CHAPTER 3

RESULTS

RESULTS

Twenty-one protein-coding loci (resolved on starch and PAGE gels) provided interpretable results (i.e. which showed sufficient activity and resolution for repeatable scoring), with 9 loci (42.86%) polymorphic in one or more of the populations studied. All the loci resolved (including monomorphic ones), locus abbreviations, enzyme commission numbers and the buffers that provided the best results are listed in Table 1.

TABLE 1: Locus abbreviations, enzyme commission numbers (E.C. No.) and buffer systems for the protein coding loci studied.

Enzyme	Locus	E.C. No.	Buffer
Adenylate kinase	AK-1*,-2*	2.7.4.3	TC
Creatine kinase	CK*	2.7.3.2	TC
Glyceraldehyde-3-phosphate dehydrogenase	GAPD	1.2.1.12	A
Glucose dehydrogenase	GLD	1.1.1.47	MF
Glyserol-3-phosphate dehyrogenase	GPD	1.1.1.8	MF
Glucose-6-phosphate isomerase	GPI-1; -2	5.3.1.9	RW
Hemoglobin	Hb*		Hb – buffer
Isocitrate dehydrogenase	IDH	1.1.1.42	A
Peptidase: Substrate: Leucyl-glycyl-glycine	LGG	3.4.-.-	A
Peroxidase	PER*	1.11.1.7	A
Post-albumin	PA-1*,-2*,-3*,-4		PAGE
Post-transferrin	P-Tf*		PAGE
Pre-albumin	Pre-A		PAGE
Sorbitol dehydrogenase	SORD	1.1.1.14	MF
Superoxide dismutase	SOD-1; -2	1.15.1.1	RW

* Polymorphic loci

Allele frequencies for polymorphic loci in populations Africanis, SPCA, Saluqi and Jericho are given in Table 2. Table 3 presents coefficients for heterozygote deficiency or excess (**d**), Chi-square (χ^2) values and degrees of freedom (**DF**) at all polymorphic loci, as well as individual heterozygosity values (**h**) for each population.

Results obtained from PAGE-systems were scored according to a study done on Japanese, Asian and European dogs by Tanabe *et al.* (1991). Loci identified with the PAGE method include post-albumin (**PA**) 1, -2 and –3, as well as pre-albumin (**Pre-A**) and post-transferrin (**P-Tf**). During the present study a fourth, monomorphic post-albumin (**PA-4**) was identified. According to the previously mentioned study, this locus was also found to be monomorphic and authors reported that the protein bands were expressed at very low densities. Tanabe *et al.* (1991) did not include this locus because the protein bands were expressed at very low densities.

3.1. VARIATION AT PROTEIN-CODING LOCI

The endemic Africanis population was found to be monomorphic for allele A at the following polymorphic loci: **AK-1**, **-2**,and **Hb**. Genotypes for this group complied to Hardy-Weinberg expectations at three of the polymorphic loci (**PA-1**, **-2** and **-3**), and fell short of ideal Hardy-Weinberg proportions at loci **CK**, **PER** and **P-Tf**. Heterozygote deficiencies were encountered at **CK** (**d**=-1.000) and **PA-1** (**d**=-0.172), with systems **PER**, **PA-2**, **-3** and **P-Tf** showing heterozygote excess.

The SPCA group displayed polymorphism at **AK-2**, **PER**, **Hb**, **PA-1**, **-3** and **P-Tf**, and was monomorphic for allele A at the remaining three polymorphic loci (**AK-1**, **CK** and **PA-2**). Hardy-Weinberg expectations were met at loci **PER**, **PA-1**, **-3** and **P-Tf**, but not at **AK-2** and **Hb**. Heterozygote deficiencies occurred at all of the polymorphic loci, except at **P-Tf** (**d**=0.199), where a heterozygote excess was found.

The Saluqi dogs were monomorphic for allele A at loci **AK-1**, **-2, CK** and **PA-2**. Polymorphism for this group occurred at the remaining five loci (**PER**, **Hb**, **PA-1**, **-3** and **P-Tf**). Hardy-Weinberg expectations were met at all loci, except **PER**, and heterozygote excess was observed at all the loci for this group, excluding the **PA-1** system.

The last group (mongrel dogs from Jericho) was polymorphic at all the loci in Table 2, except at **CK** and **AK-2** where only allele A was found. Allele frequencies at loci **AK-1** and **PER** did not comply to Hardy-Weinberg expectations, with heterozygote deficiencies at all loci, except **PER**, where heterozygote excess was observed (**d**=3.878).

3.2. VARIATION AT POLYMORPHIC LOCI

TABLE 2: Allele frequencies for polymorphic loci in four populations of dogs.

Locus	Allele	Africanis	SPCA	Saluqi dogs	Jericho dogs
AK-1	A	1.000	1.000	1.000	0.406
	B				0.594
AK-2	A	1.000	0.833	1.000	1.000
	B		0.167		
CK	A	0.963	1.000	1.000	1.000
	B	0.037			
PER	A	0.470	0.531	0.500	0.467
	B	0.530	0.469	0.500	0.533
Hb	A	1.000	0.560	0.818	0.611
	B		0.440	0.182	0.389
PA-1	A	0.655	0.658	0.656	0.625
	B	0.167	0.132	0.188	0.063
	C	0.179	0.211	0.156	0.313
PA-2	A	0.978	1.000	1.000	0.929
	B				0.071
	C	0.022			
PA-3	A	0.476	0.658	0.536	0.688
	B	0.524	0.342	0.464	0.313
P-Tf	A	0.324	0.158	0.156	0.375
	B	0.118	0.316	0.281	0.188
	C	0.559	0.526	0.563.	0.437

Two alleles (designated A and B) were scored in the four populations in the **AK-1** system (figure 6). Only the A-allele was observed in the Africanis, SPCA and Saluqi dog populations. The B-allele was the most common allele in the Jericho population, with the A allele represented in a single heterozygote. The latter displayed two bands as expected for a monomer.

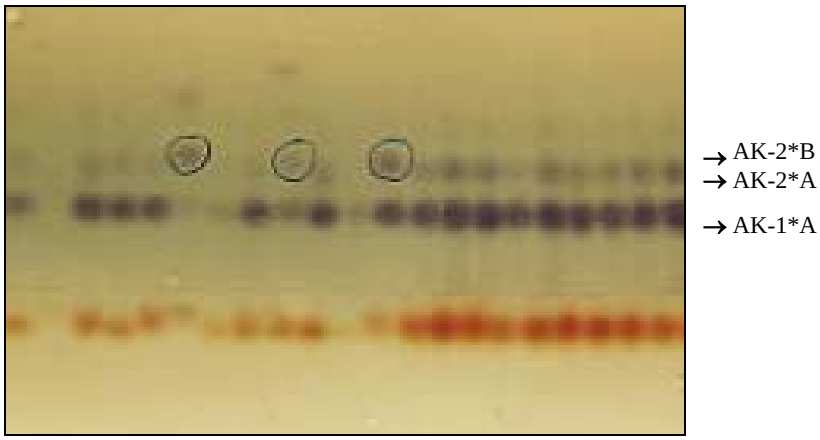

Figure 6: Adenylate kinase on a TC gel, indicating polymorphism.

Two alleles were scored for the **AK-2** system. Allele A was the common allele in the SPCA population, with the B allele occurring at low frequency (0.167). The Africanis, Saluqi and Jericho dogs were monomorphic for allele A. No heterozygotes were observed in the AK-2 system for any of the populations.

No heterozygotes were observed in any of the populations studied at the **CK** locus and two alleles were present in this system. The A-allele was the most common allele in the Africanis breed, with allele B only occurring at a very low frequency (0.037). The other three populations were monomorphic for allele A.

The presence of two alleles was shown in the **Hb** system. The A-allele was the predominant allele in the SPCA, Saluqi and Jericho groups (with frequencies ranging from 0.560 to 0.818), with monomorphism in the Africanis population. Only one heterozygote was observed in the SPCA population for this locus, which displayed two bands as expected for a monomeric locus.

Two alleles were scored in the **PER** system. The B-allele was slightly favoured for populations Africanis and Jericho, with allele A occurring at lower frequencies (0.470 and 0.467 respectively). The SPCA group had allele A as the most common allele, with a 50/50 frequency of alleles A and B in the Saluqi population. Heterozygotes were observed in all four populations, with Saluqi dogs being purely heterozygotic. These heterozygotes displayed two bands, characteristic for monomeric loci such as **PER**. These allozyme phenotypes were therefore in agreement with the quantenary structure of the corresponding enzymes (Ward, 1977).

Various loci were scored from PAGE (figure 7). In the **PA-1** system, three alleles (scored as A, B and C) were identified. Allele A was found in higher frequency, with alleles B and C appearing in much lower frequencies in all four populations (0.062 – 0.188 and 0.179 – 0.313, respectively). Heterozygotes were observed in all four the dog populations studied, which were expressed as two bands as is expected for a monomeric subunit structure. The **PA-2** system also had three alleles present. The SPCA and Saluqi dogs were monomorphic for **PA-2*A** and this allele was the most common allele by far in the Africanis and Jericho populations (allele frequencies of 0.978 and 0.929, for the latter two populations respectively). Allele B was only observed in the Jericho group (at a frequency of 0.071), and allele C (frequency 0.022) was solely found in the Africanis breed. Heterozygotes (displayed as two bands) were observed in the last mentioned group, but only one heterozygote was found in the Jericho group. Two alleles (A and B) were identified at the **PA-3** locus. With the exception of the Africanis population, allele A occurred in larger frequencies in all the populations in this system (frequencies ranging

between 0.536 and 0.688). Allele B was the most common in the Africanis, occurring at a frequency of 0.524. Double-banded heterozygotes were observed in all four populations.

Three alleles were scored in the final polymorphic system, **P-Tf**. With the exception of the Jericho population, allele C occurred in largest frequency in all the populations at this locus, with frequencies ranging from 0.526 to 0.563. Jericho dogs expressed the A allele more frequently (frequency of 0.500). Alleles A, B and C occurred in all four populations and two-banded heterozygotes were observed in all the populations. The Africanis breed had the combination of alleles A-C more than any of the other populations studied.

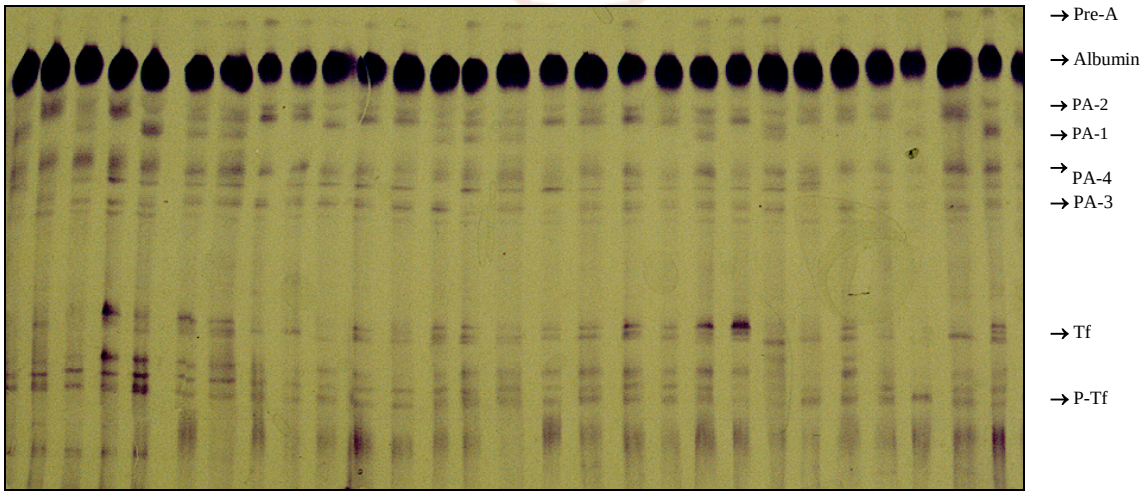

Figure 7: Pre-Albumin, Albumin, Post-Albumins, Transferrin and Post-Transferrin stains as a result of PAGE.

It should be noted that the locus **Tf** did occur throughout the analyses, but this occurrence was infrequent and not constant. Hence, the scoring results from this locus were not included in this study.

3.3. POPULATION STATISTICS

The average individual heterozygosity (**h**) values (Table 3) ranged from 0.043 to 0.569 for Africanis, 0.278 to 0.598 for SPCA, 0.298 to 0.580 for Saluqi, and 0.133 to 0.633 for Jericho. Three alleles were observed at the **PA-1**, **-2** and **P-Tf** loci, whereas all of the other polymorphic protein coding loci had only two alleles. Values of more then 0.043 indicate that nearly as many A as B alleles were encountered at loci with two alleles.

The alleles classic of Africanis did not comply to Hardy-Weinberg expectations at loci **CK**, **PER** and **P-Tf** (although they met Hardy-Weinberg expectations at loci **PA-1**, **-2** and **-3**). The alleles of the SPCA group deviated from Hardy-Weinberg proportions only at two loci (**AK-2** and **Hb**), whereas the Saluqi population showed a deviation only at one locus (**PER**). The population sampled at Jericho fell short of Hardy-Weinberg expectations at loci **AK-1** and **PER** (with conformation to Hardy-Weinberg expectations at loci **Hb**, **PA-1**, **-2**, **-3** and **P-Tf**).

TABLE 3: Heterozygote deficiency or excess (**d**), χ^2 values, degrees of freedom (**DF**) and individual heterozygosity (**h**) values for polymorphic loci in four dog populations.

Locus	Group	d	χ^2	DF	h
AK-1	Jericho	-0.874	13.117*	1	0.482
AK-2	SPCA	-1.000	44.794*	1	0.278
CK	Africanis	-1.000	53.020*	1	0.071
PER	Africanis	0.868	38.439*	1	0.498
	SPCA	-0.066	0.285	1	0.498
	Saluqi	0.909	10.000*	1	0.500
	Jericho	0.554	4.928*	1	0.498
Hb	SPCA	-0.965	55.023*	1	0.493
	Saluqi	0.167	0.392	1	0.298
	Jericho	-0.338	1.171	1	0.475
PA-1	Africanis	-0.172	5.285	3	0.512
	SPCA	-0.189	2.745	3	0.506
	Saluqi	-0.287	3.043	3	0.510
	Jericho	-0.077	0.700	3	0.508
PA-2	Africanis	0.011	0.011	1	0.043
	Jericho	0.000	0.000	1	0.133
PA-3	Africanis	0.038	0.061	1	0.499
	SPCA	-0.203	0.838	1	0.450
	Saluqi	0.246	0.914	1	0.497
	Jericho	-0.182	0.318	1	0.430
P-Tf	Africanis	0.462	29.914*	3	0.569
	SPCA	0.199	1.532	3	0.598
	Saluqi	0.148	1.192	3	0.580
	Jericho	0.111	0.619	3	0.633

** Loci where significant (P<0.05) deviations of allele combinations from expected Hardy-Weindberg proportions occur*

Table 4 gives a summary of loci where significant deviations (P<0.05) of the alleles from expected Hardy-Weinberg proportions occur. The **AK-1** system showed significant differences that distinguished the Jericho population from the rest (Africanis, SPCA and Saluqi). The Africanis breed could be distinguished from Jericho dogs at the **Hb** locus as well as from the SPCA group at loci **AK-2**, **Hb** and **P-Tf** and, finally, from the Saluqi

breed at **Hb** and **P-Tf**. Furthermore, the dogs sampled from the various SPCA's could be distinguished from the Saluqi population at the loci **AK-2** and **Hb**.

TABLE 4: Genetic differentiation – Loci where significant deviations (P< 0.05) of allele frequencies from expected Hardy-Weinberg proportions occurred in the four dog populations.

Group	Comparing group	Locus	Probability
Africanis	SPCA	AK-2	0.00008
		Hb	0.00000
		P-Tf	0.00824
	Saluqi	Hb	0.01570
		P-Tf	0.03118
	Jericho	AK-1	0.00000
		Hb	0.00014
SPCA	Saluqi	AK-2	0.01036
		Hb	0.02890
	Jericho	AK-1	0.00000
Saluqi	Jericho	AK-1	0.00000

The values for the four populations of the following parameters are indicated in Table 5: average heterozygosity (**H**), the mean number of alleles per locus (**A**) and percentage of polymorphic loci, using the 95% criterion, (**P**). The values for unbiased **H** varied between 0.106 (Africanis) and 0.182 (Jericho) and the **A** values ranged from 1.33 to 1.57 for Saluqi and Jericho respectively. The **P** values obtained were the lowest in Saluqi (23.81%) and Jericho had the highest value of 42.86%.

TABLE 5: Average heterozygosity (**H**) values and mean number of alleles per locus (**A**), with standard errors thereof and percentage of polymorphic loci (**P**).

Group	H (SE)	A (SE)	P
Africanis	0.106(± 0.046)	1.38(± 0.15)	28.57
SPCA	0.137 (± 0.050)	1.38 (± 0.15)	28.57
Saluqi	0.118 (± 0.048)	1.33 (± 0.14)	23.81
Jericho	0.159 (± 0.054)	1.43 (± 0.15)	33.33

3.4. FIXATION INDICES

Population differences were studied by calculating fixation indices for each locus as well as the mean weighted value across all loci (Tables 6 and 7). The values for $\mathbf{F_{ST}}$ in all the populations studied range from 0.005 to 0.523, with an average of 0.090 (Table 6). The mean $\mathbf{F_{IS}}$ is 0.021, while the $\mathbf{F_{IT}}$ value is 0.108 (Table 6). The **AK-1** locus contributed the most to differentiation between populations. The gene diversity analysis indicates that only 5.65% (average pairwise $\mathbf{F_{ST}}$ value) of differentiation exist *between* the populations, which translates into a 94.35% variation *within* populations.

TABLE 6: Summary of F-statistics at all loci.

Locus	**F_{IS}**	**F_{IT}**	**F_{ST}**
AK-1	0.870	0.938	0.523
AK-2	1.000	1.000	0.085
CK	1.000	1.000	0.028
PER	-0.589	-0.581	0.005
Hb	0.436	0.527	0.162
PA-1	0.152	0.162	0.012
PA-2	-0.064	-0.020	0.041
PA-3	-0.018	0.013	0.031
PA-4	0.440	0.486	0.083
P-Tf	-0.233	-0.166	0.055
Mean	***0.021***	***0.108***	***0.090***

The mean pairwise $\mathbf{F_{IS}}$ values (Table 7) across all loci range from -0.277 (Africanis and Saluqi) to 0.176 (SPCA and Jericho). The mean pairwise $\mathbf{F_{IT}}$ values (Table 7) range from – 0.258 (Africanis and Saluqi) to 0.232 (SPCA and Jericho), with mean pairwise $\mathbf{F_{ST}}$ values (Table 7) between 0.014 (Africanis and Saluqi) and 0.098 (Africanis and Jericho).

TABLE 7: Mean pairwise F-statistics across all loci between the four populations.

Population	**SPCA**	**Saluqi**	**Jericho**
Africanis	F_{IS} = 0.045 F_{IT} = 0.098 F_{ST} = 0.056	F_{IS} = -0.277 F_{IT} = -0.258 F_{ST} = 0.014	F_{IS} = -0.064 F_{IT} = 0.040 F_{ST} = 0.098
SPCA	-	F_{IS} = 0.024 F_{IT} = 0.045 F_{ST} = 0.022	F_{IS} = 0.176 F_{IT} = 0.232 F_{ST} = 0.069
Saluqi		-	F_{IS} = -0.080 F_{IT} = 0.006 F_{ST} = 0.080

3.5. GENETIC DISTANCES

Table 8 shows a matrix of genetic distance coefficients. The largest **D** value (Nei, 1978) was obtained between Africanis and Jericho (0.028) and the smallest value of 0.001 was found between the endemic Africanis and the indigenous Saluqi dogs. The values obtained using Nei's (1972) measure are slightly higher, ranging from 0.004 (between Africanis and Saluqi) and 0.035 (between Africanis and Jericho) and show the same trend. The average **D** value (Nei, 1978) is 0.015.

TABLE 8: Nei's (1978) unbiased genetic distance values (above diagonally) and Nei's (1972) genetic distance (below diagonally).

Population	Africanis	SPCA	Saluqi	Jericho
Africanis	******	0.014	0.001	0.026
SPCA	0.016	******	0.002	0.018
Saluqi	0.004	0.006	******	0.019
Jericho	0.032	0.025	0.027	******

The phylogenetic relationships between the breeds or populations studied are summarised in Figure 8. This was obtained using the UPGMA method and generated by the BIOSYS-1 computer programme. In this figure it can be seen that the Africanis and Saluqi dogs are very closely related genetically. Jericho and Africanis are the furthest apart and therefore the least genetically similar.

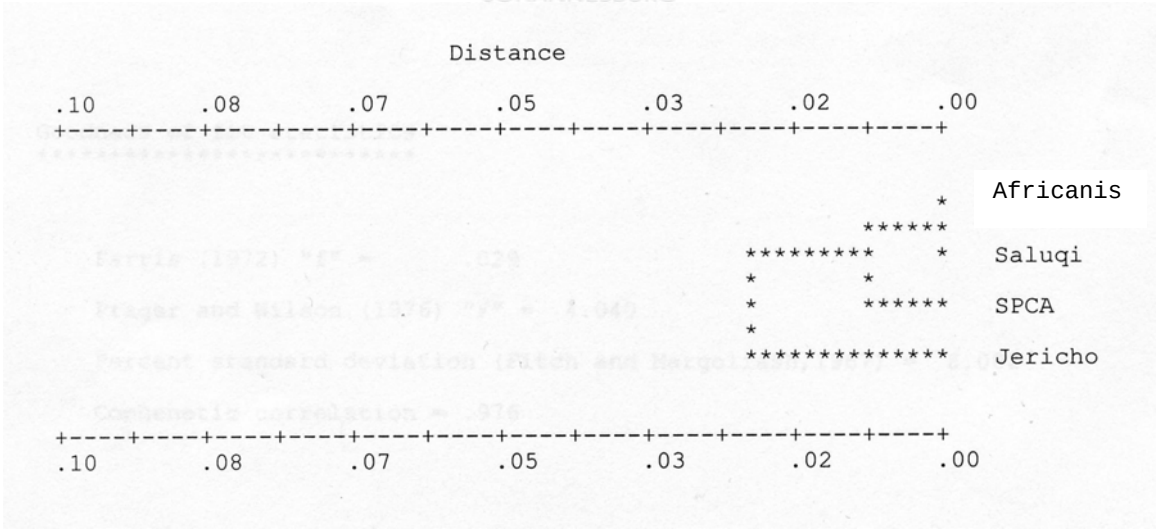

Figure 8: Phylogenetic relationships between the four dog groups (Nei, 1978).

3.6. POPULATION DIFFERENTIATION

The computer programme GENEPOP (Raymond and Rousset, 1995, 1997) was used to determine if significant (P<0.05) differences in allele frequencies (Table 9) occur between population pairs. Significant differences occurred at **AK-1** and **Hb** for all the populations. The **AK-2** locus could be used to differentiate between SPCA group and all the other populations, except Jericho, while **P-Tf** helped to differentiate populations SPCA and Saluqi from the Africanis breed.

TABLE 9: Significant differentiation (P<0.05) between population pairs using GENEPOP.

Populations	**SPCA**	**Saluqi**	**Jericho**
Africanis	AK-2 Hb P-Tf	Hb P-Tf	AK-1 Hb
SPCA	-	AK-2 Hb	AK-1
Saluqi		-	AK-1