, some fairly simply preliminary treatments of the sample being analyzed by electrophoresis are helpful for suggesting the nature of structural variation. Blumberg and Warren (1961) showed that a reduction in electrophoretic mobility of human Tf fractions was effected by enzymatic removal of sialic acid residues from the molecule. Kristjansson and Cipera (1963) subsequently applied the enzyme, neuraminidase, to swine serum samples and found that the treatment caused a similar reduction in electrophoretic mobility of Tf fractions. Sera from pigs of two different Tf genotypes were treated with neuraminidase and compared to untreated serum. That study only showed that mobilities of the Tf's were altered by neuraminidase; it did not

substantiate the concept that sialic acid residues were essential determinants of variation in the electrophoretic characteristics. More recently, three separate groups of investigators have studied Tf isolated from swine serum. Initial isolation of Tf was accomplished by essentially similar, although not identical procedures, in each study. Removal of most of the serum proteins besides Tf was accomplished by rivanol precipitation. However, there are considerable differences in scope among the three studies. Comparison of electropherogram patterns of neuraminidase-treated and untreated Tf was a technique applied by two groups. Stratil and Kubek (1974) utilized serum obtained from one pig of the phenotype Tf B. Results were interpreted as favoring the concept that none of the electrophoretically distinguishable Tf fractions contained more than two sialic acid residues per molecule. Graham and Williams (1975) also made comparisons of electrophoretic mobility before and after neuraminidase treatment. The stated conclusion from that study was in agreement on the point that most Tf molecules contain two sialic acid residues, although some apparently contain just one. The latter workers accomplished more extensive structural studies of swine Tf. Those studies indicated that carbohydrate portions of the Tf may be attached at two unlike sites along the polypeptide. Furthermore, evidence from quantitative analyses of carbohydrate contents suggested that some Tf molecules may contain a single carbohydrate chain, while others contain two. Thus, there is considerable consistency in the conclusions of these two studies. However, a somewhat earlier study provided markedly different evidence about the sialic acid content. Hudson et al. (1973) reported finding seven to eight sialic acid residues per molecule of swine Tf. Differences among conclusions of the different workers may be due to actual differences of the materials analyzed. Only Stratil and Kubek provided an explicit description of the source of the material studied, namely, serum from an individual of the phenotype Tf B. Hudson et al. studied material obtained from a commercial source.

The study by Hudson et al. (1973) provided evidence of a lack of quarternary structure in swine Tf. Measurement of molecular weight was performed under conditions known to be generally effective for

dissociating the subunit polypeptides of proteins. The value obtained was essentially equal to that found under non-dissociating conditions.

Ceruloplasmin. Studies of some fundamental structural properties of swine serum ceruloplasmin have been reported. Mukasa et al. (1968) observed the sedimentation characteristic of ceruloplasmin under various conditions in the ultracentrifuge. The observations were interpreted as evidence that the molecule consisted of two identical subunits which were non-covalently associated with each other. Later, Ryden (1972) produced the cyano ethylated derivative of swine ceruloplasmin, and subjected it to gel filtration in guanidine hydrochloride solution. Ryden concluded that dissociation into subunits did not occur with that treatment. No attempts have been made at using either of these contrasting conclusions in forming a concept about a structural basis for ceruloplasmin polymorphism. Matsunaga and Nosoh (1970) analyzed the glycopeptides obtained after tryptic digestion of ceruloplasmin. The carbohydrate portions of those glycopeptides consisted of the same hexoses and hexose derivatives as were found in the Tf molecule (vide supra). Thus, sialic acid is a component of the carbohydrate fraction of swine ceruloplasmin. Furthermore, some evidence was obtained to indicate that carbohydrate moieties might be attached at more than one specific position along the polypeptide. Despite this, there is presently no substantial reason to believe that differential content of sialic acid is the basis for electrophoretically detectable variation similar to that which is apparent with transferrin. There are no reports of attempts at comparative structural studies of the ceruloplasmin from pigs of different phenotypes.

Hemopexin. There are no reports of attempts to accomplish fundamental physico-chemical descriptions of the hemopexin of swine serum. However, methods such as those used with Tf and ceruloplasmin have been applied for study of the hemopexin of rabbits and humans (Hrkal and Muller-Eberhard, 1971). Results of both electrophoretic and ultracentrifugational studies constituted evidence that the hemopexins of both species were glycoproteins containing only a single polypeptide. The carbohydrate portions were found to contain mostly the same hexoses and hexose derivatives as were found in swine Tf and ceruloplasmin,

with no evidence of other sugars or amino sugars. No attempts were made at describing intra-species variation in structural features of those hemopexins.

6-phosphogluconate dehydrogenase. No structural studies on the Pgd of swine tissues have been reported. Information that may be of some pertinence for understanding the genetic basis for variation of the enzyme has come from a study of Pgd of human erythrocytes. Pearse and Rosemeyer (1974) studied the behavior of the purified enzyme during ultracentrifugation and electrophoresis in polyacrylamide gel. The results yielded an estimate that the molecular weight of the enzyme was 102,000 daltons, and that it was dissociable into two like subunits of apparent molecular weight of 53,000.

Phosphohexose isomerase. There are no available descriptions of the structure of swine erythrocyte phosphohexose isomerase. However, reports on the phosphoglucose isomerase of another source seem of interest. Gracy and Tilly (1975) estimated that the enzyme isolated from human erythrocytes has a molecular weight of 132,000 daltons. Dissociation into two subunits, each having an apparent molecular weight of 63,000, was effected by use of 6 M guanidine hydrochloride.

There are no reports of studies of any similar nature for the slow alpha-2 macroglobulin, nor for the component referred to as serum amylase.

Erythrocyte antigens. Improved understanding of the structural nature of cellular antigens is dependent on development of adequate knowledge of the nature of cell membranes themselves. Although the structure of the erythrocyte membrane has not been well described to date, some notable progress has been made towards structural characterization of the membrane components which confer antigenicity. Some consideration should be given to certain studies which have utilized the erythrocytes of species other than pigs. Those studies seem to provide information which is of general interest for serology and immunogenetics.

Serological relatedness has been demonstrated among antigens which are present on erythrocytes of different species. The A antigen of the human erythrocyte membrane exhibits cross-reactivity with an antiserum

identified by reactivity towards a cattle erythrocyte antigen designated as the J antigen (Stormont, 1949). An homologous component on swine erythrocytes is also designated as the A antigen. The cross-reactivity of erythrocytes of some pigs towards anti-bovine J serum was demonstrated by Sprague (1958). Evidence of the genetic basis for that reactivity or non-reactivity among pigs (Saison and Ingram, 1962; Rasmusen, 1964; Hojny, 1974) was cited in a paragraph above; the locus in pigs which determines this cross-reacting serotype may be referred to as the A-o locus.

Insight about the nature of the substance which confers this specific A serotype in humans was obtained by treatment of particular tissue substances with a crude enzyme preparation (Watkins, 1962). Observations were made on the conversion from the A serotype to an allelic serotype (H). Subsequently, simple hexoses and hexose derivatives were studied for inhibitory effect upon this apparent enzymically controlled conversion. Results were interpreted as evidence that type-A specificity was conferred by a substance which contains a polysaccharide, the terminal moiety of which is N-acetylgalactosamine. This observation served as the basis for subsequent experiments, using a different enzyme. Schenkel-Brunner and Tuppy (1973) processed tissue from humans of serotype A to obtain material which showed capability for catalyzing the transfer of N-acetylgalactosamine from the nucleotide-conjugated form to a naturally derived polysaccharide. This enzymically active preparation was applied to a reaction mixture containing the nucleotide-conjugated N-acetylgalactosamine and human erythrocytes of serotype O. The erythrocytes recovered from this incubation mixture were found to be agglutinable by anti-A serum (i.e., apparent conversion of the serotype had occurred). This result was considered as supportive of the concept that N-acetylgalactosamine is an essential structural component for the A serotype. The findings seem pertinent because they may be suggestive evidence about the nature of the gene product which controls at least one of the antigenic variants on the swine erythrocyte. Furthermore, the study which yielded this evidence may be an important "prototype" for designing experiments which will identify specific enzymes which are determinants of other specific serotypes.

Associations Among Traits

Correlated inheritance of some pairs of traits has been observed in pigs. Such correlations may be described straightforwardly when both traits involved are those with simple modes of inheritance. However, another type of association that is of much interest is that occurring between some simple trait and a more complex trait. Reports of both types of associations in pigs are described below.

Evidence of linkage in some pairs of the simple traits referred to above has come from analyses of results of backcrosses, in which mating pairs consist of one doubly homozygous pig and a doubly heterozygous mate. Linkage was apparent in the following pairs of genes (observed recombination rates are shown):

hemopexin-erythrocyte antigen K,	5.2% (Imlah, 1965),
	3.6% (Andresen, 1966a),
	3.9% (Hesselholt and Brauner-Nielsen, 1966);
amylase-erythrocyte antigen I,	0.8% (Andresen, 1966b),
	2.5% (Brauner-Nielsen, 1966);
phosphohexose isomerase-erythrocyte antigen H,	2.6% (Andresen, 1970);
phosphohexose isomerase-phosphogluconate dehydrogenase,	8.1% (Andresen, 1971).

Genotyping of the progeny from crosses of the type mentioned here is the preferred approach toward obtaining evidence of physical linkage of two genes. Verification of some such linkage relationship involving a determinant of a metric trait such as litter size is, of course, a more difficult process. Association of the metric trait with a specific single-gene trait is not necessarily sound evidence of physical linkage of the genetic determinants of the characters. Nevertheless, evidence of associations between certain single gene traits and reproductive efficiency may provide important clues toward understanding the genetic basis of the complex trait.

Some early suggestions of association of Tf genotype with reproductive ability of cattle (Ashton and Fallon, 1962) were shortly

followed by somewhat analogous findings with pigs. Kristjansson (1964) made observations on the recurrence of estrus within 60 days after mating in a population of gilts. Two Tf alleles, viz., Tf^A and Tf^B, were found among the boars and gilts used as breeding stock. Both purebred matings and crossmatings were accomplished using the Landrace and Yorkshire breeds. The pure-Yorkshire mating class which consisted of boars of the genotype Tf^B/Tf^B and gilts of type Tf^A/Tf^B was distinguished by a particularly high rate of return to estrus (45%). Another observation which is probably of pertinence was that, from crossmatings involving Landrace boars also possessing the Tf^B/Tf^B genotype, the return rate in Yorkshire gilts (of all Tf genotypes) was 38%. The mating types not falling into any of the aforementioned categories showed an average return rate of 21%. The observations were interpreted as indicative of a progressive loss of all ova, most probably due to early death of zygotes and embryos. It was proposed that the causative factor might be a locus or loci closely linked to the Tf gene.

More recently, Fesus and Rasmusen (1971) used purebred populations of the Yorkshire and Duroc breeds, and a crossbred Duroc-Yorkshire population, to compare performances among classes of matings involving the various Tf genotypes. Litter size data on 812 litters were used in that study. The Tf genotype which the earlier study by Kristjansson (1964) had associated with a high return rate, was the genotype possessed by the sires of 392 of those litters. Fesus and Rasmusen did not report the rate of unsuccessful matings. The study did not provide any evidence that Tf genotypes correlate with averages of the total number of piglets born per litter, or the averages of the number of live-born per litter.

Following the identification by King (1962), of a third allele at the Tf locus, that allele (Tf^C) was found in samples of a few of several diverse breeds surveyed by Imlah (1970). A group of related animals possessing the Tf^C in a heterozygous state were used for inter se matings which yielded indications that the gene controlling the Tf genotype was associated with an "early lethal" factor. The distribution of Tf phenotypes among offspring of those matings was compared to

results of matings among unrelated carriers of the Tf^C allele. The evidence of association between the Tf and the early lethal factor was reinforced by that comparison. The homozygous genotype Tf^C/Tf^C occurred in offspring of unrelated carriers of the Tf^C allele. However, that homozygous genotype was absent from among 81 offspring born to matings between related, heterozygous carriers of the Tf^C allele.

Evidence has been presented which suggests associations between reproductive performance and some of the genes controlling the variation in antigenic determinants. Genotypes at the G locus were determined in pigs of four different breeds involved in a total of 587 matings (Tikhonov, 1966). Averages of numbers born per litter gave some indication of lower reproductive efficiency in mating pairs consisting of mates possessing like homozygous genotypes. The particular category of homozygous matings which was at apparent disadvantage was not the same in all four breeds. Tests of statistical significance were not provided. Another study involved determination of genotypes at ten different loci controlling erythrocyte antigens, in 615 Landrace sows (Hohenbrink et al., 1970). Those sows were then classified according to the proportion of those ten loci at which heterozygous genotypes were present. Calculation of average numbers of piglets per litter born to those categories did not disclose any correlation between litter size and proportion of heterozygous loci. Additionally, Landrace boars were genotyped, and matings were controlled so as to produce litters which would consist of either all homozygous or else all heterozygous types at one of certain loci controlling erythrocyte antigens. The average number born per litter, in litters expected to be heterozygous for the E genotype, was larger than the average for litters which were expected to be homozygous at that locus, by 0.7 piglets per litter. Comparison of contrasting categories defined similarly on the basis of zygosity at the G and I loci showed increments in the same direction. However, none of the differences was shown to be statistically significant.

Data on 1126 litters were used to evaluate a possible association between reproductive ability and the H gene (Rasmusen and Hagen, 1973).

The litters were farrowed from matings within purebred Duroc and Yorkshire populations and a Duroc-Yorkshire crossbred population. One of the measurements studied was the average number of pigs per litter which survived to weaning age. Proportions of weanlings possessing the H_a phenotype were compared to proportions expected from specific mating categories on the basis of assumption of random segregation of alleles and equal viabilities of different genotypes. The observed proportions of the offspring possessing the H_a phenotype did not differ significantly from expected proportions in the litters born to heterozygous gilts (i.e., the mating classes $H^a/H^- \times H^a/H^-$ and $H^-/H^- \times H^a/H^-$). However, the mating class in which the gilt lacked the H^a allele and the boar was heterozygous (i.e., $H^a/H^- \times H^-/H^-$) produced an excess of progeny of the H_a type. Such excess was observed in each of the three breed classes. The excess was statistically significant for the Yorkshire class. Other comparisons using the data from the Yorkshires also revealed some statistically significant differences. The number of weanlings per litter from the latter mating class was lower than the value found for the reciprocal mating class ($H^-/H^- \times H^a/H^-$), and the difference was highly significant ($P<.01$). Such a significant difference was also found between those reciprocal mating categories, for the average total number born per litter, and for the number of live-born per litter. No such differences were found in the Duroc or crossbred classes. A later analysis indicated however (Rasmusen, 1975b), that the homozygous genotype containing the H^a allele was disproportionately represented in those Duroc mating pairs in which the matings were unsuccessful, or which produced litters in which all piglets died before weaning.

The effects of the A and o phenotypes on reproductive performance were evaluated in the same population (Rasmusen, 1975b). The average number of purebred piglets per litter, alive at weaning in the mating category consisting of an A-phenotype boar and an o-phenotype gilt was significantly different from the average for the reciprocal mating category (o x A). The larger average was associated with the mating of A-type boars and o-type females in Durocs, but the reverse was true

in the Yorkshire population. The difference was not significant in the crossbred population.

Electrophoretic and serological methods of analysis were applied together in a large-scale study of the individual variations in reproductive traits in Duroc and Hampshire populations (Smith et al., 1968; Jensen et al., 1968). Variants of erythrocyte antigens controlled by 12 loci and serum protein variations controlled by 5 loci were analyzed in that study. Data were gathered over a five-year period. The management plan imposed seems to have provided adequate basis for believing that the populations used were rather heterogeneous samples of the two breeds. Statistical analyses of the results attempted to identify associations between phenotypes and reproductive performance. Variance in the number of live-born per litter in Durocs appeared to have a significant association with the A, H, and E phenotypes of the dams. Variance in the same litter size trait in Hampshires appeared to be significantly associated with the E, H, and serum amylase phenotypes. Jensen et al. attempted to provide a concise summary of the results by estimating the total proportion of variance in the particular trait which was associated with variance at all of the single genes studied. The estimate indicated that in Durocs, 10.8% of the variance and in the Hampshires, 7.4% of the variance in the number of live-born per litter could be associated with the genes studied.

Molecular Bases for Heterosis

A plausible hypothesis for some of the associations between specific blood components and quantitative traits described in the foregoing sections could be that the associations are consequences of a cause-effect relationship; i.e., the variation in the latter is dependent to some extent on variation of the former. An alternative hypothesis is that the associations observed are the manifestation of linkage between genes which determine the variance in the metric trait, and those which control the simpler trait. Very little evidence exists which directly serves to support either of those concepts for any of

the associations referred to above. The evidence about those associations has been summarized here, because it seems to comprise a portion of some fundamental background knowledge which is useful for developing the means by which to interpret certain other observations on the genetic variance in litter size. Those observations, which were summarized in a preceding section, consist of evidence about effects of crossbreeding toward reproductive traits. Studies which were of a quite different nature yielded evidence which seems useful toward providing improved genetic interpretations of those crossbreeding results. Considerable background knowledge has been obtained from molecular biological studies which mostly involved sub-mammalian species. A well-studied component of human tissues is also mentioned.

It is found convenient, in most cases, to distinguish among variant forms of a single trait within a species by referring to one form as the normal (or "wild type"), and to other forms as mutants. Mutant forms occur in viruses, as well as in living cells. It has been observed that it is possible, in some cases, for a normal phenotype to be produced from the combination, within the same cell, of mutant genes from two different stocks. A well-studied bacteriophage, for which Escherichia coli is host, provided a mutant form which was the subject of an elegant study of the fine structure of genetic material (Benzer, 1957). A large number of mutants of bacteriophage T4 were identified, all of which affect a single trait of that virus. The mutants were characterized according to the effects which were observed upon combining pairs of mutant types for a mixed infection of E. coli. Results of the study of the pattern of interaction of the mutants provided an understanding of the distinction between complementing mutants and non-complementing mutants. Pairs of mutants which resulted in expression of a normal phenotype upon mixed infection were defined as a complementing pair.

There are reasons to believe that the concept of complementation is pertinent also in genetic studies of higher organisms. It is possible to recognize examples of its occurrence in the hemoglobins of vertebrate species.

Human sickle-cell anemia is a severe syndrome which is the consequence of a homozygous genotype at a single locus (Neel, 1949). Although individuals possessing the heterozygous genotype do not suffer a severe form of anemia, they were found to be identifiable by reason of the shape which their erythrocytes acquired when observed under an abnormally low oxygen pressure. Hence, the name sickle-cell trait was applied to the heterozygous "carrier state."

Pauling et al. (1949) compared characteristics of hemoglobins obtained from individuals of the three different phenotypic categories. The results were a basis for the suggestion that the sickle-cell trait is probably due to a structural feature which allows aggregation of hemoglobin molecules to such an extent that the aggregates cause a distortion of the erythrocyte. More specifically, it seemed likely that the structural feature which favored aggregation was one which occurred in deoxyhemoglobin, but not in oxyhemoglobin. Subsequent to those early studies, it was shown that the difference in characteristics is due to presence of a neutral amino acid in sickle-cell hemoglobin (Hb^S) at a site where normal hemoglobin (Hb^A) contains a dicarboxylic amino acid (Ingram, 1958; 1959). Pauling et al. (1949) had pointed out that in heterozygous individuals, the two different hemoglobin types both should be present in any individual cell. The two types should not be segregated into two erythrocyte populations, since all erythrocytes of heterozygous individuals sickle under test conditions.

Clinical observations on association of Hb^S with pathology (Allison, 1961), physiological responses to parasitization (Garnham, 1966), and physico-chemical characteristics of Hb^S (Pauling et al., 1949; Perutz and Lehman, 1968) have been assessed together, leading to a hypothesis which is considered to account for the retention of Hb^S in human populations. Presence of the Hb^S is thought to confer some degree of resistance against falciparum malaria (Allison, 1957; 1961; Vandepitte and Delaisse, 1957). The various lines of evidence seem to indicate that a tenable concept is that normal hemoglobin and Hb^S have a complementary relationship when present in individuals subjected to a particular environment. Cells containing a mixture of the two

hemoglobins evidently have characteristics which can vitiate parasite proliferation (Miller et al., 1956; Power, 1975).

The apparent complementation between normal hemoglobin and Hb^S was disclosed from careful observations on a phenotype which showed clinically evident abnormalities. Other evidence of some sort of complementation between different hemoglobin polypeptides has been obtained from an experiment involving hybridization of species. The result of the hybridization was the formation of hemoglobin which was considered to be a hybrid (Manwell et al., 1963). Hybrid offspring were obtained from crossmating of two sunfish species, viz., the green sunfish (Lepomis cyanellus) and the Warmouth (Chaenobryttus globus). Comparative electrophoretic analyses were accomplished on the hemoglobins of the hybrid and the parental species. Electrophoresis of specimens from hybrid fishes yielded a pattern of hemoglobin distribution which clearly was not simply the sum of the fractions found in the parental species. The findings which were thought to be of more essential significance however, were those indicating contrasting oxygen-binding characteristics of the hemoglobins. Oxygen equilibria curves were constructed using data from dialyzed hemoglobin solutions. The curves indicated that, over the range of physiologically significant oxygen pressures, the oxygen affinity of hemoglobin of the hybrid was less than that of the hemoglobins of either parental species. However, the oxygen pressure at which maximal oxygen binding was achieved was practically the same for all three hemoglobin solutions. The curves indicated that hemoglobin from the hybrid should be more efficient than either parental hemoglobin, in delivering oxygen to tissues. Thus, hemoglobin from the hybrid fishes has been found to possess a functional characteristic which can be described quantitatively as lying outside the range of the properties of hemoglobins found in the parental species. Presence of hemoglobin fractions which were unique to the hybrid fishes was considered to be the basis for the contrasts in oxygen equilibria characteristics. Manwell et al. proposed that the properties of the "hybrid hemoglobin" were the result of greater "heme-heme interactions."

An important prerequisite to the validation of the hypothesis described is the assurance that the hemoglobins in that population are in fact multimeric. Some progress toward verifying that property has been achieved from studies of trout hemoglobin (Brunori et al., 1973). Evidence was obtained that, at least in that particular teleost species, the hemoglobin is in fact a tetramer, and the tetramer consists of two unlike subunits having some similarities to mammalian hemoglobins.

Elucidation of the relationship between a structural variation in hemoglobin and its physiological manifestation (as in Hb^S) was probably facilitated somewhat by the relative ease of procurement of hemoglobin in abundant quantities, free of significant contamination by other single molecular species. Hemoglobin is not only present at an enormously higher concentration than most enzymes and carrier proteins, but it also occupies an obvious key role in the maintenance of metabolic processes of the whole organism. Variant forms of other proteins might be less apparent because the activity of just a single one of them is less essential to the integrity of the organism.

Studies by Markert and Moller (1959) and Cahn et al. (1962) provided much of the basis for perceiving the significance of existence of variant forms of individual specific enzymes. Awareness of the significance of the phenomenon has prompted attempts to encourage precision in the nomenclature of enzymes. Recommendations (IUPAC-IUB Commission, 1971) have been set forth regarding the usage of the term isoenzyme (alternatively, isozyme). According to those recommendations, the term should be applied to the multiple forms of enzymes which are due to genetically determined differences in primary structure. The recommendations acknowledge that at least some enzymes may exist in various forms distinguishable by non-genetic criteria. If those various forms of the protein show the same enzyme activity and occur naturally in a single species, those are to be referred to as "multiple forms of the same enzyme...."

Johnson (1974) has recently suggested a classification scheme intended for a purpose beyond maintenance of an organized nomenclature. That classification scheme is intended as being useful for assessing

the significance of polymorphism of enzymes. Although the classification scheme has a simple structure, the process of classification is heavily dependent upon the extent of knowledge of the metabolic role of the enzyme being studied. Part of the rationale is based on a suggestion by Gillespie and Kojima (1968), that the particular metabolic role of an enzyme may be a determinant of the extent to which variant functional characteristics are tolerated. Obviously, many enzymes have a high degree of substrate specificity, and act to yield products which are the substrates for other enzymes having high specificity. It was argued that, in order to maintain proper metabolic function, the natural selection process would impose quite rigid standards for the kinetic parameters of those enzymes, and they would be unlikely to be found in variant forms. However, enzymes which are "peripheral to the major energy-producing and metabolic pathways" could be relatively more likely to occur in variant forms. Nevertheless, it is suggested by Johnson (1974), that even some enzymes which must possess very strict substrate specificity might also be individually sensitive objects of the action of selective forces. Enzymology has shown that only certain reactions occurring along a pathway are potentially rate-determining for the pathway. Proper regulation of metabolism requires that an adequate level of control of output is maintained despite changes in such factors as availability of substrate, or temperature. If the environment is constant, the optimal situation is the presence of the one best-functioning form of the enzyme. However, if the environment tends to impose diverse reaction conditions, then presence of alternative forms (i.e., the heterozygous state) may be most advantageous. Potential for such flexibility in regulation may be especially good in the case of an enzyme which occupies a position such that it may exert "acute control" over the functioning of one or more entire pathways. The phenomenon of feedback regulation is cited as a well-known example of such single-step control of whole pathways. Although first described in E. coli, (Yates and Pardee, 1956), there is evidence indicating that it also occurs in vertebrates (Wynngarden and Ashton, 1959; Fox and Kelley, 1972). Another means by which one enzyme can be quite effective in controlling the rate of complex processes,

is by its involvement at a step where a particular reaction, and also the reverse of that reaction, are essential but are each controlled by a different enzyme. Johnson cites that which is a practically famous illustration, viz., the glycogen synthetase and phosphorylase activities. The former enzyme catalyzes polysaccharide synthesis (Leloir and Cardini, 1957). Phosphorylase has, as its principal intracellular function, the catabolism of polysaccharide (Schmid and Mahler, 1959; Schmid et al., 1959). The activities of the two are subject to quite different control factors.

Polymorphism of still other enzymes, not involved in the fundamental energy-transducing processes, might be a reflection of some considerable variability in the substrates which originate in the external environment. Some support for this concept has been obtained from a survey of human enzyme variants (Cohen et al., 1973).

The considerations mentioned above are the basis for Johnson's (1974) proposal, that distinction among the following categories may be useful: 1) variable-substrate enzymes, 2) regulatory enzymes, and 3) non-regulatory specific-substrate enzymes. However useful this categorization might prove to be, it is recognized that at present its application to data analysis may encounter formidable difficulties. Johnson (1974) has acknowledged the possible effects of those difficulties, and has nevertheless proceeded to apply the concept in analyses of data obtained from population surveys. Results of the analyses were interpreted as supportive of the view summarized above about the significance of enzyme polymorphism. Overall heterozygosity of the category of enzymes classified as regulatory was found to be significantly greater than the overall heterozygosity of the non-regulatory category.

There is a contrary point of view regarding enzyme polymorphism. Some theorists prefer to regard the occurrence of the polymorphism as having little or no selective significance. Zouros (1975) pointed out that the occurrence of electrophoretically detectable variants of a molecule probably is usually due to substitutions involving aspartyl, glutamyl, lysyl, or arginyl residues. Thus, certain proteins are more likely than others to be found in variant forms, because a higher

content of those four amino acids provides more potential for mutation to a primary structure having a different net charge. Such substitutions may have no significant effect toward the functional characteristics of the protein.

MATERIALS AND METHODS

Population Development

Semi-closed, purebred lines of Berkshires and Yorkshires were established at Oregon State University, Corvallis, Oregon, during the period from spring, 1959, to spring, 1961. The process of addition of new lineages to the Berkshire and Yorkshire lines is represented in Figure 1. Dates shown along the sides of the columns indicate the temporal progression of the addition of new lineages. The subpopulations were not maintained as a series of discrete generations; they are represented by separate columns in Figure 1, with as few graduations as possible along the columns, in order to avoid the impression that discrete generations were maintained.

Fourteen dams were used in the foundation stock for the Berkshire (B) line. Those females were members of a herd which had been maintained at Oregon State University for several years prior to 1959. Male members of the foundation stock were a pair of half-brothers, imported from an Iowa population which was unrelated to the Oregon State University herd. One of the boars was mated to ten females, and the other boar was mated to four females. Litters produced from those matings were farrowed during 1960 and 1961. Although 20 gilts and seven boars from among the offspring of those matings were retained for breeding, rather few inter se matings were allowed among those animals. Importations of two boars at subsequent dates served to broaden significantly the pedigree structure of the B line. The left-hand portion of Figure 1 depicts those steps. A B herd unrelated to the two founder males was the origin of an imported boar (000-300) which sired eight purebred litters by Oregon State sows in 1963. Three boars and three gilts were retained from those litters as replacement stock. Subsequently, another boar was imported from the herd from which the two founder males were obtained in 1959. That boar (000-600) was mated to three sows of the Oregon State herd, to produce litters

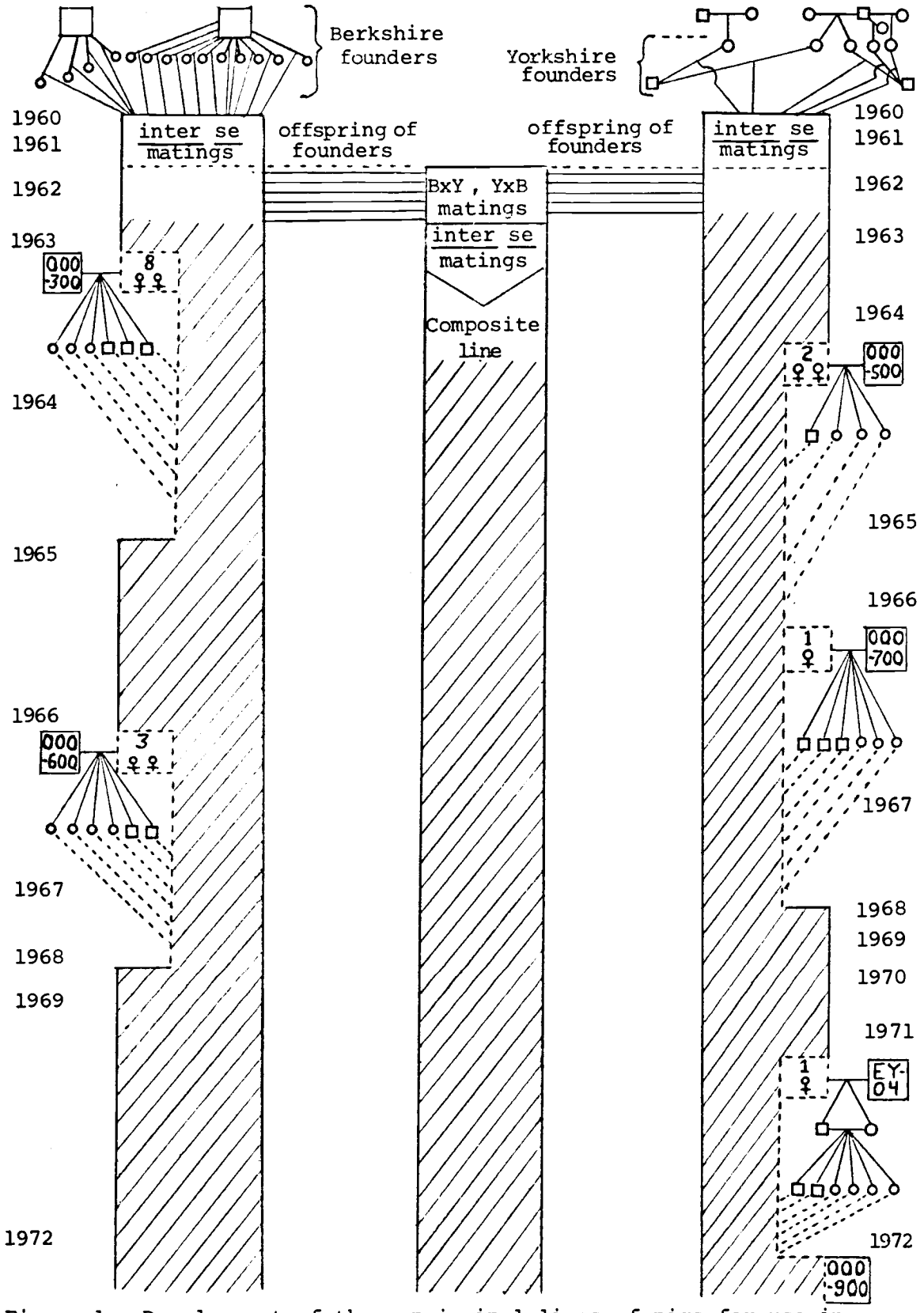


Figure 1. Development of three principal lines of pigs for use in line-crossings. Diagram indicates dates at which genetic material from outside populations was added into purebred lines.

farrowed in 1966. From among those offspring, two boars and four gilts were selected to join the breeding stock.

Foundation stock for the Yorkshire (Y) line consisted of two boars and five gilts. Four of those gilts were from a common sire, and two of those were full sisters. The two boars which were mated to those females were both obtained from a single herd, but any relationship between those boars was certainly slight. Any common ancestry between the founder gilts and the boars to which they were mated must have been several or many generations in the past. The gilts were obtained from a purebred herd located in western Oregon, and boars originated from an established purebred herd in Missouri. (Those boars had pedigrees which indicated recent parentage in Great Britain.)

The first litters produced from the foundation animals were farrowed in 1960. Subsequently, the Y population was maintained as a semi-closed line by inter se matings among the descendants of the founder animals. Exceptions to the policy of maintaining the line as a closed population are indicated in the right-hand portion of Figure 1. Those exceptions involved either matings with imported service boars, or else artificial insemination using semen obtained from other herds. 1) One boar (000-500), imported from the same herd as the founder boars, was mated to two members of the established Y line in 1964. One male and three females from among the progeny of those pigs were retained as breeding stock. 2) An indigenous sow was artificially inseminated in 1966, using semen from a boar unrelated to the herd. Three gilts and three boars were retained from the offspring of that insemination for replacement stock. 3) Another artificial insemination of a member of the local population was accomplished in 1971, using semen from another boar (EY-04) unrelated to the herd. One female produced from that litter was retained as a replacement gilt (1160-2) and a boar littermate (1160-1) was subsequently used extensively as a sire within the Y line. 4) A boar was imported in 1972 and was utilized frequently as a service boar (000-900) through 1974. That animal had no known relationship to any of the stock present in the herd.

Crossmatings between the purebred lines were begun in 1961. Inter se matings among the offspring of those crosses were begun in 1963. A third closed line was developed by means of those matings, and was designated as the Composite (C) line. The offspring from matings between B's and Y's, which served as the founder population for the C line, originated from a rather small group of purebreds. That group was comprised as follows:

two B males were mated to three Y females, and

two Y males were mated to four B females.

The seven mating pairs formed among those purebreds were the source of ten animals (F_1 offspring) used as foundation for the C line. Offspring from any inter se matings among those F_1 individuals were defined as members of the C line. However, F_1 offspring from matings of B's and Y's were considered as being a group distinct from the C line. The term "F_1 offspring" will be used throughout this thesis to denote the offspring of crossmatings between B's and Y's, and will not refer to any line-crossings involving the C line.

Some inbreeding was achieved with the breeding program described here. Spurr (1969) and Liu (1974) provided descriptions of inbreeding which have some pertinence to the present study. It is certain that at least some of the B, Y, and C sows in the present study were not included with the sows used for calculations by Liu. No attempt was made to determine what proportion of subjects of the present study were included for Liu's calculations. The averages are of interest here as a part of the fundamental descriptions of the stocks from which subjects of the present study descended.

Spurr (1969) calculated the average inbreeding of groups of B and Y dams farrowing purebred litters prior to 1969. Liu (1974) calculated the average inbreeding (± standard deviation) for groups of dams farrowing B, Y, or C litters during the period of 1962-73. Most of the B and Y dams to which Spurr's descriptions pertained were also included in the larger groups described by Liu.

Numbers of litters farrowed by the dams which were described, and the inbreeding averages of the dams were:

Spurr - dams of 149 B litters -- average inbreeding = 6%;
Liu - dams of 179 B litters -- average inbreeding = 6% ± 6%;

Spurr - dams of 181 Y litters -- average inbreeding = 10%;
Liu - dams of 280 Y litters -- average inbreeding = 13% ± 8%;

Liu - dams of 194 C litters -- average inbreeding = 13% ± 4%.

Various crossmatings were accomplished during the course of the project. B's and Y's were frequently crossmated. Routinely, F_1 gilts were selected for use as brood sows and were mated to B, Y, or C boars. Some of the daughters from those matings served as brood sows, but none which were born before 1968. Beginning with approximately the F_2 generation within the C line, a few members of that line were mated to B's and Y's. Daughters from crossmatings between the C's and either purebred line were used as brood sows beginning in 1968.

Subjects

Breeding stock in any particular breed category was comprised of only a minor proportion of all animals belonging to that sub-population. The subjects of the phenotyping studies consisted of only the breeding stock present during a late phase of the breeding program. The numbers of sows which were subjects of the phenotyping studies are shown in Table 1. Members of the B and Y lines which were phenotyped were born during the period of 1970-74 (except one member of each line, born in 1968). Members of the C line which were phenotyped were born during the period of 1970-73. Dates of birth of subjects belonging to four crossbred categories are shown in Tables 6, 7a, and 7b. Included as subjects of the phenotyping studies were all purebred and C sows and service boars present in the population during the last half of 1973. Additionally, all B's and C's subsequently selected for use as breeding stock, as well as many of the Y's subsequently selected for use as breeding stock, were included as subjects of the phenotyping studies. All of the BxC, CxB, YxC, CxY, BxF_1, and YxF_1 sows present as of early 1974 were included as phenotyped subjects, as were any members of

Table 1. Distribution of phenotyped subjects among sub-populations, and numbers of litters farrowed by those subjects.

	Breeding category												
	B	Y (natives)	Y[1] (1160- + descendants)	Y[2] (000-900 + descendants)	C	BxC, CxB	YxC, CxY	BxF_1	YxF_1	CxF_1	F_1	other	Totals
Service boars	4	1	7	5	7	0	0	0	0	0	0	0	24
Sows	14	9	26	8	17	21	11	13	15	8	4	0	146
Shoats	25	2	2	11	16	7	14	0	0	0	19	9	105
Total	43	12	35	24	40	28	25	13	15	8	23	9	275
No. litters farrowed by the above sows	39	40	85	25	37	64	44	54	51	24	17	0	480

[1] Animals in this column consist of both first-generation (1160-1, 1160-2) and second generation descendants from use of semen which was of exopatric origin.

[2] Animals in this column consist of an imported service boar (000-900) and its first-generation offspring.

those four crossbred categories which were subsequently selected as brood sows.

Breeding Management

Replacement breeding stock was selected from within the herd (except for a few imported boars and artificial inseminations mentioned above) by criteria comprising a flexible selection system (Spurr, 1969). The system was intended to insure that the herd possessed desirable genes for a variety of traits, but did not require that any individual animal should be outstanding for all traits.

Litters containing candidates for selection as breeding stock were designated at time of farrowing. Such litters were maintained following weaning, primarily as litters or as half-sib groups of 8 to 12 shoats per pen, in pens measuring 2.4 x 3.3 m. Constant access to feed and water was allowed. Pigs were raised to a weight of 81 to 91 kg under that condition of confinement, inside a building which was entirely closed or partly closed, depending on the season. Males retained as breeding stock were subsequently maintained in individual pens. Females retained as breeding stock were maintained in groups of 5 to 15 per pen, in large pens inside a semi-enclosed area. The boar to which a sow or gilt was to be mated was specifically selected prior to the breeding date. The boar and female were then penned together after signs of estrus were observed. Gravid females were maintained together in pens containing 5 to 15 individuals, until about 110 days of gestation. They were than transferred to metal-frame crates, specially constructed for sequestration of individual sows during farrowing. Sows rarely farrowed unattended. Number of piglets farrowed was recorded at the time of farrowing, with notations of obviously regressed fetuses, or of full-term fetuses born dead. Within one day of farrowing, each piglet was identified by ear-notching, and individual weights and sexes were recorded.

Maintenance Management

Content and quantity of feed provided was varied according to the animal's maturity. The feed composition also was varied in order to utilize the feed source which was most favorably priced. However, the feed was always formulated so that the ration designated for a specific age group contained a standard protein content.

Collection of Blood Samples

Preparatory to sample-drawing, each subject was driven into a steel-frame restraining crate. The medial surface of an ear was washed, and a tourniquet was applied at the base of the auricular cartilage. Blood was drawn from an auricular vein, via a 20 gauge needle fitted to a glass syringe. A portion of the sample was transferred to a slightly vacuumized glass tube containing anticoagulant, and the remainder transferred to an empty, slightly vacuumized glass tube. Anticoagulant consisted of a solution of 1.17% (w/v) sodium citrate, 0.41% citric acid, and 1.32% glucose. The sample portion with anticoagulant was placed on ice immediately after mixing the blood and anticoagulant. Ratio of anticoagulant volume to sample volume was about 1:2. The sample portion without anticoagulant was left standing at the ambient temperature for a period which was dependent on temperature. (When the ambient temperature was greater than 25°C, the coagulated specimen was placed on ice at 20 to 30 minutes after phlebotomy. When the ambient temperature was less than 17°C, the coagulating specimen was left at that temperature for about 90 minutes before being placed on ice.)

Serum was separated from the clotted fraction in a refrigerated centrifuge (2-4°C), operated at 800 x g for 20 minutes. The separated serum was immediately placed at -20°C for storage. The time interval from phlebotomy until serum separation and freezing was three to six hours.

Antisera

The antisera used as serotyping reagents were obtained as a donation from Ruth Saison of the Ontario Veterinary College at the University of Guelph. The identities of the antisera had been ascertained prior to receipt. The particular batches of anti-A serum and anti-o serum used were bovine serum, containing apparently "natural" anti-swine A or anti-swine o hemolysins.

Serotyping Protocol

The cellular fraction of anticoagulant-treated specimens was separated from the plasma and anticoagulant by centrifugation at 800 x g, using a swinging-bucket rotor in a refrigerated room (2-6°C). The packed cells were immediately re-suspended in chilled, buffered saline (0.15M NaCl + 0.015M Na_2HPO_4/KH_2PO_4, pH 7.2), sufficient in quantity to make a five to ten percent suspension of the cells. The cells were again separated using the same centrifugation conditions, and the supernatant solution was removed. This "washing" of the cells was accomplished four times. During aspiration of the supernatant solution, care was taken to also aspirate, as thoroughly as possible, the leucocyte layer from the top of the packed cells. Immediately after the fourth washing, buffered saline was used to re-suspend the cells to either a 2%, 3%, or 30% concentration. That suspension was kept under refrigeration while serotyping reagents were prepared for application.

Reaction mixtures were composed from drop-wise quantities of the components. The components were delivered using Pasteur pipettes which had been selected for uniformity of the drop volumes delivered. Reaction mixtures were contained in 0.6 x 5 cm round-bottomed tubes (excepting those tests for which the use of capillary tubes is specifically mentioned below). Concentrations of the components of the serotyping reaction mixtures varied among the different antisera

used. The details of variations of the different reaction mixtures are presented below.

Hemolytic antisera

Hemolysins were present in the reagents used to detect the A and o antigenic determinants (Saison, 1973). Prior to use, the anti-A serum was diluted five-fold, and the anti-o serum was diluted two-fold. Diluent used was the buffered saline described in the preceding paragraph, but with magnesium sulfate added, to a concentration of 0.4 mM. The magnesium-supplemented buffer was also used for re-suspending washed erythrocytes intended for testing with those two hemolytic antisera. Tests for the A-o serotypes involved use of four different reaction mixtures for each specimen:

1.	anti-A serum (2 drops)	3% cell suspension (1 drop)	rabbit serum (1 drop)
2.	anti-o serum (1 drop)	2% cell suspension (1 drop)	rabbit serum (1 drop)
3.	diluent buffer (2 drops)	3% cell suspension (1 drop)	rabbit serum (1 drop)
4.	diluent buffer (1 drop)	2% cell suspension (1 drop)	rabbit serum (1 drop)

The specific antiserum was first placed into the tube, and was followed by the cell suspension. The mixture was left to stand at room temperature for 15 minutes (re-suspended by shaking after about ten minutes). Then rabbit serum was added, within one hour after the adsorption treatment (described below) of that serum using pooled swine erythrocytes. Thorough mixing of the reaction mixture was accomplished by agitation, and the mixture was left to stand at room temperature for three hours, with occasional shaking. Mixtures in which hemolysis occurred were identified by the color of the supernatant. The degree of hemolysis was estimated macroscopically by observing the quantity of cells at the bottom of the tube in comparison to the quantity remaining in the corresponding control mixture.

The following grading system was used for describing the degree of hemolysis:

0: appearance of supernatant was not perceptibly different from control

+1: proportion of cells which were hemolyzed was definitely less than one-half

+2: about one-half of the cells were hemolyzed

+3: less than one-fourth of the cells remained un-hemolyzed

+4: total quantity of cells was hemolyzed.

Initially, it was intended that only specimens giving test results of grades +2, +3, or +4 would be considered as A or o phenotypes. However, it was subsequently decided that A and o phenotypes could reasonably be inferred also from "+1" grade test results, which occurred in only a few individuals. Control mixtures with such specimens were without any noticeable hemolysis.

Antiglobulin-dependent antisera

Reagents used to detect the H_a, I_a, and I_b antigenic determinants contained agglutinins which were apparent if anti-porcine globulin serum was used in conjunction (Saison, 1973). Buffered saline was used to dilute each of those three antisera before use. Anti-H_a serum was diluted to one-fourth, anti-I_a was diluted to one-half, and anti-I_b was diluted to six-sevenths of its original concentration. The test procedure used was that described by Saison (1958). One drop of diluted antiserum was delivered into a tube, followed by one drop of a two percent suspension of the cells. For a control test, buffered saline was used in place of antiserum. Reaction mixtures were incubated in a 37° water bath for 30 minutes with occasional shaking. The suspensions were centrifuged for six to eight minutes at 300 x g in a refrigerated centrifuge (2 to 4°). Supernatant was carefully aspirated away from the button of cells, and the cells were immediately re-suspended in chilled, buffered saline. Enough buffered saline was added to bring the level to about four mm from the top of the tube. Rinse buffer was delivered through a 22 gauge needle placed into the tube so that the entire quantity of buffer up-welled from the bottom

of the tube. The cells were again sedimented by centrifugation, and the rinsing procedure was performed a total of four times. Following the last rinsing, the sedimented cells were re-suspended by adding a single drop of buffered saline followed by one drop of diluted anti-porcine globulin serum (Fisher Scientific Co., lot #121771). After careful mixing, the suspension was incubated in a 37° water bath. Following one hour of incubation, a small quantity of cells was drawn from the bottom of the tube, gently placed onto a glass slide, and examined under a microscope at 100-fold magnification. The degree of agglutination was graded as follows (Saison, 1973):

- \- any aggregates present each consisted of less than four cells
- \+ clumps of cells consisted of only four to six cells each
- ++ clumps consisted of more than six cells
- ++v clumps were visible on the slide without magnification.

No control mixtures were observed to have a grade of "++." Reaction mixtures which were graded as "+," were considered as positive evidence of agglutination only if the degree of agglutination was distinctly greater than any agglutination observed in the corresponding control mixture.

Proteinase-dependent antiserum

Antiserum used to detect the K_a antigenic determinant was capable of producing agglutination if a proteinase was simultaneously applied to the reaction mixture (Saison, 1973). The anti-K_a serum was diluted two-fold with buffered saline before its combination with either cells or proteinase. Antiserum was mixed with an equal volume of a solution (Low, 1955) containing 1% papain (Calbiochem Co., lot #200377), 0.025 M cystein, and 0.033 M Na_2HPO_4/KH_2PO_4 (pH 5.4). Control mixtures contained proteinase diluted by buffered saline to the same concentration as the proteinase in the reagent. Vessels used for observing reactions with that reagent were disposable glass micropipetting tubes ("Yankee," Becton-Dickinson Co.) of 11 cm length and 0.7 mm inside diameter. Those tubes were utilized similarly to the method described

by Lewis et al. (1958). A two cm column of reagent was taken into the tube. The outside of the tube was wiped dry, and a two cm column of 30% cell suspension was taken into the tube. The tube was then inverted, and the unfilled end was planted into a shallow layer of modeling clay, at an angle of about 30° from the vertical plane. The planted tube was positioned before an opalescent plate which was illuminated from the opposite side by a fluorescent lamp. If no agglutination occurred within ten minutes, suspensions settled into a smooth pinnacle-like column. Agglutinated specimens also formed a pinnacle, but such pinnacles were not smooth, and resembled a stack of ellipsoids. Specimens to which anti-K_a reagent was applied were graded as positive only if the agglutination was apparent within ten minutes.

Directly Agglutinating Antisera

Reagents used to detect the G_b, N_a, N_b, and L_b antigenic determinants were applied to cell suspensions without additional components (Saison, 1973). The anti-G_b and anti-N_a sera were applied without dilution; the anti-N_b serum was diluted to three-sevenths of its original concentration before use. Reaction mixtures containing those three antisera were composed in the capillary tubes described in the preceding paragraph. Concentration of cell suspensions was 30% before mixing with antiserum. The criterion for distinguishing positive reactions from negative reactions was the same as that described in the preceding paragraph. Only agglutination occurring within ten minutes was considered to be a positive reaction. The reagent used to detect the L_b antigenic determinant also was used without additional components. That antiserum was diluted by two-fold before application. One drop of the diluted anti-L_b serum was placed into a 0.6 x 5 cm tube, and one drop of two percent cell suspension was added. The mixture was incubated in a 37° water bath for one hour. Positive reactions were distinguished from negative reactions by examination of a small quantity of the cells on a glass slide at 100-fold magnification. Grading of the degree of agglutination was by the same system described for antiglobulin-dependent agglutination. Control mixtures for

comparison to the directly agglutinating antisera consisted of cell suspensions diluted with buffered saline to the same concentration as the mixtures containing antisera.

Preparation and Evaluation of Rabbit Serum

It was mentioned above that rabbit serum was a component of the tests which involved hemolytic antisera. Rabbit serum used for these tests was from pooled slaughter-house blood. An excess of the quantity required for use in the serotyping tests was thawed about 90 minutes prior to its addition to the reaction mixture. It was used to re-suspend a one-half volume of washed, packed swine erythrocytes. Adsorption of the rabbit serum by the erythrocytes was allowed to proceed for 30 minutes at 4°. The serum was then separated from the cells by centrifugation for 15 minutes in a 4° refrigerated room. The rabbit serum processed in this way was then subjected to a measurement of its complement content before being used with hemolytic antisera. This measurement was performed on the rabbit serum each time a batch of erythrocyte specimens was tested, regardless of whether or not the serum used was a separate portion of the same original pool. Measurement of the complement was accomplished by use of a series of mixtures containing the components of a system for hemolysis of sheep erythrocytes. A constant quantity of anti-sheep erythrocyte serum (Grand Island Biological Co., lot #R4244R) was placed into each of a series of tubes, and a varying quantity of the rabbit serum was then added to that series of tubes. Total volume of each tube was made 0.55 ml, using chilled, magnesium-supplemented, buffered saline. A quantity of 0.2 ml of washed sheep erythrocytes (two percent suspension) was added to each tube and the tubes were incubated for 30 minutes at 37°. A control mixture was composed similarly, with magnesium-supplemented, buffered saline substituted for the rabbit serum. The complement titer of the rabbit serum was expressed as the smallest quantity of serum which caused complete hemolysis of the sheep erythrocytes within 30 minutes. The remaining portion of the adsorbed

rabbit serum was then diluted according to results of that titration and was used immediately with the anti-A and anti-o sera.

Refinement of Anti-porcine Globulin Serum

The anti-porcine globulin serum did not possess adequate specificity until after a refinement process. That serum was used to re-suspend an equal volume of packed swine erythrocytes. Adsorption of the serum by the erythrocytes was allowed to proceed at 37° for 15 minutes. The serum was then separated from the cells by centrifugation at 4°. This adsorption process was then repeated three times. Swine erythrocytes used for this series of adsorptions had been washed six times in buffered saline.

Evaluation of Proteinase Solution

An above paragraph described the composition of the proteinase solution used with the anti-K_a serum. Sufficiently large batches of that solution were prepared and frozen in small portions for use over several months. Consistency of the composition of the proteinase-dependent agglutination test requires that the proteinase activity should be the same in mixtures composed at different times. Therefore, the relative activities of the preparation were checked intermittently following its placement into storage. The index of activity used was the renin-like activity of the papain (Balls and Hoover, 1937). A 20 gm quantity of dried whole milk (Carnation, "Non-fat, extra grade") was ground to a smooth paste in about 20 ml of 0.12 M sodium acetate buffer (pH 4.6). The paste was diluted to 100 ml with that buffer and strained through a few layers of gauze cloth. The renin-like activity of the papain was then measured using a series of five ml quantities of the rehydrated milk. Quantities of the papain solution, ranging from 0.05 ml to 0.5 ml were placed into that series of tubes and brought to a 0.5 ml volume using buffered saline. Those tubes were left on ice until the substrate solution was delivered by pipette. The tube was then placed into a 40° water bath. Each tube was picked

up at regular intervals and tilted to determine whether or not a thickening had occurred. Relative activities of separate batches were depicted by plotting the reciprocal of the time required for "clotting" to occur, versus the concentration of papain present in the reaction mixture. The occurrence of "clotting" is somewhat prior to the time of virtual solidification of the milk. The time at which it occurred with a given papain concentration, from a single batch of papain, was found to be reasonably reproducible.

Electrophoretic Analyses

Structure and Use of Electrophoretic System

Separations of the variant forms of the six components described in Figures 2 through 7 were accomplished by use of five different procedures for starch gel electrophoresis. Starch gels were processed into electropherograms following the separation. Some of the electropherograms could be obtained by a non-specific processing method; other electropherograms were obtained only by more specific methods.

Although there were differences among the electrophoresis procedures, it was possible to use practically the same apparatus for each. A simply constructed frame supported molten starch while gelation proceeded. Four acrylic plastic slats were used to form a rectangular box (23 x 13 x 0.6 cm), the base of which was a slightly larger glass plate. These slats were held in place by placing a spring-tension clip against each one. The acrylic plastic sides and ends of the frame were sealed to the glass base by petroleum jelly.

All chemicals used in these procedures were of reagent grade, except that certain dyes were not graded by suppliers according to such a system.

The apparatus structure described below is similar to that devised by Smithies (1955) for horizontal electrophoresis. The method for forming gels is essentially that of Kristjansson (1963).

Hydrolyzed starch used for the gels was obtained from Van Waters and Rogers Co. (control #RC6540-2), except the gels used for Hx

separations, for which the starch was obtained from Connaught Laboratories (lot #305-2) of Toronto. Compositions of buffers used and concentrations of starch were different for each of the five systems employed; those differences are shown in Table 2. The method for forming the gels was the same in each case, however. A 250-ml volume of buffered solution ("gel buffer") was used for each gel. A 65-ml portion of the gel buffer was placed into a one liter-sized vacuum flask, and the required amount of starch was slowly poured into the buffer through a narrow-stemmed funnel as the flask was swirled. The remaining 185-ml portion of buffer was heated until boiling had barely begun. As this unmixed portion of buffer was being heated, the mixture of starch plus buffer was swirled to obtain a homogeneous slurry, and the heated portion of the buffer was then quickly poured into that slurry. This total quantity of buffer plus starch was placed over a Bunsen burner, the flask was stoppered and negative pressure was applied by means of a water-tap aspirator. Heating and negative pressure were continued until ten seconds after the time at which the molten mixture was obviously completely de-gassed (amount of time for this to be achieved was dependent upon the starch concentration). The molten mixture was poured into the frame described above, and a glass plate was placed over the surface of the gel. Two lead bricks were then placed atop that cover plate for compression to assure flatness of the top surface of the gel. The covered gel was allowed to cool at room temperature for about two hours. Following that initial cooling period, the gel was stored at 2 to 5°, until specimens were loaded into it. The duration of that storage period was a minimum of 11 hours; no gels were stored longer than 19 hours before being used. The plate containing the gel was kept atop a test-tube rack from the time of pouring until it was used. Such a placement was considered to permit reasonably homogeneous cooling during the gelation period.

Pieces of chromatography paper (0.5 x 0.7 cm, Whatman No. 3MM) were the medium by which specimens were applied to the gel. A portion of each specimen was pipetted into a well in a glaze-finished porcelain spot-testing plate, and the applicator paper was soaked in that specimen for at least ten minutes. Preparatory to loading specimens

Component	Starch concentration	Gel buffer	Electrode buffer	Application potential[1]	Application period	Separation potential[1]	Separation time	Staining
Hx	13.80 gm per 100 ml of buffer	0.06 M Tris[2] + 0.005 M Citric acid pH 8.6	0.30 M H_3BO_3 + 0.05 M NaOH pH 8.00 to 8.10	5.5 volts/cm	20 min.	Variation from 6.5 to 8.0 volts/cm	15.2 hrs.	Benzidine + peroxide
Cp	11.62 gm per 100 ml of buffer	0.014 M Tris + 0.003 M Citric acid pH 7.5	0.30 M H_3BO_3 + 0.1 M NaOH pH 8.7	4.5 volts/cm	20 min.	Variation from 12 to 16 volts/cm	Until borate front came to 11.2 cm beyond insert	Chromogen oxidation (see text)
Tf	Same gel as used for Cp							Amido blk.
Sa	15.60 gm per 100 ml of buffer	0.006 M Na_2HPO_4/NaH_2PO_4 pH 7.8	0.30 M Na_2HPO_4/NaH_2PO_4 pH 7.6	6 volts/cm	20 min.	8 volts/cm	7.3 hrs.	Amido blk.
Am	12.00 gm per 100 ml of buffer	0.021 M Tris/Tris-HCl pH 7.4	0.30 M H_3BO_3 + 0.1 M NaOH pH 8.7	4.3 volts/cm	15 min.	5.5 volts/cm until borate crossed insert; thereafter, 12 to 14 v/cm	Until borate front came to 10.6 cm beyond insert	De-staining effected contrast against background
Pgd	15.60 gm per 100 ml of buffer	0.01 M Na_2HPO_4/NaH_2PO_4 pH 7.0	0.20 M Na_2HPO_4/NaH_2PO_4 pH 7.0		None	12 volts/cm	4.5 hrs.	Substrate + Chromogen
Phi	Same gel as used for Pgd							Substrate + Chromogen

[1]Voltage gradients were measured over only the 5-cm segment nearest the anodal side of the insert line.

[2]Tris = Tris(hydroxymethyl)aminomethane, obtained as the "Trizma base" label from Sigma Chemicals.

Table 2. Contrasts among designs of systems for electrophoretic analyses.

into the gel, the cover plate was pried away, the support slat at one end was slid away, and a transverse slice was made at 6.0 cm from that end. This 6-cm segment was pushed away from the longer segment to form a gap of approximately 0.5 cm. The longer segment of the gel was then covered with a sheet of transparent food-wrap film to prevent drying. The applicator papers containing the specimens were placed against the cut edge of the long segment using a forceps. Those applicator papers were spaced 0.2 cm apart. Following application of the specimens, the short segment of the gel was pushed forward to close the gap at the sample insert position. Before covering the short segment of the gel with the transparent film, a seal was placed onto the top edge of each applicator paper by touching a drop of warmed petroleum jelly to it with a small platinum spatula.

Closure of the electrical circuit was achieved by wicks consisting of three layers of chromatography paper (Whatman No. 3MM). Each wick was 13 x 20 cm, and was soaked in the electrode buffer while specimens were being loaded. The upper end of each wick was placed over a 2.1-cm segment of the top surface of the gel, and the other end of the wick extended to the bottom of the electrode vessel. Mostly, the different analysis systems differed in terms of the composition of the electrode buffers, as indicated by Table 2. Each electrode vessel contained 600 ml of buffer. The platinum electrodes were wound along glass rods. The glass rod was hung from the wall of the electrode vessel. The cathode was connected to the end of the gel nearest the position of the applicator papers. The DC power supply unit was a Buchler Instruments model 3-1014A.

During the electrophoretic separation procedure, the plate which held the gel slab was positioned horizontally atop a metal plate through which coolant flowed. The coolant plate was slightly longer than the gel, but shorter than the glass plate holding the gel. Tap water at a temperature of 16 to 19° flowed through the plate at a rate of 150 ml per minute while electrophoresis was in progress, when serum specimens were being analyzed. If the specimens were hemolysates, water was circulated through the plate from a thermostatically cooled (10°) reservoir, and a tray of ice was placed on the top surface of

the gel. This special cooling system was necessitated by the higher current flow occurring with the system used for analysis of hemolysates.

The electrophoretic analyses of serum components may be described as consisting of two distinct phases, viz., the application and the separation phases. The application phase consisted of the relatively short period during which applicator papers were in place. The lengths of that phase and the potentials applied were various, depending upon the composition of the gel, as shown in Table 2. Upon completion of that phase, the applicator papers were removed from the insert position using a flat-tipped membrane-handler's forceps, and the short cathodal segment was pushed into position directly against the longer segment of the gel. The separation phase was then begun, using conditions described in Table 2. During the separation phase of the process, potential difference over the length of the gel varied, and it was necessary to frequently adjust the power supply setting to maintain conditions which were effective for achieving the desired separation of components. Duration of the separation phase, with certain discontinuous buffer systems, was determined by the amount of time required for the edge of the discontinuity to progress to a specific distance from the insert line. Those distances are indicated in Table 2, for the discontinuous systems used for analyses of Tf, ceruloplasmin, and the so-called amylase. That criterion was not applied with the discontinuous system used for separation of hemopexin variants. Although the discontinuity moved to the anodal edge of the gel after about 13 hours, electrophoresis was continued past that time, as indicated in Table 2. Unlike the techniques used for serum specimens, the analysis of hemolysate involved only a separation phase. The applicator papers were left in position until termination of the electrophoresis.

Conjugated Serum or Hemolysate as Specimens

Electrophoretic analyses of serum involved only undiluted specimens, except in the case of separation of the hemopexin variants.

Serum specimens to be analyzed for hemopexin variants were mixed with a buffered solution of hematin, prior to application to the starch gel. Hematin was prepared for mixing with serum by the following procedure: hematin was brought into solution at a concentration of 4.7 mg/ml in 0.2 M NaOH; one volume of that basic solution was diluted into ten volumes of the same buffer used for preparing the starch gel (described in Table 2). About a 7- to 9-microliter volume of that solution was mixed with a 25-microliter portion of each specimen to be analyzed. The mixtures of serum + hematin were allowed to stand at room temperature for 10 to 15 minutes, after which the conjugated specimens were absorbed onto the applicator papers and loaded into the gel, as described above.

Hemolysates used for electrophoretic analyses were prepared from washed erythrocytes. Those cells consisted of the unused portions of suspensions which had been prepared for serotyping procedures. Following the withdrawal of the portions needed for serotyping, the cells were allowed to settle at 2 to 5° and were stored for periods of five to ten days. The supernatant buffered saline was aspirated away from the sedimented cells, and hemolysis was effected by adding three volumes of cold distilled water to one volume of cells. (Usually, the hemolysis was accomplished within 12 hours prior to the electrophoretic analysis; however, repetitions of the analysis were accomplished with some hemolysates which had been stored at -20° for as long as several weeks.) Specimens for electrophoretic analyses were diluted by combining two volumes of hemolysate with one volume of the buffer solution used for preparing the starch gel.

During initial phases of the study, the hemolysates were centrifuged at 1200 x g for 15 minutes before withdrawing portions for electrophoretic analyses. It was found that omission of this step had no effect on the quality of results, and the centrifugation was not accomplished for specimens analyzed during later phases of the study.

Hemolysate was used to provide a reference marker in the separation of hemopexin. Such specimens consisted of hemolysate prepared as described above, but diluted further by addition of 40 volumes of the gel buffer.

Development of Electropherograms

Staining of the gels to visualize the distributions of variant components was accomplished after slicing the gel slab into half-slabs of equal thickness. That slicing was accomplished by placing the slab onto an acrylic plastic tray, structured similarly to the frame which supported the slab during electrophoresis. However, the sides of this slicing tray were just 0.3 cm-high. A slice was made through the slab at the level of the sides of the tray using a monofilament thread. Then, the portion of the slab protruding above the sides of the tray was removed from the portion which lay below that level. It was found that the wet gel had sufficient adhesiveness toward the plastic slicing tray, so that the halves of the slab could be separating by inverting the tray (with the gel lying on it), holding it over another tray or pan and prying a flat spatula between the two slices in order to cause one slice to fall away from that which adhered to the slicing tray. Thus, the slices could be separated without handling the gels; it was only necessary to pry the spatula between the slices along the edges.

Hematin-binding components in gel were disclosed by virtue of peroxidase activity. The staining solution which was used was a modification of that of Kristjansson (1961) and consisted of 0.16% (w/v) benzidine and 0.07% hydrogen peroxide in a 0.50% solution of acetic acid. Hydrogen peroxide from a stock solution was added immediately before pouring the staining solution onto the gel. The staining reaction was allowed to proceed for one hour at room temperature inside a darkened compartment. The staining solution was then poured off, the gel was rinsed once with water, and patterns on the gel were recorded immediately.

The serum component considered to be ceruloplasmin was detected on gels by the criterion of oxidase activity toward p-phenylenediamine dihydrochloride. The staining solution was the same as that used by Imlah (1964). The solution consisted of 0.03% (w/v) p-phenylenediamine dihydrochloride (Eastman Kodak, Organic Chemicals Div.) in 1.0 M sodium acetate (pH 7.40 to 7.50). The solution was poured onto the gel and

the staining vessel was placed into a 37° incubator. After 40 minutes of incubation, the stain was drawn off with a pipette, and the surface of the gel was blotted once by dropping a paper towel onto it. The stained patterns were observed immediately by putting the glass staining dish onto a photographic light table.

Development of patterns of distributions of Tf and Sa components was accomplished by means of non-specific staining of proteins. Amido black dye was used at a 1.2% concentration (w/v) in a solvent consisting of methanol/H_2O/glacial acetic acid in the ratio of 5:5:1. About 2 ml of staining solution was poured onto the cut surface of the gel and the gel was tilted to effect an even spreading of the stain over the surface. After two to three minutes, the stain solution was drained away from the gel and sufficient fixative solution was poured into the staining tray to cover the top surface of the gel. The fixative solution (methanol/H_2O/glacial acetic acid, in proportions of 5:5:1) also served as de-staining agent, to remove stain from portions of the gel where no protein components were present. De-staining was sufficient, after this solution was twice-replaced with new quantities.

Specimens were analyzed by the method considered by Baker (1968b) as being effective for demonstrating serum amylase variants. The different phenotypes were most easily discerned if background staining of the starch gel were accomplished. Thus, the buffer in which those gels were incubated (7.0 mM Na_2HPO_4/KH_2PO_4, pH 7.5) included p-phenylenediamine dihydrochloride at a concentration of 0.06% (w/v). Following electrophoretic separation, the sliced gel was placed into this buffer and incubated at 37°. After 15 to 17 hours of incubation, the buffer was removed and the gel was fixed using a 5:5:1 mixture of methanol/H_2O/glacial acetic acid. A period of at least seven hours was allowed for fixation. Finally, the fixative was replaced with tap water in which the gel was allowed to soak for at least two days. Phenotypes were observed after removing the gel from water and placing it onto a photographic light table. Observing the gel with transmitted light in this manner allowed those particular serum components to be observed as relatively more opalescent bands in comparison to the remainder of the gel. Even after fixation and a long de-staining

period, the remainder of the gel retained the stain which was acquired from the long incubation with p-phenylenediamine dihydrochloride.

Locations of Pgd activity were disclosed by coupling the dehydrogenation reaction with the reduction of a tetrazolium salt, resulting in production of a colored compound. Components necessary for this staining reaction were put into 2.5 ml of a buffered solution containing 0.20 M Tris-HCl, (pH 8.0) and 2.7 mM $MgCl_2$. Immediately before application to the gel, the solution was made to contain 0.62 mM nicotinamide adenine dinucleotide phosphate (NADP), 0.32 mg/ml phenazine methosulfate, 0.52 mg/ml of 3-(4,5-dimethyl thiazolyl-2) -2,5-diphenyl tetrazolium bromide (MTT) and 7.1 mM trisodium 6-phosphogluconate. That staining solution was poured onto the cut surface of the gel and the gel was placed inside a 37° incubator for one hour. Patterns on the gel were then recorded without delay.

Locations of Phi activity were disclosed by providing for dehydrogenation of the product of the isomerization and coupling the dehydrogenation with reduction of a tetrazolium salt. The staining method is similar to that used by Saison and O'Reilly (1971). Components necessary for this staining process were put into 3.0 ml of a buffered solution containing 0.06 M Tris-HCl (pH 8.0). The components were added to that buffer immediately prior to application of the mixture to the cut surface of the gel. Glucose-6-phosphate dehydrogenase was added, to a concentration of 1.2 International Units/ml. Other concentrations were as follows: 0.32 mM NADP, 0.27 mg/ml phenazine methosulfate, 0.30 mg/ml MTT, and 2.5 mM disodium fructose-6-phosphate. This mixture was then combined with 3 ml of melted 2% agar (Oxoid Ionagar No. 2) which had been maintained in a 37° water bath for about five minutes in order to reduce its temperature from that required for melting. After pouring the buffered solution into the beaker containing agar, the mixture was swirled in the water bath for several seconds and then poured over the surface of the gel. This staining mixture was applied only to the gel segment which was cathodal to the insert position. The agar-covered gel was then placed into a 37° incubator. Immediately following a 15-minute incubation period, the patterns on the gel were recorded.

The biological materials (except agar) and other organic compounds used for obtaining zymograms of erythrocyte enzymes were products of Sigma Chemicals. Glucose-6-phosphate dehydrogenase (lot no. 121C-8600) was that obtainable from yeast.

Autoradiography

Radioiron ($^{59}Fe^{++}$) was obtained from Mallinckrodt Chemicals in a buffered solution of sodium citrate/sodium chloride. Radioactivity of that solution was 0.025 millicurie/ml and specific activity was 17.6 millicuries/mg. Preparatory to addition of the isotope to serum specimens, aliquots containing 0.49 microcurie were pipetted onto a glass slide. The solution was allowed to concentrate by evaporation until the volume of each aliquot was about 2 microliters. Then, 25-microliter volumes of serum specimens were pipetted onto each aliquot of isotope. After mixing was promoted by gentle agitation of the glass slide, a piece of the chromatography paper used in application of specimens to gels was dropped onto each specimen. The slide was covered by inverting a small dish over it and soaking of the applicator papers was allowed to continue for 10 to 15 minutes. Applicator papers were then inserted to the starch gel and electrophoresis was accomplished as described above. Following the slicing of the gel and covering the cut surface with a sheet of transparent food-wrapping film, a sheet of Kodak "No-screen" x-ray film was apposed to the gel, and a 12-hour exposure period was allowed. The film was processed using Kodak "KLX" liquid developer and "KLX" fixer solution. The remaining gel slice was stained with amido black.

Hematin Procurement

Hematin was obtained from washed erythrocytes by the method of Schwartz et al. (1960). The initial step in disintegration of erythrocytes was accomplished using a solution consisting of 1 ml of saturated NaCl in 1 liter of glacial acetic acid. The solution was heated to 105°. One volume of swine erythrocytes which had settled

from a suspension in physiological saline was slowly added to four volumes of the hot, salt-supplemented acid. The temperature was maintained at 100° and the material was stirred intermittently. After 15 minutes, the heat was removed and the mixture was allowed to stand at room temperature for ten hours. The preparation was centrifuged at 1200 x g for 15 minutes and the supernatant was drawn off. The sediment was washed successively with one-half volumes of 50% acetic acid, distilled H_2O, ethanol, and diethyl ether. Following aspiration of most of the diethyl ether, the product was dried under a fume hood.

Data Analysis

The least squares method (Harvey, 1960; Pennington, 1970) was used to seek evidence that a portion of the variance in litter size was associated with phenotypes. That analysis was accomplished by means of computational programs used in tandem. The first of those programs utilized the matrix inversion method for solution of a set of linear equations. Results of those computations were then utilized by a second program which provided an analysis of variance, as well as calculation of least squares constants. Those computational programs were written by Prof. K. E. Rowe and were made available for use through the Oregon State University Computer Center's "Open Shop Operating System." Other somewhat more fundamental statistical analyses of the data were accomplished using another specially designed computational subsystem which is available through the same operating system. That subsystem has been described in a departmental technical report (Guthrie et al., 1974).

Quantitative descriptions of associations between specific phenotypes of sows and variance in number of piglets per litter farrowed were provided by least squares analysis models which included terms representing some, but not all of the genes which have been mentioned above in this section. Besides those genetic factors, the equations included a term intended to account for the effect of the varying ages of the brood sows at time of farrowing. Another term

which was included was intended to account for the effect of the semester during which the individual litter was conceived.

The several models used for these analyses are variants of one of two different basic models. The two are described separately here.

The more easily described model used for least squares analyses considered separately each phenotype at a specific genetic locus, whereas the other type of model utilized condensed phenotypic categories for certain genes. The former model is described by the following expression:

$$P_i = \mu + b_d C_d + b_{jk} G_{jk} + b_a A_i + e_i$$

where

P_i = a datum pertaining to the composition of the i^{th} litter of pigs born during the course of the project;

μ = sub-population mean which would be found in the special case where the sub-population consisted of equal numbers present in the different discrete categories defined for C_d, equal numbers in each of the phenotypic categories defined for the j^{th} genetic locus, and if the value of A_i were 40.0;

b_d = least squares constant describing relationship of the variable P to the variable denoted as C_d;

b_{jk} = least squares constant describing relationship of the variable P to the variable G_{jk};

b_a = multiple regression coefficient describing relationship of the variable P to the variable denoted as A;

C_d = discontinuous variable which indicates the semester during which the i^{th} litter was conceived;

G_{jk} = discontinuous variable which indicates whether or not the dam which farrowed the i^{th} litter possessed the k^{th} phenotype determined by the j^{th} genetic locus;

A_i = age of dam (in weeks) at date of farrowing of the i^{th} litter, and

e_i represents randomly varying factors affecting the i^{th} litter, which are assumed to be normally distributed among the litters comprising the set of data being analyzed.

The other model, which combined certain phenotypic categories, was of the following form:

$$P_i = \mu + b_d C_d + b_{jk} G_{jk} + b_{mr} H_{mr} + b_a A_i + e_i$$

where

μ = sub-population mean which would occur in the special case where the sub-population consisted of equal numbers present in the different discrete categories defined for C_d, equal numbers in each of the phenotypic categories defined for the j^{th} genetic locus, equal numbers in each of the categories resulting from pooling of certain phenotypes controlled by the m^{th} genetic locus, and if the value of A_i were 40.0;

b_{mr} = least squares constant describing relationship of the variable P to the variable denoted as H_{mr};

H_{mr} = discontinuous variable indicating whether or not the dam of the i^{th} litter belonged to the r^{th} category formed by pooling phenotypes, where the r^{th} category consisted of phenotypes controlled by the m^{th} genetic locus,

and other symbols shown in this model are as defined above.

The two equations shown in the above paragraphs describe the basic forms of analytical models used; however, various models were applied to the data. The various models differed in terms of the number of independent variables used; e.g., one model involved terms representing three phenotypes, whereas another involved terms representing 23 phenotypes (besides terms representing the age of the dam and the semester of conception).

More detailed description of the process of data analysis can be facilitated by considering the total reproductive data from all female subjects as a <u>data set</u>, consisting of subsets. A subset consisted of the data on sows of one of the breed categories. There was no attempt at analysis of the data set as a whole; instead, each subset was treated separately by the least squares method.

RESULTS

Reproductive Performance

Previous studies have provided information about performance of the Oregon State purebred lines, the C line, and a portion of the crossbred breeding stock (Spurr, 1969; Liu, 1974), as mentioned in a previous section. However, those studies did not deal with the performance of females produced from crossmatings of purebreds with C or F_1 animals. Therefore, there are presented here descriptions of the reproductive performance of those crossbred females and for purposes of comparison, data are also included about the purebred parental stocks from which those crossbreds were produced.

Crosses of purebred lines with the C line and with the F_1 population were accomplished using animals born prior to 1973. Spurr (1969) effectively described the contrasts in reproductive performances among the purebred and C lines during the period prior to 1969. It is of interest to determine whether or not such contrasts also occurred subsequently in the breeding stock. (A considerable proportion of the B, Y, C, and F_1 animals which were parents of crossbred sows were born after 1968.) Table 3 consists of data which describe contrasts among the three semi-closed lines (B, Y, and C) and the F_1 hybrid population from 1969 until mid-1973. All females of those breed categories which served as breeding stock during that period are included, regardless of whether or not they were dams of crossbred sows. "Number of fetuses per litter" refers to the total number of piglets born, either dead or alive. Comparisons of the breed category averages at each of the first three parities provide evidence that B's farrowed litters which were distinctly smaller than those of the other three categories. The contrasts between performance of B's and that of each other category were statistically significant at the first and second parities ($P<.05$) according to the criterion of Duncan's "new" multiple range test (Steele and Torrie, 1960). Data on the fourth, fifth, and sixth parities are more meager; however, it should be noted that the few

Table 3. Prolificacies of females born during 1969 through mid-1973 in purebred lines and two hybrid populations derived therefrom. Number of fetuses per litter ± standard deviation.

	Parity				
Breed category	1	2	3	4	5+6
B	7.45 ±2.80	7.56 ±2.68	7.70 ±3.40	9.33 ±.59	8.83 ±3.66
No. of litters	24	16	10	3	6
Standard error	±.57	±.67	±1.08	±.34	±1.49
Y	10.21 ±2.36	9.67 ±2.97	10.07 ±3.53	11.25 ±3.19	12.29 ±2.02
No. of litters	56	42	27	24	17
Standard error	±.32	±.46	±.68	±.65	±.49
C	9.23 ±2.59	9.72 ±2.71	10.59 ±3.37	10.63 ±3.16	12.00 ±6.16
No. of litters	40	29	17	8	4
Standard error	±.41	±.50	±.82	±1.12	±3.08
BxY + YxB	9.04 ±1.95	11.00 ±2.85	12.33 ±2.00	11.14 ±3.39	11.00 ±2.00
No. of litters	25	21	9	7	3
Standard error	±.39	±.62	±.67	±1.28	±1.16
Category pairs found significantly different*	B-Y B-C B-(BxY + YxB)	B-Y B-c B-(BxY + YxB)	None	Not tested	Not tested

*Differences were tested using Duncan's "new" multiple range test for the 5% probability level.

data on B's are quite consistent, in being lower than corresponding averages for Y's and C's. As mentioned above, Spurr (1969) found that after adjusting for the ages of the sows, the average number of fetuses per litter for B's was 2.23 less than the values found for both Y's and C's. The table also shows that averages for the F_1 females were consistently higher than the mid-values between the B and Y averages at any specific parity.

There is evidence in Table 4 about the effects of crossbreeding on reproductive performance. Separate averages are shown for individual parities. Data are arranged so as to facilitate comparisons of performances of crossbred categories with the performances of respective parental groups. Averages for the B, Y, and C groups were calculated using only data on animals born during specific periods, as is indicated in the line showing years of birth. It was intended that the B, Y, and C averages shown should be indicative of the characteristics of the particular sub-populations as they existed during the period when the crossbred breeding stock was being produced, instead of populations present earlier or later than the parents of the crossbreds. Therefore, data for the average on any particular parental population (viz., B, C, or Y) consisted only of that from specific calendar years during which the parents of the crossbreds were born. For example, B boars and sows born in the years 1966 and 1968 through 1971 were parents of the crossbred brood sows produced from matings of B's with C's. Berkshires born in other years did not produce any of the brood sows of that crossbred type. Thus, the averages shown in Table 4 for B's for comparison to performances of those crossbreds were calculated using data from only the years 1966 and 1968-71. This criterion for data selection does permit inclusion of data on some quite distant relatives of the animals which were parents of the crossbreds, rather than data only on parents of crossbreds; no effort was made to assure that each brood sow or service boar in the parental lines produced crossbred brood sows.

Data contained in Table 4 are from all brood sows produced by crossmatings among the semi-closed lines. Calculation of averages for daughters from the matings of C's to B's or Y's was performed in such

Table 4. Performances of crossbred daughters of purebred and Composite pigs compared to parental groups.

Breed category	BxC	CxB	B	C	YxC	CxY	Y	Cc
Year of birth[a]	1967-71	1969-72	1966,1968-71	1967-71[b]	1967-72	1967-72	1967-71	1967-70
			Parity 1				Parity 1	
Fetuses/litter±std. dev.	8.18	8.81	7.76±2.76	9.23±2.35	9.88	11.14	10.12±2.19	9.49±2.34
Number of litters	17	27	33	44	16	28	57	35
Reciprocal categories,pooled	8.57±3.04				10.68±3.00			
Standard error	±.46				±.45			
Confidence interval (95%)	7.65 - 9.49				9.77 - 11.59			
Mid-parent value			8.50				9.81	
			Parity 2				Parity 2	
Fetuses/litter±std. dev.	8.77	9.72	7.86±2.78	10.09±2.76	11.75	11.95	10.66±3.31	10.52±2.53
Number of litters	13	18	21	32	12	21	38	27
Reciprocal categories,pooled	9.32±3.02				11.88±2.51			
Standard error	±.54		±.61	±.49	±.44		±.54	±.49
Confidence interval (95%)	8.21 - 10.43				10.99 - 12.77			
Mid-parent value			8.98				10.59	
			Parity 3				Parity 3	
Fetuses/litter±std. dev.	10.50	10.00	8.21±3.64	11.35±3.29	11.78	11.63	10.13±3.22	11.62±3.20
Number of litters	12	13	14	23	9	16	24	21
Reciprocal categories,pooled	10.24±3.06				11.68±4.34			
Standard error	±.61		±.97	±.69	±.87		±.60	±.70
Confidence interval (95%)	8.98 - 11.50				9.89 - 13.47			
Mid-parent value			9.78				10.88	
			Parity 4				Parity 4	
Fetuses/litter±std. dev.	11.40	12.00	9.83±1.72	10.55±2.47	12.50	13.00	11.94±2.73	9.91±2.43
Number of litters	10	6	6	11	6	14	18	11
Reciprocal categories,pooled	11.63±2.39				12.85±2.92			
Standard error	±.60		±.70	±.74	±.65		±.64	±.73
Confidence interval (95%)	10.36 - 12.90				11.49 - 14.21			
Mid-parent value			10.19				10.93	
			Parities 5 + 6				Parities 5 + 6	
Fetuses/litter±std. dev.	10.00	11.00	10.25±3.20	10.83±6.77	13.00	13.11	12.05±2.54	10.83±6.77
Number of litters	9	4	12	6	7	9	21	6
Reciprocal categories,pooled	10.31±2.36				13.06±2.67			
Standard error	±.65		±.92	±2.76	±.67		±.55	±2.76
Confidence interval (95%)	8.88 - 11.74				11.64 - 14.48			
Mid-parent value			10.54				11.44	

[a] "Year of birth" is that of the crossbred dams, in columns headed BxC, CxB, YxC, CxY, and that of their respective parental stocks, in columns headed B, C, and Y.

[b] One dam born prior to 1967 is also included for these averages.

[c] One C dam and one sire were born prior to 1967; the record for that dam is included for these averages.

a way as to obtain evidence on whether or not the particular breed of dam used in a crossmating was a determinant of the reproductive ability of the daughters. Separate averages were calculated on the daughters from the mating of B boars to C sows (BxC) and on the daughters from mating of C boars to B sows (CxB), besides a single average for all daughters produced from matings between B's and C's. Averages for daughters from those reciprocal types of matings suggest that daughters of the CxB mating may have been somewhat superior to those from BxC matings, but the difference was neither large nor entirely consistent over the different parities. Hence those reciprocal categories were pooled before comparing crossbreds to parental averages. The data obtained by pooling the two reciprocal categories will be referred to below as the data on the (BxC + CxB) category. Evidence of heterosis was sought, by comparing average number of fetuses per litter for the crossbred dams with the mid-value of the averages of respective parental populations.

The differences by which the (BxC + CxB) exceeded the mid-parent estimate showed a progressive increase, over the first through the third parities. That contrast was not maintained into the fifth and sixth parities. It should be noted that estimates of mid-parental performance lie well within the respective 95% confidence intervals of the average performance of the (BxC + CxB) subjects, except at the fourth parity.

Data shown in the right-hand portion of Table 4 were used in seeking evidence of heterosis in daughters from matings between Y's and C's. There was less indication of contrasts between the reciprocal categories of such matings, except at the first parity, at which YxC daughters farrowed 9.88 fetuses per litter, in comparison to 11.14 fetuses per litter farrowed by the CxY category. Since the contrast was much less at subsequent parities, data for the reciprocal categories were pooled for comparisons to parental averages. The pooled reciprocal categories are referred to below as the (YxC + CxY) category. It was found that at the different parities, the averages for the (YxC + CxY) not only exceeded the mid-values of the parental lines, but also exceeded the value for the higher parental line. The

estimates of mid-parental performance were less than lower limits of the 95% confidence interval for averages of the (YxC + CxY) at each parity except the first and third.

Data in Tables 5a, 5b, and 5c on matings of B, Y, and C males to F_1 females were thought to be of interest in view of the fact that the F_1 and C populations were both produced by means of crossmatings of members of the same two parental breeds. However, comparisons considered to be most meaningful were not simply those involving the parental populations. It was evident that a heterotic effect toward reproduction was occurring in the daughters of B-Y matings (Table 3). It was not of real interest to find simply that performances of BxF_1 and YxF_1 sows did not exceed the performance of the more prolific of the parental populations. Therefore, evidence of heterotic effects toward reproduction in the BxF_1, YxF_1, and CxF_1 was sought by making comparisons to performances of the inbred populations which were sources of the genes present in the daughters produced from crossing of F_1 females. Performances of the backcross daughters were compared to the apparent reproductive capabilities of the stocks from which they descended by calculating an estimate using a two-step averaging process. The mean value of the performances of the maternal grandparental stocks was used as the estimate of performance to be expected of the F_1 parents, on the assumption that the variance in performance was only due to additively expressed genetic factors. Then, the mean value between that estimate and the average performance of the paternal stock was obtained, to serve as the estimate of the performance expected of the backcross daughters (i.e., the BxF_1 or YxF_1). Those predictions were found to be remarkably close to the performances of the BxF_1 sows at the first and second parities. This method of comparison provided some suggestions of heterosis after the second parity in the BxF_1 and after the first parity in the YxF_1. However, it is necessary to note that there were large standard errors (±.71 or greater) associated with nearly all of the means used for estimating performances in paternal and grandparental groups after the second parity, and this raises considerable doubt about the usefulness of these suggestions of heterosis.

Table 5a. Performances of backcross daughters of B boars and F_1 hybrid sows compared to parental and grandparental groups.

Breed category	BxF_1	B (paternal population)	B (grand-parental population)	Y (grand-parental population)
Year of birth	1970-73	1966 1968 1970	1966-68	1967-70
		Parity 1		
Fetuses/litter±std. dev.	8.37±2.97	7.94±2.99	7.35±2.37	10.12±2.18
Number of litters	19	17	23	43
Standard error	±.68	±.73	±.50	±.33
Confidence interval(95%)	6.94-9.80			
Mean value of backcross predicted by additivity[a]	8.34			
		Parity 2		
Fetuses/litter±std. dev.	8.73±3.73	8.18±2.56	8.11±2.71	10.54±3.27
Number of litters	15	11	9	28
Standard error	±.96	±.77	±.90	±.62
Confidence interval(95%)	6.66-10.80			
Mean value of backcross predicted by additivity	8.75			
		Parity 3		
Fetuses/litter±std. dev.	10.36±3.72	9.30±3.16	8.83±3.71	9.41±2.94
Number of litters	11	10	6	17
Standard error	±1.12	±1.00	±1.51	±.71
Confidence interval(95%)	7.87-12.85			
		Parity 4		
Fetuses/litter±std. dev.	11.78±3.67	10.00±2.61	10.00±2.17	11.17±2.76
Number of litters	9	5	4	12
Standard error	±1.22	±1.17	±1.08	±.80
Confidence interval(95%)	8.96-14.60			
		Parities 5 + 6		
Fetuses/litter±std. dev.	11.64±2.98	10.10±3.41	11.83±1.94	11.58±2.84
Number of litters	11	10	6	12
Standard error	±.90	±1.08	±.79	±.82
Confidence interval(95%)	9.64-13.64			
Mean value of backcross predicted by additivity	10.91			

[a] Additivity predictions were obtained from a two-step averaging process:
1) calculation of mid-value of maternal grandparental groups, then
2) calculation of mid-value between (1) and paternal group.

Table 5b. Performances of backcross daughters of Y boars and F_1 hybrid sows compared to parental and grandparental groups.

Breed category	YxF_1	Y (paternal population)	B (grand-parental population)	Y (grand-parental population)
Year of birth	1970-73	1968[a]	1966-68[b]	1967-70
		Parity 1		
Fetuses/litter±std. dev.	9.06±2.26	10.50±1.55	7.71±2.89	10.12±2.18
Number of litters	33	16	24	43
Standard error	±.39	±.39	±.59	±.33
Confidence interval(95%)	8.26-9.86			
Mean value of backcross predicted by additivity	9.71			
		Parity 2		
Fetuses/litter±std. dev.	10.68±2.82	10.00±3.16	8.11±2.71	10.54±3.27
Number of litters	25	14	9	28
Standard error	±.56	±.84	±.90	±.62
Confidence interval(95%)	9.52-11.84			
		Parity 3		
Fetuses/litter±std. dev.	10.00±3.18	9.83±3.49	8.83±3.71	9.41±2.94
Number of litters	17	6	6	17
Standard error	±.77	±1.42	±1.51	±.71
Confidence interval(95%)	8.36-11.64			
		Parity 4		
Fetuses/litter±std. dev.	12.40±3.31	11.33±2.89	10.00±2.16	11.17±2.76
Number of litters	10	3	4	12
Standard error	±1.05	±1.67	±1.08	±.80
Confidence interval(95%)	10.03-14.77			
		Parities 5 + 6		
Fetuses/litter±std. dev.	9.60±1.67	11.00±2.65	11.83±1.94	11.58±2.84
Number of litters	5	3	6	12
Standard error	±.75	±1.53	±.79	±.82
Confidence interval(95%)	7.51-11.69			

[a] Four of the YxF_1 sows were sired by a Y boar born in 1972; the "paternal" Y averages therefore include data on four littermates of that boar, in addition to the data on 1968 Y sows.

[b] Data also include one grand-dam born in 1971.

Table 5c. Performances of daughters of C boars and F_1 hybrid sows compared to parental and grandparental groups.

Breed category	CxF_1	C (paternal population)	B (grandparental population)	Y (grandparental population)
Year of birth	1969-73	1967-68, 1970[a]	1965-66, 1968, 1970	1966, 1968-70
		Parity 1		
Fetuses/litter±std. dev.	9.16±2.12	9.55±2.41	8.17±2.75	10.21±2.52
Number of litters	25	29	29	38
Standard error	±.42	±.45	±.51	±.41
Mean value of backcross predicted by additivity	9.37			
		Parity 2		
Fetuses/litter±std. dev.	9.44±3.31	10.68±2.53	7.68±2.75	10.96±2.95
Number of litters	16	22	19	24
Standard error	±.81	±.54	±.63	±.60
Mean value of backcross predicted by additivity	10.00			
		Parity 3		
Fetuses/litter±std. dev.	10.11±3.48	11.33±2.89	9.37±2.58	9.88±3.07
Number of litters	9	15	16	16
Standard error	±1.16	±.75	±.65	±.77
Mean value of backcross predicted by additivity	10.48			
		Parity 4		
Fetuses/litter±std. dev.	10.83±2.23	10.67±1.63	9.45±2.46	10.70±2.75
Number of litters	6	6	11	10
Standard error	±.91	±.67	±.74	±.87
Confidence interval(95%)	8.49 - 13.17			
Mean value of backcross predicted by additivity	10.37			
		Parities 5 + 6		
Fetuses/litter±std. dev.	12.80±2.05	10.50±6.19	10.60±3.09	11.00±2.16
Number of litters	5	4	15	4
Standard error	±.91	±3.10	±.80	±1.08

[a] Averages for the C group do not include data on any females born in 1971, although the sire of one CxF_1 brood sow was born in 1971.

There were no such suggestions of heterosis in the data used to compare CxF_1 sows with the parental and grandparental stocks (Table 5c). However, it should be noted that as with means in the two preceding tables, there were large standard errors (±.65 or greater) associated with the means found at parities later than the second.

Also notable however, is another pattern of consistency which is seen by comparison among the five crossbred categories described in Tables 4, 5a, 5b, and 5c. The average performance in the (YxC + CxY) at any specific parity was greater than the average performances of the (BxC + CxB), BxF_1, and YxF_1 breed categories. Likewise, the performance of the (YxC + CxY) exceeded that of the CxF_1 breed category at each parity except the fifth and sixth.

The data in Tables 4, 5a, 5b, and 5c are from the entire population of each crossbred category listed. Contents of Tables 6, 7a, and 7b were intended for the same sort of comparisons as the contents of the four preceding tables. However, Tables 6, 7a and 7b consist of data pertaining to only the subjects of the phenotyping studies described under MATERIALS AND METHODS or to the parental populations from which those subjects were produced. The phenotyped subjects in the (BxC + CxB), (YxC + CxY), BxF_1, and YxF_1 breed categories were born during a relatively shorter time span than were the total subpopulations comprising those four categories. Members of those four categories which were excluded from Tables 6, 7a, and 7b by reason of unavailability for phlebotomy were those which either were born at a relatively early stage of the line-crossing program or were not retained in the herd after bearing one or two litters. Calculations which provided the results in Tables 6, 7a, and 7b were accomplished in order to determine whether or not, in that subset of sows available for phenotyping studies, there were any indications of heterosis.

Some interesting consistencies are seen in the data shown in Table 6. The average performance of the (BxC + CxB) subjects at each parity was greater than the mid-value of the parental stocks and was greater than the average of either parental group at each parity after the second. The average performance of the (YxC + CxY) subjects was as great as or greater than that of either parental group at each

Table 6. Performances of crossbred phenotyped daughters of purebred and Composite pigs compared to parental groups.

Breed category	BxC + CxB	B	C	YxC + CxY	Y	C
Year of birth[a]	1970-72	1966,1968, 1970-71	1967-71	1971-72	1968-69, 1971	1967, 1969-70
		Parity 1			Parity 1	
Fetuses/litter±std. dev.	8.79±2.88	7.92±2.65	9.21±2.37	10.91±1.64	10.03±2.10	9.89±2.58
Number of litters	19	26	43	11	32	27
Standard error	±.66	±.52	±.36	±.49	±.37	±.50
Confidence interval (95%)	7.40 - 10.18			9.81 - 12.01		
Mid-parent value		8.56			9.96	
		Parity 2			Parity 2	
Fetuses/litter±std. dev.	9.56±3.05	8.27±2.22	10.00±2.76	11.82±2.64	10.48±3.38	10.26±2.61
Number of litters	16	15	31	11	23	23
Standard error	±.75	±.57	±.50	±.80	±.70	±.54
Confidence interval (95%)	7.96 - 11.16			10.05 - 13.59		
Mid-parent value		9.13			10.37	
		Parity 3			Parity 3	
Fetuses/litter±std. dev.	11.57±2.90	8.62±3.45	11.14±3.21	12.11±4.96	10.31±3.86	11.37±3.27
Number of litters	14	13	22	9	16	19
Standard error	±.78	±.96	±.68	±1.65	±.97	±.75
Confidence interval (95%)	9.90 - 13.24			8.20 - 16.02		
Mid-parent value		9.88			10.84	
		Parity 4			Parity 4	
Fetuses/litter±std. dev.	12.44±1.67	10.00±1.87	10.10±1.97	11.57±2.23	11.58±3.09	9.89±2.52
Number of litters	9	5	10	7	12	9
Standard error	±.56	±.83	±.62	±.84	±.89	±.84
Confidence interval (95%)	11.16 - 13.72			9.51 - 13.63		
Mid-parent value		10.05			10.73	
		Parities 5 + 6			Parities 5 + 6	
Fetuses/litter±std. dev.	11.83±1.60	10.10±3.41	10.83±6.77	14.20±1.48	12.15±2.15	12.25±6.65
Number of litters	6	10	6	5	13	4
Standard error	±.65	±1.08	±2.76	±.66	±.60	±3.32
Confidence interval (95%)	10.15 - 13.51			12.37 - 16.03		
Mid-parent value		10.46			12.20	

[a] "Year of birth" is that of the crossbred dams, in columns headed BxC + CxB and YxC + CxY, and that of their respective parental stocks, in columns headed B, C, and Y.

Table 7a. Performances of backcross phenotyped daughters of B boars and F_1 hybrid sows compared to parental and grandparental groups.

Breed category	BxF_1	B (paternal population)	B (grand-parental population)	Y (grand-parental population)
Year of birth	1971-73	1966, 1968, 1970	1966-68	1967-70
		Parity 1		
Fetuses/litter±std. dev.	8.77±3.14	7.94±2.99	7.35±2.37	10.12±2.18
Number of litters	13	17	23	43
Standard error	±.88	±.73	±.50	±.33
Confidence interval(95%)	6.86-10.68			
Mean value of backcross predicted by additivity[a]	8.34			
		Parity 2		
Fetuses/litter±std. dev.	9.18±3.43	8.18±2.56	8.11±2.71	10.54±3.27
Number of litters	11	11	9	28
Standard error	±1.03	±.77	±.90	±.62
Confidence interval(95%)	6.88-11.48			
Mean value of backcross predicted by additivity	8.75			
		Parity 3		
Fetuses/litter±std. dev.	9.90±3.57	9.30±3.16	8.83±3.71	9.41±2.94
Number of litters	10	10	6	17
Standard error	±1.13	±1.00	±1.51	±.71
Confidence interval(95%)	7.34-12.46			
Mean value of backcross predicted by additivity	9.21			
		Parity 4		
Fetuses/litter±std. dev.	11.78±3.67	10.00±2.61	10.00±2.17	11.17±2.76
Number of litters	9	5	4	12
Standard error	±1.22	±1.17	±1.08	±.80
Confidence interval(95%)	8.96-14.60			
		Parities 5 + 6		
Fetuses/litter±std. dev.	11.64±2.98	10.10±3.41	11.83±1.94	11.58±2.84
Number of litters	11	10	6	12
Standard error	±.90	±1.08	±.79	±.82
Confidence interval(95%)	9.64-13.64			
Mean value of backcross predicted by additivity	10.90			

[a] Method of calculating additivity prediction is described in footnote to Table 5a.

Table 7b. Performances of backcross phenotyped daughters of Y boars and F_1 hybrid sows compared to parental and grandparental groups.

Breed category	YxF_1	Y (paternal population)	B (grand-parental population)	Y (grand-parental population)
Year of birth	1971-73	1968[a]	1966-68[b]	1967-70
		Parity 1		
Fetuses/litter±std. dev.	9.53±2.39	10.50±1.55	7.71±2.89	10.12±2.18
Number of litters	15	16	24	43
Standard error	±.62	±.39	±.59	±.33
Confidence interval(95%)	8.20-10.86			
Mean value of backcross predicted by additivity	9.71			
		Parity 2		
Fetuses/litter±std. dev.	10.77±2.39	10.00±3.16	8.11±2.71	10.54±3.27
Number of litters	13	14	9	28
Standard error	±.66	±.84	±.90	±.62
Confidence interval(95%)	9.33-12.21			
Mean value of backcross predicted by additivity	9.66			
		Parity 3		
Fetuses/litter±std. dev.	11.00±2.83	9.83±3.49	8.83±3.71	9.41±2.94
Number of litters	11	6	6	17
Standard error	±.85	±1.42	±1.51	±.71
Confidence interval(95%)	9.10-12.90			
		Parity 4		
Fetuses/litter±std. dev.	13.86±2.79	11.33±2.89	10.00±2.16	11.17±2.76
Number of litters	7	3	4	12
Standard error	±1.05	±1.67	±1.08	±.80
Confidence interval(95%)	11.28-16.44			
		Parities 5 + 6		
Fetuses/litter±std. dev.	9.60±1.67	11.00±2.65	11.83±1.94	11.58±2.84
Number of litters	5	3	6	12
Standard error	±.75	±1.53	±.79	±.82
Confidence interval(95%)	7.51-11.69			

[a] Four of the YxF_1 sows were sired by a Y boar born in 1972; the "paternal" Y averages therefore include data on four littermates of that boar, in addition to the data on 1968 Y sows.

[b] Data also include one grand-dam born in 1971.

parity. However, it is apparent that the standard errors associated with mean values in Table 6 were nearly all so large that it is unlikely that crossbred averages would be found to be significantly greater than averages for parental groups.

Comparisons between performances of BxF_1 subjects and performances of parental or maternal grandparental populations are shown in Table 7a. It is interesting to note that the averages for the BxF_1 subjects were greater than the values predicted using the additivity assumption at each parity. This sort of contrast was also found with data on YxF_1 subjects (Table 7b) at the second, third, and fourth parities. (The values predicted with the additivity concept were not shown at certain parities in Tables 7a and 7b, because the averages for the backcross subjects were found to be greater than the averages for the paternal group and either maternal grandparental group.)

As with preceding tables, some comparisons among the different crossbred categories are of interest. Average performance of the (YxC + CxY) exceeded the performances of the (BxC + CxB), BxF_1, and YxF_1 at each parity except the fourth. That pattern of contrasts is similar to the pattern found with the larger subsets of data in Tables 4 through 5c. However, standard errors of means in Tables 6, 7a, and 7b at all parities beyond the first were large (nearly all were distinctly greater than ±0.50).

It is apparent that the data in Tables 4 through 7b would have little usefulness for statistically significant evidence of heterosis. Standard errors associated with most of the mean values were so large as to indicate that predictions of crossbred performances based on assumptions of additivity of gene expression are imprecise estimates. Therefore, there probably would be no useful information gotten from application of any commonly used method of testing significance of differences between mean values. However, it is appropriate to make further comments regarding the statistics describing the variance. The standard errors at the first parity tended to be smaller than those at subsequent parities. The generally large values of standard errors were to a great extent the simple consequence of the sizes of samples which are described by the values. However, inspection of the

standard deviations is worthwhile. Considering all the samples described in Tables 4 through 7b, those for which the standard deviation was less than ±2.00 were a small portion. It was by no means apparent what factor or factors might have been the most significant causes of such variance within samples. However, it was quite plausible that an important cause might have been that of genetically determined variance in reproductive capability among individuals within breed categories.

The comparisons of crossbred performances to the predicted performances estimated from parental populations yielded suggestive evidence that certain crossbred types possessed reproductive capabilities which were greater than those expected on the basis of performances of respective parental groups. Furthermore, the quite sizeable variances in performances within breed categories seemed to deserve some scrutiny. Thus, efforts at adding a new dimension to the study of the various breed categories seemed justified.

Phenotyping Criteria

The criteria used for determining serotypes were described under MATERIALS AND METHODS. Phenotypes controlled by six other loci were determined from the patterns observable on starch gel electropherograms. Those phenotypes are shown in photographs comprising Figures 2 through 7.

Hemopexin

Variation among specimens on the zymogram shown in Figure 2 is evident in the segment labelled as "Hp-Hb." However, the variation at that position among individual specimens was not found to have a genetic basis. The component is probably the haptoglobin-hemoglobin complex. The means of distinguishing hemopexin from other hematin-containing substances in the samples is demonstrated by comparison among specific channels on the gel. The material contained in the channels labelled by "**" consisted only of hemolysate diluted with buffer. Channels labelled by "*" contained specimens to which no

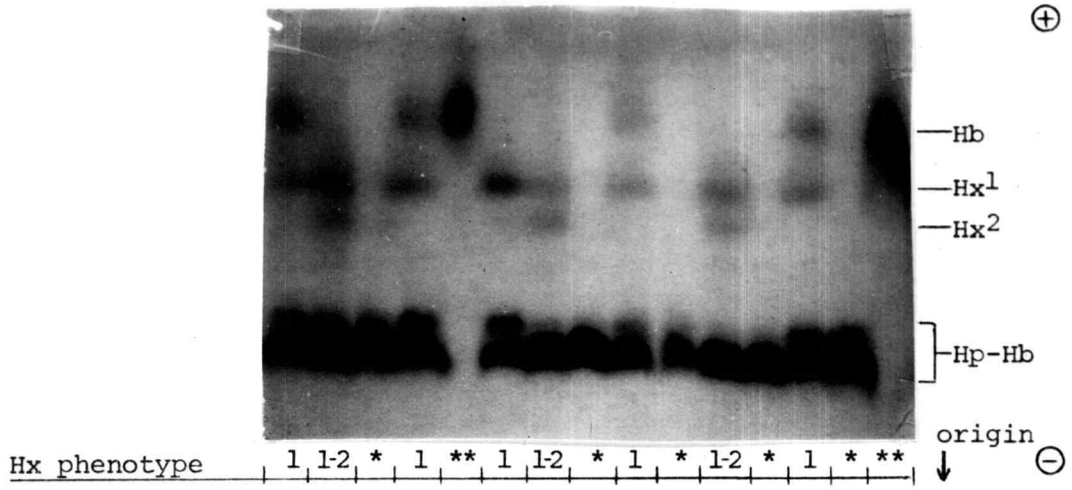


Figure 2. Electropherogram demonstrating hemopexin (Hx) phenotypes, and distinction of Hx from hemoglobin (Hb) and the haptoglobin-hemoglobin complex (Hp-Hb). Stained for peroxidase activity, using benzidine/peroxide. Channels labelled with numerals are serum specimens containing exogenous hematin (0.2 mg/ml).

*Indicates specimens which consisted of serum without exogenous hematin.

**Indicates channels which contained hemolysate, without serum or exogenous hematin.

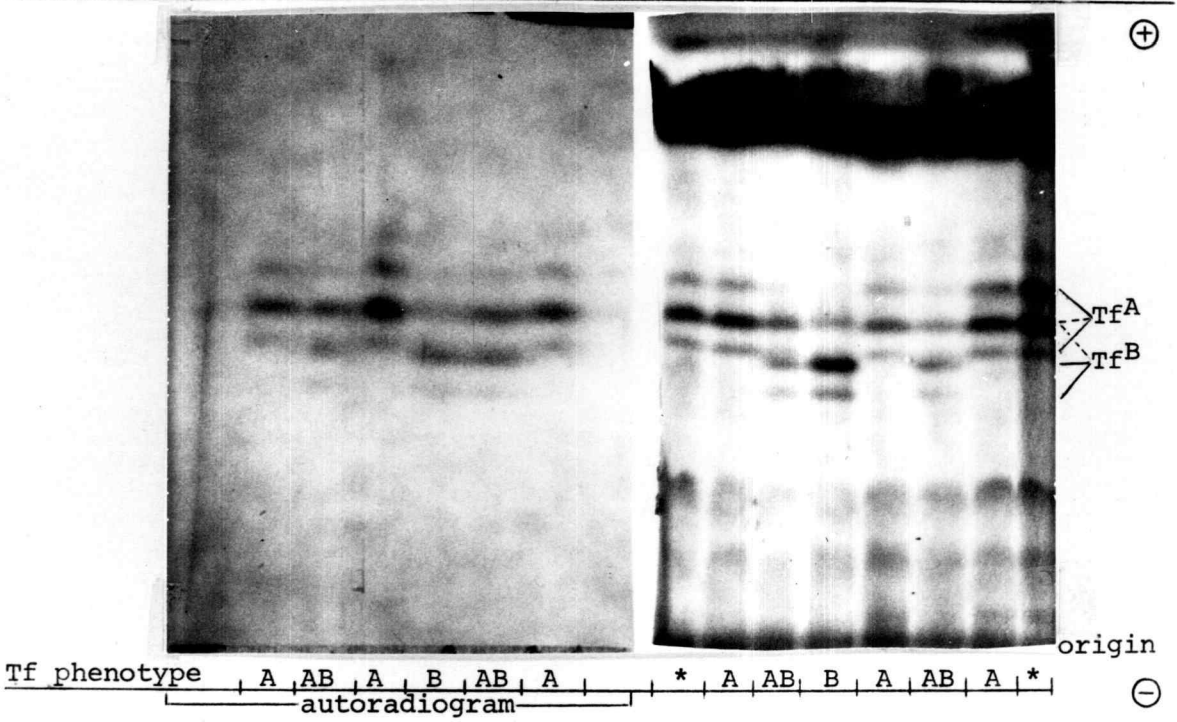


Figure 3. Autoradiogram, with corresponding electropherogram demonstrating transferrin phenotypes, stained with amido black. Correspondence of the autoradiogram produced from the electropherogram is that of a mirror image. Specimens contained radioiron (^{59}Fe), except those labelled by (*).

exogenous hematin had been added. The other channels contained serum specimens which were treated alike, by adding a buffered hematin solution prior to application of the serum to the gel, as described under MATERIALS AND METHODS. No stained material is evident at positions labelled as "Hx^1" and "Hx^2" in those channels containing specimens to which hematin solution had not been added. Stained material at positions besides the "Hb" and "Hp" segments is evident in channels containing specimens to which hematin was added prior to electrophoresis. Such channels contain stained bands at either one or both of the segments labelled as "Hx^1" and "Hx^2." A phenotype consisting of stained material at only one of those positions represents the only homozygous Hx genotype found in the population, *viz.*, Hx^1/Hx^1. A different phenotype, in which staining occurred instead at two of those positions, was designated as Hx 1-2, and is considered as being due to the genotype Hx^1/Hx^2.

Variation among individual serum specimens also may be observed at the position corresponding to that of free hemoglobin (i.e., the component found in the channels labelled "**"). A component was found at that position with some serum specimens but not with others. The possibility that this variation has a genetic basis is given some further consideration below.

Ceruloplasmin

The serum component, *para*-phenylenediamine oxidase, is synonymously referred to as ceruloplasmin (Holmberg and Lurell, 1948; Imlah, 1964). Variation with respect to this component was observed among the specimens analyzed. However, it was not possible to conclude that any of the evident variation had a genetic basis. Some of the specimens appeared to contain a component which was located nearer the insertion position on the gel than was the ceruloplasmin contained in other specimens. That particular fraction appeared in some specimens after they had been in frozen storage for several weeks or months prior to electrophoresis. However, that more cathodal

component was observed in practically none of the specimens, when analyzed after storage for less than one month. The variation observed was most probably the consequence of some denaturation during storage.

Transferrin

The right-hand portion of Figure 3 shows two different types of pattern in which three bands are present in the beta-globulin segment. The pattern containing three bands nearer the anodal end is labelled as Tf "A" and that which contains three bands nearer the cathodal end is labelled as Tf "B." Those patterns represent respectively, the homozygous genotypes Tf^A/Tf^A and Tf^B/Tf^B (Kristjansson, 1963; Baker, 1968a). A different type of pattern, in which four bands are found in the beta-globulin segment of the gel, is the phenotype Tf AB, resulting from the genotype Tf^A/Tf^B. Except for two of the channels on that gel, the sera contain radioiron, added shortly before insertion into the gel. The left-hand half of the photograph shows an autoradiogram which was developed from this gel according to the procedure summarized in MATERIALS AND METHODS. The autoradiogram served as the basis for identifying the bands labelled as "Tf^A" and "Tf^B." The arrangement of the autoradiogram relative to the electropherogram is as a mirror image. "Channels" nearest the side edges of the autoradiogram show no photo-development; those two channels are the portions which, during exposure, overlay the gel channels labelled by "*." Specimens inserted to those two channels were applied without any radioiron.

Slow alpha-2 macroglobulin

Figure 4 displays the three phenotypes denoted as Sa B, Sa C, and Sa BC. Those phenotypes are distinguished from each other by differences at the anodal extremity of a fraction found near the cathodal end of the gel. Phenotypes Sa B and Sa C indicate the presence of homozygous genotypes Sa^B/Sa^B and Sa^C/Sa^C, respectively

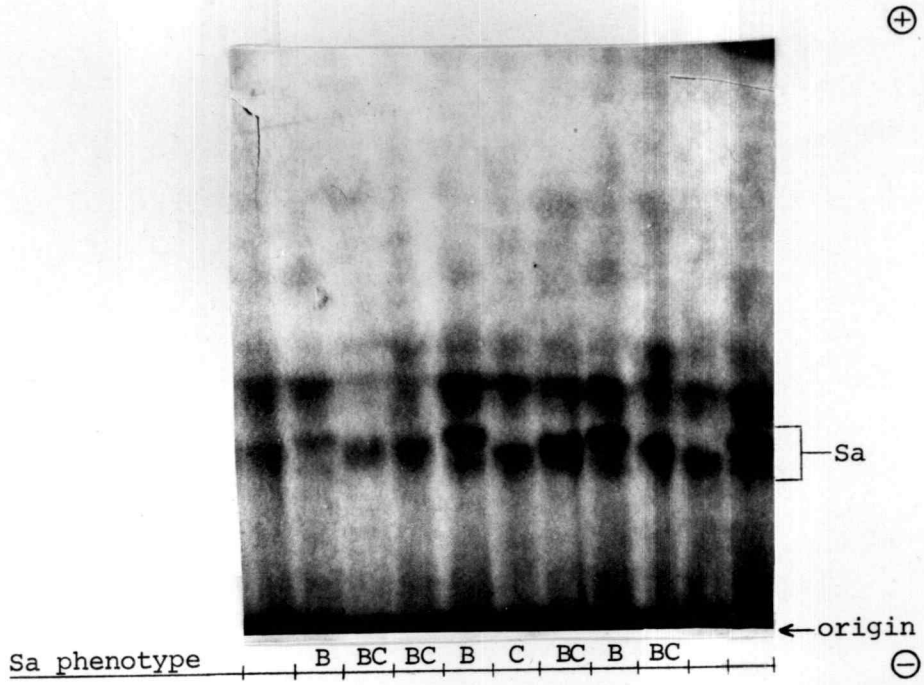


Figure 4. Electropherogram demonstrating slow alpha-2 macroglobulin phenotypes, stained with amido black.

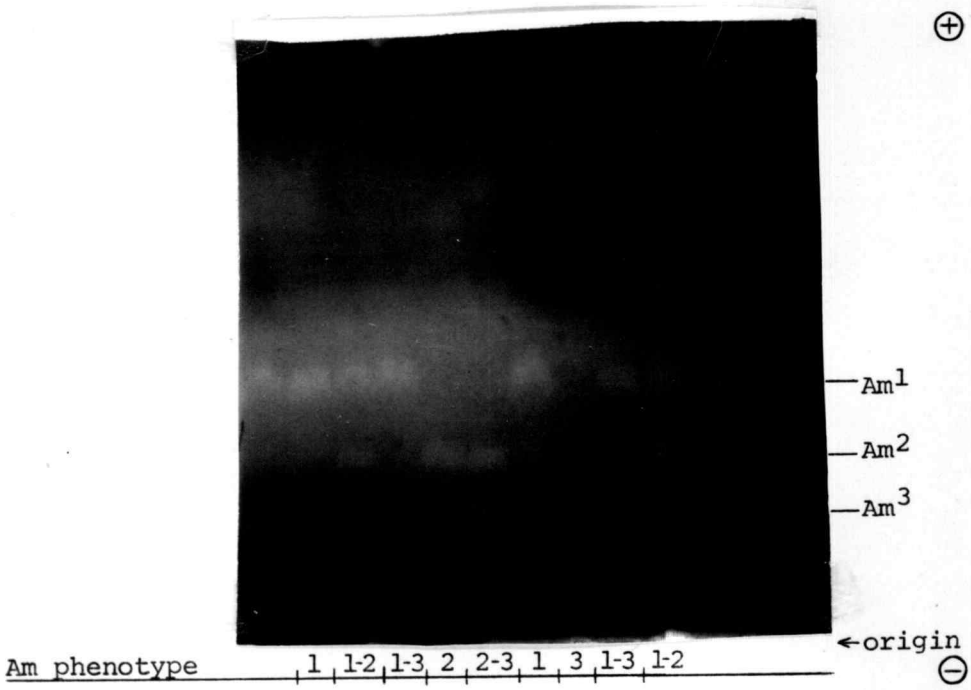


Figure 5. Electropherogram demonstrating amylose-modifier phenotypes. Amylose-modifier locations were disclosed by staining gel with para-phenylenediamine and subsequently de-staining.

(Schroffel, 1965). The pattern labelled as "BC" is the phenotype resulting from the heterozygous genotype, Sa^B/Sa^C.

Amylose-modifier

Six different phenotypic patterns are shown on the photograph in Figure 5. The method of electrophoretic separation, development of the gel pattern, and the patterns themselves are quite similar to those those reported by Graetzer et al. (1965). Moustgaard and Hesselholt (1966) decided that the components could be denoted as the amylase of swine serum. However, the present study did not subject the components described in Figure 5 to tests which could provide any substantial indication of functional properties. Thus, there has been no verification of true amylolytic activity of the variants described here. Due to this fact, it seems advisable to avoid the use of such a specific term as amylase in referring to this material. Throughout the following sections of this thesis, the set of variant components displayed in Figure 5 will be referred to as the amylose-modifier. The abbreviation "Am" will be retained for reference to specific phenotypes or genotypes. Thus, the nomenclature here is that originated by Graetzer et al. and Moustgaard and Hesselholt. The single-banded patterns labelled in Figure 5 as Am "1", Am "2", and Am "3" indicate the presence of the homozygous genotypes Am^1/Am^1, Am^2/Am^2, and Am^3/Am^3, respectively. Likewise, the phenotypes labelled as Am "1-2", Am "1-3", and Am "2-3", each consisting of two bands, are representative of the heterozygous genotypes Am^1/Am^2, Am^1/Am^3, and Am^2/Am^3, respectively.

6-phosphogluconate dehydrogenase

The developed zymogram shown in Figure 6 contains single-banded patterns which are of two different types. The single band located at the more anodal position constitutes the phenotype Pgd 1. A single band located at a more cathodal position constitutes the phenotype Pgd 2. Those phenotypes are indicative of the presence

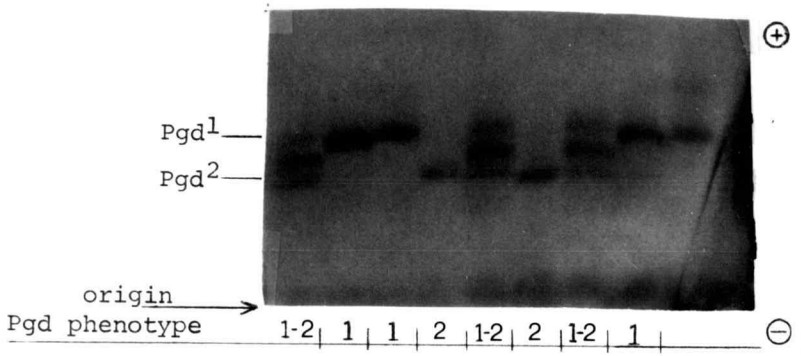


Figure 6. Electropherogram demonstrating 6-phosphogluconate dehydrogenase phenotypes. Zymogram was obtained by coupling enzyme activity to reduction of tetrazolium salt.

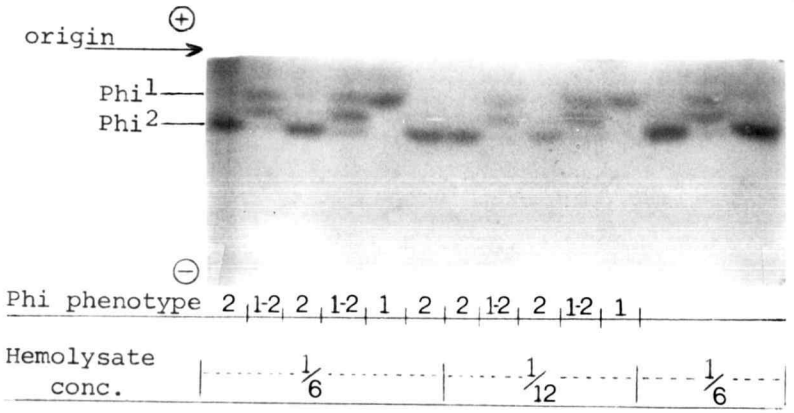


Figure 7. Electropherogram demonstrating phosphohexose isomerase phenotypes. Zymogram was obtained by coupling oxidation of enzyme reaction product to reduction of tetrazolium salt.

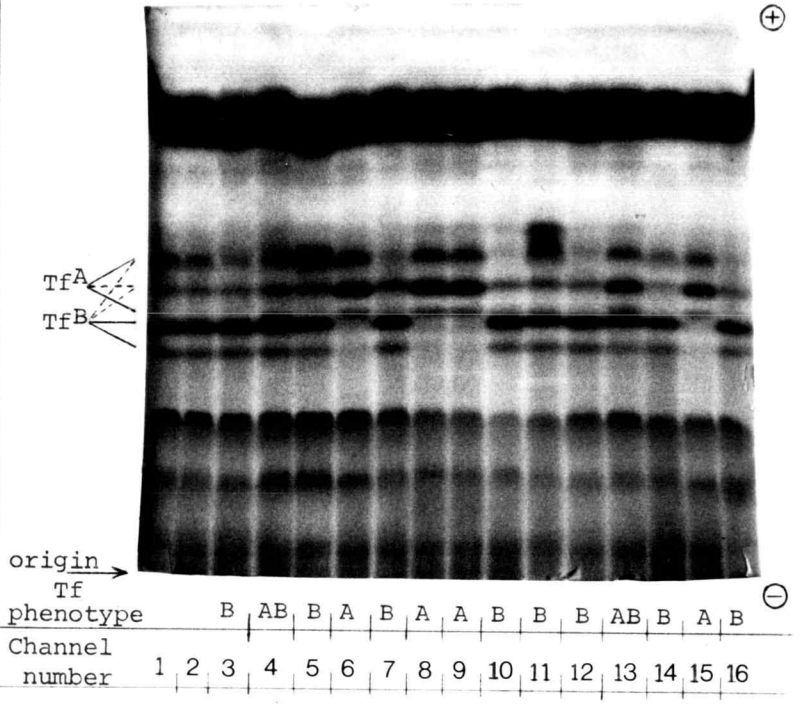


Figure 8. Electropherogram demonstrating inconstancy of Tf B phenotype. Specimens in channels 10 and 16 were obtained from two gilts at ages of ten and six months. Specimens in channels 11 and 7 were obtained from the same pigs at ages of 18 and 13 months, and contain a more anodal Tf fraction not seen in the earlier specimens.

of the genotypes Pgd^1/Pgd^1 and Pgd^2/Pgd^2, respectively. The heterozygous genotype, Pgd^1/Pgd^2, is indicated by a phenotype consisting of three bands (Pgd"1-2"), two of which are found at the same positions as the bands comprising the homozygous phenotypes. When the method for zymogram development described for this component under MATERIALS AND METHODS was modified simply by omission of trisodium 6-phosphogluconate from the developer mixture, the result was total absence of the bands shown in Figure 6.

Phosphohexose isomerase

Results of the zymogram development method used for detecting Phi activity are shown in Figure 7. That enzyme behaved as cationic fractions in the analytical system described in MATERIALS AND METHODS. The phenotype consisting of the single band that is located nearest the cathode (viz., the phenotype Phi 2) represents the homozygous genotype Phi^2/Phi^2. A single band located at a lesser distance from the insertion line (denoted as Phi 1) is due to another homozygous genotype, Phi^1/Phi^1. A heterozygous genotype, Phi^1/Phi^2, is evidenced by a phenotype (Phi 1-2) consisting of components with mobilities identical to those denoted as "Phi^1" and "Phi^2", and also a component with intermediate mobility. Figure 7 does not make apparent the presence of the more cathodal component in some of the specimens in which the intermediate component is present. Consequently, some samples denoted as Phi 1-2 appear to consist of two components instead of three, but each such phenotype does contain the distinctive intermediate component. When the zymogram developer mixture described under MATERIALS AND METHODS lacked disodium phosphofructose, the bands shown in Figure 7 did not appear.

Tests of Phenotype Continuity

Additional blood samples were taken from ten of the subjects at dates varying from 6 to 20 months after the initial specimen was obtained. Those subjects were of ages ranging from 6 to 32 months

at the time of the first sample-drawing. Those specimens were used in seeking evidence as to whether or not the phenotypes observed might be dependent on season of the year or age of the animal.

The phenotypes found were the same at each age in each of the pigs included in this sub-group, except that some variation was found with the Tf patterns. The Tf B phenotype seems to be a somewhat inconsistent pattern, in that it may consist of three bands, or a fourth band may occur in some specimens. That fourth band had practically the same mobility as the most anodal Tf band found in the Tf A and Tf AB phenotypes. This variation is demonstrated in specimens shown in Figure 8. Specimens in channels 10 and 16 were obtained from two gilts which were, respectively, ten and six months of age at the time of the first sampling. Subsequent samples, drawn from the two pigs at 18 months and 13 months of age, respectively, are shown in channels 11 and 7. Those two later samples revealed the presence of a more anodal component. A total of 36 subjects were found to possess such a four-banded phenotype. The anodal band was quantitatively less intense in most of the specimens than that shown in channel 11 on Figure 8. The evidence of this inconsistency among specimens from single individuals was the basis for denoting both a three-banded and a four-banded pattern as the phenotype Tf B. Those two phenotypes are distinguishable from the Tf AB phenotype on the basis of the most cathodal member of the set of bands labelled as "Tf^{A}" in Figure 8. (That segment actually appears contiguous with one of the segments labelled as "Tf^{B}" when seen in a Tf AB phenotype, e.g., channel 4.) That most cathodal "Tf^{A}" segment is absent from the Tf B phenotype.

Initial Phenotyping Studies

Capability for demonstrating individual differences in serotypes and on electropherogram patterns was utilized initially in an attempt to discern differences among the B, Y, and C lines within the subject population. Those analyses comprised the portion of the study referred to in the INTRODUCTION as the "initial phase."

Electrophoretic and serological analyses were accomplished on blood samples from all purebred and C brood sows and service boars present in the population during the summer and autumn of 1973. All purebred and C animals selected as replacement stock during the subsequent period were descendants of the phenotyped subjects. Besides brood sows and service boars, several sibships consisting of pigs which never joined the breeding stock were also phenotyped. Those phenotypings were accomplished because one or both parents of the sibships had been marketed prior to the time when sample-drawing was begun.

Results of the phenotyping study of the B, Y, and C stock referred to here are shown in Table 8. The data have not been reduced to allele frequencies; the intention of describing genetic contrasts among the three lines is served adequately by tabulation of the numbers of individual phenotypes found. It is possible to infer directly from certain phenotypes that the individual possessed a heterozygous genotype. This was not possible for the G, H, K, and L genes, because of lack of the antisera which detect certain known serotypes controlled by those genes. Thus, the phenotypes controlled by the G, H, K, and L genes, for purposes of this study, consisted of the reactivity or non-reactivity of erythrocyte suspensions toward specific reagents. Reactivity of an individual's erythrocytes toward the specific anti-G_b serum constitutes the G_b phenotype. Non-reactivity of a specimen towards the anti-G_b serum is denoted as the G^- phenotype. Similarly, use of the specific anti-H_a, the anti-K_a, and the anti-L_b sera provided the means for distinguishing between the H_a and H^- phenotypes, between the K_a and K^- phenotypes, and between the L_b and L^- phenotypes. Also, direct inference of heterozygous genotypes was not possible from phenotypes controlled by the A-o and S genes, because of dominance-recessiveness relationships. Two specific antisera were used to determine the phenotypes controlled by the A-o locus and the epistatically acting S locus (described in the Background Literature). The A and o phenotypes were determined by those specific antisera; non-reactivity with both of those antisera constitutes the "-" phenotype, which is due to homozygosity of a

Table 8. Numbers of phenotypes found in Berkshire, Yorkshire, and Composite pigs by initial survey.

Gene	Phenotype	B	"Native" Y	Y's of exopatric origins†	Y progeny of 1160-1††	C
A-o	A	4	0	2	6	0
	o	0	12	1	6	36
	"_"	7	0	0	0	0
G	G_b	11	1	3	5	34
	G^-	0	11	0	7	2
H	H_a	0	0	3	2	10
	H^-	11	12	0	10	26
K	K_a	11	0	0	9	0
	K^-	0	12	3	3	36
I	I_a	11	12	0	6	36
	I_{ab}	0	0	2	6	0
	I_b	0	0	1	0	0
L	L_b	10	4	2	8	35
	L^-	1	8	1	4	1
N	N_a	1	0	0	0	0
	N_{ab}	7	0	1	8	6
	N_b	3	12	2	4	30
Hx	Hx 1	8	12	3	12	36
	Hx 1-2	3	0	0	0	0
Tf	Tf A	3	0	1	4	3
	Tf AB	8	9	2	5	12
	Tf B	0	3	0	3	21
Sa	Sa B	11	5	1	6	36
	Sa BC	0	7	1	6	0
	Sa C	0	0	1	0	0
Am	Am 1	0	0	0	3	0
	Am 1-2	0	8	3	3	0
	Am 2	2	4	0	6	2
	Am 1-3	0	0	0	0	0
	Am 2-3	7	0	0	0	11
	Am 3	2	0	0	0	23
Pgd	Pgd 1	7	2	1	0	35
	Pgd 1-2	4	7	1	11	1
	Pgd 2	0	3	1	1	0
Phi	Phi 1	5	6	0	0	20
	Phi 1-2	2	5	0	1	16
	Phi 2	4	1	3	11	0

† Animals described in this column were either imported or produced by artificial insemination, as explained in the text.

†† Animals described in this column were second-generation descendants of a semen donor which was of exopatric origin (sire of 1160-1), as described in the text.

recessive allele at the S locus. Available antisera were sufficient so that heterozygous genotypes at the I and N loci were apparent from certain observed phenotypes.

Certain electropherogram patterns also allow direct identification of individuals possessing heterozygous genotypes. The phenotypes which permit such direct inference have been mentioned in preceding sub-sections.

Sub-categorization of Yorkshire Line

It is convenient to sub-categorize the Y line and to note the distribution of alleles among those sub-categories. The basis for this sub-categorization was the fact of the importation of breeding stock in 1971 and 1972 (as indicated in Figure 1). Artificial insemination of one indigenous sow in 1971 produced one gilt and one boar (identified, respectively, as 1160-2 and 1160-1), both of which were retained as replacement stock. Subsequently, a Yorkshire boar without any known relationship to the Oregon State herd was imported in 1972. That individual is identified by the number 000-900. Both of the boars mentioned here have been used extensively for servicing of sows of the Y line.

There were genetic contrasts between that group which was of foreign extractions and descendants from closed line matings within the Y line (Table 8). Phenotype A was not found among the subjects which were Oregon State Y's. The "natives" also lacked the alleles H^a, I^b, and N^a, which were found among the three animals of exopatric origins. Furthermore, at least some "native" Y's possessed the Phi^1 allele, but that allele was not present in the three pigs which were of exopatric origin.

Several sibships sired by boar 1160-1 were included in the initial phase of the study, because dams of those sibships had been removed from the herd prior to initiation of the study. Because of the demonstrated genetic contrast between boar 1160-1 and the indigenous Y's, it is appropriate to consider the offspring of that boar as another category, separate from the "native" Y's. Results

of phenotyping that group of offspring are shown under the heading "Y progeny of 1160-1" in Table 8. This categorization proved useful. First, it provided evidence that boar 1160-1 possessed similarities to the "native" Y's which are not apparent from serological testing of the boar itself. Specifically, the animal apparently possessed the a^o allele at the A-o locus and the G^- allele at the G locus. Perhaps of more importance however, is the evidence that the K^a allele was present in Y sows which had been removed from the herd shortly before this study began.

Contrasts Among the Composite and Purebred Lines

The matter of essential concern at this stage of the study was whether or not the C's could be found to differ from the purebred lines, by use of the criterion of differences at the loci listed in Table 8. That table provides evidence of contrasts among the B, Y, and C lines.

The C's available for sampling were found to differ from the B's which were available in terms of phenotypes determined by the A-o, S, H, K, Hx, and Pgd genes. B's were shown to possess the A, S^s, K^a, and Hx^2 alleles, and these alleles were not observed to be present in the C's. But, the C's were found to possess the H^a allele, which was not present in any of the B's studied. A noteworthy contrast in allele frequencies was found at the Pgd gene, since the Pgd^2 allele found in B's was found in only one of the C's. This might be indicative of a considerable contrast in gene frequencies which also existed at the Pgd locus during previous generations.

Differences between the C's and Y's seem most readily apparent at the G, K, Sa, Pgd, and the Am loci. The G_b phenotype was present in nearly all C's, but was found in only one of the "native" Y's. The K^a allele was not found in the C's, but that allele apparently was possessed by some "native" Y's no longer present at the time of this study. That conclusion is indicated by the results of analyses on progeny which had been produced by an earlier mating between native Y sows (not available for sampling) and a boar known to lack

the K^a allele. The results comprise part of the data in Table 8, under "Y progeny of 1160-1." Y's also possessed the Sa^C, Pgd^2, and Am^1 alleles, whereas those alleles were not found in any of the C subjects, except one individual which possessed the Pgd^2 allele. C's were found to possess the Am^3 allele, which was not observed in any of the Y's studied.

The results suggest differences between the C and Y lines at other genes also, but that evidence is more equivocal. The I^b allele was found only in Y's, but it is uncertain whether its presence in the two progeny from artificial insemination was a result of inheritance from the indigenous dam, or instead from the imported semen. Perhaps the presence of the H^a and N^a alleles in the same offspring of the artificial insemination was due to inheritance from the indigenous dam. Therefore, there is uncertainty about whether or not the native Y's differed from the C's in composition of the I, H, and N loci over the period prior to the additions of stock of exopatric origin, which is described in Table 8 under the heading "Y's of exopatric origin."

The study which yielded the results shown as Table 8 was accomplished because of interest about whether or not the B, Y, and C lines differed for specifically identifiable genes. It is apparent that the findings have rather limited implications, and a major reason for this is the structure of the population of subjects available for study. The major portion of the B's, Y's, and C's which were parents of crossbred breeding stock were not available for sampling by the time of this study. It can be seen from Table 4 that 44 daughters of crossmatings between B's and C's were selected to be bred at least one time, and the same number of daughters of crossmatings between Y's and C's also farrowed at least one litter. However, among the B and C animals which were phenotyped, there were parents of only 15 of the BxC and CxB sows; among the Y and C animals which were phenotyped, there were parents of only 14 of the YxC and CxY sows. This observation makes it clear that, although those initial phenotyping studies indicated that contents of certain loci were dissimilar among the parental lines, there is no substantial assurance

that this was true throughout the period of crossmating of C's with B's and Y's.

It was intended that the results of the initial phase should be used only for predicting the usefulness of further studies involving a more diverse population of subjects. The observations regarding that initial phase encouraged the postulate that associations existed between some phenotypes being studied and the reproductive performance. Averages shown in Table 3 indicate that the average reproductive performance of B's was distinctively different from that of Y's and C's. It can be seen from Table 4 that many of the daughters from crossmatings of B's and C's displayed reproductive performance which was different from that which would be expected if this trait were determined mostly by additively acting genes. That this was also true of daughters from crossmatings of Y's and C's is also indicated by data in Table 4. Results in Table 8 support the idea that crossing of C's with B's or Y's produced heterozygous genotypes which either were not formed from intra-line matings or else occurred at distinctly lower frequencies compared to occurrence due to crossmatings. Crossmating of at least some B's and C's would have permitted formation of an H genotype (viz., H^a/H^-) which apparently did not occur among the B's. Furthermore, it seemed plausible that a heterozygous K genotype, resulting from crossing B's with C's, was one which did not occur within the B line. The basis for that idea was the finding of the K_a phenotype in each of the B's, which suggested that a homozygous genotype prevailed within that line.

The data in Table 8 permitted the expectation that crosses between C's and Y's could result in formation of a genotype which was not apparent in the subjects described in that table. An Am^1 allele from the Y line and an Am^3 allele from the C line would have produced the heterozygous genotype Am^1/Am^3. Contrasts between the C's and Y's at the Sa and Pgd genes also seemed of significance. More than one-half of the "native" Y subjects possessed the Sa^C allele and/or the Pgd^2 allele, as shown in Table 8. Therefore, crossing of C's and Y's would be expected to result in formation of genotypes at the Sa and Pgd loci

which either did not occur or were of insignificant frequency in offspring from intra-line matings of C's.

Expanded Subject Population

The results above provided the motivation for proceeding to a study involving six categories of crossbred descendants of the parental lines, as well as additional members of the parental lines. No designed attempt was made to use the subjects of the initial phenotypings to produce significant numbers of crossbred daughters for the subsequent phase of the study. Instead, the studies on crossbred populations utilized offspring from matings which were made without knowledge of the results of the initial phenotyping studies. In fact, a majority of the crossbred subjects were offspring from matings in which one or both parents were not subjects of the phenotyping studies.

The composition of the total population used, including the subjects of the initial phenotyping studies, along with the various crossbred categories used in the subsequent phase, are described in Table 1. It may be seen from that table that a sizeable number of subjects were shoats not retained for use as breeding stock. Reasons for including individuals of such a status in the study are presented below.

Family Studies

It is apparent from remarks presented in a previous section that many of the crossbred sows studied were offspring of boars and sows which were also subjects of the blood component studies. Results of analyses of those crossbred sows and the several sibships of shoats mentioned in the preceding paragraph constituted useful data about the modes of inheritance of the components being studied. Furthermore, results on at least some families provided evidence about the presence of recessive alleles in sub-populations in which the alleles were not apparent from the initial phase of the study.

Evidence from earlier studies, about the modes of inheritance, was cited above (see Background Literature). However, those studies mostly were carried out with only a rather restricted variety of breed types. It is appropriate to utilize the data from the present study in evaluating whether or not the modes of inheritance proposed earlier do in fact apply to the subject population. Furthermore, lack of specificity of the serotyping reagents or inadequacy of electrophoretic separation systems for distinguishing among the different phenotypes might be made evident by the family studies.

However, matings involving certain combinations of parental phenotypes are absent from the data presented below. The study involved 14 polymorphic genes, and the frequencies of some phenotypes were rather small. In order to include litters produced from all possible mating combinations, it would have been necessary either to use a much larger group of subjects than was used, or to choose subjects primarily on the basis of the particular phenotypes of the parents.

The analysis of results of the blood component studies yielded indications of excesses of heterozygous genotypes at certain loci. However, confirmation of the occurrence of non-equilibrium ratios of genotypes would have required a considerable expansion of the subject population, and that was not attempted.

Distributions of Phenotypes Within Families

The major portion of the results from analyses on families is presented as Tables 9, 10, and 11. However, data on other families are contained in Tables 12 through 15. This separation is considered appropriate because of the particular implications of the latter set of data.

The portions of the family studies which pertain to inheritance of the determinants of erythrocyte antigens are shown in Table 9. The subjects for these studies were a group of 124 individuals, born to 33 different mating pairs, except that a smaller group was used for one particular component. That exception is explained below.

Table 9. Distribution of phenotypes among offspring of mating pairs in which phenotypes of erythrocyte antigens were known.

Gene	parental phenotypes[a]	Number of mating pairs	Number of offspring typed	Progeny phenotypes		
A				A	o	"-"
	o x o	4	16	0	14	2
	A x o	14	41	16	20	5
	o x A	3	18	7	10	1
	A x A	4	21	13	6	2
	A x "-"	2	6	2	1	3
	"-" x A	1	6	2	1	3
	o x "-"	4	13	9	4	0
	"-" x "-"	1	3	0	0	3
G				G_b	G^-	
	G_b x G_b	19	84	80	4	
	G_b x G^-	10	20	17	3	
	G^- x G_b	4	20	18	2	
H				H_a	H^-	
	H_a x H_a	2	19	12	7	
	H_a x H^-	17	56	39	17	
	H^- x H^-	14	49	0	49	
K				K_a	K^-	
	K_a x K_a	6	26	26	0	
	K_a x K^-	4	15	10	5	
	K^- x Ka	9	31	25	6	
	K^- x K^-	14	52	0	52	
L				L_b	L^-	
	L_b x L_b	22	81	78	3	
	L_b x L^-	10	39	24	15	
	L^- x L_b	1	4	3	1	
I[†]				I_a	I_{ab}	I_b
	I_a x I_a	14	46	46	0	0
	I_{ab} x I_a	7	27	10	17	0
	I_{ab} x I_{ab}	1	7	3	3	1
	I_b x I_a	6	11	0	11	0
N				N_a	N_{ab}	N_b
	N_{ab} x N_{ab}	7	29	2	18	9
	N_{ab} x N_b	7	23	0	14	9
	N_b x N_{ab}	7	25	0	11	14
	N_a x N_b	1	9	0	9	0
	N_b x N_b	11	38	0	0	38

[a] Order of phenotype designators is "sire x dam."

[†] Family studies on I phenotypes involved fewer individuals than did studies of other components, because of a limited quantity of one antiserum.

A-o phenotypes. Observations of some critical importance are the following: The A phenotype was not observed among progeny from matings in which both parents possessed the o phenotype; the A phenotype was found among progeny of matings in which neither parent possessed that phenotype, but this only occurred in the o x "-" mating class; the single mating of two parents possessing the "-" phenotype yielded only offspring which also possessed the "-" phenotype.

G phenotypes. A major portion of the matings presented in Table 9 involved either a B or C parent, or both. The G_b phenotype was found in nearly all B and C pigs analyzed (see Table 8). The G_b phenotype was present in all except nine of the progeny used for the family studies. The family studies did not include progeny of any matings in which both parents lacked the G^b allele.

H phenotypes. Mating pairs in which both members of the pair possessed the H_a phenotype yielded a group of offspring in which about one-third of the individuals lacked the H_a phenotype. The matings which involved only pigs which lacked the H_a phenotype produced only offspring which also lacked the H_a phenotype.

K phenotypes. Only the K_a phenotype was found among offspring of matings in which both parents possessed the K_a phenotype. The category involving parents which were both of the K^- phenotype was the only category which permitted critical testing of the hypothesized mode of inheritance of K phenotypes. The K_a phenotype was absent from offspring of the 14 matings between pigs lacking the K_a phenotype.

L phenotypes. The L^- phenotype was possessed by at least some of the offspring of each mating category shown in Table 9. Only three individuals with the L^- phenotype were observed among the subjects produced from matings between L_b pigs. Among the 43 subjects produced by matings in which only one parent was of the L_b phenotype, 16

possessed the L^- phenotype. The family studies did not include progeny of any matings in which both parents possessed the L^- phenotype.

I phenotypes. It is evident from Table 9 that fewer data were obtained for family studies pertaining to the I phenotypes. The quantity of available anti-I_b serum was somewhat limited. Consequently, certain sibships were not tested for presence of the I_b phenotype. Five fewer sibships were used for the family studies on the I phenotype than for studies of the other erythrocyte antigens. (All except one of those five sibships were born from matings between parents possessing the I_a phenotype.) The I_b phenotype was not found among any of the subjects born from matings in which one or both parents possessed the I_a phenotype. Only the I_{ab} phenotype was found among subjects born to the mating pairs consisting of one I_a and one I_b parent.

N phenotypes. Three different phenotypes were found among the progeny of mating pairs consisting of only the N_{ab} phenotype. However, nearly two-thirds of the 29 individuals possessed the N_{ab} phenotype. This proportion is in excess of that to be expected if the mode of inheritance were that of two co-dominant alleles at a single locus and the genotype frequencies were at equilibrium. The chi-square test of significance of the deviation from the expected distribution of genotypes shows that the deviation approached statistical significance ($P = 0.08$). The N_a phenotype was absent from among progeny of the mating pairs in which one or both members of the pair showed the N_b phenotype. Only the N_{ab} phenotype was observed among the progeny from the matings between the N_a and N_b phenotypes. The mating category consisting only of pigs possessing the N_a phenotype is absent from these data.

Results of family studies pertaining to inheritance of the polymorphic forms of erythrocyte enzymes and serum components are shown in Tables 10 and 11. The source of these data was the same group of subjects from which the results in the preceding table were obtained.

Hemopexin. Among the offspring described in Table 10, no phenotypes were found which were not possessed by one or both parents of the individual subject. The Hx 1-2 phenotype was not observed among the progeny of mates both possessing the Hx 1 phenotype. It is apparent from Table 10, that phenotypes other than Hx 1 were relatively uncommon among the breeding stock used for the matings yielding these data. There were no subjects produced from mating pairs in which both members possessed the Hx 1-2 phenotype.

Transferrin. Mating categories consisting of pairs in which both members had the Tf A phenotype, or in which both members had the Tf B phenotype produced 16 of the progeny used for these data. Those progeny were each of the same phenotype as the respective parental pairs. The Tf A phenotype was not found among offspring of mating pairs in which one member possessed the Tf B phenotype. Likewise, the Tf B phenotype was not found among subjects produced from matings involving one parent possessing the Tf A phenotype. The data do not contain any progeny known to have been produced by matings in which one parent possessed the Tf A, and the other, the Tf B phenotype.

Slow alpha-2 macroglobulin. Progeny from the mating category consisting of pigs possessing only the Sa B phenotype were found to possess only that phenotype. However, progeny from the mating category consisting of pigs only possessing the Sa BC phenotype were found to possess either the Sa BC or the Sa B phenotype. The Sa C phenotype was not observed among the progeny of any of these mating pairs. The only individual possessing that phenotype was mated to a boar possessing the Sa B phenotype. All offspring from that mating which were analyzed were found to possess the Sa BC phenotype.

Amylose-modifier. Data which describe the distribution of Am phenotypes among the subjects constitutes a comparatively large array. However, several essential observations can be stated concisely. The progeny produced from any particular mating category were found to

Table 10. Distribution of phenotypes among offspring of mating pairs in which phenotypes of serum proteins were known.

Gene	Parental phenotypes[a]	Number of mating pairs	Number of offspring typed	Progeny phenotypes					
Hx				Hx 1	Hx 1-2				
	Hx 1 x Hx 1	28	106	106	0				
	Hx 1 x Hx 1-2	1	5	4	1				
	Hx 1-2 x Hx 1	4	13	4	9				
Tf				Tf A	Tf AB	Tf B			
	Tf A x Tf A	1	3	3	0	0			
	Tf A x Tf AB	1	6	2	4	0			
	Tf AB x Tf A	8	34	17	17	0			
	Tf AB x Tf AB	17	47	14	23	10			
	Tf AB x Tf A	1	12	0	8	4			
	Tf B x Tf AB	3	9	0	5	4			
	Tf B x Tf B	2	13	0	0	13			
Sa				Sa B	Sa BC	Sa C			
	Sa B x Sa B	20	80	80	0	0			
	Sa B x Sa BC	4	9	2	7	0			
	Sa BC x Sa B	3	19	11	8	0			
	Sa BC x Sa BC	5	9	3	6	0			
	Sa B x Sa C	1	7	0	7	0			
Am				Am 1		Am 2		Am 2-3	
					Am 1-2		Am 1-3		Am 3
	Am 1 x Am 1-2	1	4	3	1	0	0	0	0
	Am 1-2 x Am 1	1	12	4	8	0	0	0	0
	Am 1-2 x Am 1-2	8	22	6	8	8	0	0	0
	Am 1-2 x Am 2	4	17	0	9	8	0	0	0
	Am 1-2 x Am 2-3	2	10	0	2	3	1	4	0
	Am 2-3 x Am 1-2	1	1	0	1	0	0	0	0
	Am 2 x Am 2-3	1	6	0	0	2	0	4	0
	Am 2-3 x Am 2	3	10	0	0	4	0	6	0
	Am 2-3 x Am 2-3	7	27	0	0	8	0	15	4
	Am 2-3 x Am 3	1	5	0	0	0	0	5	0
	Am 3 x Am 2-3	3	8	0	0	0	0	3	5
	Am 2 x Am 1-3	1	2	0	0	0	0	2	0

[a] Order of phenotype designators is "sire x dam."

belong to not more than two phenotypic categories, except for the following mating categories: Am 1-2 x Am 1-2, Am 1-2 x Am 2-3, and Am 2-3 x Am 2-3. The Am 1 phenotype was not found among the progeny from mating pairs in which one member possessed either the Am 2, the Am 3, or the Am 2-3 phenotype. The Am 2 phenotype was not found among the progeny from mating pairs in which one member possessed either the Am 1, the Am 3, or the Am 1-3 phenotype. The Am 3 phenotype was not found among the progeny from mating pairs in which one member possessed either the Am 1, the Am 2, or the Am 1-2 phenotype. At least some of the progeny from any particular mating category showed a phenotype identical to one of the parents, except for those produced by the mating pair consisting of the Am 2 and Am 1-3 phenotypes. It is necessary to point out that the data lack any mating categories in which both members of the pair are of the phenotype Am 1, or both of the phenotype Am 2, or both of the phenotype Am 3.

Pgd. The mating categories in which at least one parent was of the Pgd 1 phenotype did not produce subjects which possessed the Pgd 2 phenotype. Likewise, mating categories in which a parent was of the Pgd 2 phenotype did not produce subjects which possessed the Pgd 1 phenotype. Mating pairs consisting only of pigs of the Pgd 1 phenotype produced 20 subjects and all of those possessed the parental phenotype. Mating pairs in which one member was of the Pgd 1 phenotype and the other member of the Pgd 2 phenotype produced 29 of the subjects, all of which possessed the Pgd 1-2 phenotype. It should be noted that the mating category involving only individuals of the Pgd 2 phenotype is absent from these data.

Phi. Each of the possible combinations of Phi phenotypes is represented by the mating categories shown in Table 11. Subjects produced from mating pairs consisting only of the Phi 2 phenotype each possessed the parental phenotype. Subjects produced from the pairs consisting only of mates possessing the Phi 1 phenotype also possessed only the parental phenotype, but the number of those subjects was quite small. A group of subjects in which all three of the known

Table 11. Distribution of phenotypes among offspring of mating pairs in which phenotypes of erythrocyte enzymes were known.

Gene	Parental phenotypes[a]	Number of mating pairs	Number of offspring typed	Progeny phenotypes		
Pgd				Pgd 1	Pgd 1-2	Pgd 2
	Pgd 1 x Pgd 1	7	20	20	0	0
	Pgd 1 x Pgd 1-2	8	23	13	10	0
	Pgd 1-2 x Pgd 1	7	23	13	10	0
	Pgd 1-2 x Pgd 1-2	2	11	2	5	4
	Pgd 2 x Pgd 1-2	4	18	0	6	12
	Pgd 2 x Pgd 1	3	11	0	11	0
	Pgd 1 x Pgd 2	2	18	0	18	0
Phi				Phi 1	Phi 1-2	Phi 2
	Phi 1 x Phi 1	1	2	2	0	0
	Phi 1 x Phi 1-2	5	17	9	8	0
	Phi 1-2 x Phi 1	4	15	5	9	1
	Phi 1-2 x Phi 1-2	1	9	2	4	3
	Phi 1-2 x Phi 2	2	5	0	2	3
	Phi 2 x Phi 1-2	7	25	0	11	14
	Phi 1 x Phi 2	4	14	1	13	0
	Phi 2 x Phi 1	5	20	0	19	1
	Phi 2 x Phi 2	4	17	0	0	17

[a] Order of phenotype designators is "sire x dam."

phenotypes were present was produced from the mating pair consisting only of the Phi 1-2 phenotype; however, this was also true of another mating category. The category designated as Phi 1-2 x Phi 1 produced 14 offspring which each possessed one of the two parental phenotypes, but one other offspring from this mating category possessed the Phi 2 phenotype. The mating categories designated as Phi 1 x Phi 2 and Phi 2 x Phi 1 produced, respectively, 13 and 19 offspring possessing the Phi 1-2 phenotype; but, in addition, both of these mating categories produced one offspring which had the same phenotype as the sire (viz., Phi 1 and Phi 2, respectively). Those three individuals which seem to be exceptional among the offspring of these three mating-type categories are described further below. None of those three individuals were used as breeding stock.

Pertinence of Family Studies Toward Genotype Assignment

The significance of results which follow can be better appreciated, if interpretations of some of the foregoing results are stated at this point. The results of the family studies described above are consistent with the concept that the A phenotype may be the result of either a homozygous or a heterozygous genotype, in which the A^A is present, whereas the o phenotype is due to a homozygous genotype containing the a^o allele (viz., a^o/a^o). The G_b phenotype may be the result of either a heterozygous or a homozygous genotype in which the G^b allele is present. Likewise, the H_a, K_a, and L_b phenotypes can result from respectively, the H^a, K^a, and L^b alleles, present in either homozygous or heterozygous genotypes. The results are further consistent with the concept that for the other components listed in Table 8 (with the apparent exception of the Phi), genotypes may be correctly inferred from phenotypes.

Exceptional Inheritance of Phi Phenotypes

It seems appropriate at this point to present some additional description of the three individuals described in the preceding

subsection as having unexpected Phi phenotypes. Further consideration of the identities of those individuals is helpful for assessing the usefulness of observations on the Phi variation in the population.

The three individuals in which unexpected Phi phenotypes were observed were all born within a six-month period (January through July of 1974). A conspicuous fact about the identities of the three subjects was that two were purebred members of the B line (identified as 1700-4 and 1831-4), and the third was a crossbred maternal half-sibling (identified as 1705-4) of one of the other two. Furthermore, the two purebreds possessed quite obvious co-ancestry, to be described more explicitly under DISCUSSION. Another pertinent fact is that 11 other subjects which were littermates of those three exceptional subjects possessed only the phenotypes which could be expected on the basis of the parental phenotypes.

It is proposed that these findings are explainable on the basis of the existence of a third allele at the Phi locus. That allele may be denoted by the symbol Phi^0 for the present. If enzyme molecules containing the translation product from the Phi^0 allele have no phosphohexose isomerase activity, then animals possessing the heterozygous genotypes Phi^0/Phi^1 or Phi^0/Phi^2 might display phenotypes which are indistinguishable from animals of the genotypes Phi^1/Phi^1 and Phi^2/Phi^2, respectively. This interpretation of the findings is detailed under DISCUSSION by presenting the hypothesized pattern of inheritance of the phenotypes.

Families Suggesting Mis-assignment of Paternity

It is appropriate at this point to describe another group of families in which phenotypes were found which were not to be predicted on the basis of the phenotypes of the parents.

The preceding paragraphs and Tables 9 through 11 described the major portion of that group of phenotyped subjects in which the parents as well as progeny were analyzed. However, four sibships besides the ones described above were also phenotyped subjects, as were their parents. Results found from those four sibships are

described here separately from the other subjects of family studies, because the implications seem quite different from implications of the results above. The phenctypes of individual mating pairs and the distribution of phenotypes found among the offspring which were phenotyped are described in Tables 12 through 15.

Phenotypes of the Y boar identified as 1160-1, a B sow, and a litter identified as offspring from the mating of those two pigs are shown in Table 12. The progeny were identified as litter 1571. Previously, evidence had been obtained showing that boar 1160-1 possessed a heterozygous H genotype. That boar was the recorded sire of 36 phenotyped subjects (other than those described in Table 12); 16 of those possessed the H^- phenotype. Considering the evident heterozygosity of the sire at the H and I loci, the occurrence of H^a and I^b alleles in all of the eight progeny described suggested that some type of highly non-random process(es) was (were) responsible for the distribution of genotypes as it was found. Besides the apparent non-random inheritance of the H^a and I^b alleles within litter 1571, it was found that one litter-member (1571-2) possessed the Pgd 1 phenotype. Occurrence of that phenotype in the litter is not to be expected if there is exact correlation between phenotypes and genotypes. That is, the finding is contrary to the expectation that all members of litter 1571 should have inherited the Pgd^2 allele from the sire-of-record, 1160-1. The individual possessing the exceptional phenotype was a male. However, this should not be considered as evidence of sex-linkage of the Pgd gene; one littermate of that animal was also a male (1571-3), and it possessed the Pgd 1-2 phenotype. The foregoing observations permit the suggestion that boar 1160-1 was not the sire of all members of litter 1571, and perhaps was not the sire of any members of that litter.

A litter of pure B pedigree identified as 1615 is described in Table 13. Each member of that litter was found to possess one or two phenotypes which would not be predicted on the basis of the phenotypes of the parents. The phenotypes are identifed in Table 13 by underlining. The N_a phenotype was found in one member of the litter, despite the finding that the sire had the phenotype N_b. The Hx 1-2

Table 12. Family of phenotyped subjects in which one unexpected phenotype was observed. Offspring of the dam described should not possess the Pgd 1 phenotype, if the sire possessed the genotype Pgd^2/Pgd^2.

Gene	A-o	G	H†	K	I	L	N	Hx	Tf	Sa	Am	Pgd	Phi	Sex	Recorded Parent ident.
								Litter #1571							
Sire phenotypes	A	G_b	H_a	K^-	I_{ab}	L_b	N_{ab}	Hx 1	Tf AB	Sa B	Am 1-2	Pgd 2	Phi 2		1160-1
Dam phenotypes	A	G_b	H^-	K_a	I_a	L_b	N_{ab}	Hx 1	Tf AB	Sa B	Am 2-3	Pgd 1-2	Phi 1		1054-8
Individual offspring															
piglet #2	A	G_b	H_a	K_a	I_{ab}	L_b	N_{ab}	Hx 1	Tf AB	Sa B	Am 1-2	Pgd 1	Phi 1-2	M	
3	A	G_b	H_a	K_a	I_{ab}	L_b	N_{ab}	Hx 1	Tf A	Sa B	Am 2-3	Pgd 1-2	Phi 1-2	M	
4	A	G_b	H_a	K^-	I_{ab}	L_b	N_b	Hx 1	Tf A	Sa B	Am 1-3	Pgd 1-2	Phi 1-2	F	
5	o	G_b	H_a	K_a	I_{ab}	L_b	N_b	Hx 1	Tf AB	Sa B	Am 1-3	Pgd 1-2	Phi 1-2	F	
6	A	G_b	H_a	K^-	I_{ab}	L_b	N_{ab}	Hx 1	Tf B	Sa B	Am 1-3	Pgd 1-2	Phi 1-2	F	
7	A	G_b	H_a	K_a	I_{ab}	L_b	N_b	Hx 1	Tf AB	Sa B	Am 1-3	Pgd 1-2	Phi 1-2	F	
8	o	G_b	H_a	K^-	I_{ab}	L_b	N_{ab}	Hx 1	Tf AB	Sa B	Am 1-3	Pgd 1-2	Phi 1-2	F	
9	A	G_b	H_a	K_a	I_{ab}	L_b	N_{ab}	Hx 1	Tf AB	Sa B	Am 2-3	Pgd 1-2	Phi 1-2	F	

†Sire was demonstrated to be of heterozygous genotype at the H locus. Animal was the recorded sire of 36 other phenotyped subjects; 16 of those possessed the H^- phenotype.

Table 13. Family of phenotyped subjects in which unexpected phenotypes were observed among six offspring. Progeny phenotypes which are underlined were not expected to occur among offspring of the mating pair described.

Gene	A-o	G	H	K	I	L	N	Hx	Tf	Sa	Am	Pgd	Phi	Sex	Recorded Parent ident.
								Litter #1615							
Sire phenotypes	A	G_b	H^-	K_a	I_a	L_b	N_b	Hx 1	Tf AB	Sa B	Am 2-3	Pgd 1-2	Phi 1		1054-2
Dam phenotypes	"-"	G_b	H^-	K_a	I_a	L_b	N_{ab}	Hx 1	Tf AB	Sa B	Am 2-3	Pgd 1	Phi 2		1125-5
Individual offspring															
piglet #1	"-"	G_b	H^-	K_a	I_a	L_b	N_{ab}	Hx 1	Tf AB	Sa B	Am 2-3	Pgd 1	Phi 2	M	
3	A	G_b	H^-	K_a	I_a	L_b	N_b	Hx 1-2	Tf AB	Sa B	Am 2-3	Pgd 1-2	Phi 1-2	M	
4	"-"	G_b	H^-	K_a	I_a	L_b	N_{ab}	Hx 1-2	Tf A	Sa B	Am 2-3	Pgd 1	Phi 2	M	
5	"-"	G_b	H^-	K_a	I_a	L_b	N_a	Hx 1	Tf AB	Sa B	Am 2-3	Pgd 1	Phi 2	F	
6	"-"	G_b	H^-	K_a	I_a	L_b	N_{ab}	Hx 1-2	Tf A	Sa B	Am 2-3	Pgd 1	Phi 2	F	
7	"-"	G_b	H^-	K_a	I_a	L_b	N_b	Hx 1	Tf A	Sa B	Am 2-3	Pgd 1	Phi 2	F	

phenotype was found in three members of the litter, and the Phi 2 phenotype was found in five members, but neither of the pigs recorded as parents possessed the Hx^2 allele, and the sire lacked the Phi^2 allele. The most likely explanation for these observations is that the paternity designation was incorrect, and the actual sire possessed the phenotypes N_{ab} and Hx 1-2. The Phi phenotype of the true sire could have been Phi 1, if the genotype was Phi^1/Phi^0. But, the alleged sire (1054-2) was the sire-of-record for 15 other phenotyped subjects besides those described in Table 13. Those subjects were farrowed by sows possessing either the Phi 1-2 or Phi 2 phenotypes. No evidence was found from the latter matings that Phi^1/Phi^0 might be the sire's genotype. Thus, it is likely that Phi phenotypes found in litter 1615 are evidence that the sire was not 1054-2.

A litter which was recorded as having been sired by a B possessing the Hx 1-2 and the N_{ab} phenotypes is described in Table 14. However, none of the nine members of that litter was found to possess phenotypes indicating the presence of either the Hx^2 or the N^a allele. Each of the nine offspring possessed one, two, or three phenotypes which would not be predicted on the basis of the phenotypes of the parents-of-record. Four of the offspring possessed the I_b phenotype, although the sire-of-record had the I_a phenotype. Four of the offspring possessed the H_a phenotype, although neither parent possessed the H_a phenotype. The Tf AB phenotype was present within the litter, although both parents-of-record possessed the Tf A phenotype. Also, two of the progeny possessed the Am 1 phenotype, but the sire-of-record lacked any genotype containing the Am^1 allele. The phenotypes which were observed could have occurred if the sire possessed the phenotypes H_a, I_{ab}, Tf AB, and Am 1-2. It is concluded that boar 1219-1 was not the actual sire of this litter, and it is likely that the true sire possessed the N_b and Hx 1 phenotypes, not the N_{ab} and Hx 1-2 types.

Observations from a family where only one parent of subject animals had been phenotyped are contained in Table 15. Inspection of such cases revealed a crossbred sibship possessing phenotypes of two components which would not be expected among progeny of the sire-of-record. The sow identified as 1274-6 possessed the Am 1 and Phi 2

Table 14. Family of phenotyped subjects in which unexpected phenotypes occurred in nine offspring. Progeny phenotypes which are underlined were not expected to occur among offspring of the mating pair described.

Gene	A-o	G	H	K	I	L	N	Hx	Tf	Sa	Am	Pgd	Phi	Sex	Recorded Parent ident.
								Litter #1837							
Sire phenotypes	"-"	G_b	H^-	K_a	I_a	L_b	N_{ab}	Hx 1-2	Tf A	Sa B	Am 2-3	Pgd 1	Phi 2		1219-1
Dam phenotypes	A	G_b	H^-	K_a	I_{ab}	L_b	N_b	Hx 1	Tf A	Sa BC	Am 1-2	Pgd 1	Phi 2		1546-9
Individual offspring															
piglet #1	A	G_b	H_a	K^-	I_b	L^-	N_b	Hx 1	Tf A	Sa B	Am 1-2	Pgd 1	Phi 2	M	
2	A	G_b	H^-	K_a	I_b	L_b	N_b	Hx 1	Tf AB	Sa BC	Am 1-2	Pgd 1	Phi 2	M	
3	A	G_b	H^-	K^-	I_{ab}	L_b	N_b	Hx 1	Tf A	Sa BC	Am 1	Pgd 1	Phi 2	M	
4	o	G^-	H^-	K_a	I_b	L_b	N_b	Hx 1	Tf AB	Sa BC	Am 1-2	Pgd 1	Phi 2	M	
5	o	G_b	H^-	K_a	I_b	L_b	N_b	Hx 1	Tf A	Sa B	Am 1-2	Pgd 1	Phi 2	M	
6	A	G_b	H_a	K_a	I_a	L_b	N_b	Hx 1	Tf AB	Sa BC	Am 1-2	Pgd 1	Phi 2	M	
7	A	G_b	H_a	K^-	I_{ab}	L_b	N_b	Hx 1	Tf AB	Sa BC	Am 2	Pgd 1	Phi 2	F	
8	o	G_b	H^-	K_a	I_{ab}	L_b	N_b	Hx 1	Tf AB	Sa BC	Am 2	Pgd 1	Phi 2	F	
9	A	G_b	H_a	K^-	I_{ab}	L_b	N_b	Hx 1	Tf AB	Sa BC	Am 1	Pgd 1	Phi 2	F	

Table 15. Sisters possessing phenotypes indicating absence of alleles possessed by sire-of-record. Am 1 phenotype would not be expected among offspring of the boar described. Progeny testing indicated that boar probably lacked the Phi^0 allele.

Gene	A-o	G	H	K	I	L	N	Hx	Tf	Sa	Am	Pgd	Phi	Sex	Recorded Parent ident.
Sire phenotypes	o	G^-	H^-	K^-	I_a	L_b	N_b	Hx 1	Tf AB	Sa B	Am 2	Pgd 1-2	Phi 1		730-1
Dam phenotypes	Unknown	-	-	-											
Individual offspring															
1274-6	o	G^-	H_a	K^-	I_a	L_b	N_b	Hx 1	Tf B	Sa B	Am 1	Pgd 1	Phi 2	F	
1274-9	o	G_b	H_a	K^-	I_a	L_b	N_b	Hx 1	Tf B	Sa B	Am 2-3	Pgd 1	Phi 2	F	

phenotypes. A full sibling identified as 1274-9 also was found to have the Phi 2 phenotype. It is suggested that the paternity of that sibship was incorrectly recorded, since the sire-of-record was found to possess phenotypes which indicate absence of the Am^1 and Phi^2 alleles.

Results of the family studies described here were considered as having important implications regarding the population being studied. The evidence of mis-assignments of paternity seemed to cast doubt upon the value of any data analysis which considers the service boar as a source of variance in litter sizes or in utero viability.

Recombination of Phi, Pgd, and H Alleles

Efforts were made at evaluating relationships of genes which were studied. However, those efforts were quite limited in scope. It seemed likely that only very little evidence about linkages could be obtained with data from this population of subjects, unless the evidence were sought by means other than simple examination of distributions of genotypes among offspring of appropriately selected parents. It was felt that application of methods other than the simple, direct method, would be outside the scope of this study. However, due to results of analyses of correlations involving performance data, certain linkage relationships came to be of more direct interest.

It was considered worthwhile to seek evidence that the Pgd, Phi, and H genes were present within a single linkage group in this population. Such was the linkage pattern indicated from the Duroc population studied by Andresen (1970, 1971). Distributions of Pgd, Phi, and H phenotypes were inspected among mating pairs included in the family studies data. That search did not identify mating pairs which were appropriate for direct testing of linkage between the Pgd and H genes. However, a few families were found which were useful for evidence about linkage of the Phi with the H, or with the Pgd locus. Among sibships used for family studies, there were 19 individuals sired by either one C boar or one Y boar possessing the heterozygous Phi genotype and the H_a phenotype. Those 19 individuals were farrowed by

five B sows which possessed homozygous Phi genotypes and the H^- phenotype. Presence of the H^- phenotype in some of those offspring was considered evidence that the sires possessed the H^a/H^- genotype. Among the offspring, 18 of the 19 individuals possessed combinations of Phi and H phenotypes which indicated that the Phi and H alleles were not assorting independently. Besides those individuals, another sibship had been produced from the mating of a boar possessing the homozygous Pgd and Phi genotypes with a sow possessing heterozygous genotypes at those loci. Five offspring were phenotyped, and all of those displayed combinations of phenotypes indicating the assortment of the Pgd and Phi alleles as a linked couple.

Phenotypic Distributions Within Total Subject Population

The distribution of the total population of phenotyped subjects was described in Table 1. The distribution of phenotypes within that population is presented next. It may be seen from Table 16 that certain alleles were absent from some breed categories but not from others. The observation that was of most significance to the design of a method of data analysis was that the phenotyping results demonstrated genetic variation at two different levels.

First, the absence of certain alleles from some breed categories, but not from others, is an indication of genetic contrasts among the breed categories. The A^A allele was found in B's, and in Y's sired by boars of exopatric origin. However, that allele was absent from the C's and the "native" Y's, and was also absent from nearly all progeny of crossmatings between C's and Y's. The H^a allele was not observed in any purebred B's, but was found in the other two parental lines and in most crossbred categories. None of the B's or C's were found to possess the I^b, Sa^C, or Am^1 alleles, and those alleles were also absent from subjects produced from crossmatings of B's and C's. The I^b, Sa^C, and Am^1 alleles were found in individuals described in the column labelled "other," but this observation is of doubtful significance. All individuals to which that column pertains were members of a litter described above (Table 14) for which the paternity

Table 16. Distribution of phenotypes within various breed categories comprising the total subject population.

Locus	Phenotype	B	Y (native)	Y (Outbred lineages)	C	BxC, CxB	BxF_1	YxC, CxY	YxF_1	BxY, YxB	CxF_1	other[a]	Total
A-o	A	14	0	26	0	18	6	1	5	16	0	6	
	o	6	12	28	40	9	4	21	9	6	6	3	
	"-"	23	0	5	0	1	3	3	1	1	2	0	
													275
G	G_b	43	1	45	37	28	13	23	12	23	8	8	
	G^-	0	11	14	3	0	0	2	3	0	0	1	
													275
H	H_a	0	0	38	10	12	1	8	2	15	0	4	
	H^-	43	12	21	30	16	12	17	13	8	8	5	
													275
K	K_a	43	0	11	0	20	8	2	8	16	4	5	
	K^-	0	12	48	40	8	5	23	7	7	4	4	
													275
I	I_a	26[b]	12	15[c]	39[b]	20[b]	13	25	12	7	8	1	
	I_{ab}	0	0	30	0	0	0	0	3	16	0	4	
	I_b	0	0	2	0	0	0	0	0	0	0	4	
													237[b,c]
L	L_b	42	4	41	39	26	11	21	12	21	8	8	
	L^-	1	8	18	1	2	2	4	3	2	0	1	
													275
N	N_a	3	0	0	0	0	0	1	0	1	0	0	
	N_{ab}	26	0	22	8	13	5	12	3	11	1	0	
	N_b	14	12	37	32	15	8	12	12	11	7	9	
													275
Hx	Hx 1	28	12	59	40	25	13	25	15	23	8	9	
	Hx 1-2	15	0	0	0	3	0	0	0	0	0	0	
													275
Tf	Tf A	15	0	16	3	6	3	4	5	6	1	3	
	Tf AB	25	9	31	12	19	7	8	9	13	4	6	
	Tf B	3	3	12	25	3	3	13	1	4	3	0	
													275
Sa	Sa B	43	5	28	40	28	12	20	11	21	6	2	
	Sa BC	0	7	30	0	0	1	5	3	2	2	7	
	Sa C	0	0	1	0	0	0	0	1	0	0	0	
													275
Am	Am 1	0	0	14	0	0	0	1	3	0	0	2	
	Am 1-2	0	8	28	0	0	0	7	2	3	0	5	
	Am 2	4	4	17	2	8	4	10	3	3	1	2	
	Am 1-3	0	0	0	0	0	0	2	0	6	1	0	
	Am 2-3	33	0	0	12	14	6	5	6	11	1	0	
	Am 3	6	0	0	26	6	3	0	1	0	5	0	
													275
Pgd	Pgd 1	23	2	8	37	23	8	11	8	9	7	9	
	Pgd 1-2	16	7	35	3	5	5	14	6	14	1	0	
	Pgd 2	4	3	16	0	0	0	0	1	0	0	0	
													275
Phi	Phi 1	13	6	0	22	9	2	5	3	3	2	0	
	Phi 1-2	20	5	18	18	12	6	15	5	16	4	0	
	Phi 2	10	1	41	0	7	5	5	7	4	2	9	
													275

[a] Data in this column pertain to the same litter described in Table 14.

[b] The I phenotypes were not determined in certain litters born to parents both known to possess the I_a phenotype.

[c] The I phenotypes were not determined in certain Y progeny born to parents whose I phenotypes were known.

should be considered uncertain. The A^A and K^a alleles were not observed in any of the C's analyzed, but those two alleles were present in nearly all of the other eight breed categories. The Pgd^2 allele was found in only one family of C's, although that alelle was fairly common in the B's and the Y's.

Secondly, the data in Table 16 demonstrate variation among individuals within the separate breed categories. Some of the breed categories were groups which seemed to display monomorphism at certain loci, but this was not true for most of the loci in any of the nine breed categories. There were some cases where two alleles were present at a locus, with the frequency of one allele being sufficiently low so that very little polymorphism was observed at that locus. However, it seems much more significant to note that in a large proportion of the cases, the heterozygote/homozygote ratio was greater than 0.5. Furthermore, it is of interest to note that, in a considerable number of cases and at several loci, the heterozygote/homozygote ratio exceeded 1.0. Examples of this were the proportions of individuals found to possess the Tf AB phenotype in the B and Y purebred groups and in the crossbreds denoted as BxC + CxB, BxF_1, YxF_1, and BxY + YxB.

It was speculated that the genetic effect of crossmating might be displayed by means of comparing sub-group averages of the proportion of heterozygous loci per individual. It was immediately obvious that that method would be of limited usefulness because of dominance-recessiveness relationships at one-third of the loci which were studied. That fact obviated use of information on those loci in heterozygosity calculations. Nevertheless, casual inspection of distributions of phenotypes within the population made it seem of interest to compare the average heterozygosities. The average was based on the individual values of the proportion of heterozygous loci among each of nine loci at which only co-dominant alleles were present. The ceruloplasmin locus was included among the nine loci, although no variation of the ceruloplasmin genotype was evident in the population, and the ceruloplasmin phenotype is not referred to in any of the other tabulated data. Calculations were accomplished using the data on all subjects

in the separate breed categories, including shoats. Results of the data analyses are shown in Table 17.

It may be seen that the only crossbred category for which average heterozygosity exceeds those of both purebred categories, is the (BxY + YxB) category (0.444). The average heterozygosities for each of the other crossbred categories are all values which are intermediate between those of the C and purebred lines. "Least significant differences" (Steel and Torrie, 1960) were calculated for the purpose of evaluating statistical significance of the contrasts of crossbred categories relative to respective purebred categories. There were no evaluations of statistical significance of contrasts among the breed categories, other than the comparisons of crossbreds to respective parental categories.

Comparisons of average heterozygosities were accomplished due to interest in the possibility that the averages for crossbred categories could be found to be larger than those of either of the parental categories. Such a finding then could be viewed as supportive of the idea that the superior performance of, e.g., the YxC + CxY (see Tables 4 and 6) was achieved by reversal of inbreeding depression. However, the comparisons referred to here provide evidence that the heterozygosities of the (YxC + CxY) and (BxC + CxB) were only greater than the C parental group, and not greater than those of the purebred parental groups. Interpretation of this observation should include consideration of the fact that certain contrasts among breed categories were evident at loci not considered in the calculation of the averages (e.g., A-o and H). If zygosities could have been ascertained for all loci at which there were evident differences among breed categories, the resulting values for at least some crossbred categories might have been considerably different from the values shown in Table 17. However, it is uncertain whether any of the average heterozygosities would have been higher than those found on the basis of nine loci. For example, if loci controlling additional erythrocyte antigens had been used, those would have included the A-o and K loci. There is considerable likelihood that practically all (YxC + CxY) subjects inherited the same

Table 17. Proportions of polymorphic loci within categories, and average individual heterozygosities. Individual heterozygosities were the proportions of heterozygous loci among nine of the loci analyzed. Excluded from calculations were the genotypes at the A-o, S, G, H, K, and L loci.

	B	Y (natives)	Y's of exopatric origin	Y offspring of exopatric males	C	BxC, CxB	BxF_1	YxC, CxY	YxF_1	BxY YxB	CxF_1
No. of subjects	43[a]	12	3	56[b]	40	28[a]	13	25	15	23	8
Proportion of analyzed loci found polymorphic	10/15	8/15	11/15	13/15	9/15	11/15	11/15	12/15	13/15	13/15	8/15
Polymorphic loci among the loci where only co-dominant alleles were found	6/9	5/9	6/9	7/9	5/9	6/9	6/9	6/9	7/9	7/9	6/9
Average heterozygosity per individual (±standard deviation)	0.349 .130	0.333 .125	0.370 .064	0.376 .124	0.147 .124	*/** 0.262 .139	**/* 0.256 .131	o/** 0.302 .152	**/o 0.274 .145	**/* 0.444 .154	N.T. 0.194 .078

[a] Values on 16 of the B subjects and 8 of the CxB subjects depended on an inference of homozygosity at the I locus, on the basis of finding the same homozygous genotype in all parents of those individuals.

[b] For one sibship consisting of 12 pigs, individual values were based on 8 loci, because of a limited supply of one antiserum.

*/**: crossbred average is significantly different from both highest ($P<.05$) and lowest ($P<.01$) parental group.

**/o: crossbred average is significantly different ($P<.01$) from highest (but not lowest) parental group.

o/**: crossbred average is significantly different ($P<.01$) from lowest (but not highest) parental group.

N.T.: indicates contrasts among CxF_1 and parental groups were not tested for statistical significance.

allele from both parents, and therefore those loci probably would not have been shown to be heterozygous.

Further comment is appropriate regarding the observation that the C line average contrasts rather strikingly with the values of nearly all other breed categories, including the purebreds. This seems indicative that maintenance of the C's as a strictly closed line was effective for achieving a relatively higher proportion of homozygous loci, compared to the purebred lines; in the purebred lines, the occasional introduction of males from unrelated populations may have been the cause of the relatively higher heterozygosity. The observation is that which would be expected if inbreeding of the C line had been causing more rapid increase in proportions of homozygotes at various loci, compared to the rate of increase of homozygotes in the purebred categories. Thus, a plausible basis for certain contrasts of performances among breed categories might be that, along with achievement of relatively high frequencies of homozygous genotypes at loci being studied, alleles at other loci, having unfavorable effects toward performance, were being eliminated from the C line. If so, homozygous combinations containing such unfavorable alleles would be absent from or uncommon in offspring of crossmatings of C's with B's and/or Y's, although such homozygous configurations might be common in purebred females. Such purging within the C line could have provided a significantly positive effect toward performance of crossbreds. The effect could have been particularly notable if unfavorable alleles eliminated from the C line were recessive alleles which remained present in at least moderate frequencies within one or both purebred lines.

There is of course no substantial evidence about alleles present at loci in the same linkage groups with the loci being analyzed. It seemed reasonable to attempt to obtain some clearer understanding of those associations, by seeking to demonstrate correlations between performance and the phenotypes being studied.

The results shown in Tables 16 and 17 demonstrate that much genetic variability existed not only among sub-populations distinguishable as separate breed categories, but within those sub-populations

as well. Thus, there was justification for attempts to determine whether or not some portion of that genetic variation correlates with the variation in the reproductive performance.

Phenotyping Results and Performance Data

Least squares analyses were accomplished on the body of data which consisted of the phenotyping results, measures of reproductive performance of females, and selected items of information about variation in the management practices used with the herd. It was intended that this analysis would effectively identify management factors which had influenced reproductive performance, and identify genes which may have been linked to loci which were determinants of variance in that performance.

Formulation of Model

It has been pointed out (e.g., Snedecor and Cochran, 1967) that the purpose of such analyses is not well-served if independent variables included are excessive in number. It seemed apparent that certain genes which had been involved in the phenotyping studies could reasonably be omitted from the analysis. It is fairly apparent from the distribution shown in Table 16 that the G_b phenotype was present in nearly all subjects. Most individuals which lacked that phenotype were purebred Y's. This pattern of distribution of the G phenotypes made it seem unlikely that these phenotypes would be found to be correlates of contrasts in reproductive performance. This consideration led to the decision to omit the G phenotypes from the statistical analysis. The pattern of distribution of the I^b allele was the basis for deciding to omit the I phenotypes from the model. It seemed that the I^b allele was not sufficiently distributed so as to require its inclusion in a least squares analysis to evaluate significance of allelism at that locus. The I^b allele was useful only as an indicator of the exopatric ancestry of certain Y's (and a very few crossbred daughters of Y's). The Hx 1-2 phenotype was found in three sows,

and other subjects possessing that phenotype were either shoats or service boars. Therefore, the Hx variants were not considered in this model. Certain other genes which were included in the least squares model were considered by means of somewhat simplified categorizations. The Sa C phenotype was observed in only two pigs (Table 16). It would be of no practical value to consider those two individuals as a separate category in the analysis; thus, they were combined with the other phenotypic category which possessed a Sa^C allele, viz., Sa BC. Somewhat similar considerations were applied for the H and K genes. The zygosity of individuals possessing the H_a and K_a phenotypes was not treated as a variable in this analysis. It was possible to infer that a large proportion of the sows having such phenotypes were heterozygous, by knowledge of phenotypes of either a parent or progeny, but it was decided not to utilize that knowledge for this analysis. The group of individuals for which the H and/or K genotype was uncertain could be expected to consist of both heterozygous and homozygous pigs. It was considered reasonable to combine such individuals with those known to possess heterozygous genotypes.

The composition of the group of females which provided performance data for this analysis is shown in Table 1. That table provides some indication of a management factor which seemed to require particular consideration. It is apparent from the ratios of numbers of litters farrowed to number of dams, that the numbers of reproductive data obtained are not the same for each of the dams. A number of dams were retained long enough to farrow only one litter, whereas others farrowed as many as seven or eight litters.

Other management procedures used with this herd have not been entirely uniform. Consideration was given to certain of these as possibly significant causes of variance in individual performance.

Mating records and performance data were inspected to the extent that it became possible to decide that it was unlikely that the breed of service boar could be shown to be an important determinant of variance in the size of litters born to the subjects of this particular study.

An attempt was made at demonstrating a cause-effect relationship between the performance at various parities and the age at which the first litter was farrowed. Scatter diagrams were constructed to depict the relationship between the age of the dam at the date of its first parity and the number of fetuses per litter. A separate set of diagrams was constructed for each of three groups. The compositions of the three groups are shown in Table 18. The separate analyses of these sub-populations involved construction of a diagram for each parity through the fourth. Correlation coefficients were calculated for the body of data used for each scatter diagram. The coefficients serve to express the correlations between age of the dam at first parity and number of fetuses farrowed at particular parities. Although one of the coefficients shown in Table 18 is as large as 0.38, notable deviations from zero are quite inconsistent. Coefficients of 0.38 and 0.28 were found for two different parities in the YxC + CxY + YxF_1 subgroup, but those two values are not for consecutive parities. A value of -.34 was found for the fourth parity in the Y line, but the coefficients were distinctly less in absolute value at the other three parities. An analogous procedure was followed to evaluate the relationship between the age of the dam at its first parity and the number of stillborn per litter. However, scatter diagrams of the data indicated that the frequencies of stillborn fetuses were not sufficient so as to evidence a correlation with age of the dam at first parity. The observations described here served as indication that it would not be necessary to consider the age at first parity as a source of variance in performances at subsequent parities.

Table 18. Coefficients of correlation between age of dam at first parity and number of fetuses farrowed at various parities. Sub-populations consist only of phenotyped sows.

Sub-population	Parity 1	Parity 2	Parity 3	Parity 4
BxC, CxB, BxF_1	-.09	-.003	.13	-.001
YxC, CxY, YxF_1	.11	.38	-.03	.28
Y line	-.07	-.07	-.07	-.34

Total Fetuses per Litter

Application of the method of least squares was focused on the problem of identifying both genetic and non-genetic factors which were significant correlates of total number of fetuses per litter. The method for distinguishing real associations from spurious associations caused difficulty with respect to assigning levels of statistical significance to those associations. Nevertheless, attempts have been made to describe the specific modes of expression of prolificacy determinants which seemed to be genuinely correlated to the blood components which were studied.

I. Models including only non-genetic variables.

Two non-genetic factors were considered. Table 19 consists of the results from use of the model consisting only of factors representing a.) the semester during which conception occurred, and b.) the age of the dam at the date of farrowing.

Considerable variation was found among breed categories. Some of the variation is evident from the least squares (L.S.) means for total fetuses per litter (Table 19). The mean value was found to be lowest in the B line (7.33). L.S. means differed only slightly (between 8.40 and 8.85) among the C, (BxC + CxB), and BxF_1 categories. The L.S. means found for the Y line and the YxF_1 category were equal (9.70 and 9.68). The L.S. mean for the (YxC + CxY) category was 10.78, a value 1.08 fetuses greater than the mean found for the next highest category.

The effect of the particular semester of the year differed among the breed categories, not only in magnitude, but also in the direction of the effect. There was variation in the effect of age of dam, as shown by the regression coefficients (the dimension of the constants describing that effect is "fetuses per litter per week of dam's age"). However, effects of non-genetic factors by themselves were of little pertinence to the study. Consideration of effects of non-genetic

Table 19. Least squares means and constants for non-genetic variables found to be correlates of variance in total fetuses per litter.

Breed	mean[a]	semester[b]		Dam's age at farrowing[c]
		early	late	
B	7.33	-.41	.41	.014
Y	9.70	-.37	.37	.018
C	8.85	-.75	.75	.039
BxC, CxB	8.70	.14	-.14	.035
BxF_1	8.40	.76	-.76	.030
YxC, CxY	10.78	-.35	.35	.024
YxF_1	9.68	-.20	.20	.024

[a] Means are values adjusted to dam's age of 40.0 weeks.

[b] "Early" semester was 23 September through 24 March. "Late" semester was 25 March through 22 September.

[c] Dimension of constant for dam's age is number per litter per week.

variables was worthwhile, in order to avoid confounding those effects with the effects of genetic variables.

II. Models including non-genetic and/or genetic variables.

Attempts were made to estimate the effects of various genetic, as well as non-genetic variables. Tables 20a and 20b consist of results from analyses on the various breed categories. Any specific sub-section of Table 20a or 20b pertains to a specific breed category, and contains analyses of variance results from three or four models. Each of the models was of a different level of complexity.

- The results from the model including only non-genetic factors were described above.
- The most complex model (I) used on data from a specific breed category was not necessarily the same for each breed category. Certain genetic variables were not considered, even in the most complex model applied, but the genes which were omitted were not the same for each breed category. The decision to omit a specific locus from a model was based on whether or not there was a sufficient distribution of phenotypes controlled by that locus.
- One of the models having complexity which is intermediate between the aforementioned types is of particular concern. That which is denoted as the select (S) model differed from other models described in the same table by consisting only of variables for which the "F" ratios were greater than the critical "F" value for the 5% level of statistical significance. The "S-model" for each specific breed category was arrived at by a process of selectively eliminating individual variables from the more complex models. Elimination of a variable from the model was based on the size of the "F" ratio found for its contribution to the total variance. Models with successively reduced degrees of complexity were obtained by simply omitting the term for which the "F" ratio was found to be smaller than those for

Table 20a. Results of least squares analyses to identify sources of variance in total fetuses per litter, from dams in the B, Y, and C lines[a].

Breed[b]	Model[c]	Semester	Dam's age	A-o	H	K	N	Tf	Sa	Am	Pgd	Phi	R^2	Error Mean Square
B	I	.74	.09	1.99			1.80	.18		1.08	.11	1.80	.297	6.45
	S									4.20			.102	6.45
	NG	.94	2.44										.091	6.72
Y	I	4.77	6.16	1.51			2.68	3.26	.51	.40	1.33	2.72	.235	7.62
	S	5.05	7.38				7.96	4.61					.179	7.73
	NG	4.22	9.95										.082	8.46
C	I	5.07	9.38		.50		.64	1.79		.06		.56	.422	3.96
	S	5.40	11.70					3.21					.401	3.60
	NG	5.07	9.30										.279	4.08

[a] Results are expressed as the "F" ratios (mean square attributed to individual variable)/(mean square of deviation from least squares prediction). Blank spaces indicate that the specific variable was not included in the model.

[b] Breed categories, listed as separate sections, were each treated by separate series of analyses.

[c] Coded indicators of level of complexity of the model used:
I = initial model (i.e., the most complex model);
S = select model (i.e., "F" values for all sources of variation are greater than critical "F" values at the 5% significance level);
NG = model containing only terms for L.S. mean and non-genetic variables.

Table 20b. Results of least squares analyses to identify sources of variance in total fetuses per litter, from dams belonging to four crossbred categories.[a]

Breed[b]	Model[c]	Semester	Dam's age	A-o	H	K	N	Tf	Sa	Am	Pgd	Phi	R^2	Error Mean Square
BxC, CxB	I	1.15	15.03	.23	.21	.17		6.35		2.67	.03	1.12	.499	5.53
	S		19.12					8.04		3.53		5.81	.483	5.20
	NG	.17	12.24										.179	7.57
BxF_1	I	5.40	4.90	.07		9.26	.40	1.05		1.30	1.00	.58	.475	8.26
	P	6.15	5.34			20.52		2.64		2.71			.441	7.68
	S	4.18	5.21			9.39							.293	8.91
	NG	2.97	7.16										.163	10.35
YxC, CxY[d]	I	1.07	.54		1.11			2.12	.46	2.85	.49	.60	.376	7.42
	P							6.65		2.93	2.76		.266	7.35
	NG	.63	3.91										.108	8.26
YxF_1	I	0	2.31	3.24		1.20		.02	.02	.52	1.10	2.61	.316	6.89
	P		4.60	5.96								3.10	.248	6.26
	S		4.64										.086	7.14
	NG	.28	3.92										.091	7.25

[a,b] See footnotes to Table 20a.

[c] Coded indicators of level of complexity of the model used:
I, S, and NG are as defined in footnote to Table 20a;
P = model which included terms for one, two, or three genetic variables in addition to those included in the select model ("F" ratios for the additional variables are less than the critical "F" values).

[d] Select model was not obtained for the (YxC + CxY) category; "F" ratios for all variables considered were found to be less than the values required by criteria for developing a select model.

the other terms in the model. The process of successive simplification was continued until the model contained no terms representing sources of variance for which the "F" ratio was less than the critical "F" value for the 5% level of significance.

- Models having complexity intermediate between types "I" and "S" were of considerable interest in the case of certain crossbred groups. Thus, included in certain sub-sections of Table 20b are models denoted as pre-select, which include one, two, or three more genetic variables than the "S" model. Such models were included, because the additional genetic variables are factors for which the "F" ratios are only slightly less than the critical "F" values for the 5% significance level.

A. Initial models.

Applications of the most complex models yielded some "F" values (Tables 20a, 20b) which are statistically significant. The "F" ratio for the variance attributed to the dam's age in the (BxC + CxB) category, and the "F" ratios for both of the non-genetic variables in the Y, C, and BxF_1 categories are either statistically significant or highly significant. The "F" value found for the Tf in the (BxC + CxB) category, and that for the K phenotype in the BxF_1 category are highly significant ($p<.005$).

B. Select models.

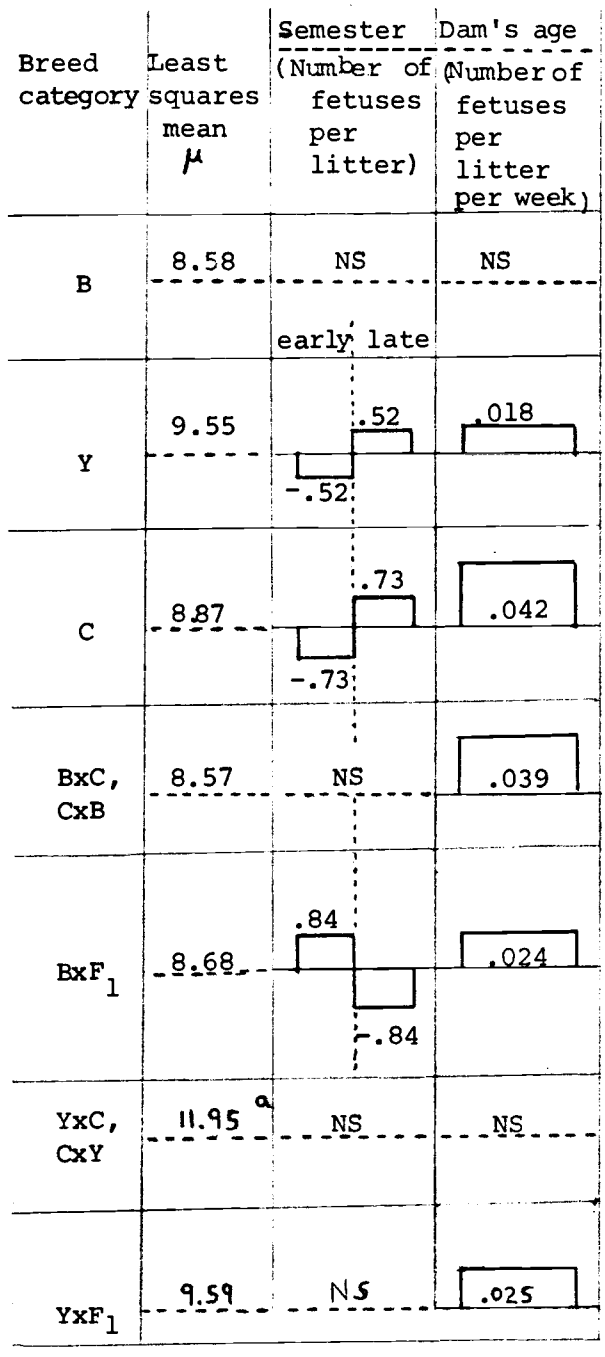
Other components of Tables 20a and 20b were utilized in evaluating the relative effectiveness of models used in analyses on separate breed categories. Thus, there can be comparisons of relative effectiveness of, e.g., select models applied to the various categories, by reference to the error mean square and the measurement designated as "R^2." The symbol "R^2" denotes the proportion of the total sum of squares which is

attributed to the variables represented in the least squares model, i.e., (sum of squares due to regression)/(total sum of squares).

Regarding the values of R^2, it seems interesting to note that in some of the breed categories, the value obtained from the select model was less by only a rather small increment, in comparison to the value of R^2 found from using the more complex model which was the "first approximation." That is, the R^2 values found with the initial and select models applied to the Y category were 0.235 and 0.179, values which differ by an increment of only 0.056. The R^2 values from initial and select models applied to data from the C line were 0.422 and 0.401. The R^2 values from the initial and select models used with the data from the (BxC + CxB) were 0.499 and 0.483. It is apparent from Table 20b that an opposite extreme occurred with data from the (YxC + CxY) category; strict adherence to the policy of eliminating variables having "F" values which were less than the critical values at the 5% level resulted in elimination of all variables which were considered by the analyses. The values of R^2 found with the select models used on data from the B and YxF_1 categories also were rather low (0.102, 0.086). The select model applied to the data from the BxF_1 yielded an R^2 value (0.293) which was somewhat larger than those found with the B, Y, and YxF_1 categories.

Also, it may be seen from Tables 20a and 20b, that the error mean square found with each of the select models remained fairly large. None of the various models which were applied were effective in reducing, by any major proportion, the error mean square found from using the model which contained only non-genetic terms.

Least squares constants and regression coefficients for non-genetic terms, as found by application of select models, are depicted in Figure 9. The results with data on certain breed categories showed that in those categories, one or both non-genetic factors were unimportant relative to certain genetic factors in accounting for the variance. Breed categories with which this was found are identifiable by absence of the L.S. constant and/or the regression coefficient in Figure 9. Also shown for each category is the L.S. mean, as defined under MATERIALS AND METHODS.



[a]Data analyses did not yield a model which fulfilled criteria for "select" models. Therefore, mean value shown for (YxC + CxY) is the population mean, instead of a L.S. mean.

Figure 9. Non-genetic variables indicated by least squares analyses as correlates of variance in total fetuses per litter. Constants depicted are a portion of results obtained using models defined as "select". Separate rows show values for separate breed categories. Scale for dam's age is different from scale for semester during which conception occurred. "NS" is shown where analysis indicated that a trivial amount of variance was accounted for by linear association with that variable.

L.S. constants obtained using the select models are depicted in Figure 10. "XX" within the space intended for the constant for a particular blood component indicates that none of the models which were applied to the data subset included a term representing that component. The abbreviation "NS" is used to indicate that an "F" ratio less than the critical (5%) value was obtained from using a model that was more complex than the select model.

It may be seen from Table 20a (as well as from Figure 9) that neither non-genetic factor was found to be useful as a component of the select model for accounting for variance in the B line. It is also evident (Table 20b and Figure 9) that both non-genetic factors were eliminated from consideration as important correlates of performance in the (YxC + CxY) category. Also, the effect of the semester of conception was not found to be useful as a component of the select models applied to the (BxC + CxB) and YxF_1 breed categories.

Tables 20a and 20b and Figure 10 show that the select models used for the different breed categories were of differing complexities in terms of the genetic factors included. The select model applied to the (BxC + CxB) category included terms representing three loci. Two loci were represented in the select model applied to the Y line. The select models applied to the B, C, and BxF_1 categories each involved only one genetic variable. The select model developed for the YxF_1 category contained only a term representing a non-genetic variable.

Results which are of primary interest are the relative values of L.S. constants found using the select models. The select models identified genetic loci which seem worthy of consideration as real correlates of prolificacy variance. The patterns of contrasts among L.S. constants for phenotypes controlled by certain genes are suggestive of genetic determinants of prolificacy which show an additive mode of gene expression; patterns of contrasts among L.S. constants found for other loci are suggestive of determinants of prolificacy having a non-additive type of gene expression. The variables identified by the select models as correlates are categorized in Table 21, according to the mode of expression which these results suggest.

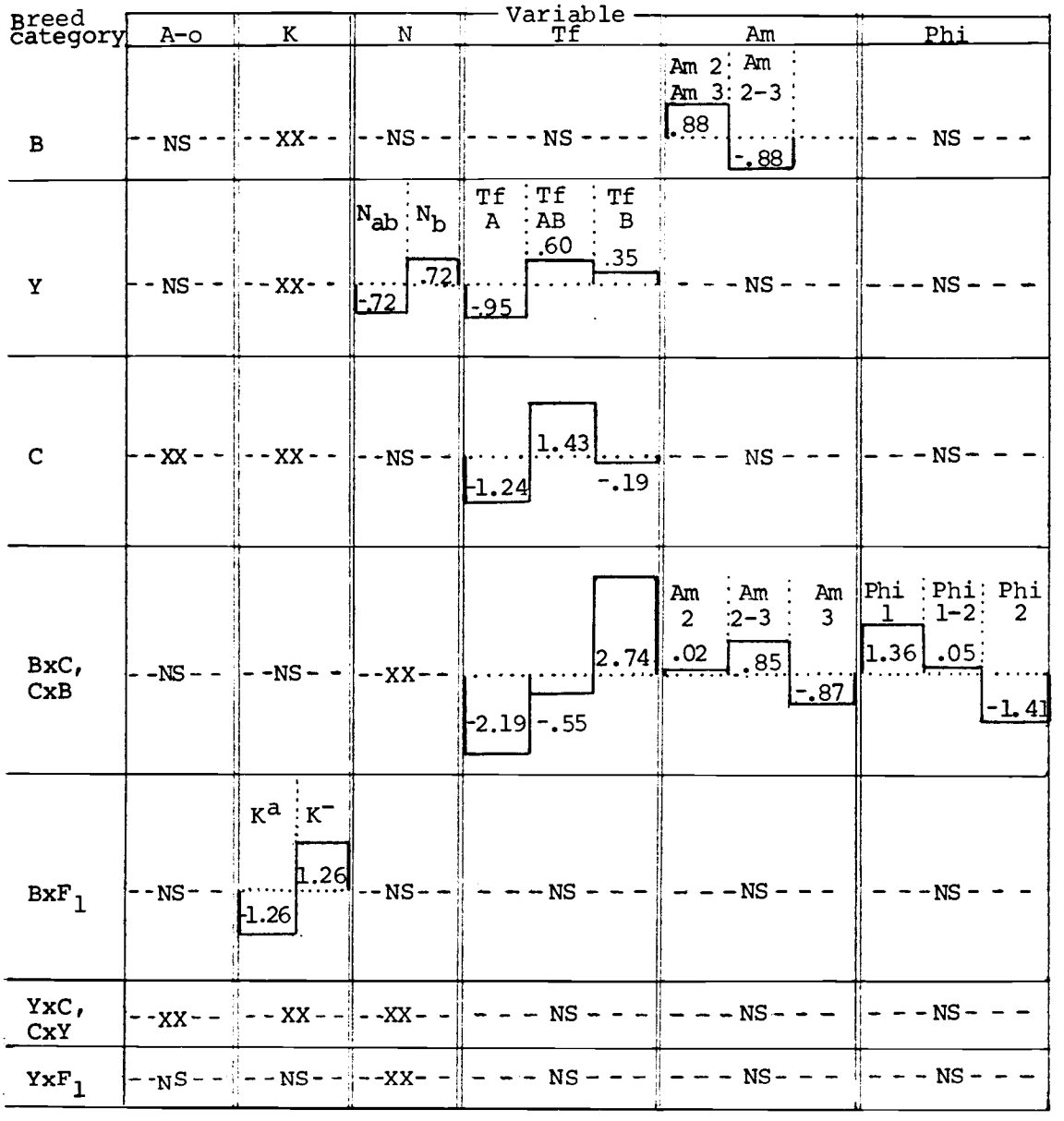


Figure 10. Genetic variables indicated by least squares analyses as correlates of variance in total fetuses per litter. Least squares models defined as "select" were used to compute the constants shown. Separate rows depict magnitudes of least squares constants within individual breed categories Dimension of constants is "number of fetuses per litter". "NS" is shown where analyses indicated that amount of variance accounted for by linear association with that variable was insufficient for it to be included in the select model. "XX" is used to indicate that variable was omitted from least squares analyses due to inadequate distribution of phenotypes.

Table 21. Categorization of apparent modes of gene expression.

Breed	Additive	Overdominant	Indefinite
B			Am
Y		Tf	N
C		Tf	
BxC, CxB	Tf, Phi	Am	
BxF_1			K

Additively expressed alleles. 1.) Among the Tf phenotypes (Figure 10) within the (BxC + CxB) category, the Tf B was the variant having the L.S. constant of highest value (2.74). The lowest constant among Tf phenotypes within the (BxC + CxB) was that found for Tf A (-2.19). The value of the constant found for the Tf AB (-.55) was an intermediate value. 2.) The L.S. constants for the Phi 1 and Phi 2 phenotypes in the (BxC + CxB) category were found to be quite similar in absolute value, but have opposite signs (1.36 and -1.41, respectively). The constant for the Phi 1-2 phenotype (0.05) was intermediate between the constants for the other two phenotypes.

Overdominance. 1.) The select model applied to the (BxC + CxB) indicated also that the Am phenotype was an important correlate of performance. Contrasts among the L.S. constants found for the three Am phenotypes had resemblance to contrasts described above, in that the constant for only one of the phenotypes was a value distinctly greater than zero. However, the finding that the highest L.S. constant (0.85) was that associated with the heterozygous genotype (Am^2/Am^3), was a distinct contrast to the findings mentioned in the preceding paragraph. 2.) Among L.S. constants for the Tf phenotypes in the C line, only that found for the Tf AB was a positive value (1.43). This value was a distinct contrast to L.S. constants found for the Tf A (-1.24) and the Tf B (-.19). 3.) As with the pattern of contrasts found with the Tf phenotypes in the C line, it was found that in the Y line, the L.S. constant for the Tf AB phenotype was a higher value

than the constants for either the Tf A or Tf B phenotypes (-.95 and 0.35, respectively).

Indefinite modes of gene expression. Several of the components of the select L.S. models represented loci at which some but not all of the known genotypic combinations were in evidence. Similarly, certain other phenotypic categories were represented by such few data that it seemed that only some pooling of phenotypes could permit meaningful results to be obtained by L.S. analyses. The pooled phenotypic categories used with those distributions are described next. Such incomplete distributions of genotypes prevented categorization of the type of interaction of alleles involved.

Some pooling of Am phenotypes was found to be necessary for analyses of data from the B line. One of the classes was formed by pooling the Am 2 and Am 3 phenotypes, and the other class consisted of the Am 2-3 phenotype. The positive L.S. constant (0.88) found by the analysis was that of the class consisting of Am 2 plus Am 3 phenotypes. It was also necessary to pool two of the phenotypes controlled by the N locus in order to include those phenotypes in the L.S. analyses. One of the classes was formed by pooling the N_a and N_{ab} phenotypes, and the other class consisted of the N_b phenotype. Terms representing N phenotypes were contained in the select model used for the Y category. However, it happened that pooling of N phenotypes was unnecessary with that particular breed category, since only two phenotypes were found among Y subjects.

The largest "F" ratio found with any of the loci considered by select models was that for the K phenotypes (9.39) in the BxF_1 category. Categorization on the basis of the K phenotypes depended on the simple presence or absence of the K^a allele. It should be noted that sufficient variability in K phenotypes was present so that those phenotypes could also be considered among the variables involved in development of select models for the YxF_1 and (BxC + CxB) categories. However, the proportions of sums of squares found associated with K phenotypes in the latter two categories were not sufficient to permit inclusion of the K phenotypes in the select models.

C. Pre-select models.

Results from analyses on certain categories made it seem worthwhile to include data obtained from other models besides those designated as I, S, and NG. One such model was added to Table 20b for each of three crossbred categories, viz., the BxF_1, (YxC + CxY) and YxF_1. It should be noted that those three models contained genetic variables for which "F" ratios were considerably greater than 1.00. However, not all of those "F" ratios were greater than the "F" values which are critical at the 5% level of statistical significance.

A reason for including results of the models denoted as pre-select may be appreciated from Table 20b. The pre-select model applied to the BxF_1 data yielded "F" ratios of 2.64 and 2.71 for the Tf and Am, respectively. Those values are certainly less than the critical "F" value for the 5% level of statistical significance (3.2); deletion of only the terms representing those two loci yielded the model denoted as select. The pre-select model applied to the (YxC + CxY) contained only terms representing the Tf, Am, and Pgd phenotypes. The stepwise elimination of variables for which "F" ratios were less than the critical values (viz., Am and Pgd) yielded a model containing terms representing only a single locus (Tf), and the "F" value for that source of variation was reduced to a sub-critical value. Therefore, no select model was obtained for the (YxC + CxY) category. A mostly different combination of variables comprised the pre-select model used for the YxF_1 category. Genetic loci considered in that model were the A-o and Phi. The "F" ratio for the A-o (5.96) was distinctly greater than the critical value, while that for the Phi (3.10) was slightly less than the critical value (3.2).

An observation which may be of some value is that the three pre-select models mentioned here each included factors representing one or two loci which were represented in select models used for other breed categories. "F" ratios found for the Tf and Am in the pre-select models for the BxF_1 and (YxC + CxY) seem to give indication of associations of those phenotypes with performance, although all

except one of those values are somewhat less than the critical values. Findings from those two pre-select models are therefore consistent with the evidence from select models developed for the B, Y, C, and (BxC + CxB), indicating that there were real associations of the Tf and Am phenotypes with performance. Also, the pre-select model for the YxF_1 contained terms representing the Phi phenotypes; the size of the "F" ratio (3.10), although sub-critical, is consistent with the presence of the Phi phenotypes in the select model for one other breed category (BxC + CxB). Furthermore, the Pgd also was included in the pre-select model used for the (YxC + CxY), and the "F" ratio was sizeable (2.76), although less than the critical value. This observation may be viewed as important because of the evidence of linkage of the Pgd with the Phi locus, which was mentioned in a section above (see Associations Among Traits).

L.S. constants for the Tf phenotypes in the BxF_1 (Figure 11) seem indicative of an incompletely dominant mode of gene expression. Constants for the Tf A and Tf AB are similar in value (0.84 and 0.60), and are distinctly greater than the constant for the Tf B (-1.44). Interestingly, this is a distinct contrast from the distribution of L.S. constants for Tf phenotypes in the (BxC + CxB), in two respects. Firstly, with the (BxC + CxB) the distribution of constants (Figure 10) is characteristic of an additive mode of gene expression, and secondly, the L.S. constant of highest value is that of the Tf B, while that of lowest value is the constant for the Tf A. However, the L.S. constants for Tf phenotypes found using the pre-select model for the (YxC + CxY) show one aspect of similarity to results with the (BxC + CxB). The highest L.S. constant (Figure 11) is that for the Tf B (1.37) in the former category (cf. 2.74, shown for the latter category in Figure 10). It remains uncertain whether the Tf was also associated with additive gene expression in the (Yx C + CxY), because only two Tf phenotypes (viz., Tf B and Tf AB) were found among those sows.

L.S. constants for the Am phenotypes in the BxF_1 show a pattern of contrasts which is indicative of an additive mode of gene expression. Constants for the Am 2 and Am 3 are nearly equal in absolute value (-1.97 and 1.92) and that for the Am 2-3 is nearly zero (0.05).

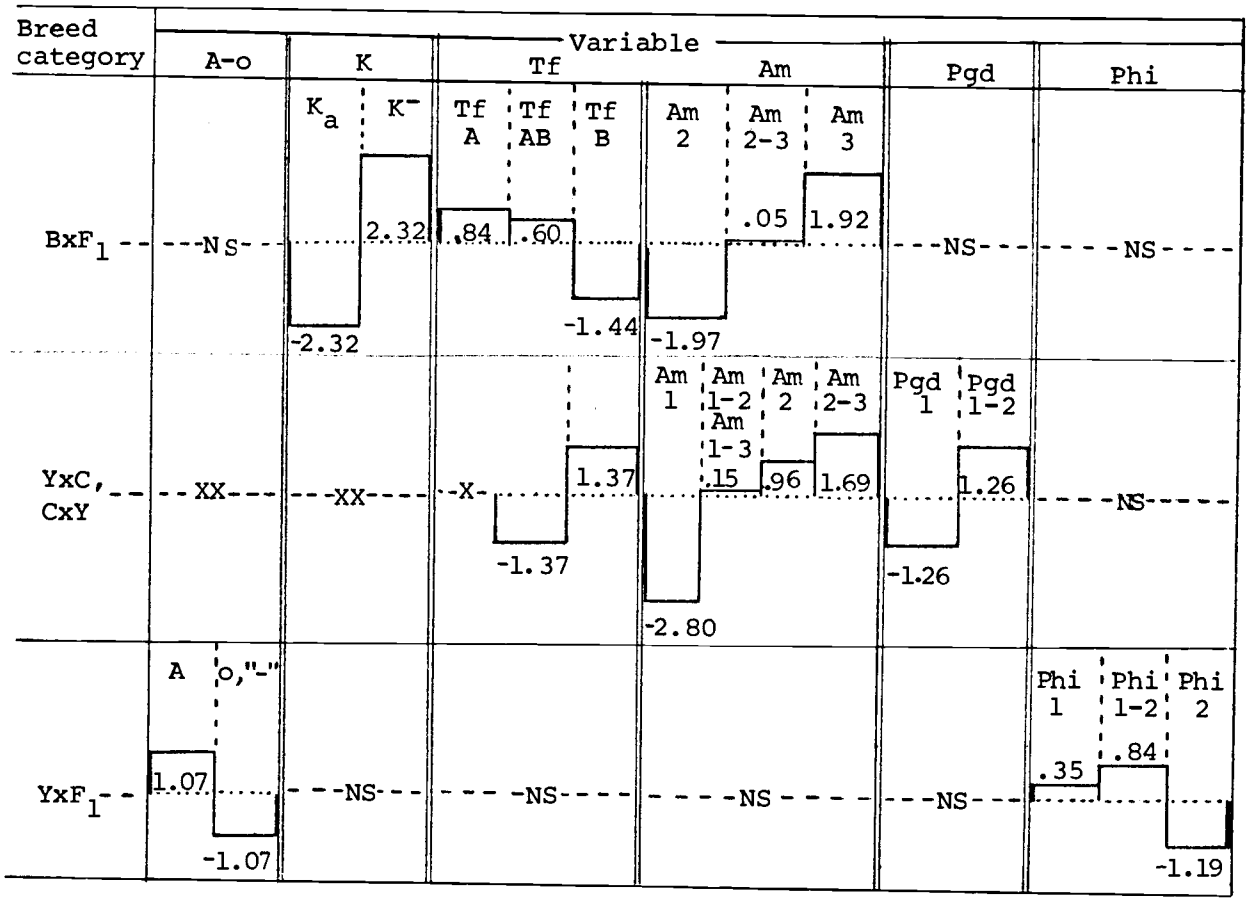


Figure 11. Least squares constants obtained using the pre-select models developed for three crossbred categories. Constants describing effects of non-genetic variables are omitted from the data shown. "NS" indicates that "F" value considerably less than the critical value was found using a model more complex than the pre-select model. Scale is same as in Figure 10.

These findings are a distinct contrast to results from the select model used with the (BxC + CxB), which show that the lowest constant is that for the Am 3 and the highest constant is that for the Am 2-3. The results from the pre-select model used with the (YxC + CxY) are of some interest in this regard (Figure 11), in that the highest of L.S. constants for the Am phenotypes is that of the Am 2-3 (1.69).

Finally, it may be noted that from the pre-select model used with the YxF_1, L.S. constants for the Phi 1 and Phi 1-2 were found to be positive, and that for the Phi 2 was found to be negative (-1.19). Such is also the pattern with Phi constants found with the select model used for the (BxC + CxB), as is shown in Figure 10. However, while the pattern of variation of Phi constants in the (BxC + CxB) indicates an additive mode of gene expression, the pattern of variation found in the YxF_1 using the pre-select model (Figure 11) is that of an overdominant mode of gene expression; the highest of L.S. constants for Phi is that of Phi 1-2 (0.84).

Distribution of Regressed Fetuses

Regressed fetuses were found in 72 of the 480 litters which served as the data set for this study. The distributions of such litters within individual breed categories were scrutinized. It was noted that in nearly every single breed category, only one or two of the litters containing regressed fetuses were litters in which the total number of fetuses was less than the mean value for that breed category. More than two of the litters farrowed by the BxC or CxB sows and containing regressed fetuses, were litters in which the total number of fetuses was less than the mean value for the (BxC + CxB), but these litters actually comprised only 27% of the total number of the litters farrowed by BxC and CxB sows and containing regressed fetuses. Considering the litters containing regressed fetuses as a particular subset (N = 72) of the total data, it was found that 14 of those litters (19.4%) were litters in which the total number of fetuses was less than the particular sub-population average.

DISCUSSION

Prior to the single-gene analyses, evidence was available which indicated contrasts in reproductive performance among separate breed categories comprising the O.S.U. swine herd. Although statistical significance was not demonstrated for those contrasts, certain aspects of the performance data gave motivation for a study of genetic contrasts within the population.

It was necessary to give careful consideration to the question of validity of criteria for distinguishing among the variant forms of the blood components which were studied. Family relationships among subjects made possible a useful amount of corroboration of the previously suggested concepts of genetically determined variation of those components. Attention was given to the limitations of the usefulness of the study due to the evidence that paternity assignments have been incorrect for some litters.

Certain aspects of the distribution of phenotypes among the various breed categories provided a motive for investigating correlations of blood component phenotypes with variance in reproductive performance. Statistical analyses of reproductive performance and individual phenotypes were focused primarily toward obtaining insights about modes of expression of individual genes. It was found appropriate to make some comparisons between results of this study and studies of other populations. The extent to which results of the separate studies are corroborative was given consideration, with intention of deriving efficient approaches to reproductive physiology in this species.

Preliminary Evaluations of Performance

Liu (1974) presented evidence that litters consisting of larger numbers of piglets were produced by certain types of crossmatings among three semi-closed lines, in comparison to intra-line matings within the O.S.U. swine population. Litters produced by crossmating of the two

purebred lines (B and Y) were larger in terms of both number of live-born and total number of fetuses, compared to litters produced from intra-line matings. Such a result also was found from the crossmating of C females with Y boars. However, mating of C females with B boars did not yield such an increase. Instead, about seven percent fewer live-born and total fetuses were produced in comparison to the averages of litters produced from the intra-line matings of C's. A reasonable interpretation of these observations could be that certain categories of crossbred conceptuses possessed phenotypes which conferred a superior level of in utero viability. Direct tests of this hypothesis were not designed. Instead, other performance data have been analyzed in search of other evidence about crossbreeding effects.

Reproductive performance of crossbred females was compared to performances of parental populations. Little attention was given to the performance of daughters produced from matings between B's and Y's. Evidence of heterotic effects with such matings had been presented earlier (Spurr, 1969; Liu, 1974). Instead, the focus of the present study was toward describing the performance characteristics of daughters produced from mating pairs either consisting of a purebred and a C, or else, of a purebred boar and a daughter of a B-Y cross.

It was pointed out under RESULTS, that there are certain patterns of consistency in the data which describe performances of crossbred categories (Tables 4 through 7b). The most prominent consistency is that of the differences by which performances of (BxC + CxB) and (YxC + CxY) sows exceeded the mid-values of the respective parental (B, C, and Y) groups (Tables 4 and 6). The fact that the contrasts were in the same direction at each parity in the (YxC + CxY) and at all except one parity in the (BxC + CxB) was viewed as persuasive evidence that heterosis was occurring. The persuasiveness of that evidence was diminished by the sizes of the standard errors of the average performances of parental groups. Those particular statistics were practically sufficient reason to disregard the possibility of demonstrating statistical significance in the contrasts of crossbred means with estimates of mid-parent performances. The same sort of contrasts of BxF_1 and YxF_1 performances in comparison to predicted

values were found at certain parities; however, it was also found that with the performances of the purebred populations on which predicted performances were based, standard errors were comparable in size to those pertaining to the parents of the (BxC + CxB) and (YxC + CxY).

It was pointed out that, of the samples described in Tables 4 through 7b, the standard deviations of only a small proportion were less than ±2.00. Thus, although the data were segregated according to breed category and age group (parity), there was much variance not accounted for by those two factors. It should be evident from MATERIALS AND METHODS (above), that the management procedures for the herd were intended to minimize variation of environmental factors likely to affect reproductive performance. It seemed reasonable to suppose that the population offered an opportunity to discern some genetic factors which were causes of the variance which is evident from the standard deviations in Tables 4 through 7b. There were no indications from those data, as to whether or not most of the variance was due to non-genetic factors which were not controlled.

Aside from comparisons of crossbred groups to semi-closed lines, comparisons among the crossbred categories also revealed patterns of contrasts which were notable. It was apparent that sizes of standard errors of the mean values were such that statistically significant differences would be found with only few, if any, of the comparisons between breed categories. The large standard errors notwithstanding, it was of considerable interest to find that (YxC + CxY) performance exceeded performances of the (BxC + CxB), BxF_1, and YxF_1 at each parity. That observation served as a basis for the hypothesis that a genotype was present in the YxC and CxY females which determined a higher degree of prolificacy than that of other breed categories which were considered. Many of the animals on which the hypothesis was based were no longer available at the time of the phenotyping studies. Therefore, emphasis should be given to indications that the subjects available for phenotyping studies were useful for evaluating the hypothesis. The portions of data in Tables 6, 7a, and 7b which pertain to crossbred females were obtained only from phenotyped subjects. Comparisons involving the phenotyped subjects seemed to indicate sufficient

similarity to those found with larger data subsets, so that the subjects were expected to be useful samples.

Descriptions of the composition of the performance data on the phenotyped subjects (Table 1) make it apparent that a larger portion of those data pertain to sows of the Y line (i.e., 150 litters) than to any other single breed category. Inclusion of that relatively large amount of Y performance data was mostly the consequence of efforts to accomplish as thorough a sampling as possible of each of the breed categories. This apparent disproportion was not considered necessarily undesirable. The results of the initial survey of the population (Table 8) indicated that an H phenotype (H_a) was present among the Y's at a considerable frequency, whereas most if not all brood sows in certain other breed categories lacked that phenotype. Furthermore, the Am^1 allele was found among the Y subjects at a considerable frequency, as well as in crossbred subjects of Y parentage, but was absent from subjects belonging to the other lines. These observations caused the Y population to be an object of considerable interest, because H as well as Am phenotypes had been suggested by previous studies (Jensen et al., 1968; Rasmusen, 1975b) as apparent correlates of variance in reproductive performance. The Am 1 phenotype was not included in those studies, however.

Phenotyping Criteria

It was considered reasonable to infer the presence of specific genotypes at certain loci, using the results of the tests for phenotypes attributed to those loci. This policy was followed with the I, N, Sa, Tf, Am, and Pgd genes, and with some reservations, also with the Hx and Phi genes. An obvious fact from the outset was that direct inference of genotypes could not follow from knowledge of phenotypes at the G, H, K, and L loci, simply due to lack of antisera which detect certain previously identified co-dominant alleles at those loci. Some elaboration is necessary here regarding reservations about the results which were obtained from studies of certain of those genes, and also regarding results from study of the A-o phenotypes.

Knowledge of the A-o phenotypes is to be regarded as having perhaps more ambiguous application than knowledge of other phenotypes. This is partly due to the apparent epistasis which may affect expression of those phenotypes. The finding of the "-" phenotype prevents ascertainment of the presence or absence of either the A^A or a^o alleles at the A-o locus, and instead provides some information about the composition of a different locus, as pointed out in a previous section. Also, the occurrence of the A phenotype prevents direct ascertainment of presence or absence of the a^o allele, due to the dominance-recessiveness relationship. Thus, with some specimens, no information about the A-o locus was obtained from tests using anti-A and anti-o sera, and with specimens in which the A phenotype is detected, the genotype may remain uncertain.

Some comments are appropriate regarding results obtained from use of the anti-K_a serum. Recommended protocol (Saison, 1973) for use of that antiserum might be suspected of vitiating the specificity of the reagent. The recommended protocol required that the components of the reaction mixture were combined as two portions; one portion consisted of the erythrocyte suspension, and the other consisted of a mixture of proteinase and the antiserum. The two separate portions were drawn into a capillary tube as two distinct segments, and were mixed by inverting the tube to allow erythrocytes to settle downward through the proteinase and antiserum. Knowledge of the protein nature of antibodies leads to the suspicion that this protocol may be undesirable, in that some degree of degradation of antibodies may be accomplished. (This protocol differs in that respect from the protocol which was originated by Low (1955) for application of proteinase-dependent antisera.) Results from use of the former protocol with other antisera, not mentioned anywhere above, yielded results which were not compatible with a co-dominant pattern of inheritance. Those latter results were the basis for initially holding some reservation about results with the anti-K_a serum. However, the application of that reagent to groups consisting of parents as well as offspring yielded results which were compatible with the concept that the K_a phenotype which is observed, is simply determined by a co-dominant allele. The K_a phenotype was

not detected in any of the 52 offspring of parental pairs which lacked the K_a phenotype (Table 9). The reactivity toward the anti-K_a serum, found with the cells of all 26 subjects which were offspring of pairs in which the K_a phenotype was possessed by both the dam and sire, could be due to the homozygous K^a/K^a genotype in at least one member of each of those six parental pairs. Also pertinent with respect to this evaluation may be the observed distributions of the K_a phenotype within breed categories. The K_a phenotype was absent from subjects belonging to the C line. The only Y subjects which possessed the K_a phenotype were either first- or second-generation descendants of a single boar-sow pair. The K_a was found in all 43 members of the B line which were analyzed. Perhaps, significant degradation of the anti-K_a immunoglobulins was avoided by means of assuring a short time-period between mixing of the antiserum with proteinase and the time of application to specimens. None of the specimens was tested with antiserum + proteinase mixture which had been mixed more than ten minutes prior to time of application.

It is uncertain whether or not the Hx phenotypes described under RESULTS are properly identified with the phenotypes which have been denoted by others as Hx 1 and Hx 1-2 (Imlah, 1965; Graetzer et al., 1965). Those reports, which provided the early descriptions of ten Hx phenotypes (involving four alleles), did not utilize free Hb as a reference marker for comparing relative mobilities of the other heme-binding substances. It seemed most reasonable to denote the Hx phenotypes found in this study as Hx 1 and Hx 1-2. The bases for this identification were comparisons to phenotypes described by Kristjansson (1961), who used the same separation method as that used here. That report included an electropherogram on which free Hb was present as a reference marker. An additional uncertainty regarding the Hx phenotypes is due to the incomplete evaluation of the possibility that the Hx 0-1 phenotype may be present in several subjects. The report by Kristjansson provides no assistance on this point, since all subjects of that study apparently lacked the Hx^0 allele. The component described by Graetzer et al. as the product of the Hx^0 allele was shown in the electropherogram obtained from that study, at a sufficiently

anodal position so that it seems unlikely that it has been mistaken in the present study as the product of either the Hx^1, Hx^2, or Hx^3 allele. However, Graetzer et al. did not provide a comparison of mobilities of the Hx 0 fraction and free Hb. Consequently, it is necessary to remain open to the possibility that the product of the Hx^0 allele is in evidence in the subjects of the present study. That variant may be the component having mobility indistinguishable from that of free Hb, but appearing in certain specimens only if exogenous hematin has been added (see Figure 2). Thus, this amounts to a suggestion that more heterozygosity existed at the Hx locus than is indicated in the results shown in Table 16. This possibility was not explored, however. The component which might be the product of the Hx^0 allele was not found in any specimens without the presence of the product of the Hx^1 allele. Perhaps the explanation for this finding is a low frequency of the Hx^0 allele, so that the Hx 0 phenotype was not found in any of the subjects. It would be of interest to accomplish family studies using mating pairs wherein both mates yielded serum specimens showing the presence of the most anodal hematin-binding component. Lack of results of such studies made it advisable to disregard the possibility that the product of the Hx^0 allele is present. Thus, although subjects possessed the hematin-binding component which co-migrated with Hb, they were considered as possessing the Hx 1 phenotype.

Certain findings from the family studies have been interpreted as evidence that some variation at the Phi locus has not been disclosed by the analytical method used. The basis for this interpretation can be better appreciated by reference to Figure 12, which is a depiction of relationships among the subjects considered to possess the hypothesized genotypes Phi^0/Phi^1 and Phi^0/Phi^2. Subjects of the phenotyping are distinguished within Figure 12 by the inclusion of the symbol for the observed Phi phenotype, next to the identification number. (The pedigree shown also includes several pigs which were not subjects of this study.) It is evident that, if Phi alleles are strictly codominant, then the phenotypes observed in pigs identified as 1700-4, 1705-4, and 1831-4 would not be expected to occur.

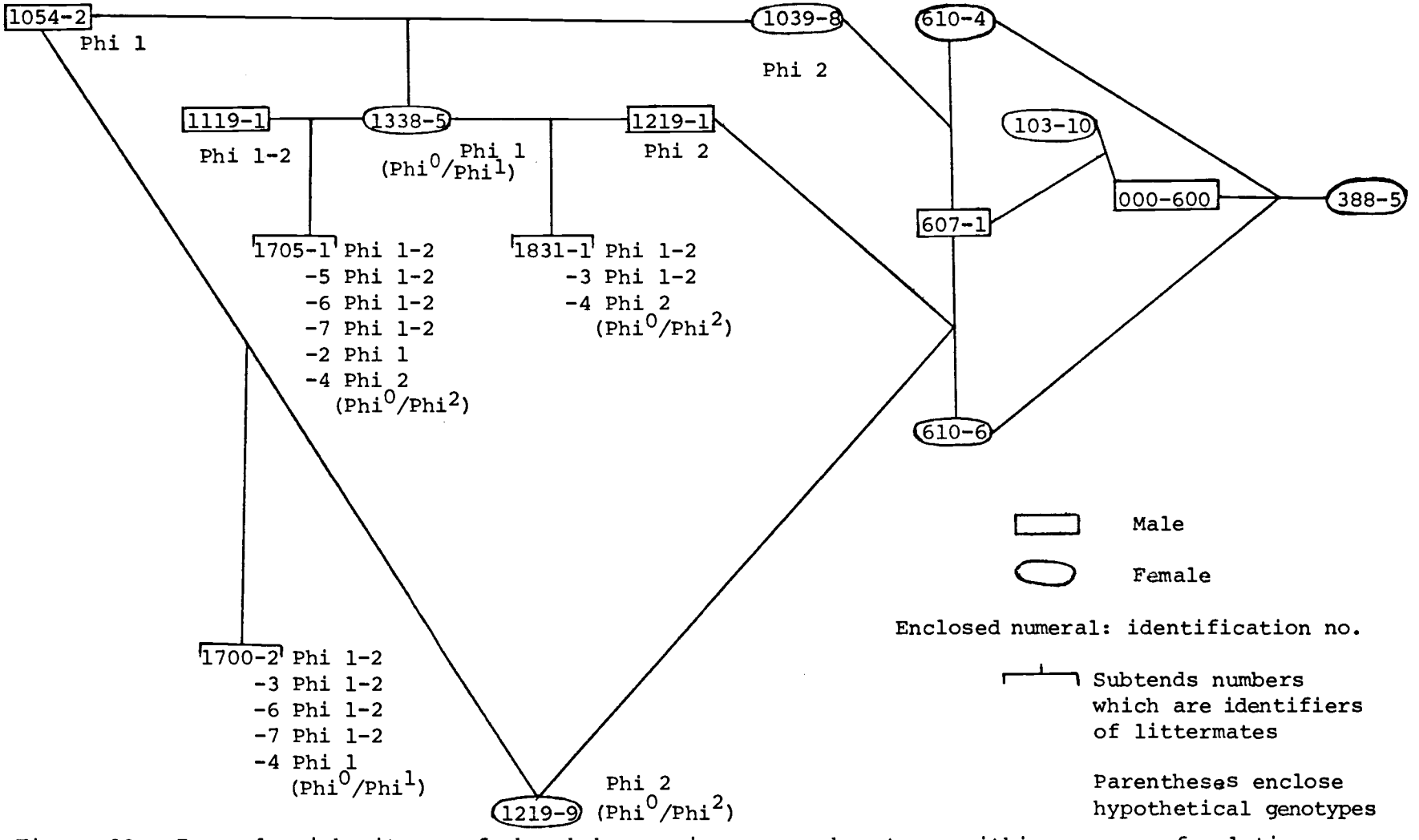


Figure 12. Irregular inheritance of phosphohexose isomerase phenotypes within a group of relatives.

Pig #1700-4 could display the Phi 1 phenotype if it had not inherited a Phi^2 allele from the dam (1219-9). Individuals identified as 1705-4 and 1831-4 could display the Phi 2 phenotype if they had not inherited the Phi^1 allele from their dam (1338-5). These considerations were the basis for the hypothesis that the two dams mentioned here (1219-9 and 1338-5) possessed, respectively, the genotypes Phi^0/Phi^2 and Phi^0/Phi^1. It is further proposed that the Phi^0 allele was then inherited by one member of each of three litters farrowed by those sows. The offspring supposed to have inherited the Phi^0 allele are indicated by showing the hypothetical genotypes beneath the observed phenotypes. "Phi^0" is used here to represent an allele, the product of which was functionally distinct from the products of both the Phi^1 and Phi^2 alleles, in at least one respect. The evidence of a dimeric structure of erythrocyte phosphoglucose isomerase in humans (Gracy and Tilley, 1975) is pertinent to this concept. That finding can be a basis for the expectation that swine erythrocyte phosphohexose isomerase also is a dimeric molecule. If this is in fact the true quarternary structure, then this immediately presents a likely, straightforward explanation for the pattern of electrophoretically detectable variations. Thus, if a functional heterodimer, as well as homodimers, can exist, then the single-banded patterns of the phosphohexose isomerase described as the Phi^1 and Phi^2 phenotypes were probably homodimers. If so, the former phenotype consisted of the polypeptide product of the Phi^1 allele and the latter consisted of the product of the Phi^2 allele. The three-banded pattern denoted as the Phi 1-2 phenotype could then be considered as due to the presence of both types of homodimer and also the heterodimer. The latter would presumably contain two non-identical subunits which are the products of the Phi^1 and Phi^2 alleles. The heterodimeric fraction would be that band which is of intermediate electrophoretic mobility on Figure 7. The hypothetical third allele, denoted here by "Phi^0", might yield a polypeptide product which either does not participate in formation of dimeric molecules, or else causes dimers in which it is present, to be enzymically non-functional.

A pedigree involving the aforementioned subjects is presented in Figure 12, because it shows some support for the hypothesis. That is, the records indicate that sows 1219-9 and 1338-5 were, respectively, first- and second-generation descendants of boar 607-1. Furthermore, those two sows were also first- and second-generation descendants of siblings, viz., 610-6 and 610-4. Thus, it is reasonable to suggest that sows 1219-9 and 1338-5 both inherited the Phi^0 allele from the same ancestor. That ancestor may have been boar 607-1, boar 000-600, or sow 388-5. The origination of the Phi^0 allele from a single ancestor is not essential to the validity of this hypothesis; however, if true, it would be consistent with the suggestion that the Phi^0 allele was not frequent among the subjects of this study. Obviously, substantiation of existence of the Phi^0 allele requires family studies which involve offspring from mating pairs consisting of mates which are both apparent possessors of that allele.

Existence of a Phi^0 allele is not the only reasonable hypothesis by which to explain the observations. Deletion of the Phi locus from one of the members of the pair of chromosomes carrying that gene could produce the effect which is described here. Thus, for the individuals described in Figure 12 as possessing genotypes containing the Phi^0 allele, it may be appropriate to consider the symbol Phi^0 as indicating deletion of the Phi gene from one chromosome.

The foregoing may have rather specific implications toward other portions of the results reported here. The concept of deletion of the Phi gene implies that possessors of a chromosome with that deletion were correctly identified by the phenotyping procedure as possessors of only one, not two, alleles at the Phi locus. The concept of an inactive Phi gene product from one of the alleles implies however, that heterozygosity existed at least in a structural sense, at the Phi locus, and that heterozygosity was not being detected by the analytical procedure used here. Therefore, it seems important to note that no other cases of irregular occurrences of Phi phenotypes were found within the subject population [except those in which occurrences of additional irregularities make it rather apparent that there was mis-assignment of paternity (i.e., families described in

Tables 13 and 15)]. Another highly pertinent fact may be the indication that those irregular occurrences of Phi phenotypes discussed here were due to inheritance from only one individual ancestor of certain B and (BxC + CxB) subjects.

Much as with the Hx phenotypes, denotations given to the variants of the Sa component (Figure 4) must be considered only tentative. Schroffel (1965) described six different phenotypes, attributed to three alleles at the Sa locus. Only three different Sa phenotypes were observed among the subjects studied here, and it seemed that those phenotypes showed most resemblance to those denoted by Schroffel as the Sa B, Sa BC, and Sa C. It seems necessary to remain open to the possibility that the phenotypes displayed in Figure 4 are not correctly identified as the Sa B, Sa BC, and Sa C. However, the family studies described under RESULTS are compatible with the conclusion that the three phenotypes found are due to presence of two alleles in the population.

Evidence was presented under RESULTS favoring use of the denotation Tf B for not only one, but two distinct phenotypes. The remarks regarding that evidence are considered as only the first explicit argument in favor of regarding a four-banded phenotype as the Tf B. However, careful examination of the literature indicates that this particular assignment of identity has been implicit with studies on other populations. The electropherogram presented by Kristjansson (1963) shows Tf B phenotypes on which there are faint but perceptible stained fractions, at the same position as the most anodal Tf fraction in the Tf A and Tf AB phenotypes (see Figure 3). Also, Schroffel (1966) presented an electropherogram containing phenotypes of individuals possessing a Tf^B allele and lacking the Tf^A allele. The more anodal band that is evident on some but not all of the Tf B phenotypes in Figure 8 was quite obvious in at least two of the specimens shown by Schroffel.

Tests of Phenotype Continuity

Efforts were made to demonstrate that the same phenotype was found consistently in specimens obtained from the same individual at different dates. However, those efforts involved only a small number of subjects. Below is a discussion of reasons for not attempting a more extensive verification of temporal consistency of phenotyping results.

Second samples were drawn from ten subjects for reasons which varied somewhat among those individuals. Most typically, the motive for obtaining secondary specimens from particular individuals was the finding that in some individual specimens, there was a Tf phenotype which did not truly resemble those displayed in the literature as being typical (Kristjansson, 1963; Baker, 1968a). Thus, certain subjects were selected as sources of secondary specimens, based on the Tf phenotype and often, also on the fact of the subject's relatively young age (six to ten months) at the date of initial sampling. Although the motives for the additional samplings did not require repeated analyses for each component being studied, it seemed desirable to utilize those secondary specimens as opportunities to obtain evidence of repeatability of the results with each blood component.

The group of subjects from which specimens were obtained at different dates amounted to a total of only nine sows and one boar. The size of that group and the distribution of phenotypes of certain components were such as to cause the group to have only limited usefulness for tests of the stability of phenotypes with age.

Those comparisons of serial samples provided evidence that an additional serum component may appear at the anodal extremity of the Tf segment of the electropherogram, although the component was not previously evident in serum from that individual. The finding is not of particular significance to the task of assigning phenotypes, since that particular band does not by itself cause ambiguity in the identification of the Tf phenotypes. Data obtained were too limited

to permit worthwhile speculation as to whether the appearance of the additional component may have been dependent upon age, physiological state, or season of the year. Furthermore, this study did not consider the question of whether or not the component is a Tf fraction. Appearance of the component, as found in the later specimen from sow 1369-10 (Figure 8), suggests that it may have been something other than Tf. The fraction seen in that particular specimen is more diffuse than the band having similar electrophoretic mobility and seen on Tf A and Tf AB phenotypes.

Substantial evidence exists that most of the antigenic determinants studied here are present on the erythrocytes of neonates (Smith et al., 1968). The A and o determinants are not evident at birth, but are detectable within only a few weeks after birth (Saison, 1962; Rasmusen, 1964). The three studies cited here provided no evidence regarding the minimum age for detection of the N_a and N_b antigens. The present study provided some evidence that those two antigens are also detectable well before adulthood. The study by Smith et al. (1968) also observed the variations of the Tf and Hx phenotypes, and also three of the Am phenotypes, at six weeks of age. A small proportion of the subjects of the present study were phenotyped while still at juvenile stages. One litter of five pigs was phenotyped at four and one-half months of age; other litters, totalling 28 pigs, were phenotyped at ages of five to six and one-half months. Results with those subjects amounted to some assurance that previously described variants controlled by the Phi, Pgd, and Am loci are detectable in samples from juveniles.

Thus, there did not seem to be any obvious need for careful evaluation of age-dependency of the phenotypes for purposes of this study.

Evidence of Inheritance of Phenotypes

Certain comments seem necessary regarding the value of the inheritance studies which have been reported to date. These are

followed by comments regarding the usefulness of the family studies which were possible with the population used for the present study.

The Background Literature section contains specific citations to studies in which there were analyses of the phenotypes of mating pairs, and the phenotypes of offspring produced from those matings. The results of those studies were the essential basis for the fundamental expectation from the outset of the present study that the phenotypes being analyzed are genetically determined. It was on the strength of the studies which have been cited, that the modes of inheritance of the phenotypes within the subject population were not more thoroughly investigated. However, this dependence on previously reported evidence is not to imply a total disregard for the possibility that the modes of inheritance which were apparent in other populations may be different from modes of inheritance in the population studied here. It was pointed out in a section above, that thorough studies of modes of inheritance in this population would require that it be considerably expanded, or else that it be of a different composition. It was possible, nevertheless, to accomplish at least some degree of verification that the modes of inheritance of the genotypes studied here are those which had been proposed previously (see Background Literature).

Observations presented under Family Studies (Tables 9 through 11) are supportive of the previously proposed modes of inheritance.

A-o Phenotypes. Epistatic control of expression of the A and o phenotypes, as described by Saison and Ingram (1962), is evidenced by the results shown in Table 9. A specific genotype (or genotypes) present at a "suppressor" locus (S) can prevent expression of the A or o phenotype. A "suppressor" genotype (inferred from the "-" phenotype) was present in one or more of the offspring born in each mating category shown, except one (o x "-"). The apparent inheritance pattern of the "suppressor" genotype is typical of a homozygous recessive allele. Individuals with a "suppressor" genotype occurred in offspring of mating types where neither parent possessed such a genotype, viz., the offspring of the o x o, A x o, o x A, and A x A

categories; the "suppressor" genotype was present in each of the offspring from the mating pair in which both mates possessed the "suppressor" genotype. Regarding alleles at the A-o locus itself, dominance of the A^A allele relative to the a^o allele was evident. The A phenotype was not found among the offspring of the o x o mating category. Offspring showing the A and o phenotypes were produced from the mating category in which both mates possessed the A phenotype.

Epistasis of the gene determining occurrence of the "-" phenotype was indicated by two types of findings: 1.) individuals possessing each of the three phenotypes (A, o, and "-") were found among the offspring of the mating categories consisting of one mate having the A phenotype and one mate having the "-" phenotype, and 2.) both the A and o phenotypes were found among the offspring from matings of an o phenotype and a "-" phenotype. It would be difficult to reconcile these findings with a concept that the A, o, and "-" phenotypes are all controlled by alleles at only a single locus. There is additional support for the concept of this epistatic relationship. Rasmusen (1972) and Hojny (1974) have presented evidence that the gene controlling the "-" phenotype is linked with the H gene, yet no evidence has been found to indicate linkage between the H and the A-o genes.

$\underline{G^b}$. Lack of reaction of a specimen with the anti-G_b serum is denoted as the G^- phenotype for purposes of this study. The G^- phenotype is probably indicative of presence of the G^a/G^a genotype (Andresen, 1962). Anti-G_a serum was not available for this study. Therefore presence of the G^a must be considered uncertain, although there have been no reports of specimens which react with neither the anti-G_a nor the anti-G_b serum. It may be seen in Table 9 that offspring possessing the G^- phenotype were produced from each of the three mating classes. This may be interpreted as evidence that at least some of the parents possessing the G_b phenotype did not possess the homozygous G^b/G^b genotype. It is apparent from Table 9 that no critical testing of specificity of the anti-G_b serum was accomplished

with these studies, because none of the mating pairs consisted only of mates possessing the G^- type.

$\underline{H^a}$. Alleles other than the H^a are known to be present in subjects of other studies by reason of reaction with other antisera (Hojny, 1973). None of those antisera was available for the present study. Thus, no evidence is available to indicate whether individuals represented in Table 9 as possessing the H^- phenotype are all of one genotype or are of various genotypes. Pigs possessing the H^- phenotype comprised a considerable proportion of the offspring of the mating classes where one or both mates possessed the H_a phenotype. This may be interpreted as evidence that at least some of the parents possessing the H_a phenotype did not possess the homozygous H^a/H^a genotype. Some critical testing of specificity of the anti-H_a serum was possible with this population. Matings between two individuals of the H^- phenotype produced 49 offspring which were also subjects of these studies. Each of those 49 offspring were found to possess the H^- phenotype, a finding which supports the concept that the H^a allele is co-dominant with all other H alleles present in this population.

$\underline{K^a}$. Antisera which detect antigens controlled by K alleles other than K^a were not available for this study. Hence, it is uncertain whether or not individuals possessing the K^- phenotype were all of one genotype. Several pigs possessing the K^- phenotype were found among the offspring of the mating classes wherein one mate possessed the K_a phenotype. This was considered as evidence that at least some of the parents possessing the K_a phenotype were not of the homozygous K^a/K^a genotype. Critical testing of specificity of the anti-K_a serum was feasible because 52 of the subjects were offspring of mating pairs where both parents possessed the K^- phenotype. Each of those 52 offspring were also of the K^- phenotype, an observation which is consistent with the concept that the K^a allele was co-dominant with other K alleles in this population.

L^b. Lack of reaction of a specimen with the anti-L_b serum, denoted as the L^- phenotype, is probably indicative of the presence of the L^a/L^a genotype (Andresen, 1962). But, because anti-L_a serum was not used for study of this population, it is not appropriate to assume the presence of the L^a allele. Table 9 shows that offspring possessing the L^- phenotype were produced from each of the three different mating classes. This observation is interpreted as evidence that at least some of the parents possessing the L_b phenotype were not of the homozygous L^b/L^b genotype. No critical testing of the specificity of the anti-L_b serum was accomplished, because none of the mating pairs consisted only of mates possessing the L^- phenotype.

I Phenotypes. It is obvious from Table 9 that mating categories containing certain combinations of I phenotypes were not included in the family studies. Absent from these studies were mating pairs wherein one mate possessed the I_b phenotype and the other possessed the I_{ab}, and also the type of mating wherein both mates possessed the I_b phenotype. The limited supply of anti-I_b serum, mentioned in a previous section, was not a cause of this incompleteness. Four of the five sibships for which anti-I_b serum was not used were selected for this omission by reason of knowledge that all of the parents possessed the I_a phenotype. The fifth sibship omitted (which was the last sibship selected for inclusion in the study) was produced from parents belonging to the $I_{ab} \times I_a$ category. Several sibships produced from parents in that particular category had already been included in the family studies data. Inclusion of one or more families belonging to the $I_b \times I_{ab}$ parental category could have been accomplished by more prejudicial selection of the family studies subjects. However, failure to include families wherein both parents possessed the I_b phenotype was almost certainly due to the fact that, until near the conclusion of the phenotyping program, none of the brood sows in the population possessed the I_b phenotype. Consequently, this portion of the family studies yielded quite meager evidence on the matter of specificity of the anti-I_a serum, That is, the I_b

phenotype was found in only one of the offspring included in the data in Table 9 and furthermore, the same individual boar was included in each of the six mating pairs comprising the I_b x I_a category. Therefore, it is appropriate here, to point out one feature of the data included in Table 14. The sibship described in Table 14 contained four pigs which possessed the I_b phenotype. This is strong evidence of the real specificity of the anti-I_a serum. That is, the I_b phenotype was detected within a sibship in which there was no reason to expect that it would be found, since it was known that the sire-of-record possessed the I_a phenotype. Additional comments are appropriate regarding the distribution of I phenotypes shown in Table 9. Evidence of specificity of the anti-I_b serum is seen in the observation that only the I_a phenotype was found among 46 offspring of parents possessing only the I_a phenotype. This latter observation is also taken as evidence of the true co-dominance of the I^b allele relative to the I^a allele. Further evidence of that co-dominance is the absence of the I_b phenotype among 27 offspring of matings between an I_a and an I_{ab} phenotype. No evidence was found that the I^b allele was functioning as a recessive factor in this population. The patterns of inheritance are entirely consistent with those described by Andresen (1964) in the Duroc and Hampshire breeds.

N. The family studies data do not include families in which both parents possessed the N_a phenotype. Thus, there has been no critical testing of the specificity of the anti-N_b serum. Clearly, such critical testing would be necessary before attempting to attach any significance to the finding that the N_a phenotype was present in only a small proportion (2/29) of the offspring of mating pairs consisting only of mates possessing the N_{ab} phenotype. It is apparent that the anti-N_b serum had some degree of specificity, since a total of five individuals were found in this population (see Table 16) whose erythrocytes did not react with that reagent (i.e., the N_a phenotype). However, it is necessary to guard against using only inadequate evidence for concluding that the anti-N_b is a sufficiently specific reagent. It is possible that in some but not all possessors of the

N^a/N^a genotype, the N_a antigenic determinant was simply modified sufficiently (perhaps by epistatically acting genes) so as to prevent cross-reaction with the anti-N_b serum. These studies, on the other hand, present strong evidence of the specificity of the anti-N_a serum. Only the N_b phenotype was found among 38 offspring of parents which possessed only the N_b phenotype. The results of these studies are consistent with the concept that the N^a and N^b alleles are co-dominant as described by Hojny et al. (1966).

Hx. Only two Hx phenotypes were found among the subjects (Table 10). The less frequent of those two phenotypes (Hx 1-2) was found in only a small proportion (5/33) of the mating pairs comprising the family studies data. Consequently, the data have only limited usefulness for evaluating the proposed modes of inheritance (Imlah, 1965; Schroffel et al., 1965) of that serum component. The data support the concept that the Hx^2 allele is co-dominant with the Hx^1 allele, since only the Hx 1 phenotype was found among 106 offspring of parents which possessed only the Hx 1 phenotype, and the Hx 2 phenotype was not found among the offspring of any of those mating categories in which at least one parent possessed the Hx 1 phenotype. An additional hematin-binding component, found as a co-migrant of the hemoglobin, was mentioned under Phenotyping Criteria. The apparent pattern of inheritance of that component is not considered here. It seems preferable that there should be more positive evidence of the distinction between that component and hemoglobin before consideration is given to a genetic basis for its presence.

Tf. Results shown in Table 10 are in agreement with the concept of co-dominance of the Tf^A and Tf^B alleles. A quite small proportion of the subjects of the family studies were families in which parents possessed only the Tf A or else only the Tf B phenotype. Only the Tf A was found among the three offspring of the mating pair consisting of parents possessing only the Tf A phenotype, and only the Tf B was found among the 13 offspring of parents which possessed only the Tf B phenotype. Co-dominance is further supported by the observation that

the Tf B phenotype was not found among the 40 offspring of matings in which just one parent possessed the Tf A phenotype, and also by the observation that the Tf A phenotype was not found among 21 offspring of matings in which just one parent possessed the Tf B phenotype.

Sa. Data shown in Table 10 do not contradict the concept that the Sa^B and Sa^C alleles are co-dominant. However, the distribution of phenotypes among families which were studied is such that the data do not constitute an effective test of the concept of single-locus control of the phenotypes via two co-dominant alleles. It would be possible to explain the distribution of phenotypes among offspring of pairs wherein at least one parent possessed the Sa BC phenotype, and the explanation would not require the concept that the Sa BC phenotype represents a heterozygous genotype. However, no elaboration of the alternatives is presented here. Instead, it is more essential to point out that the most useful observation obtained was the finding of the Sa BC phenotype in the seven offspring of the mating of a boar of the Sa B phenotype with a sow of the Sa C phenotype. This latter observation is important evidence in favor of the hypothesis that the Sa BC phenotype is due to heterozygous combination of the Sa^B and Sa^C alleles. Further significance of the results presented here is their original nature. There have been no previous reports on phenotypic distributions in families produced from the types of matings shown in Table 10. The study by Schroffel *et al.* (1965) originally suggested that the phenotypes described here are due to presence of two alleles, Sa^B and Sa^C. However, the test of inheritance patterns of those alleles was accomplished at that time using mating pairs wherein one or both members of any particular pair possessed a genotype containing a third allele, *viz.*, Sa^A. Beyond results reported here, useful testing of the hypothesized mode of inheritance would also involve the phenotyping of the offspring from matings between pigs each possessing the Sa C phenotype.

Am. Results with this population are in agreement with the concept that the Am phenotypes are determined by co-dominant alleles

at a single locus. Each of three alleles previously described by Graetzer et al. (1965) were found in this population, viz., Am^1, Am^2, and Am^3. The phenotypes found among offspring from any specific mating category were phenotypes which would be expected on the basis of this concept of the pattern of inheritance. The subjects produced from three of the specific mating categories listed in Table 10 did not include individuals having certain phenotypes which could be expected from those matings. These were the mating categories denoted by Am 2-3 x Am 1-2, Am 2-3 x Am 3, and Am 2 x Am 1-3. This is not a significant observation, because none of those three mating categories included more than five offspring. Disregarding the separation of reciprocal mating categories, it is seen from Table 10 that there were a total of eight different combinations of Am phenotypes within the group of parents described there. Therefore, these observations constitute a useful although incomplete test of the hypothesized pattern of inheritance. A more thorough testing of the mode of inheritance of determinants of these phenotypes would require larger numbers of offspring from the three mating categories mentioned above (Am 2-3 x Am 1-2, Am 2-3 x Am 3, Am 2 x Am 1-3, and/or reciprocals of these categories), and would also require inclusion of offspring from mating categories which were not represented in these data. Absent from among the subjects of these family studies are offspring from 13 different mating categories.

Pgd. Distribution of phenotypes among the subjects produced from parents with known Pgd phenotypes (Table 11) is in agreement with the concept of control by a single locus and presence of two co-dominant alleles within the population. Only the Pgd 1 phenotype was found among 20 offspring of parents which possessed only the Pgd 1 phenotype. The Pgd 1 phenotype was not found among 47 subjects known to be offspring of mating pairs in which one member possessed the Pgd 2 phenotype. The Pgd 2 phenotype was not found among 75 subjects known to be offspring of mating pairs in which just one mate possessed the Pgd 1 phenotype. Thus, neither allele appeared to function as a

recessive factor, nor was there evidence of any other alleles which had a recessive nature.

Phi. The various possible pair-wise combinations of three Phi phenotypes were represented among the mating pairs listed in Table 11. Only two subjects of the family studies were known to be offspring of parents which both possessed the Phi 1 phenotype, and both of those offspring possessed the parental phenotype. That observation is of little significance, because of the small number of individuals. The number of subjects which were offspring of parents known to possess the Phi 2 phenotype was 17, and each of those possessed the same phenotype as the parents. These observations are the results expected according to the concept that two co-dominant alleles at a single locus were the determinants of the three phenotypes (Saison and O'Reilley, 1971). A somewhat more significant feature of the distribution of Phi phenotypes shown in Table 11 was pointed out in the RESULTS section above, and the implications were discussed in the present section under the heading Phenotyping Criteria. It seems necessary to add a further comment about the interpretation of these observations on the subjects of the family studies. No evidence was obtained which suggested the presence of the hypothetical "Phi^0" allele in any subjects other than those five which were identified in the earlier sections. Lack of evidence of the presence of the hypothetical "Phi^0" allele in other subjects of the family studies cannot be considered as assurance that it in fact was absent. However, no attempts are made here at estimating the likelihood that the hypothetical "Phi^0" allele was present in other subjects without having been detected. Attempts to correlate performance variance with variation at the genes studied here utilized the knowledge of distribution of the three phenotypes, viz., Phi 1, Phi 2, Phi 1-2, and disregarded the evidence which suggested that more than two alleles may have been present in the population. If the "Phi^0" allele was in fact carried by only the few animals explicitly mentioned above, then homozygous genotypes have been incorrectly inferred for just two of the subjects of the least squares analyses which were accomplished. Those two subjects were the sows

identified as 1219-9 and 1338-5, each of which produced three of the litters used as data for the statistical analyses.

Paternity Assignments

There was at least one criterion available to the management as an aid to detection of errors in recording the breed-of-sire for individual litters. That criterion had quite limited usefulness however, since it is only applicable to matings involving some, not all, of the breed categories of the dams which were used. The uncertainty about the records of paternity leads to uncertainty about the designation of the breed categories to which individuals belonged. Consequences of this uncertainty should be weighed carefully when data from this population are used for conclusions regarding the effect of crossbreeding.

Early studies of the inheritance of coat color in pigs (Spillman, 1906; Severson, 1917) showed that with crosses between Berkshires and Yorkshires, the F_1 offspring possessed the Yorkshire coat color, i.e., non-pigmented, except that on rare individuals, some black spotting was observed. Observations during the present study are in agreement with those early observations. It appears as though a factor which is characteristic of Yorkshires acts as a nearly complete dominant in the inhibition of the black pigmentation which is characteristic of Berkshires. The rare occurrence of spotting in the offspring of the crosses between Berkshires and Yorkshires was indicative that this dominance was not absolute. Among members of the C line, considerable variety of coat patterns was evident; individuals with non-pigmented coats were found at moderate frequency, and black pigs with only small non-pigmented areas were also common. The frequent occurrence of totally non-pigmented individuals in the C line could have been due to incorrect paternity recordings. However, in absence of evidence from studies with additional marker genes involving adequate numbers of C pigs, it seems appropriate to attribute those occurrences to the retention in the C gene pool of the same genetic factor which causes inhibition of pigmentation in Y's. Data

obtained by observing coat colors of subjects of this study are not considered adequate in number for drawing conclusions about the mode of inheritance of this trait. Nevertheless, it seems reasonable to hypothesize that the determinant which effects inhibition of pigmentation behaved as a dominant factor, but that by some means, its inhibitory effect may have been removed. Perhaps this might have been brought about by action of modifier genes, or perhaps by means of interaction of the heterozygous genotype with non-genetic determinants of the trait. This concept is compatible with the findings that there was complete absence of pigmentation in some pigs which were neither Y's nor crossbred offspring of one Y parent, (viz., Composites), and furthermore, some pigmentation was found to occur in pigs which were first generation offspring of only one pure Y parent. The import of the tentative conclusions stated here is that observations on coat colors of the members of a particular litter probably would have had only limited usefulness toward detection of errors in recording the breed categories of sires.

Contents of Tables 9 through 11 are results from 33 sibships which were used for the family studies. Evidence presented in another section above was considered as effectively identifying four other sibships for which paternity records are apparently incorrect. Phenotype analyses were accomplished on both of the parents-of-record in three of those four sibships. However, the data should not be considered as a basis for estimation of the rate of incorrect recording of paternity in this population. Most of those 33 sibships comprising data for Tables 9 through 11 consisted of fewer than four individuals; four of those sibships consisted of three individuals, seven consisted of only a pair of siblings, and there were seven phenotyped pairs of parents which were each represented by only one offspring. The latter group of seven is included under the denotation "sibships" for convenience in description and discussion of the results. Individuals, or sibships consisting of only a few individuals, should be expected to be frequently ineffective for disclosing actual cases of misassigned paternity which have in fact occurred. The effectiveness of

a single pig or pair of siblings for such disclosures will vary according to the distribution of genotypes at the loci being used as the criteria for detection.

Although the results of family studies reported here cannot be used as reliable estimators of the frequency of mis-assigned paternity, such mis-assignment does not seem to have been a very rare occurrence. Furthermore, the results seem to indicate that not only were the identities of sires occasionally recorded erroneously, but also that those errors have resulted in some incorrect recording of breed categories. The sire-of-record for the litter described in Table 14 was a member of the B line. The birth date of that litter was more than one year after the phenotype analyses had been completed on each service boar and brood sow present within the B line. Results presented in Table 16 show that neither the H^a nor the Am^1 alleles were found within the B line. Those findings effectively exclude not only the sire-of-record from consideration as the true sire of the litter described in Table 14, but also exclude all Berkshires in the population. Four members of the litter possessed the H^a allele, but that allele was not possessed by the dam. Two members of the litter possessed the Am 1 phenotype, which should be interpreted as being due to inheritance of an Am^1 allele from each parent. The results shown in Table 14 do not exclude the Y line as the breed-of-sire of that litter.

Linkages Among Phi, Pgd, and H Genes

Evidence from members of some specific breed, indicating linkage of genes, might reasonably be viewed as only suggestive evidence that the same linkage occurs in other breeds within the species. Evidence from the Duroc breed indicating that the Phi, Pgd, and H genes are present within a single linkage group (Andresen, 1970; 1971) came to be of special interest for this study. This particular interest was due to the finding from L.S. analyses, that either the Pgd or the Phi phenotypes were correlates of variance in reproductive performance in

some of the sub-populations which were studied here. It is desirable to ascertain that the Phi, Pgd, and H genes were linked in the population studied here, instead of only supposing that the linkage was the same as that described by Andresen.

Some limited information was obtained from the present study regarding such linkage. There were only a few families in which parents possessed genotypes appropriate for providing direct evidence about linkage of these three genes. Among offspring of the mating pairs used for such direct evidence of linkage of the Phi and H genes, just one of 19 individuals showed evidence of recombination of those genes. Within the sibship used for direct evidence of linkage of the Phi and Pgd, none of the five subjects displayed evidence of recombination. These results are in agreement with the concept that the Phi gene is linked with the Pgd and the H genes. Obviously, such numbers of subjects do not provide useful measurements of the degree of linkage. Thus, it must be acknowledged that actual recombination rates in the subjects of the present study may be quite different from the estimates presented by Andresen (1970, 1971) (Phi-H recombination, 2.6%; Phi-Pgd recombination, 8.1%).

Rationale for Data Analyses

Critical consideration has been given to the usefulness of the data which have been gathered as descriptions of the genetic nature and the performance of the subject population. An observation which may be of primary importance in this respect is the finding of variance among the breed categories in terms of reproductive performance. The apparent superiorities of the (YxC + CxY) and (BxC + CxB) categories relative to respective parental lines (Tables 4 and 6) seemed worthy of attention.

Interest in that evidence about variance in performance is intensified by the additional evidence of contrasts among breed categories in terms of blood component phenotypes. Those contrasts are apparent from comparison of the (YxC + CxY) to certain other categories. Table 16 shows the contrasts. Those which are evident

from comparison between the B and the (YxC + CxY) categories are particularly interesting, because of the quite distinct contrast between the performance of those groups. The o phenotype was possessed by nearly all (YxC + CxY) subjects, but was the more uncommon of the three phenotypes present in the B line. The H^a, Sa^C, and Am^1 alleles each were found in significant fractions of the total (YxC + CxY) group, but were absent from the B line. Fewer than one-half of the (YxC + CxY) subjects, but nearly all B subjects, possessed the Tf^A allele. Consequently, it became of interest to make comparisons of performance, within the B line, among the groups of sows which possessed different A-o phenotypes, and among groups which possessed different Tf phenotypes. Similarly, comparisons which were of interest within the (YxC + CxY) category, were a.) between sows which possessed and those which lacked the H^a allele, b.) between sows which possessed and those which lacked the Sa^C allele, c.) among groups of sows which possessed different Tf phenotypes, and d.) among groups which possessed different Am phenotypes. It was apparent that, because of small numbers of subjects possessing some of the phenotypes at the loci mentioned here, it could not have been useful to attempt comparisons among each of the phenotypes which were present. Nevertheless, it was considered worthwhile to attempt comparisons among categories formed by pooling of certain phenotypes.

Applying the same line of reasoning as above, various comparisons were considered to be of interest within other lines. When distributions of individual phenotypes are compared between the (YxC + CxY) and the (BxC + CxB), it is seen that the Sa^C and Am^1 alleles were absent from the latter category, and the o and K^- phenotypes were distinctly less frequent than in the former category. Those distributions of the o and K^- phenotypes were a basis for interest in comparisons within the (BxC + CxB) category, between the sow groups categorized according to presence or absence of the A phenotype, and between the sow groups categorized on the basis of presence or absence of the K^a allele. The absence of the Sa^C and Am^1 alleles from within the (BxC + CxB) served to intensify interest in the comparisons among

(YxC + CxY) sow groups which possessed either different Sa phenotypes or else different Am phenotypes. A few of these motives for comparisons were considered to apply as well to data on the Y, YxF_1, BxF_1, and C categories.

Design of Data Analyses

It was considered necessary to apply the L.S. method for analyses of associations between phenotypes and performance, because it was the best means available for avoiding the confounding of the associations of the phenotypes controlled by separate loci. The distribution of phenotypes among breed categories as shown in Table 16 provided some indication that a common approach to each breed category would be a rather ineffective means of data analysis. This was made more apparent by considering the distribution of phenotypes among sows alone, without boars and shoats. That distribution of phenotypes (although not shown herein) was the principal reason for applying various L.S. models to separate sub-populations, instead of to the population as a whole.

Results presented as Tables 20a and 20b display the forms of the various L.S. models which were applied to the separate breed categories. It is apparent that the most complex model applied to the data on any particular breed category was not necessarily of the same structure as the most complex models applied to other breed categories. The reasons for such dissimilarities are at least partly obvious from the distributions of phenotypes (Table 16) which have been discussed. For example, none of the L.S. models applied to data on the B line involved the phenotypes controlled by the H, K, or Sa loci, because phenotypes controlled by those loci were found to be invariant among the B sows. (Table 16 shows that this monomorphism happened to be true of the entire B line, as well as the B sows.) However, the H phenotypes were included in the initial models for the C, (BxC + CxB), and (YxC + CxY) categories, and the K phenotypes were included in the initial models used for the (BxC + CxB), BxF_1, and YxF_1 categories (Tables 20a and 20b).

It was desirable to show results on any one variable in all seven breed categories on a single page. Therefore, although the non-genetic and genetic factors were included together in the more complex models applied to the various breed categories, L.S. constants for non-genetic variables are shown as Figure 9 and L.S. constants for genetic variables are shown as Figure 10.

There were successive modifications of the L.S. models for each breed category, accomplished by eliminating the genetic (or non-genetic) factor for which the value of the mean squares was lowest, until the model involved no factors with an "F" ratio less than the critical value for the 5% significance level. That elimination process pertained to analyses in which the dependent variable was the total fetuses per litter. Reasons for focusing on total fetuses per litter seem straightforward. It was considered most likely that viability of fetuses through the parturition process must be dependent upon more variables in comparison to viability over the period up to but not including parturition. There was no basis for expecting that one or a few genetic loci might exert a much greater determinacy toward number of live-born than toward total fetuses born. It was most consistent with the goal of the study to concentrate on the measurement for which variance could be most thoroughly accounted.

No L.S. analyses were attempted with performance data obtained on sows belonging to the (BxY + YxB) and CxF_1 categories. Those particular subsets of data were considered to be too small (17 litters and 24 litters, respectively) to be useful with that analytical technique.

Determinant of Regressed Fetuses

Findings from this study indicated that the occurrence of regressed fetuses was positively correlated with the number of fetuses carried to the end of gestation. This finding is consistent with the suggestive evidence of Perry and Rowell (1969) that the individual piglet's intra-uterine development may have a significant dependency upon the pattern of distribution of the arterial branches which supply

blood to the different segments of the uterine horn. Those authors found that along at least some uteri, the pattern of anastomoses among arteries supplying a uterine horn was such as to indicate that better blood supply is provided to fetuses located nearest the extremities of the horn, in comparison to those located at intermediate segments. Although quantitative evidence has not been presented to support this explanation of variance in pre-natal viability, it seems reasonable to hypothesize that any advantage from particular positions along the uterine horn due to blood supply may be more pronounced when relatively large numbers of fetuses are present.

Analyses of Variance in Total Fetuses per Litter

Non-genetic factors were found to be correlates of variance in performance in most of the breed categories studied. Attempts to identify markers of genetic factors which show a non-additive mode of action met with only slight success. However, the study has yielded evidence of associations between certain genes and variance in performance. There was a notable consistency among sub-populations with respect to the association of genes in certain linkage groups with variance in performance. Evidence of associations of those genes with performance is consistent with evidence which has been reported from other studies.

Non-genetic Correlates

Terms representing both of the non-genetic correlates of total fetuses per litter (Figure 9) were found to be useful components of the select L.S. models developed for most of the breed categories. However, one or both of those terms were absent from the models developed for certain categories, and the sizes and even the signs of the L.S. constants were found to vary among the breed categories.

Inclusion of a factor which accounted for semester of conception was based on the consideration that the physiological state of the gilt or sow around the date of conception might be dependent upon climatic

factors, and these would therefore be a determinant of the number of ova shed, and/or the extent of zygote wastage due to implantation failure. The demarcation dates between the semesters were chosen so that litters which were classified in the "early" category should have been conceived during the period of 23 September through 24 March; those litters which were classified in the "late" category should have been conceived during the period of 25 March through 22 September. Results do not seem to indicate that correlations between the semester of conception and number of fetuses had a biological basis. Positive values of L.S. constants for the early semester were found for the (BxC + CxB) and BxF_1 categories, but that finding seems inconsistent with the finding of a negative L.S. constant in the C line, and only a trivial association with semester in the B line. Perhaps these results are due to inadequate choice of demarcations of this discontinuous variable, although it actually did have an effect. Perhaps the L.S. method would provide a clearer interpretation of the effect, if the categorizations used were not semesters but instead categorizations of breeding dates into three periods: 1.) cold season, 2.) warm season, and 3.) transitional periods (i.e., both spring and autumn might have equal effects). However, the results found here might be an indication that the associations with semester are only apparent and are due to factors which had no actual dependence on the climatic conditions.

Although the age of dam at time of farrowing in the B subjects was not found to be a useful component of the L.S. model, this is probably due to the composition of the data subset pertaining to the B line. A majority of the B sows which served as subjects of this study were culled after farrowing not more than three litters. It was apparent from inspection of data on the B subjects that second litters farrowed by most sows were not larger than were first litters. Thus, if an age-dependent increase in litter size began in B sows after the age of about two years, the data used for this analysis would not disclose that regression. A somewhat opposite effect is evident with the C line. A relatively large value of the coefficient of regression on sow's age was found. However, most of the C sows

which were subjects of this study were culled after farrowing only two litters. Simple inspection of the data showed a rather consistent increase in sizes of second litters in comparison to first litters. Thus, if regression of total fetuses per litter on age of dam was actually exponential to some degree in the C line, instead of strictly linear, the data subset used for this analysis would not have permitted disclosure of that non-linearity.

The effect of age of dam at first parturition on performance at later parities was omitted from the L.S. models on the basis of results of plotting total fetuses per litter vs. age of dam at first parity. (Those results are represented by the correlation coefficients in Table 18.) It should be acknowledged that this particular evaluation of the regression of performance on age of dam at first parity may have been inadequately designed. The pooling of certain breed categories [(BxC + CxB) with BxF_1, and (YxC + CxY) with YxF_1] may have had the effect of obscuring regression which was occurring within particular breed categories. The pooling was done however, because it seemed unlikely that data for individual parities within most cross-bred categories were adequate for demonstrating such an association.

Genetic Correlates

Evidence of apparent overdominance was found within some breed categories for certain phenotypes. Not more than one locus within any single breed category was identified as a correlate of such over-dominant interactions (Table 21). Both of the loci which were so identified (Tf and Am) were found to be polymorphic within each of the seven breed categories. However, evidence of heterozygote advantage associated with the Tf was found in only two of the breed categories (Y and C), and such an association of the Am was found in only one breed category (BxC + CxB). Aside from those findings, evidence of heterozygote advantage associated with the Phi was found in the YxF_1. However, the evidence from the YxF_1 was obtained using a pre-select model and, due to the size of the "F" ratio (3.10), it is of less

credibility than the aforementioned evidence about heterozygote advantage.

Failure to demonstrate consistency of association of apparent overdominance with particular loci, despite the polymorphism at those loci, is considered as evidence that any true overdominance which was occurring was mostly or perhaps entirely due to loci other than those which were objects of these phenotyping studies. Perhaps the most straightforward interpretation of the findings is that genes which are determinants of variance in litter size are located together within the same linkage groups with the genes which control the Tf and Am phenotypes. More precisely, considering either the Tf or Am separately, the variance found associated with that marker locus is not to be attributed to the effects of only a single locus. Instead, the determinants of performance linked to that marker were probably two or more individual gene loci, and those loci may even have been separated by several or many transcribable loci. Probably, the finding of apparent overdominance in only one or two breed categories is evidence that the genes which were the prolificacy determinants were dissimilar among at least some of the breed categories studied. That is, within the Y and C lines, the functional qualities of the prolificacy determinants linked to the Tf were such that an overdominant combination could occur, whereas different alleles were present at those determinants in other breed categories, and those alleles did not result in overdominant combinations. It is not necessary to suppose that the determinants linked to the Tf, which yielded an apparently overdominant effect in one sub-population, were allelic to those which, in another sub-population, yielded an additive effect. A line of reasoning which is analogous to the foregoing could account for the finding that heterozygote advantage is associated with the Am in the (BxC + CxB) but not in other categories.

It is evident that this study has not resulted in successful identification of specific loci which are the basis for the non-additive effects of crossbreeding. The results of the phenotyping studies could have been considered as indications that L.S. analyses would not be effective for disclosing non-additive effects from

combinations of alleles. That is, only very few crossbred sows were shown to possess a phenotype not found in parental lines (viz., Am 1-3). However, the L.S. analyses were accomplished despite that finding. Part of the motivation for more detailed study was the observed distribution of phenotypes. Relatively few of the genes which were studied were found to be monomorphic within breed categories. Another reason for proceeding to the L.S. analyses was the variance in performance that was evident within, as well as among, breed categories. A section above made reference to the high frequency of standard deviations greater than ±2.00 found for the averages describing performances of crossbred categories and respective parental or grandparental populations. That observation is just as valid with respect to the averages for only crossbred subjects of the phenotyping studies (Tables 6, 7a, and 7b). All of the standard deviations which were less than ±2.00 were of averages on samples consisting of not more than 11 litters. The finding that most of the loci studied in any category were polymorphic was encouraging for the idea that a considerable proportion of the variance was due to genetic contrasts among individual sows.

Although the overdominance which was apparent from these studies was not consistent among breed categories, there were some results obtained which were interesting in other respects. The genes which seemed indicative of heterozygote advantage in certain breed categories were found to be associated with variance in total fetuses per litter in other breed categories as well. Those correlations were of interest despite the fact that in those cases, there was no evidence of overdominance.

I. Tf

Association with additive effects was found for the Tf in the (BxC + CxB). The "F" ratio found using the select model was a quite large value (8.04, which was greater than the critical value for the 0.5% level of significance). The results from pre-select models

applied to two other breed categories also seem pertinent to the matter of association between Tf phenotypes and performance.

The pre-select model applied with the (YxC + CxY) may be indicative of a real correlation between the Tf phenotype and variance in total fetuses per litter (Table 20b). That model included terms representing the Tf, Am, and Pgd loci, and the "F" value obtained for the Tf phenotypes (6.65) was greater than the critical value for the 5% level of significance. When the pre-select model was modified by deleting the terms for which "F" ratios had been found that were less than the critical value (5%), then the "F" ratio for the Tf also was found to be less than the critical value at the 5% level (data not shown). Thus, if some less stringent criterion had been used in deciding whether or not a model should be classified as select, then a select model probably would have been obtained for the (YxC + CxY), and it would have included the Tf phenotypes. As with the (BxC + CxB), it was found from the model applied to the (YxC + CxY), that the Tf B was the phenotype with the higher L.S. constant (1.37). Evidence from this study is inadequate to indicate whether an additive or a non-additive mode of gene expression was occurring. Only two Tf phenotypes were found among the sows in the (YxC + CxY) breed category.

The pre-select model applied to the BxF_1 seemed of interest for somewhat similar reasons. That model included terms representing the K, Tf, and Am phenotypes, besides the non-genetic variables (Table 20b). The "F" value found for the Tf phenotypes is slightly greater than the critical value for the 10% level of significance (2.4). Thus, the pre-select model for the BxF_1 yielded results which are consistent with those cited for four other breed categories indicating that the Tf phenotypes were associated with variance in total fetuses per litter. However, the pattern of contrasts among L.S. constants in the BxF_1 (Figure 11) was unlike contrasts found with models applied to those four other categories. Among the Tf phenotypes in the BxF_1, that for which the highest L.S. constant was found was the Tf A (0.84), and the constant of lowest value (-1.44) was that of the Tf B.

The finding that the Tf phenotype was a correlate of performance in five of the breed categories is viewed as forceful evidence that

its association with prolificacy variance is real, and not coincidental. That conclusion seems reasonable despite the finding that the order of the values of L.S. constants for the Tf phenotypes (Figures 10 and 11) was not the same in each of those five sub-populations. The latter observation is compatible with the concept that the association reflects a relationship such as linkage to loci of prolificacy determinants. Hence, the interpretation of the observed inconsistency among breed categories could be simply that a prolificacy determinant having a positive effect (i.e., greater number of fetuses per litter) was coupled with the Tf^B allele in the (BxC + CxB) and (YxC + CxY) categories, but that particular prolificacy determinant was coupled with the Tf^A allele in the gene pool of the BxF_1. Finally, the additional inconsistent aspect, the heterozygote advantage associated with the Tf in the Y and C lines, may be interpreted as evidence that, coupled to the Tf within the Y and C gene pools, there were combinations of prolificacy determinants which were unlike the determinants in the other three sub-populations mentioned here. That is, the effects of the determinants in the Y and C gene pools were such that non-additive combinations were formed. It is not necessary to suppose that the Tf-linked determinants of variance in the (BxC + CxB) and (YxC + CxY) were allelic to the Tf-linked determinants of variance in the Y and C lines.

Another possible explanation should be suggested. The variance may be at least partly due to differences in viabilities of conceptuses possessing different genotypes, rather than due to differences in characteristics of individual sows. That is, genetic factors affecting litter size may have been genes which were expressed in the early stages of gestation. (Perhaps the period of expression was even prior to implantation of the embryo.) Perhaps, within a specific breed category, sows of a particular genotype farrowed relatively small litters because the genotype causing lowest viability was present in a greater proportion of conceptuses. The dams possessing other Tf genotypes farrowed relatively larger litters because a smaller proportion of conceptuses possessed the genotype associated with lowest viability. Certain aspects of results of the study are

consistent with this idea, although by itself it does not satisfactorily account for the findings. It is useful to consider the hypothesis that in sows of the Y, C, and (BxC + CxB) categories, zygotes with the Tf^A/Tf^A genotype possessed lower in utero viabilities than zygotes with other genotypes.

It seems reasonable to suppose that there was no assortative mating with respect to the Tf genotype. That is, the proportion of sows of a particular Tf genotype which were mated to boars of the Tf^A/Tf^A type was presumably the same for each sow type. The same presumably was true about matings with boars possessing the Tf^A/Tf^B genotype. (It was evident from the phenotyping studies that most service boars were of the Tf A and Tf AB phenotypes.) If it is further supposed that in individuals of the Tf^A/Tf^B type, gametes of the Tf^A haplotype were produced at the same rate as those of the Tf^B haplotype and both types were equally fertile, then the conceptuses of sows possessing the Tf^A/Tf^A genotype would have consisted of a greater proportion of Tf^A/Tf^A genotypes, compared to the proportions conceived by sows possessing other genotypes. Thus, if conceptuses possessing the Tf^A/Tf^A genotype had the lowest early in utero viability, then sows possessing the genotype Tf^A/Tf^A would have carried fewer fetuses to the end of gestation than would other sows. Evidence which is suggestive of this idea is that in the Y, C, and (BxC + CxB) breed categories, the L.S. constants for the Tf A were less than the constants for the other Tf types (Figure 10). The concept is also consistent with the finding that in (YxC + CxY) category, the L.S. constant for the Tf AB was lower than that for the Tf B (Figure 11). Since boars of the Tf A and Tf AB types were in service, (YxC + CxY) sows of the Tf AB type would be expected to conceive some zygotes possessing the Tf^A/Tf^A genotype. However, the concept does not satisfactorily account for the fact that in the Y and C categories, the L.S. constants which are of highest values are those for the Tf AB type. Applying the assumptions set forth above, it would be expected that Tf^A/Tf^A genotypes would occur among zygotes carried by the Tf AB sows. Thus, if the Tf^A/Tf^A conceptuses were of lowest early in utero viability, this would have had the effect of decreasing the number of

fetuses carried to the end of gestation by Tf AB sows, but would not have affected the number carried to the end of gestation by Tf B sows. The sizes of L.S. constants for Tf AB relative to other phenotypes is not necessarily a serious contradiction of the concept of differing viabilities of conceptuses, however. It may have been, that the maternal effects of the sort suggested in previous paragraphs were superimposed upon the effects of the conceptus genotype, so that the viability of the Tf^A/Tf^A conceptuses was higher in Tf AB dams than in Tf A dams.

There was no attempt to verify the hypothesis outlined above. It did not seem that such an attempt would be worthwhile. It was pointed out that the family studies which were accomplished allowed the conclusion that there was a remarkable frequency of cases of mis-assigned paternity. It was necessary to admit the possibility that, with all litters used for measuring reproductive performance, the rate of mis-assignment of paternity might have been at least as great as the rate found among the families which were phenotyped. Besides this consideration, some of the data on reproductive performance in certain breed categories were litters for which the sire-of record was a boar which was not phenotyped.

II. Am

Am phenotypes, mentioned above as displaying a pattern of heterozygote advantage in one breed category (BxC + CxB), also were found to be associated with variance in performance in the B line (Table 20a). Evidence was inadequate for indication of the mode of gene expression associated with the Am in the latter breed. Data were obtained from only one B dam having the Am 3 phenotype. It is possible that a simple additive mode of expression was operating, a more positive effect being associated with the Am^2 allele and a negative effect associated with the Am^3 allele. Pertinent to the evidence from the B and (BxC + CxB) breed categories, are indications of correlation of the Am phenotypes with variance in prolificacy in two other breed categories.

The pre-select model applied to data from the (YxC + CxY) was one which very nearly met the criterion for being classified as select. The model consisted of terms representing the Tf, Am, and Pgd, and only the "F" value for the Pgd phenotypes (Table 20b) was less than the critical value (5% level). Thus, if some less stringent criterion had been used in deciding whether or not a model should be classified as select, then a select model probably would have been obtained for the (YxC + CxY), and it would have included the Am phenotypes. It is necessary to hold reservations about the indications which the data provide regarding the mode of gene expression associated with the Am in this breed category. The distribution of phenotypes was not sufficient to allow an analysis which could yield clear evidence on this point. The results (Figure 11) are consistent with either of two concepts:

a.) there were purely additive effects associated with the three alleles found in the (YxC + CxY) category, and the prolificacy determinant with the most positive effect was coupled with the Am^3 allele;

b.) heterozygote advantage was occurring, and the greatest advantage was associated with the Am 2-3 phenotype.

The pre-select model applied to the BxF_1 (Table 20b) was one which fell short of the criterion for select models. The "F" value for the Am phenotypes (2.71) was less than the critical value for the 5% significance level, but was greater than the critical value for the 10% level (2.4). The pattern of contrasts among L.S. constants was that of an additive mode of gene expression (Figure 11) with the most positive effect associated with the Am^3 allele. Evidence regarding the associations of Am phenotypes with performance is less persuasive in the BxF_1 than in the other three breed categories [B, (YxC + CxY), and (BxC + CxB)], because of the size of the "F" ratio found with the model referred to here.

Evidence of correlation of Am phenotypes with prolificacy variance in four of the breed categories is a consistency which seems indicative of real, rather than apparent associations. However, inconsistencies

in the ordering of values of the L.S. constants cause uncertainty about the nature of the determinants which were correlated with the Am phenotype. An overdominant combination seems the most likely basis for the variance found in the (BxC + CxB) category and overdominance also may have been the basis for the variance found in the (YxC + CxY). But in contrast, there is lack of evidence for an overdominant combination in the BxF_1 category. A reasonable concept, in view of those observations, is one analogous to that presented in a paragraph above, i.e., the results may be due to the presence of the Am locus within the same linkage group with some combination consisting of two or more individual gene loci which were determinants of performance. Thus, additivity was evident in one or two breed categories, but non-additivity was apparent in at least one other, depending upon the functional qualities of the determinants which were coupled with the specific Am alleles.

III. Other Components of Select Models

Sufficient distributions of N phenotypes were found in four of the breed categories so that those phenotypes could be included in the initial models (Tables 20a and 20b). The distributions were not sufficient so that subjects possessing the N_a phenotype could be treated as a separate category. Data from sows possessing the N_a and N_{ab} types were pooled to form a single category. The select model obtained for the Y category yielded an "F" ratio for the N phenotypes (7.96) which was greater than the critical value for the 1% significance level (6.8). There were no notable correlations of N phenotypes with performance in other breed categories. The finding of such correlation in the Y line but not in other categories may be of most interest with regard to the effect of additions of new lineages to the Y line in recent years. The positive L.S. constant was that for the N_b phenotype (0.72). The N^a allele was not found in any subjects in the "native" Y sub-group. Careful consideration of the distribution of the N_{ab} phenotype among Y's provided strongly suggestive evidence that the N^a allele was introduced by sires which were of exopatric

origin. One such service boar was unrelated to the herd, and it is likely that a second such service boar inherited the N^a allele from its sire, which was also unrelated to the herd. Conclusive evidence of that origin was not obtained, because the latter boar was produced from artificial insemination of an indigenous sow not available at the time of the study. It appears that within the Y line, the N^a allele was coupled with a locus which had a negative effect compared to the effect of a factor which was coupled with the N^b allele. The N^a allele apparently was not serving as such a marker in other categories.

Among components of the select model used with the (BxC + CxB) were terms representing the Phi phenotypes. The "F" ratio obtained for the Phi (5.81) was considerably greater than the critical value for the 5% significance level (3.2). The pattern of contrasts among L.S. constants for the Phi phenotypes was characteristic of an additive mode of gene expression.

One of the L.S. analyses using data on the YxF_1 category may also be of interest with respect to the Phi. Results from the pre-select model used with that category were included in Table 20b, because the "F" ratio for the Phi (3.10) was barely less than the critical value for the 5% significance level (3.2). This may be viewed as evidence which supports that found with the (BxC + CxB). However, findings from these two categories are inconsistent with respect to the pattern of contrasts among the L.S. constants (Figure 11). The pattern of contrasts is characteristic of a simple additive mode of expression in the (BxC + CxB), but the pattern in the YxF_1 indicates overdominance. These findings with the Phi are best interpreted as evidence of linkage of the Phi locus with the locus of a determinant of performance. Results do not require a concept involving more than one gene locus as the linked determinant. However, there is no evidence which indicates that the locus of the factor effecting an additive mode of expression in the (BxC + CxB) was allelic to the locus at which there was apparent heterozygote advantage in the YxF_1.

The "F" ratio found for the K phenotypes in the BxF_1 is larger than the "F" ratios found for any other genetic variables studied (Tables 20a and 20b). Statistical significance ($P<.005$) was indicated

for this correlation of the K phenotypes by the initial model applied to the BxF_1 category. Distributions of K phenotypes were found to be sufficient in two other breed categories so as to permit inclusion of the K phenotypes in L.S. models applied to those breed categories (viz., YxF_1 and BxC + CxB). However, very little of the variance was correlated to the K phenotypes by analyses on those latter two categories. The findings are compatible with the following concept about these contrasts in associations of the K phenotypes with performance. A prolificacy determinant perhaps was present in the same linkage group as the K gene. It may be that the K^a allele in the Y gene pool was coupled with a prolificacy determinant which was different from that coupled with the K^a allele in the B gene pool. Furthermore, it seems quite likely that none of the (BxC + CxB) subjects inherited a K^a allele from a C parent (because the K_a was not found among any C subjects). Thus, it seems highly pertinent to note the likelihood that, among those three breed categories, only members of the BxF_1 would have inherited two K-linked prolificacy determinants from the B gene pool (although some of them, perhaps about one-half, probably inherited just one). It is uncertain whether or not many of the K_a individuals in the YxF_1 category possessed the homozygous genotype. However, it may be that K^a-linked prolificacy determinants descended from the Y gene pool were dissimilar from those originating from the B gene pool. The high correlation between K phenotypes and performance in the BxF_1 but not in the YxF_1 may therefore have resulted from presence of two K^a-linked determinants from the B gene pool in many of the BxF_1, whereas the other two categories mentioned here possessed not more than one K^a-linked determinant descended from a B parent or grandparent. Lack of apparent correlation of performance with K phenotype in the B category perhaps was correlated with a near total absence of variation of K genotype in that sub-population.

An alternative having some similarity to the above concept is also satisfactory. The results might be the consequence of presence in the BxF_1 category of phenotypes controlled by the K locus itself, which were absent from, or present at insignificant frequencies, in the YxF_1 and (BxC + CxB). At least one other allele has been found at

the K locus which yields an immunogenic phenotype (Andresen, 1962). Availability of antiserum which detects that factor might have been helpful for determining whether or not this latter explanation is correct.

Efficacy of Multigenic Analyses

Evidence of the shortcomings of the select models is that the error standard deviations for all except one breed category (C) were in the range from 2.28 to 2.98 (calculated from Tables 20a and 20b). However, considerable efficacy of most of the select models is indicated from comparisons between R^2 values from select models (R^2_s), and those obtained with models containing only non-genetic variables (R^2_{ng}). Values of the ratio R^2_s/R^2_{ng} varied considerably among the six categories for which select models were developed. The value of R^2_s found for the YxF_1 was about the same as R^2_{ng} ($R^2_s/R^2_{ng} = 0.95$). The value of R^2_s found for the B category was barely larger than R^2_{ng} (i.e., $R^2_s/R^2_{ng} = 1.12$). Also, the values of R^2_s/R^2_{ng} found for the C and BxF_1 categories were only 1.44 and 1.80, respectively. Ratios of R^2_s to R^2_{ng} found with the Y and YxF_1 categories were about equal (2.18 and 2.20, respectively). The ratio found from the (BxC + CxB) data (2.70) was larger than the aforementioned ratios.

The select model which yielded the smallest value of R^2_s was that of the YxF_1. It was the select model of least usefulness for this thesis. No genetic correlates of number of fetuses per litter were identified for the YxF_1 using the select model. A select model yielding an R^2_s value practically as small as the aforementioned was that of the B line (0.102). Results of L.S. analyses with the B data are unlike results from most of the other categories in at least two respects. First, as shown in Table 20a, application of model NG indicated that neither non-genetic variable was a useful correlate of performance. Secondly, neither non-genetic variable was included in the select model. So it is perhaps worthwhile to point out that from a model which consisted of one genetic variable but omitted both non-genetic variables (viz., the select model), the R^2_s value (0.102) was

slightly greater than the value of R^2_{ng} (0.091). However, comparisons between values of R^2_s and R^2_{ng} found with four other breed categories should be evaluated somewhat differently; the select models for those four categories each contained one or both non-genetic variables. Therefore, the ratio R^2_s/R^2_{ng} is an indicator of the extent to which inclusion of one, two, or three genetic variables along with non-genetic variables served to form models which more effectively accounted for performance variance. Also, it seemed that useful information was obtained from the pre-select model used with the (YxC + CxY) even though those results fell short of the criterion for select models. Therefore, it is worth noting that the ratio of the R^2 for the pre-select model (R^2_p) to R^2_{ng} was 2.46, a value which is within the range of those found for the four categories referred to immediately above.

Potential Application to Breeding Program

Sizes of L.S. constants found for certain phenotypes were such that it might be expected that knowledge of those phenotypes could be useful for selection of breeding stock. Within certain breed categories of the population described here, the knowledge of phenotypes at only one or two loci might permit efficient selection of separate groups of gilts having extremes of reproductive potential. That selection could be accomplished prior to puberty.

Differences between the highest and lowest L.S. constants among phenotypes of an individual component, in several cases, were nearly as great as or greater than the standard deviation in the breed category. The outstanding example of this was the difference between the highest and lowest L.S. constants (Figure 10) among Tf phenotypes in the (BxC + CxB) category (4.93), which was distinctly greater than the standard deviation found in that breed category (2.99). Comparable examples may be seen in other breed categories, and most of those were found with phenotypes controlled by various other loci. (Standard deviations in all of the other six breed categories were in the range from 2.31 to 3.45.)

It has been contended (Jensen et al., 1968) that a reasonable expectation is that selection on the basis of litter size would require up to ten generations in order to achieve an increase of one piglet per litter. That prognosis did not take into consideration the possibility of an improved approach which might be adopted, based on observations by Revelle and Robison (1973). The latter workers suggested that in females having highest genetic potential for prolificacy, there is frequently a diminished expression of that potential because those individuals have been disadvantaged by being born into relatively large litters. If that concept is valid, it follows that selection of breeding stock based on performance of the grand-dam would be more efficient than selection based on the performance of the dam. Even if such an approach were proven to be more effective, it would be expected that about seven generations of selection would be required for an increase of one piglet per litter. The bases for that expectation are the same selection differential as was implied by Jensen et al., and the estimate of heritability (0.28) found from regression of performances of grand-daughters on those of grand-dams (Revelle and Robison, 1973).

Although there is an impressive difference between the contrasting groups identified in this study, it is necessary to be cognizant that the correlations indicated above under RESULTS are pertinent to specific populations. Results obtained from the data analysis described probably have only limited application in selection of breeding stock. An essential prerequisite to the occurrence of the correlations described above may have been the maintenance of the parental breed categories (B, Y, and C) as closed or semi-closed lines for several generations before the crossing of lines. That particular program perhaps was effective for restricting the variety of couplings of specific marker alleles with specific prolificacy determinants, within each parental category. As indicated (see MATERIALS AND METHODS), the two purebred lines were founded using groups within each of which, there was obvious co-ancestry; also, only a rather small number of individuals served as founders of the C line. Comments above pointed out that the correlations with genotypes were probably due to

linkage between markers and prolificacy determinants. It follows therefore, that findings from this study probably could not be consistently applied with success in exclusively choosing gilts which would show superior lifetime performance. Most populations probably would contain a variety of coupled combinations of the marker alleles and the alternative forms of the prolificacy locus.

Comparisons to Other Populations

Other studies yielding evidence of correlations of blood component variation to reproductive performance were cited in a section above. Following are comparisons between portions of those earlier studies and some specific aspects of results of the present study.

Jensen et al. (1968) found correlations of H phenotypes with reproduction in two breed groups. It was found from L.S. analyses that the H_c phenotype was significantly correlated with higher numbers of live-born per litter in populations of the Duroc and Hampshire breeds. Apparently the H_c phenotype in that Duroc population was due to homozygosity of the H^c allele, and there was persuasive evidence that the effect associated with the H^c allele in the Duroc population was additive. The Hampshire population studied by Jensen et al. possessed one or more H alleles besides the H^a and H^c (no other antisera were used for attempting identification of alleles other than the H^a and H^c). The H^a and H^c alleles were of minor frequencies in the Hampshire population. Because of this, it was unclear whether or not the favorable effect associated with the H^c was expressed in an additive manner.

More recently, Rasmusen and Christian (1976) presented evidence of association of H phenotypes with occurrence of a pathologic state referred to as the Porcine Stress Syndrome. Considerable effort has been given to the study of this syndrome as it is found in certain populations (Christian et al., 1968; Jones et al., 1972). There are reasons to suspect that susceptibility to this malady may be distinctly more frequent than are individuals which develop the symptoms. Rasmusen and Christian (1976) suggest that variation in some component

of muscle cell membranes may be controlled by the H gene and that component may be a basis for the variation in individual susceptibilities to the syndrome. There was fairly strong indication of correlation between the H genotype and susceptibility. Whether or not the H gene itself is the determinant remains uncertain. Thus, although the present study yielded very little indication of correlation of H phenotypes with performance, it is pertinent to recall the evidence that the H locus is linked with the Pgd and Phi loci. Association of the H phenotypes with variance in live-born per litter, association with variation in susceptibility to the Porcine Stress Syndrome, and the evidence presented herein of correlation of Phi phenotype with variance in total fetuses per litter, amount to an impressive pattern of indications that the Pgd-H-Phi linkage group is the locus of one or more important determinants of variance in fitness. Identification of those determinants remains to be accomplished. That one or more of them may determine a component of cell membranes in certain tissues, as suggested by Rasmusen and Christian, remains a tenable concept. Other tissue components, besides cell membranes, should not be disregarded as the possible location of this variant.

Some evidence was presented for a correlation between Am phenotype and variance in the number of live-born in the Hampshire population studied by Jensen et al. (1968). Distribution of phenotypes within that population was only sufficient to demonstrate existence of correlations, without permitting insights about whether additive or non-additive gene expression was occurring. Two phenotypic categories were considered in the analysis of that population. A positive L.S. constant (0.48) was found for the category consisting of the heterozygous genotype, and a negative constant was found for the homozygous genotype (I.S.U. Staff, 1975). It should be mentioned also that in the other population (Durocs) in which Jensen et al. (1968) sought correlations with performance, only one Am phenotype was found. The evidence of correlation with variance within the Hampshire population supports results of the present study indicating that the Am locus was associated with variance in total fetuses per litter.

Further remarks are appropriate here regarding the study referred to in the preceding two paragraphs (Jensen et al., 1968), from which evidence was obtained for correlation of the Am phenotype with reproductive performance in the Hampshire breed and correlation of the H phenotype with reproductive performance in both the Duroc and Hampshire breeds. The approach used for genetic analysis of the subjects of that study was quite similar to the approach used in the present study. However, there were contrasts between the two studies, which should be pointed out. There were considerable differences with respect to population structures. Data were collected by Jensen et al. from a herd which was maintained using a breeding program (USAEC Contr. Rep., 1969) quite different from that of the Oregon State herd. The Hampshire and Duroc populations studied both originated from larger founder populations. Subsequent to establishment of the latter two populations, there was a continuing program of introduction of service boars procured from stocks usually having no relationship to the founder stocks. Such twice-annual procurements were the rule over the entire duration of the data collection period, which lasted four and one-half years. There was continuing attention towards attempting to obtain, at each breeding season, boars which were unrelated to previously obtained stock. It is apparent from the description of the breeding program, that the subjects of that study comprised populations which were inbred to a practically insignificant degree. Furthermore, there were no crossbred subjects studied. The evidence from analyses of data did not preclude the possibility that the H and Am loci were actual determinants of the performance variance. Considering the indications that the populations had rather broad genetic bases, it seems remarkable that there was evidence of significant correlations between performance and the Am phenotypes in Hampshires, and evidence of highly significant correlations between performance and the H phenotypes in both breeds. Results of the analyses of variance were compatible with the interpretation that the Am and H loci themselves were determinants of variance in performance. Results from the study of the Oregon State population do not provide evidence that the H locus was an actual determinant of variance in performance. Those data do

make it seem unlikely that the Am locus was a determinant of performance variance (i.e., note the dissimilarity between the patterns of L.S. constants found with the B category and the pattern found in the (BxC + CxB)).

Associations of specific Tf phenotypes with determinants of reproductive success were found in certain populations as cited earlier (Kristjansson, 1964; Imlah, 1970). Results of those studies gave no persuasive evidence that the Tf phenotype itself was a determinant of the variance in reproductive success. It was suggested instead that the observations were due to linkage between the Tf gene and the locus of a recessive, early-acting lethal factor. There is no evidence that the factors involved were of the same locus in the populations studied by Kristjansson (Canadian populations of the Yorkshire and Landrace breeds) and Imlah (population descended from a mixture of breeds, including Yorkshire and Landrace), although that is an interesting possibility. Association of the Tf phenotype with variance in reproductive performance in the Oregon State population was less apparent than in other populations cited above; the Tf-associated determinant of performance variance in the O.S.U. population was made evident by means of L.S. analyses which simultaneously considered several genetic variables. There was no indication of presence of factors so extreme as a totally lethal factor. Certainly there is no substantial reason for supposing that the determinant in the subjects of the present study is allelic to those in the other populations. Nevertheless, it is important to note the coincidence of the evidence of these correlations between fitness and Tf phenotypes. Although the determinants of reproductive performance in these three studies probably are not allelic, the evidence from them may be indicative that the Tf gene is located within a linkage group containing a complex of genes which are important determinants of reproductive performance. Likely means for exploiting this possibility are discussed in a subsection below.

Significance Toward Future Experimental Designs

Results of the present study are important for indication of favorable population structures to be used in studies of the genetics of reproductive performance. Previously, there has been skepticism toward usefulness of phenotypes controlled by a single locus as a selection criterion. It has been felt that prolificacy is a trait which is dependent on such a large number of loci that the determinant(s) found correlated with any single marker would not account for a worthwhile proportion of the variance in the trait. Still, it remains reasonable to hypothesize that the usefulness of at least some markers may be strongly dependent on the extent to which the breeding program has diminished the variety of couplings between markers and prolificacy determinants. Results reported herein might serve as background information quite useful in designing a method of testing this hypothesis.

It is proposed that future studies of the genetic basis of prolificacy should be directed toward describing contrasts in functional characteristics of alleles present at some few loci which have apparent effects on variance in reproductive performance. The population to be used for such a study could be of a structure different from that of populations typically used in the past for studies of reproductive performance.

Blocks of subjects could be produced, each subject serving as a single test of the effect associated with a specific phenotype. A single block would consist of full sisters; each of the subjects within a block would possess a particular marker phenotype. They would have either one or two specific marker alleles and any such allele among all subjects would be of monogametic origin. This particular uniformity could be effected by means of certain full-sib matings during the production of the subjects. Figure 13 is intended to clarify details of this plan by illustrating the method for production of data obtained from an individual block.

Marker gene = M; alleles = M_1, M_2

M represents a gene which is evidently linked to the locus of a prolificacy determinant

GENERATION 1 Selection of carrier of marked chromosomes

$M^1/M^2 \times M^2/M^2$ genotypes of mating pair

GENERATION 2 M^1/M^2, M^2/M^2 genotypes of offspring†

GENERATION 3 Production of homozygous configuration of marked gene

$M^1/M^2 \times M^1/M^2$ (intermating of heterozygous siblings from generation 2)

M^1/M^1, M^1/M^2, M^2/M^2 (select M^1/M^1 genotype as grandparent of test subjects)

GENERATION 4 Combination of M^1 allele with M^2 allele of monogametic origin

$M^1/M^1 \times M^1/M^2$ (outcrossing of member of generation 3, to genotype M^1/M^2)

M^1/M^1, M^1/M^2

GENERATION 5 Production of subjects for testing

$M^1/M^2 \times M^1/M^2$ (select and mate heterozygous siblings, from generation 4, using only one male)

M^1/M^1, M^1/M^2, M^2/M^2

TESTING Test subjects from generation 5, by mating to an unrelated boar.

Analyses of results: analyses of variance in litter size, with classes consisting of data from dams possessing genotypes M^1/M^1, M^1/M^2, M^2/M^2.

†Conclusiveness of results might be enhanced by attention at certain steps to genotypes at other genes besides M; genes previously identified as correlates of variance in prolificacy could be made monomorphic by careful selection of offspring used at generation 3.

Figure 13. Production of a sibship for use as a block of subjects in testing relative values of genotypes controlled by a single locus. Diagram only describes a single block of subjects; additional blocks could be produced, as replicates, by analogous procedure.

The essential advantage to the use of such a population structure would be a greatly increased probability that a prolificacy determinant linked to a specific marker allele is the same among several individuals possessing that marker allele. That is, if an allele designated as P_1 is present as a prolificacy determinant linked to a marker allele M_1, then subjects comprising a block would inherit the M_1P_1 couple. Of course, recombination occurring between the marker and the locus of the prolificacy determinant could diminish the usefulness of the scheme. If that rate of recombination were very sizeable, the effect would be to lower the probability that the coupled combination of marker allele and prolificacy allele is the same in different subjects (and on homologous chromosomes present in individual homozygotes). If the rate of recombination were insignificant, then there would be an insignificant proportion of subjects possessing some couple in which the M_1 marker is linked to some prolificacy allele other than P_1. The parents of the subjects could be selected to assure that monomorphism is present at other marker genes known to be correlates of variance in prolificacy. Choosing full siblings as parents of the subjects could be effective for preventing polymorphism at such a large number of prolificacy determinants that the effect of an individual locus is obscured. According to results of the present study, it should be possible to produce blocks of subjects in which certain "marker phenotypes" are correlates of extremes in reproductive performance.

It is appropriate to give consideration to the fact that the subjects (and certain progenitors) involved in the proposed plan will be inbred to the extent of 25%. Studies involving various breeds have yielded evidence that the degree of inbreeding of individual females is negatively correlated with the reproductive potential (Chambers and Whatley, 1951; Urban et al., 1966). According to this point of view, the 25% level of inbreeding in subjects of the proposed program could have considerable consequences. However, it is by no means certain that there would be a seriously negative effect. The study of heterotic effects carried out by Squiers et al. (1952) involved one Hampshire and two separate Poland China populations. Average levels of inbreeding in those three lines were 30, 25, and 45%, respectively.

Although most of the data obtained in that study were from observations on excised reproductive tracts (described under Background Literature), during one phase of the study a group of gilts from each line were allowed to farrow litters. Since no information was provided about levels of inbreeding in those particular samples from the separate lines, it is assumed that levels of inbreeding of the dams comprising those three samples were about the same as the averages mentioned for the whole populations. Squiers et al. found that the average litter sizes were 8.1 piglets in the most highly inbred line and 7.5 and 7.0 piglets in the other two. A somewhat smaller amount of data has been obtained on performances of Oregon State sows which were 25% inbred. Those data are the sizes of 26 litters farrowed by 10 different sows (mostly the offspring of brother-sister matings among Y's). One of those litters was spontaneously aborted during the last trimester and another consisted of only three live-born. However, the median value of the 26 litters was 10 live-born per litter. These observations are indicative that, whether or not there would be some depressive effect from the inbreeding occurring with the program proposed above, the inbreeding effect would not seriously diminish the practicability. It should be kept in mind that the principal goal of the plan would not be that of obtaining subjects with exceptionally high prolificacy, but rather, the intent would be to dichotomize the subject population into groups showing opposite extremes in performance. (Hopefully, the extremes will be correlated with the marker phenotypes.)

It is expected that sows produced by the program proposed above would be useful as subjects of certain comparative studies utilizing molecular biological techniques. Likely examples of such studies are outlined in paragraphs below. Results of previously reported studies, which had a variety of objectives, serve as the rationale for suggestions for future studies.

Assays of Hormones

A radioimmunoassay technique has been developed for measurement of human follicle-stimulating hormone (FSH) in blood serum (Faiman and

Ryan, 1967). It seems reasonable to expect that procedure to be readily adaptable for assays of FSH of porcine tissue fluids. A method of radioimmunoassay of serum concentration of porcine luteinizing hormone (LH) has also been developed and found to be effective for demonstrating rather wide variations of serum concentration of LH over the course of an estrous cycle (Niswender et al., 1970). Assays for the gonadotropins mentioned here might be usefully applied to serum specimens obtained at various stages of the estrous cycle from contrasting groups of subjects. Those groups of subjects would differ by the criterion of reproductive performance; the program for selection which is outlined above could be more efficacious than a program of mass directional selection toward obtaining contrasting groups. Thus, those groups of subjects could be useful for testing the hypothesis that individual ovulation rates are correlated with some degree of genetic control of the rate of output of FSH or LH by the pituitary.

Functional Identity of Gene

Results reported here have the effect of intensifying interest in determination of the molecular bases for polymorphism of certain components and in further exploration of linkage relationships of those genes to other polymorphic genes. Although it seems improbable that associations between phenotypes and performance which were reported here are evidence of cause and effect relationships involving the phenotypes, nevertheless it may be of some importance to ascertain the functional characteristics of the loci which are responsible for the phenotypic variation. Results of this study were interpreted as indicating that certain loci controlling blood component phenotypes might be co-located within the same linkage groups with genetic determinants of reproductive performance. The possibility exists that at least some of those linkages are such that genetic determinants of performance and the locus controlling blood component phenotypes are members of a single complex of loci which are functionally related.

Hx and Am phenotypes consisting of two bands with differing electrophoretic mobilities are considered as representing heterozygous

genotypes (Imlah, 1965; Graetzer et al., 1965). Hx and Am phenotypes which consist of only single bands are considered as representing homozygous genotypes. It seems evident that each separate band comprising a heterozygous phenotype consists of the product from expression of a separate allele.

Pgd and Phi phenotypes consisting of single bands are considered as representing homozygous genotypes (Saison, 1968; Saison and O'Reilly, 1971). Heterozygous Pgd and Phi genotypes are evidenced by phenotypes consisting of three bands. A previous section (see RESULTS, Phenotyping Criteria) contains the suggestion that the individuals with homozygous Phi genotypes possess only homodimeric Phi molecules, and those with the heterozygous genotype possess heterodimeric as well as homodimeric Phi molecules. Precisely the same concept could be applied to the Pgd phenotypes, and that would be entirely consistent with the evidence that the Pgd from human erythrocytes is a dimeric structure (see BACKGROUND LITERATURE, Molecular Bases for Polymorphism).

These concepts about the bases for the observed phenotypes are lacking in details about the functional characteristics of the genes controlling those phenotypes. It would be only speculative to suggest that the polymorphism of any of the four aforementioned components is due to variant forms of the structural genes. The observed phenotypic variations could have other functional bases. It is as likely that, in populations displaying polymorphism of the Hx, Am, Pgd, or Phi, the structural genes are monomorphic, and the phenotypic variation is actually the consequence of action of modifier genes. Certain phenotypes might be the result of a post-translational modification which does not occur in individuals possessing another phenotype. A type of post-translational modification which could be the basis for Hx or Am polymorphism is that which might be effected by some protease having a narrow substrate specificity (e.g., see Steiner et al., 1968). The heterogeneity that is characteristic of individuals which are heterozygous at those loci could result from differential expression of the gene which codes for the protease. Within any cell in which the Hx or Am is synthesized, there might be sites along the endoplasmic reticulum where a polypeptide modification apparatus contains the specific

protease, while at other sites the alternative state (i.e., absence of the specific protease) exists. Since there is reason to believe (Redman and Cherian, 1972) that proteins to be secreted from the cell are "vectorially released" from microsomes across the membrane of the endoplasmic reticulum, it may be that such proteins are processed through only a single post-translational modification site, without coming into contact with other modification sites after being released into the lumen of the endoplasmic reticulum. Thus, two separate populations of Hx or Am molecules could be produced, viz., those which were modified by the specific protease, and those which were not modified by it. Rather than further detailing this concept, it is appropriate to point out another possible basis for polymorphism, which might be pertinent to at least some of the components studied here.

At least some of the serum proteins contain non-protein subunits. Evidence of such non-protein components was cited under BACKGROUND LITERATURE. It is plausible that either protein or non-protein components could become associated with proteins studied here at a post-translational step, so as to result in the phenotypic variation which is observed. As suggested above, within heterozygous individuals, there might be two different types of sites at which molecules undergo modification. Any single newly completed polypeptide might be processed through just one of those types of sites during release to the lumen of the endoplasmic reticulum, without coming into contact with other modification sites. Thus, two separate populations of Hx and Am molecules could be produced.

The types of post-translational modification described above could apply to the enzymes in which three-banded phenotypes occur, if the concept of the modification process were altered slightly. The modification of the Pgd and Phi would most likely occur at some site other than the endoplasmic reticulum.

The phenotypic variation of the Tf is a pattern which is different from those of the aforementioned components, in that homozygous individuals show patterns consisting of three or four bands, and heterozygous individuals show five bands. There is evidence about

structural features of the swine Tf which is pertinent to concepts about the bases for polymorphism. Such results seem particularly interesting in view of knowledge about associations of Tf phenotypes with variance in metric traits.

Associations of Tf polymorphism with variance in reproductive performance have been reported in other species. Reports of such associations in cattle were cited above under Background Literature. Subsequent to the reports by Kristjansson (1964) and Imlah (1970) regarding associations in pigs, Rasmusen and Tucker (1973), studying several breeds of sheep, found evidence of selective advantage of a particular Tf phenotype at the pre-weaning stage. Thus, the evidence at this time is suggestive that in these three artiodactylan species, the Tf genes may be located in the same linkage groups with determinants of variance in reproductive performance. It seems quite apparent that the genes which determine Tf polymorphism in cattle and pigs are not the determinants of variance in performance. Evidence presented by Ashton (1965) favored the concept that a heterozygous Tf genotype was a correlate of superior viability and also a correlate of superior male fertility in cattle. The concept of superior viability of a heterozygous genotype was contradicted by the findings of Cooper and Rendel (1968). Using data obtained from other populations, the latter workers instead found evidence of superior viability of homozygous genotypes. Imlah presented evidence that the Tf gene was linked with a factor responsible for pre-natal deaths in pigs. Evidence from the present study did not indicate a consistent association of a specific phenotype with superior performance in each of the breed categories where the Tf was a correlate of variance in performance.

It would be of considerable interest to determine whether or not the structural gene for the swine Tf is the basis for the polymorphism of the Tf. There are reasons for suspecting that the polymorphism is at least partly if not entirely due to a determinant(s) other than the locus specifying the polypeptide sequence. Nevertheless, there would seem to be quite good prospects for characterization or actual identification of the locus or loci controlling the polymorphism.

Stratil and Kubek (1974) found that heterogeneity still remained after applying sialidase to remove sialic acids from the Tf obtained from a pig possessing the Tf B phenotype. Hudson et al. (1973) reported finding seven or eight sialic acid residues per molecule of swine Tf, in contrast to the conclusion by Stratil and Kubek that none of the fractions of their Tf preparation contained more than two sialic acid residues per molecule. The contrasts between those findings could be due to a difference in phenotypes of individuals from which Tf preparations were obtained. (The source of material used by Hudson et al. was an individual, or individuals, of unknown phenotype.) Pertinent to this idea is the description by Graham and Williams (1975) of structures of the glycopeptides isolated following enzymic degradation of swine Tf. The only amino acid found to be glycosylated was asparagine. [Glycosylated asparagine is represented in the following discussion, as asparagine (CHO).] However, studies of primary structures of the glycopeptides indicated that there were two different amino acid sequences containing asparagine (CHO). One of those sequences is arginine-lysine-asparagine(CHO)-arginine-serine, and the other is serine-aspartate-asparagine(CHO)-leucine-serine. Therefore, it is apparent that the porcine Tf molecule contains at least two sites at which carbohydrate moieties may be covalently attached.

Other aspects of the structure of the carbohydrate portion make it apparent that considerable structural variation in that portion of the molecule is at least plausible. Graham and Williams (1975) found that each of the two glycopeptide types which were isolated apparently contained hexoses, sialic acid, and another hexose derivative. Estimated composition of each of the two de-sialylated glycopeptides (moles of sugar per mole of glycopeptide) was as follows: mannose, 3.6; galactose, 2.6; N-acetylglucosamine, 4.2; fucose, 1.2. The estimated carbohydrate composition of intact Tf, in comparison, was such that it seemed unlikely that each molecule of Tf contained more than one oligosaccharide. However, the data suggested that a fraction (not more than one-third) of the Tf molecules might contain two polysaccharide groups. Thus, Graham and Williams have obtained evidence of a possible molecular basis for heterogeneity of swine Tf. No

information was provided as to the phenotype of the individuals which were the source of the Tf used in that study, nor was there any assurance that the material came from only one individual. Nevertheless, evidence which has been presented is such that it is open to the hypothesis that variation in the carbohydrate portion of Tf could be the sole basis for the Tf polymorphism. Hudson et al. (1973) estimated that the total carbohydrate content of swine Tf is considerably greater than the estimate by Graham and Williams (1975). Hudson et al., without attempting to isolate intact glycopeptides, obtained results which suggested that each Tf molecule contains four oligosaccharide units. As pointed out above, these contrasts may be due to real differences in the materials which were used for the two studies.

Presently, it remains altogether reasonable to postulate that the gene which controls sialylation of Tf is simply a member of a complex of genes which control the sialylation of various tissue components. The polymorphism of Tf may be merely an indicator of much more extreme variations in the nature of the glycosylation apparatuses which exist within the species.

There is no basis for a supposition that the structures of oligosaccharides, such as that found in Tf, are determined by a linear coded sequence which is translatable in the same manner as genes which determine polypeptide primary structure. That particular concept of the means for control of synthesis of oligosaccharides not only is without any basis in experimental evidence, but actually would be difficult to reconcile with the evidence that in at least one species, the carbohydrate component of Tf is a branched heterosaccharide (Jamieson et al., 1971). Heterosaccharide components of a variety of tissues have been studied. Those studies have led to a concept about control of synthesis of heterosaccharides such as the carbohydrate components of serum proteins. It has been proposed that assembly of those components is controlled by one or more multiglycosyltransferase systems (Roseman, 1970). According to that concept, each step in the assembly of an oligosaccharide is controlled by the action of a glycosyltransferase, and the glycosyltransferase involved at any step

has specificity for a particular acceptor molecule, as well as specificity for the monosaccharide unit which it adds to the nascent heterosaccharide. (More precisely, the actual process is known to involve nucleoside phosphate-complexed monosaccharide units, instead of free monosaccharides.) The product of each glycosyltransferase reaction serves as the substrate for the next enzyme within an ordered multi-enzyme array; thus, the complex of glycosyltransferases has the property of "cooperative sequential specificity." The specification of the sequence of a heterosaccharide does not depend on existence of a series of codons which specify individual monosaccharides. The genetic information necessary for synthesis of an oligosaccharide chain is translated by means of synthesis of a particular multiglycosyltransferase complex. There is uncertainty as to details of the structure of multiglycosyltransferase complexes. However, it is evident that variation in structure of those complexes may be the basis for variation in the structures of glycoproteins present in different individuals.

Possible Basis for Correlation of Phenotype with Performance

Implications from variations in the glycosylation apparatus may be extended to tissues of the reproductive tract. There is evidence that sialic acid may have an important function in uterine tissue.

Carlborg and Gemzell (1969) found strong and consistent correlation between rate of excretion of estrogen in human urine and the sialic acid content of the cervical mucus. Ganguly et al. (1976) compared sialic acid contents of endometrial biopsy specimens obtained from women of various fertility categories. Specimens were obtained from presumably fertile women at different stages of menstruation or in early pregnancy. Compared to those specimens, the average sialic acid contents were found to be three- to five-fold greater in specimens from women who either used a steroidal contraceptive, copper intrauterine contraceptive devices, or displayed lactational amenorrhea. Rajalakshmi et al. (1972) assayed whole uterine horns from rats for sialic acid concentration. That study showed that, although uterine sialic acid

fell steadily from the first up to the fourth day post-mating, a significant increase in sialic acid concentration occurred late on the fourth day. It was known that in the population studied by Rajalakshmi et al., blastocysts arrive in the uterus during the time period from late on the fourth day to early on the fifth day post-mating. This was considered to be a significant correlation of events. Findings from the three studies mentioned here are indicative of a probable role of sialic acid as a correlate of the fertility status. However, the nature of the association remains unclear. A relatively high content was found in the rat uterus at the date of blastocyst entry into the uterus, but the distinctly highest values found in human endometrial specimens were those of apparently infertile individuals. Probably it is unrealistic to seek evidence of a common basis for these observations on two quite dissimilar species, especially since whole uteri were analyzed in rats, whereas the human material consisted of endometrial biopsy specimens.

However, it may be inappropriate to interpret these observations as being evidence that sialic acid of the endometrium is somehow a determinant of the fertility level. It may be that endometrial sialic acid variation is only coincidental with variation of some component which actually is involved as a primary determinant of fertility. The ovary could be considered as a likely site of such a component.

Elegant studies of interactions of proteins with specific tissues have provided a basis for hypotheses about involvement of sialic acid as a determinant of differentiation processes occurring in the cycling ovary. First, it was shown by Morell et al. (1971) that various glycosylated proteins, after intravenous injection, disappear from the peripheral blood of rats at rates which can be altered by sialidase treatment. Within one-half hour after injection of radiolabelled intact ceruloplasmin, orosomucoid, alpha-2 macroglobulin, or FSH, the radioactivity in the plasma decreased by a fairly minor increment. However, if those radiolabelled proteins were subjected to sialidase treatment before injection, the rate of disappearance of label from the plasma was markedly greater. Furthermore, it was found that a major proportion of the recoverable radioactivity could be detected in the

liver when that organ was removed following the plasma samplings. Subsequent efforts at clarifying the basis for this apparent dependency on the presence of sialic acid involved the use of orosomucoid, ceruloplasmin, haptoglobin, and chorionic gonadotropin (Pricer and Ashwell, 1971). Thos glycoproteins and derivatives obtained from sialidase treatment were used in studies of in vitro binding to a fraction of rat liver. Those studies indicated that desialylated derivatives of the aforementioned glycoproteins could be effectively bound by what was apparently the membrane fraction obtained from rat liver slices. The conclusion from that series of studies was that sialic acid has a reciprocal sort of role in controlling the uptake of glycoproteins by the parenchymal cell membrane. The presence of a sialic acid residue on the glycoprotein prevents the binding of the protein to the membrane. Binding of the protein to the cell membrane occurs, when the sialic acid is absent from the glycoprotein but present on the membrane.

The in vitro studies mentioned here involved neither FSH, LH, nor tissues from the reproductive tract. It remains mostly speculative whether or not presence of sialic acid on gonadotropins and its coordinated removal from gonadal cell membranes are a basis for follicle-stimulating or luteinizing effects. Nevertheless, that concept has at least some basis by reason of the known characteristics of those two hormones. Sialic acid has been found as a component of the carbohydrate portions of the FSH and LH, at least in sheep and humans (Justisz and de la Llosa, 1972). Furthermore, it is apparent that in at least one other tissue besides liver, the presence of sialic acid is important in the mediation of the interaction of a polypeptide hormone with the cell membrane (Cuatrecasas and Illiano, 1971).

These speculations about variance in ovarian function need not be restricted to the consideration of interactions between hormones and the target cells. Obviously, the occurrence of ovum release from a follicle must reflect a changing interrelationship among the cells comprising the follicle. There is evidence that carbohydrate components of the cell surface are important, at least in some tissues,

for determining the adhesiveness properties of the cells (Roseman, 1970). It is reasonable to theorize that an important determinant of whether a developing follicle yields a viable ovum, or instead becomes atretic, is a genetically determined capability for glycosylation of the cell surface after uptake of LH by the follicular cells. There might be variation in functional properties of multiglycosyltransferase complexes present in different individuals, the variation being such that sialic acid-containing components on the gonadal cells are of varying structure among different individuals. Perhaps the oligosaccharide "stem" containing the terminal sialic acid residue, if relatively short, does not permit such strong polarity to be achieved by the cell surface, compared to that which is possible with a longer oligosaccharide "stem." A sufficient polarity of the cell surfaces may be requisite for assuring that the ovum is effectively shed, without being retarded by "stickiness" of the cell surface.

SUMMARY

Comparisons of female reproductive performance were made among seven different breed categories within a swine population descended from a small founder population consisting of members of the Berkshire and Yorkshire breeds. One of the breed categories comprising this population was a closed line (denoted as the Composite line) developed by inter se matings of offspring from crossmatings of Berkshires and Yorkshires. Other breed categories consisted of offspring from outcrossings of the Berkshire, Yorkshire, and Composite lines. Two such categories were produced by crossmatings among those three lines; two other crossbred categories were produced by backcrossing daughters of crosses between Berkshires and Yorkshires (i.e., F_1 offspring) to either Berkshire or Yorkshire boars. Breed compositions of the four outbred categories are represented by the symbols (BxC + CxB), (YxC + CxY), BxF_1, and YxF_1.

Heterosis was apparent with the reproduction data from the (YxC + CxY) category. There were also indications of a heterotic effect in the (BxC + CxB), but evidence was less persuasive. There were less consistent indications of a heterotic effect on reproductive performance in the other two outbred categories. Contrasts among performances of the seven categories were sufficient to motivate more detailed analyses of genetic contrasts among those breeds.

Demonstrations of genetic contrasts among the categories were accomplished by means of electrophoretic analyses of hemolysates and blood serum and by serology of erythrocytes. Those methods were used to detect phenotypic variations in four serum proteins, two erythrocyte enzymes, and seven antigenic determinants located on the erythrocyte membrane. Genetic control of variation of each of those blood components had been indicated from studies of other populations. Among 275 pigs from which specimens were obtained for those analyses, there were 146 dams which produced a total of 480 litters.

Parentage records indicated that among the subjects of this study, there were sibships produced from boar-sow pairs which were also

subjects of the study. This indicated the feasibility of accomplishing at least some limited testing of previously proposed modes of inheritance of the phenotypes. The median size of such sibships was three pigs; nevertheless family studies yielded evidence that paternity designations were incorrect for three of those sibships. Additionally, records indicated that either the dam or the sire of some other sibships were subjects of this study. Comparisons of the phenotypes in such sibships to those of the phenotyped parent indicated that for one such sibship, the paternity designation was incorrect. Among the sibships comprising either complete or partial family data, it seemed that those which yielded evidence of incorrect paternity recording amounted to a significant proportion.

Certain alleles were found to be heterogeneously distributed among the seven breed categories mentioned above. Alleles which were present at three different loci within the B line were not found within the C line; an allele at one other locus was present within the C line but was absent from the B line. Some contrasts were also found between the Y and C lines by this criterion. Alleles which were found at five different loci within the Y category were not found within the C category; an allele at one other locus was present within the C category but was absent from the Y category.

The pattern of distribution of phenotypes among the B, Y, and C categories indicated that crossmatings could result in formation of heterozygous combinations of alleles which were found only in the homozygous state in the B or C categories. Furthermore, distribution of alleles at one locus was such that crosses of Y's with either B's or C's could produce a phenotype which was not present in any of the B, Y, or C subjects. Besides heterogeneity among the breed categories, there was phenotypic variation within categories with respect to most of the components studied. These observations were motivation for attempts to demonstrate correlations between phenotypes and variance in reproductive performance.

One or more regressed fetuses were present in 72 of the 480 litters comprising the measures of reproductive performance. Within each individual breed category, the sizes of the litters containing

regressed fetuses were compared to the within-category mean of the total number of fetuses per litter. Those comparisons indicated that the occurrence of regressed fetuses was positively correlated with the total number of fetuses per litter.

The least squares (L.S.) method was used for analyses of reproductive performance and the results of blood component phenotyping. Primarily, the analyses focused on total number of fetuses per litter as the measure of reproductive performance. Phenotypic variation of the various blood components was sufficient so that L.S. analyses involved phenotypes of six components in the B and the (YxC + CxY) categories, five in the C, and seven in the Y, (BxC + CxB), BxF_1, and YxF_1 categories.

Insight about the mode of gene expression occurring at loci which control reproductive performance was a principal goal of the study. Consequently, there were sequential simplifications of the compositions of the L.S. models used. The purpose was to eliminate in a step-wise manner the factors representing genes having seemingly non-significant correlations to performance. It was expected that this would be effective for providing insight as to whether additive or non-additive modes of gene expression were occurring at loci which have real associations with the blood component genes. The analyses also considered two non-genetic sources of variance, viz., the age of dam at date of farrowing, and the semester during which conception occurred. The L.S. models developed by this process are designated as the select models. Select models were developed by L.S. analyses on performance data from six of the seven breed categories mentioned above. The select model obtained for the breed category for which the smallest amount of data was available included one genetic and two non-genetic sources of variance. Select models developed for other breed categories included zero, one, or two non-genetic, and as many as three genetic sources of variation. Thus, considerable variation occurred among the sub-populations in the extent to which correlates of reproductive performance were identified.

The erythrocyte antigens controlled by the loci denoted as N, K, and A-o, and also the phenotypic variants of the phosphohexose

isomerase, were found to be correlated with performance in one of four breed categories. None of those four components was identified by the select model as a correlate in more than one breed category. Certain consistencies in the correlations of other blood components with prolificacy were considered noteworthy. The transferrin phenotype was correlated to the prolificacy in three breed categories, and the amylose-modifier phenotype was a correlate in two categories.

Results from L.S. analyses using select models were scrutinized to discern the modes of gene expression associated with performance-correlated phenotypes. Heterozygote advantage was evident with the transferrin in two of the breed categories in which it was a correlate of performance. There was an apparent additive mode of gene expression with the transferrin in the third breed category in which it was a correlate. Heterozygote advantage seemed apparent with the amylose-modifier in one of the breed categories; distribution of phenotypes was not sufficient for evidence about the mode of gene expression in a second breed category in which the amylose-modifier was a correlate of performance. The phosphohexose isomerase was associated with an additive mode of gene expression in the single breed category in which it appeared to be a correlate of performance. It was not possible to obtain evidence about modes of gene expression associated with the N, K, or A-o phenotypes due to insufficient distributions of those phenotypes.

Models denoted as pre-select differed from those referred to above by a single criterion. The proportion(s) of variance associated with one or two of the sources of variation was (were) less than the proportion required according to the criterion for a combination of variables comprising a select model; however, the variance proportion(s) associated with such components of pre-select models in three breed categories was (were) less than that of a "select" component by only a rather small proportion. Because of the nearness of such proportions to the "select criterion," those particular pre-select models were considered as being useful for insights about correlations and about modes of gene expression. Obviously, each pre-select model included one or two sources of variation which are somewhat more likely

to have been only coincidentally associated with performance variance, in comparison to any of the sources of variation included in select models.

Results with the pre-select models in two of the breed categories suggested that both the transferrin and amylose-modifier were associated with performance. Results from one of those same pre-select models suggested association of the phosphogluconate dehydrogenase with performance. Results with a third pre-select model suggested that the phosphohexose isomerase was associated with performance.

Results of the study were not effective for demonstrating that any of the genes which were studied were actual determinants of the prolificacy variance. Instead, the associations were probably of some other nature. There was either a moderate or a high degree of heterozygosity at a considerable proportion of the loci studied. However, there were very few loci which were identified as significant correlates of prolificacy in more than one of the breed categories. Regarding phenotypes which were found to be significant correlates in more than one breed category, there was very little consistency in the relative values of the phenotypes when values found for different breed categories were compared. For example, an amylose-modifier phenotype found to be correlated with a positive effect toward litter size in the (BxC + CxB) was instead associated with a negative effect in the Berkshire line.

One or more of the genetic variables in each breed category was associated with performance to the extent that those phenotypes should be effective as criteria for selection of breeding stock within the particular breed category. The differences between L.S. constants of highest and lowest value for those variables were at least one-half the standard deviations in total number of fetuses per litter. Also, the difference between highest and lowest L.S. constants for at least one of the genetic variables was even greater than the standard deviation in the C and (BxC + CxB) breed categories.

Skepticism towards selection programs based on single traits is appropriate with respect to heterogeneous populations. However, the results with the present study are consistent with the concept that

selection based on genotypes at only a few loci, if applied to sufficiently homogeneous populations, can be as effective as, or perhaps more effective than, mass directional selection. (Homogeneity, as used here, is intended to refer to the extent to which distinct markers are specifically coupled to distinct genetic determinants of performance.) Since matings between close relatives (e.g., full siblings) have not consistently proved to be deleterious toward reproduction, it is anticipated that entire groups of subjects can be produced which possess homozygous configurations of specific single alleles which are of monogametic origin. If genes controlling blood component phenotypes are present in the same linkage groups with determinants of prolificacy, then the strategy proposed here could provide for effective marking of specific prolificacy alleles, rather than allowing ambiguous marking of such alleles.

Validation of a selection system based on genes identified here could be of interest for other purposes in addition to selection of breeding stock. The system could be useful for obtaining a population in which a major proportion of genetic variance in prolificacy is due to only a few loci. Such a population might then be highly useful as the object of studies using certain elegant quantitative methods now available for reproductive physiology.

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