Biochemical polymorphism in five pig breeds in South Africa

F.H. van der Bank*, M. Groenewald and A. Kotze**
Rand Afrikaans University, Department of Zoology, P.O. Box 524, Auckland Park, 2006 South Africa

Received 1 May 1997; accepted 21 January 1998

Electrophoresis was used to study the genetic diversity within and between five pig breeds (Large White, Landrace, Duroc, Wild pig and Chester White) in South Africa. They were compared with regard to gene and phenotypic allele frequency differences at 11 protein and enzyme coding loci. Two monomorphic loci were identified namely haemoglobin and ceruloplasmin. The average heterozygosity values ranged from 0.091 to 0.381; the percentage of polymorphic loci ranged from 18.18 to 81.82 in the Wild pig and the Large White breeds respectively, and the mean number of alleles per locus ranged from 1.18 (Wild pig) to 2.91 (Landrace). The genetic distance values were between 0.056 and 0.288; with the smallest distance between the Large White and the Landrace breeds and the largest distance between the Duroc and Wild pig. The results are discussed with specific reference to genetic divergence between breeds within this species where interbreeding often occurs.

Elektroforese is gebruik om die genetiese diversiteit te bepaal binne- en tussen vyf varkrasse (Groot Wit, Landras, Duroc, Wilde vark en Chester Wit) in Suid Afrika. Hulle is vergelyk ten opsigte van hul geen en fenotipiese alleelfrekwensieverskille by 11 proteïen- en ensiemkoderende lokusse. Twee monomorfiese lokusse is geïdentifiseer naamlik hemoglobien en seruloplasmien. Die gemiddelde heterosigositeitswaardes strek vanaf 0.091 tot 0.381; die persentasie polimorfiese lokusse is tussen 18.18 en 81.82 in die Wilde vark en die Groot Wit rasse respektiewelik, en die gemiddelde aantal allele per lokus strek vanaf 1.18 (Wilde vark) tot 2.91 (Landras). Die genetiese afstandwaardes is tussen 0.056 en 0.288; met die kleinste afstand tussen die Groot Wit en die Landras rasse en die grootste afstand tussen die Duroc en Wilde varke. Die resultate word bespreek met spesifieke verwysing na genetiese divergensie tussen rasse van hierdie spesie waar inteling dikwels voorkom.

Keywords: Pig, swine, allozyme, enzyme, genetic variation, genetic distance, protein polymorphism

*To whom correspondence should be addressed

**Present address: Animal Improvement Institute, Private Bag X2, Irene, 1675 South Africa

Introduction

The study of polymorphic proteins, together with blood groups, has been used for parentage control, studies of genetic structure of breeds and populations (i.e. estimation of heterozygosity in populations, estimation of genetic distances between breeds, linkage studies and gene mapping), and in the search for relationships between genotypes at polymorphic loci and production traits (Stratil 1995). The theoretical basis for this field of application is that, from a genetical point of view, breeds can be defined as populations which differ from each other in the relative distribution and frequencies of certain genes (Hesselholt 1969).

Breed comparisons in pigs are greatly facilitated by the large number of genetically controlled polymorphic systems. In the present study, five pig breeds are compared with regard to gene and phenotype frequencies with biochemical genetic markers used for standard routine parentage control analysis. Not only are these results of academic importance, but the amount of inbreeding and/or the extent of outbreeding during the two and a half decades since the previous estimate by Meyer (1972) should give an indication of the effect on active selection since some of the loci studied are directly linked to the production characteristics selected for, with differences in allele frequencies indicating each breed's particular identity (Van Zeveren 1995).

Materials and Methods

The genetic relationships between South African pig breeds were studied using gene frequency data obtained from electrophoretic analysis at the following 11 structural gene loci that code for soluble blood and serum proteins and enzymes: transferrin (**TF**), glucose phosphate isomerase (**GPI**), phosphogluconate dehydrogenase (**PGD**), haemoglobin (**HB**), protease inhibitor 1, 2 (**PI-1, -2**), postalbumin-1A (**PO-1A**), -2 (**PO-2**), serum amylase (**AM**), haemopexin (**HPX**) and ceruloplasmin (**CP**).

Blood samples were collected from pigs over a period of four years at: 11 Large White stud pig breeders (four from the Western Cape, four from Kwazulu Natal and three from Gauteng); 10 South African Landrace stud pig breeders (five, four and one breeder each from the provinces named above); and six Duroc stud breeders (two, three and one breeder each from the different provinces). Population sizes were as follows: Large White (1123), Landrace (845), Duroc (302), Wild pig (2) and Chester White (12). The Duroc is a relatively recent breed and was introduced into this country in 1980/81. Of the four domestic pig breeds, the largest of the herds had in excess of 500 sows whereas the smallest herd consisted of only 16 sows. The ratio of boars to sows was 50:50 in all the domesticated breeds except for the Landrace where the ratio was 40:60. A minimum of 44 animals, from the larger herds per breeder was evaluated and the characteristics for which they were selected included feed conversion ratio, average daily gain, backfat thickness and reproductive traits. The sample size for Chester White was small as there is only one herd in South Africa, and only a few Wild pig individuals exist.

Samples were analysed by horizontal starch, agarose and polyacrylamide gel-electrophoresis (PAGE) using the buffer systems and gel concentrations described by Shaw & Prasad (1970) and Gahne & Juneja (1985). Tris was used in the buffer in the two-dimensional PAGE system (pH 8.6–9.0) to separate allele products for **TF, PI, PO, CP** and **HPX. AM** and **HB** were separated on starch and **GPI** and **PGD** were separated on agarose gels using a phosphate buffer with EDTA and magnesium chloride (pH 7.2).

The **BIOSYS-1** computer programme (Swofford & Selander, 1981) was used to calculate individual heterozygosity values per locus (*h*), average heterozygosity (*H*), coefficients of heterozygote deficiency or excess (*d*), Chi-square (χ^2) values, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), Wright's (1978) fixation (*F*) indices and genetic distances (*D*) between all five pig breeds. Genetic distance and phylogenetic analysis (**DISPAN**; Ota 1993) was used to construct a phylogenetic tree using the unweighted group-method with the arithmetic mean (**UPGMA**) of Sneath & Sokal (1973), the genetic distance measure of Nei *et al.* (1983) and neighbour-joining and bootstrap tests (1 000 replications).

Results

Two monomorphic loci were encountered namely **HB** and **CP**. The relative allele frequencies for the five pig breeds, *d*, *h* and *F* values for polymorphic loci are listed in Table 1. Gene diversity (*d*) values ranged from –1.0 (Wild pig: **PO-1A**) to 0.5 (Wild pig: **PI-2**) and *h* values ranged from 0.121 (Landrace: **TF**) to 0.811 (Large White: **PO-1A**). The relative allele frequencies for the Wild pig were fixed, with only heterozygotes at **PI-2** and homozygotes at the other enzyme coding loci, with the exception of **PO-1A**, where each individual studied was monomorphic for alternative alleles. The highest allele frequencies common to most of the breeds studied were: the B-allele at **TF, GPI,** and **AM**; the S-allele (**PI-2**) and the F-allele (**PO-1A**) (Table 1). Distinct allele frequency differences between breeds were obtained at the other polymorphic loci studied (e.g. the highest allele frequency values were obtained at **PI-1*s** and **HPX*d** in the Duroc compared to **PI-1*a** and **HPX*b** for the other breeds). In the domesticated breeds, the Large White breed had the highest allele frequency for the A-allele at **PGD** and the Landrace at **PO-2*f**. The Wild pigs had the least genetic variation, followed by Chester White, then Duroc, Large White and Landrace breeds. At **PI-1** the S-allele occurred most frequently in the Duroc; at **PI-2** the I-allele was found most frequently in the Chester White breed; at **PO-2** the S-allele had the highest frequency in the Large White, Duroc and Chester White, while the D-allele occurred most frequently in the Duroc at the **HPX** locus. The **HPX*a, b, c** and **d** alleles corresponded to **HPX*0, 1, 2** and **3**, respectively, as referred to in some literature (e.g. Vacková *et al.* 1996). The loci where allele frequencies conformed to expected Hardy-Weinberg proportions are marked with asterisks in Table 1 (i.e., Landrace: **TF**; Duroc: **TF, GPI** and **PO-2**; Wild pig: **PI-2**; Chester White: **TF, GPI, PGD, PI-2, PO-2** and **HPX**). Deviations that occurred, were a result of deficiencies of heterozygotes, except at the **PI-1** locus in the Large White breed where a slight excess of heterozygotes ($d = 0.071$) was found (Table 1).

At the stress susceptibility markers, the following allele frequencies were observed: in the **PGD** system the Landrace, Duroc and Chester White breeds showed high frequencies for the B-allele, whereas in Large White and Wild pigs, the A-allele was predominant; at **PO-2** the S-allele occurred most frequently in the Large White, Duroc and Chester White breeds; whereas at the **GPI** system the B-allele occurred frequently in all the domesticated breeds, but was monomorphic in the Wild pigs.

Wright's measure of differentiation among all breeds (F_{ST}) ranged from 0.061 to 0.366 (Table 1). The loci that contributed most to population differences were **PGD, PI- 1, PO-2** and **HPX,** where F_{ST} values were 0.366, 0.273, 0.306 and 0.244 respectively. Mean *F* values of populations relative to the total population, F_{IS}, is 0.177; 0.341 for the total population and its subpopulations (F_{IT}) and F_{ST} = 0.200 for the amount of differentiation among subpopulations relative to the limiting amount under complete fixation.

The *H* value was the highest (38.1%) for the Large White and only 9.1% for the Wild pigs; *P* ranged from 18.18-81.82 for these breeds and *A* was only 1.18 for the Wild pigs compared to 1.91–2.91 for the domesticated pigs (Table 2). The unbiased *D* (Nei 1978) values between breeds ranged from 0.056 to 0.288 (Table 3); with the smallest distance between the Large White and the Landrace breeds and the largest distance between Wild pigs and Duroc. The mean genetic distance is 0.112, and the phylogenetic relationships between the breeds studied are illustrated in the dendrogram (Figure 1). Figure 1 reflects the above-mentioned results, with the Wild pig as basal, the Large White and Landrace breeds (which were the most outbred) grouped together and the domesticated breeds linked in the order of greatest to least genetic variation.

Discussion

Genetic variation

Ideal Hardy-Weinberg populations do not actually occur in nature owing to various factors which can shift the equilibrium and disrupt the stability of a population, giving rise to change in the genetic structure. Furthermore, significant deviations of allele frequencies may occur owing to crossing and linking, inbreeding, sampling error, population bottle-necks and random genetic drift. Since the pig breeds studied do not represent natural populations and because of directed selection of domesticated stock, it came as no surprise that allele frequencies deviated from the expected Hardy-Weinberg proportions at most loci (Table 1). However, it is interesting to note that the Wild pigs displayed very little polymorphism at enzyme coding loci (probably owing to the small sample size studied), and that distinct differences were encountered for allele frequencies at the stress susceptibility markers (**GPI, PGD** and **PO-2**). Deficiencies of heterozygotes were encountered at these loci (Table 1). All of the breeds had similar allele frequencies at five of the polymorphic loci studied (**TF, GPI, PI-2, PO-1A** and **AM**), and the remaining polymorphic loci may, therefore, serve as markers to define the breeds (Table 1). This is also reflected by the high F_{ST} values obtained at these loci.

Our results not only compare well with those of

Table 1 Relative allele frequencies, ***F*** values for polymorphic loci, coefficients of heterozygosity deficiency or excess (***d***) and individual heterozygosity (***h***) values are listed after each locus for five pig breeds in South Africa

Locus	Allele	White	Land.	Duroc	Wild	Chest.	F_{IS}	F_{IT}	F_{ST}
TF	A	0.302	0.060*	0.172*		0.042*	0.011	0.115	0.106
	B	0.692	0.936	0.823	1.000	0.917			
	C	0.006	0.004	0.005		0.042			
	d	–0.030	0.025	–0.042		0.022			
	h	0.430	0.121	0.293		0.156			
GPI	A	0.497	0.127	0.311*		0.318*	–0.064	0.103	0.157
	B	0.503	0.873	0.689	1.000	0.682			
	d	–0.070	–0.095	–0.109		0.400			
	h	0.500	0.222	0.428		0.434			
PGD	A	0.674	0.485	0.145	1.000	0.273*	0.123	0.444	0.366
	B	0.326	0.515	0.855		0.727			
	d	–0.122	–0.123	–0.190		–0.125			
	h	0.440	0.500	0.248		0.397			
PI-1	F	0.604	0.581	0.430	1.000	1.000	0.064	0.320	0.273
	S	0.396	0.419	0.570					
	d	0.071	–0.140	–0.124					
	h	0.478	0.487	0.490					
PI-2	F	0.098	0.115	0.008			0.008	0.077	0.069
	I	0.354	0.442	0.316	0.500*	0.708*			
	S	0.548	0.443	0.676	0.500	0.292			
	d	–0.381	–0.144	–0.128	0.500	–0.420			
	h	0.565	0.595	0.444	0.500	0.413			
PO-1A	A	0.021	0.007				0.624	0.662	0.101
	B	0.083	0.016	0.003					
	D	0.223	0.379	0.139	0.500	0.167			
	E	0.035	0.074	0.003					
	F	0.273	0.203	0.434	0.500	0.750			
	G	0.003	0.005						
	I	0.124	0.086	0.137					
	R	0.039	0.034	0.161		0.083			
	S	0.199	0.196	0.123					
	d	–0.602	–0.457	–0.589	–1.000	–0.603			
	h	0.811	0.763	0.733	0.500	0.403			
PO-2	F	0.341	0.604	0.270*	1.000	0.292*	0.089	0.368	0.306
	S	0.659	0.396	0.730		0.708			
	d	–0.156	–0.143	–0.052		–0.034			
	h	0.449	0.478	0.394		0.413			
AM	A	0.101	0.094				0.119	0.173	0.061
	B	0.899	0.902	1.000	1.000	1.000			
	C		0.004						
	d	–0.105	–0.135						
	h	0.181	0.178						
HPX	A	0.016	0.037				0.156	0.362	0.244
	B	0.795	0.542	0.176	1.000	0.750*			
	C	0.018	0.062	0.384		0.167			
	D	0.171	0.359	0.440		0.083			
	d	–0.160	–0.105	–0.192		–0.207			
	h	0.338	0.573	0.628		0.403			
Mean							0.177	0.341	0.200

* Polymorphic loci where allele frequencies conformed to expected Hardy-Weinberg proportions; White: Large White; Land.: Landrace; Wild: Wild pig; Chest.: Chester White.

Table 2 Average heterozygosity (*H*) values and mean number of alleles per locus (*A*), with standard errors thereof and percentage of polymorphic loci (*P*)

Breed	*H*(SE)	*A*(SE)	*P*
Large White	0.381 (±0.073)	2.82 (±0.67)	81.82
Landrace	0.356 (±0.079)	2.91 (±0.67)	81.82
Duroc	0.333 (±0.076)	2.45 (±0.51)	72.73
Wild pig	0.091 (±0.061)	1.18 (±0.12)	18.18
Chester White	0.238 (±0.061)	1.91 (±0.25)	63.6

Table 3 Nei's (1978) unbiased genetic distance values (above diagonal) and genetic identity values (below diagonal)

Breed	Large White	Landrace	Duroc	Wild pig	Chester White
Large White	–	0.056	0.105	0.118	0.082
Landrace	0.946	–	0.077	0.078	0.082
Duroc	0.900	0.926	–	0.288	0.102
Wild pig	0.889	0.925	0.750	–	0.127
Chester White	0.921	0.921	0.903	0.881	–

populations of the same breed in other countries, but also with th results previously reported by Meyer(1972) for 131 Large White and 578 Landrace pigs in South Africa. For example, the highest allele frequencies were obtained at the following loci: A-allele (**PGD**: Large White); B-allele (**GPI**: Large White, Landrace, Duroc; **PGD**: Duroc; **AM, HPX, TF** and **CP**: Large White, Landrace, Duroc); F-allele (**PO-2**: Landrace; **PI-1**: Large White; **PO-1A**: Large White, Duroc) and the S-allele (**PO-2**: Large White, Duroc; **PI-2**: Large White, Duroc) (Imlah, 1965; Hesselholt *et al.*, 1966; Hesselholt, 1969; Meyer, 1972; Oishi *et al.*, 1979; Gahne & Juneja, 1985; Vögeli *et al.*, 1985; Kamyczek *et al.*, 1995; Vacová *et al.*, 1996). We have obtained more variation compared to Meyer's results at the **AM** locus (e.g. allele frequency for the A-allele = 0.101 in the present study compared to 0.023); the **HPX** locus (0.016 and 0.171 for the A- and D-alleles respectively compared to 0.004 and 0.053); and at the **TF** locus (0.302 compared to 0.99 for the A-allele, and 0.006 compared to zero for the C-allele); with less variation at the **HPX** B-allele and no variation at the **CP** A-allele in the Large White breed (Table 1). In the Landrace breed, we have obtained less variation at **AM** and **CP**, but more at **TF** and similar frequencies at the **HPX** locus compared to values reported by Meyer (1972). These minor differences may be the result of directed selection, different populations studied, improved analytical methods and/or different sample sizes studied.

The values of *A*, *P* and *H* were the lowest in the Wild pigs (probably because of the small sample size and inbreeding) and were increasingly higher for the Chester White, Duroc, Landrace and Large White breeds (Table 2). This is the result of a lack of alleles at one locus (**AM**) in Landrace and in progressively more loci in Duroc and Wild pigs respectively, compared to that of the Chester White (Table 1). Nevertheless, sufficient genetic variation exists within the domesticated breeds for selection purposes. This result is in agreement with results reported by Cepica *et al.* (1995) for corresponding breeds (i.e. *H* values for Duroc, Landrace and Large White were 0.326, 0.370 and 0.382 respectively).

Genetic differentiation

Genetic data, produced by protein electrophoresis, can be used by systematists to determine whether samples are from different gene pools, representing different species (review: Thorpe & Solè-Cava 1994). This review gives details of methods for distinguishing and identifying cryptic and sibling species. The distinction is due to genetic differentiation, which is to be expected for populations that are geographically separated so that little or no gene flow can occur between them. The criterion which must be applied if electrophoretic data is to be used, is to assess the level of differentiation found between populations (i.e. a test of whether or not they are from the same gene pool). Wright's (1978) fixation index is such a measure. The mean F_{ST} value of 0.200 for polymorphic loci (Table 1), is an indication of the relatively large genetic differentiation between the populations studied. The F_{IS} value (0.177) obtained in the present study also reflects the above phenomenon since values of F_{IS} are less (close to zero) in most natural populations where random mating within subpopulations occurs (Nei 1986). In addition, the F_{IT} value of 0.341 (which quantifies inbreeding owing to population subdivision), is indicative of relatively effective barriers to gene flow between the populations studied. This is not surprising because each breeder protects his breed from outbreeding in order to maintain typical breed characteristics.

Another statistically based measure to describe genetic differentiation is *D* values. We have obtained the largest *D* value between the Wild pig and Duroc breeds (0.288; Table 3) and the smallest value between the Landrace and Large White breeds (0.056). Our results are also similar to those of researchers for corresponding breeds in other countries (e.g. Cepica *et al.*, 1995; Janik *et al.*, 1995), with large *D* values

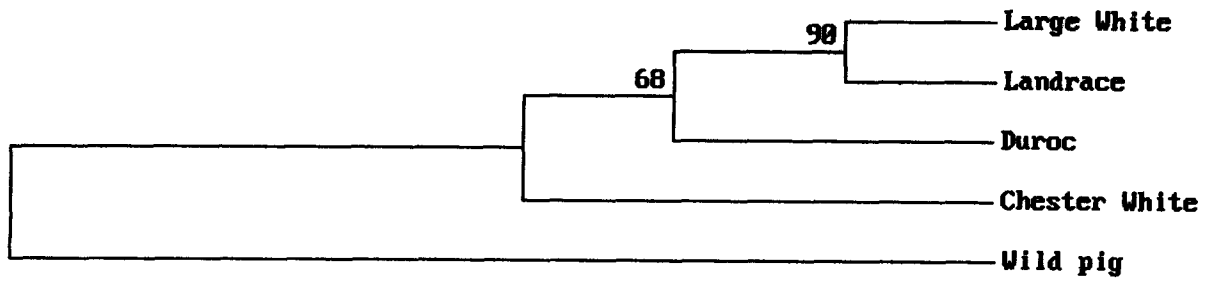

Figure 1 Phylogenetic relationships between five pig breeds in South Africa. Bootstrap numbers are listed at nodes.

between the Duroc and Large White compared to smaller values between the Duroc and Landrace breeds.

The phylogenetic relationships between the breeds studied are summarised in Figure 1. The groupings reflect the allozyme results, showing the Wild pig as basal, with the other breeds diverging progressively as a result of more alleles gained. The grouping of the Large White and Landrace breeds is expected because South African pig stud breeders use F_1 sows (Landrace × Large White) to form the pivot of the crossbreeding programme in the majority of commercial pig farms. This practice has invariably narrowed the genetic base of these breeds.

Conclusions

The present study shows that our results compare well with those of other researchers, and that domesticated pig breeds in South Africa have not changed drastically regarding their allele frequencies at polymorphic enzyme loci. This is an important result since uncontrolled interbreeding would narrow the gene pool, to render previous selection efforts futile. Furthermore, the results for the pig breeds where large sample sizes were involved are important for the future monitoring of gene flow in populations to determine levels of inbreeding and crossbreeding in each breed, and to enhance the global information on domestic animal diversity.

References

ČEPICA, S., WOLF, J., HOJNÝ, J., VACKOVA, I. & SCHRÖFFEL J.Jr., 1995. Relations between genetic distance of parental pig breeds and heterozygosity of their F_1 crosses measured by genetic markers. *An. Genet.* 26, 135.

GAHNE, B. & JUNEJA, R.K., 1985. Prediction of the halothane (*Hal*) genotypes of pigs by deducing *Hal, Phi, Po2, Pgd* haplotypes of parents and offspring: results from a large-scale practice in Swedish breeds. *An. Bl. Gr. Biochem. Genet.* 16, 265.

HESSELHOLT, M. 1969. Serum Protein Polymorphisms in Swine - Electrophoretic Identification, Genetics and Application. Munksgaard, Copenhagen 156 pp.

HESSELHOLT, M., LARSEN, B. & NIELSEN, P.B. 1966. Studies on serum amylase systems in swine, horse and cattle. Annual Report, Royal Veterinarian Agricultural College, Copenhagen, 1966, 78.

IMLAH, P., 1965. A study of blood groups in pigs. Blood groups in animals *Proceedings of the 9th European Conference on Animal Blood Groups*, Prague, 1964, 109.

JANIK, A., RYCHLIK, T., PARYS, A. & KAMYCZEK, M. 1995. Preliminary investigation on genetic distances between pig breeds raised in Poland. In: *Proceedings of the Pig-Workshop 15–17 November 1995 Gent, Belgium.* Abstracts published by the International Society for Animal Genetics. pp. 26.

KAMYCZEK, M., KWACZYNSKA, A. & JANIK, A., 1995. Genetic polymorphism of GPI and GPD in some swine breeds and lines raised in Poland. In: *Proceedings of the Pig-Workshop 15-17 November 1995 Gent, Belgium.* Abstracts published by the International Society for Animal Genetics. pp. 20.

MEYER, E.H.H., 1972. Serum Protein Polymorphism in pig breeds in South Africa. In: *Proceedings of the XIIth European Conference on Animal Blood Groups, Biochemical Polymorphism and Blood proteins* pp. 315–322. Publishing House of the Hungarian Academy of Sciences.

NEI, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89, 583.

NEI, M., 1986. Definition and estimation of fixation indices. *Evolution* 40, 643.

NEI, M., TAJIMA, F. & TATENO, Y., 1983. Accuracy of estimated phylo trees from molecular data. *J. Mol. Evol.* 19, 153.

OISHI, T., KOMATSU, M. & ABE, T., 1979. Breed variations of gene frequencies in pig red cell PHI, 6PGD, PGM and ADA enzyme systems and their availability for paternity test. *Jap. J. Zootech. Sci.* 50, 879.

OTA, T., 1993. **DISPAN**. Pensylvania State University, Philadelphia.

SHAW, C.R. & PRASAD, R., 1970. Starch Gel Electrophoresis of Enzymes — A Compilation of Recipies. *Biochem. Genet.* 4, 297.

SNEATH, P.H. & SOKAL, R.R., 1973. Numerical Taxonomy. Freeman, San Francisco.

STRATIL, A., 1995. Biochemical polymorphisms in the pig. In: *Proceedings of the Pig- Workshop 15-17 November 1995 Gent, Belgium.* Abstracts published by the International Society for Animal Genetics. pp. 3–5.

SWOFFORD, D.L. & SELANDER, R.B., 1981. **BIOSYS-1**: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* 72, 281.

THORPE, J.P. & SOLÈ-CAVA, A.M., 1994. The use of allozyme electrophoresis in invertebrate systematics. *Zool. Scripta* 23, 3.

VACKOVÁ I., ČEPICA, S., WOLF, J., HOJNÝ, J. & SCHRÖFFEL, J.Jr., 1996. A genetic blood-marker study on six pig breeds bred in Czechia. *J. An. Breed. Genet.* 113, 63.

VAN ZEVEREN, A. 1995. A genetic study of four Belgian pig populations by means of seven microsatellite loci. In: *Proceedings of the Pig-Workshop 15-17 November 1995 Gent, Belgium.* Abstracts published by the International Society for Animal Genetics. pp. 14.

VÖGELI, P., SCHWÜRER, D., KÜHNE, R. & WYSSHAAR, M., 1985. Trends in economic traits, halothane sensitivity, blood group and enzyme systems of Swiss Landrace and Large White pigs. *An. Bl. Gr. Biochem. Genet.* 16, 285.

WRIGHT, S., 1978. Evolution and the Genetics of Populations. Vol 4.Variability within and Among Natural Populations. University of Chicago Press, Chicago.