Syarahan INAUGURAL

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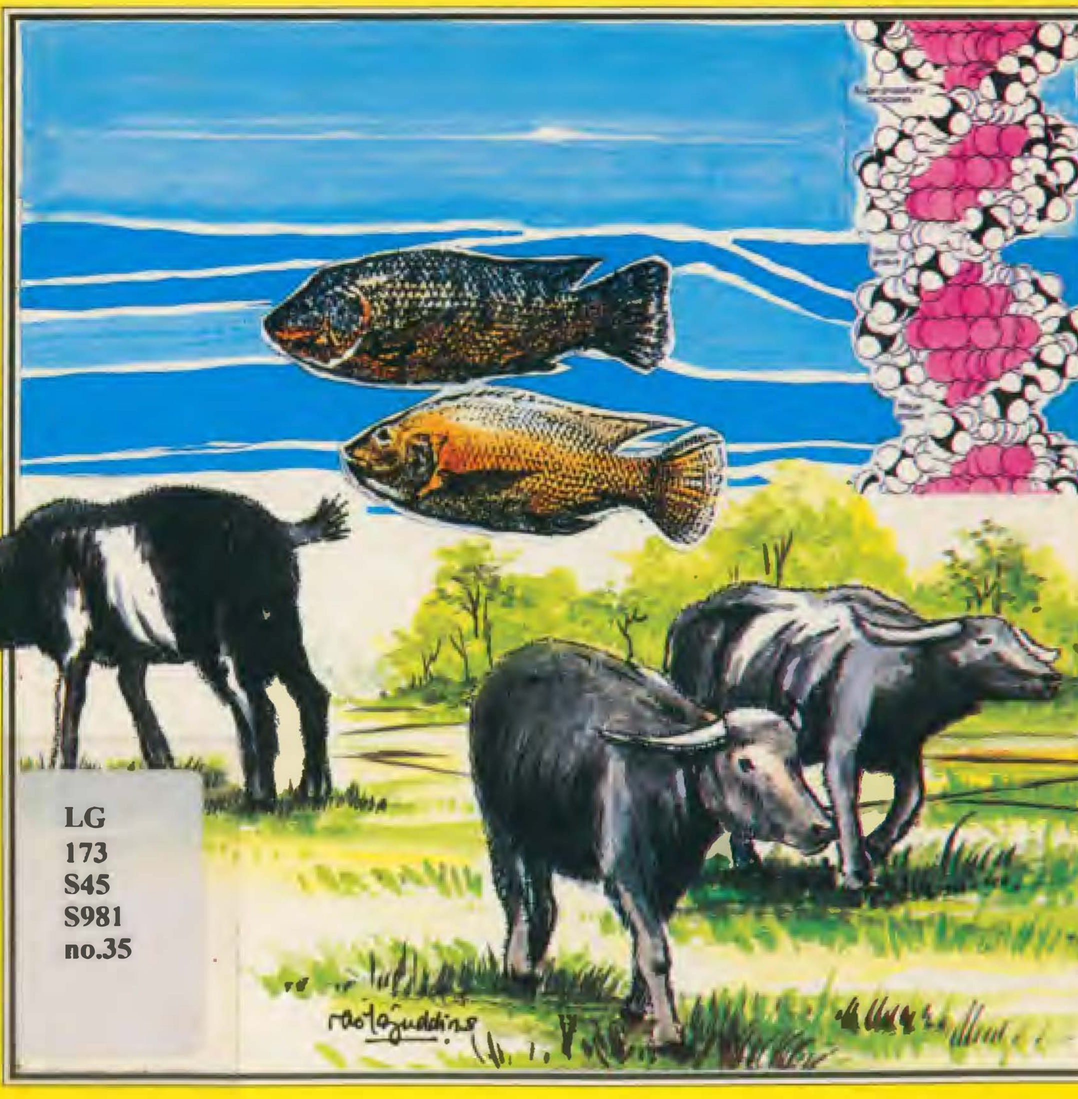

TAN SOON GUAN

Tan Soon Guan was born 49 years ago in Melaka, Malaysia. From 1955 to 1960 he attended Anglo Chinese Primary School and from 1961 to 1965 he obtained his secondary education in Anglo Chinese Secondary School, both in Jalan Tengkera, Melaka. From 1966 to 1967 he attended 6th. form classes at the Melaka High School.

In 1968 Tan Soon Guan directly entered the second year of study for the B.Sc. degree programme in the Biological Sciences at the University of Malaya in Kuala Lumpur. He graduated with a general degree in Biology in 1970 and with an honours degree in Genetics in 1971. He worked for one year as a Tutor in Genetics at the University of Malaya before being awarded an East West Centre Fellowship to further his studies to the PhD level at the University of Hawaii, Honolulu by the Government of the United States of America. He graduated with a PhD degree in Genetics in 1975.

He was appointed as a lecturer at the Department of Biology, Universiti Pertanian Malaysia in mid 1976. He was promoted to Associate Professor in 1985 and to Professor of Genetics in 1995. He is currently Head of the Biology Department at the Faculty of Science & Environmental Studies, UPM.

Tan Soon Guan has been very active in research and teaching since starting his academic career. His earlier research work was on human biochemical population genetics of Malaysians in collaboration with the University of California San Francisco International Centre for Medical Research group at the Institute for Medical Research, Kuala Lumpur. Subsequently, he worked on a project entitled "Genetic variation in natural populations of the *Anopheles balabacensis* complex and *An. maculatus* complex of Southeast Asia" in collaboration with Mahidol University and the United States of America Component of the Armed Forces Research Institute for Medical Research, both in Bangkok, Thailand as well as with the State Entomologist of Sabah. He also worked on the genetics of cocoa pod borers and padi hoppers in collaboration with entomologists at UPM. In 1988 Tan Soon Guan started on a collaborative project "Genetic identification of strains and genotypes of buffaloes and goats from Southeast Asia." This is a collaborative project involving UPM and the University of Malaya, in Malaysia and the University of New England and the University of Queensland in Australia. This project took 9 years to complete and it is currently the most comprehensive biochemical (protein level) and molecular (DNA level) population genetics study on the various indigenous Southeast Asian populations of these two important livestock species. From 1993 to 1995 he collaborated with the University of Malaya and the Queensland University of Technology on a project "Genetic identification of different strains of *Oreochromis* (tilapia) breeds and selection based on progeny testing of broodstocks for improved production." His interest in fish genetics continues until today and he is now involved in a project on "Population genetics for management and conservation of aquatic resources and DNA fingerprinting in fishes" with other researchers at UPM and the University of Malaya.

GENETIC DIVERSITY OF SOME SOUTHEAST ASIAN ANIMALS: OF BUFFALOES AND GOATS AND FISHES TOO

INTRODUCTION

The genetic relationship between any two populations is a function of differences between them in allele frequencies, with this relationship usually expressed in terms of a genetic distance. Thus, if two populations are homozygous for different alleles at a particular locus, the distance is the maximum possible while if the allele frequencies in the two populations are identical, the distance is zero. The estimated genetic distances may vary among loci and the most accurate measures of relationships will be obtained by averaging over many loci (Nei, 1978). In animal breeding, it is expected that a cross between two animals originating from two populations with a large genetic distance between them will result in maximum heterosis or hybrid vigour. Although many estimates of genetic distances are available, Nei's standard genetic distance, D, (Nei, 1972) had been extensively used in studies of livestock populations and breeds. The genetic relationships among livestock populations can also be represented graphically by means of a dendrogram based on the genetic distances and clustered by methods such as the neighbour joining method of Saitou and Nei (1987) or the unweighted pair group method with arithematic averaging (UPGMA) of Sneath and Sokal (1973). In order to get good estimates of genetic relationships, Lewontin (1974) suggested that 50 or more loci be studied while Barker (1994) demonstrated the marked effects using a small number of loci can have on estimates of relationships for populations within a species. Since in the past, many studies of genetic relationships among livestock breeds worldwide have not used more than 15 loci (Gonzalez et al., 1987; Tunon et al., 1989; Zanotti Casati et al., 1990) their interpretations need to be taken with caution.

In order to remedy the situation, we embarked on a project in 1988 to study the genetic relationships among 17 geographical populations of the Asian water buffaloes (*Bubalus bubalis*) and among 11 populations of the indigenous Katjang goat (*Capra hircus*) of Southeast Asia and the feral goat of New South Wales, Australia using 53 and 42 biochemical genetic loci respectively from the blood. In addition for buffaloes we also typed a subset of these populations by using DNA level markers namely 21 nuclear microsatellite loci and for the mitochondrial DNA cytochrome b gene and the D-loop control region.

Studies on the different geographical populations of the various livestock species in Asia were initiated by members of the Society for Researches on Native Livestock of Japan in the nineteen sixties. Since then this group of researchers had studied domestic livestock species such as cattle, pigs, sheep, goats, Asian water buffaloes, chicken and horses as well as their wild relatives whenever possible from countries throughout the region for example Japan, China, the Philippines, Thailand, Indonesia, Malaysia, Sri Lanka and Bangla Desh. They usually used morphology, morphometrics, blood groups and blood protein polymorphisms in their studies. The results of their research had been published in Japanese with English abstracts as Reports of the Society, usually one for each country for example Report No. 14 published in 1992 was for Nepal.

As far as the use of blood protein polymorphisms to study genetic relationships is concerned, most of the extensive work were done by Dr. Amano and his collaborators. Since they were studying many species it is understandable that their results for each species were based on sampling relatively small numbers of animals from each locality for example in water buffaloes this ranged from 2 to 28 animals and for a modest number of loci ranging from 8 in their earlier reports to 25 in the later ones (Amano et al., 1992 and references therein). Apart from this group of dedicated Japanese researchers who studied many livestock species and populations from many east Asian countries, other studies were usually confined to a single country and species. As an example, Chen et al.(1990) examined the various geographical populations of the yellow cattle in China using the same approaches as the Japanese group. In their biochemical polymorphism studies they used only six blood protein loci although their sample sizes were large (2217 animals from 20 Chinese cattle populations were typed). Chen et al. (1990) found evidence that genes from Bali cattle (*Bos banteng*) had been introduced into Chinese cattle just as they had been introduced into Southeast Asian cattle populations but not into the zebu group cattle of the Indian subcontinent (Namikawa et al., 1986).

The water buffalo is an important Asian livestock species. In 1990 136.3 million of the world's 141 million buffaloes were found in Asia (Chantalakhana, 1992). There are two types of Asian buffaloes (Macgregor, 1941), the River buffalo of the Indian subcontinent, the Mediterranean countries and the Balkans usually used for meat and milk and the Swamp buffalo of Southeast Asia which are found from Assam in India to the Yangtze valley of China, traditionally used for draught. In Japan, Swamp buffalo can be found in Okinawa. Behaviourally, the River wallows in clear water while the Swamp in muddy water. Morphologically, the Swamp is slate-black in colour with white-grey chevrons on the brisket below the neck and has long horns growing horizontally from the head curving backwards to form a cresent. The Rivers are larger and jet black in colour with relatively short horns that curl forward, downward and backward. Swamp buffalo has traditionally been used for draught power in Southeast Asia and the River for milk and meat. However, the Swamp is being increasingly used for meat as

Southeast Asia develops and in the United States of America, Swamp buffalo meat is being promoted as a health food since its calorie and fat contents are lower than those of cattle beef (Johnson and Charles, 1975). While the River buffalo has been selected to form about 18 improved breeds with high milk yield and differing in horn form, the Swamp has not been divided into different types although some geographical populations, especially in China, have been given local names. The Swamp has retained the low milk yield and primitive horn shape of the wild Arni buffalo found in Northern India and Indochina (Cockrill, 1987). Buffaloes are believed to have been domesticated in India about 5000 years ago and in China about 4000 years ago (Cockrill, 1974) but Cockrill (1987) stated that domestication in China may have occurred 7000 years ago. Chromosomal studies showed that the morphologically Swamp indigenous buffalo of Sri Lanka (the Lankan buffalo) shared the same diploid chromosome number of 50 with the River buffalo while the Southeast Asian Swamp had 48 (Bongso et al., 1977).

Goats are believed to have been domesticated 7000 years ago in west Asia. Asia has 56% of the world's population of 506 million goats in 1987 (Nozawa, 1991) and of these more than half were found in the Indian subcontinent where many breeds such as the Jamnapari and the Anglo-Nubian had been developed. These breeds have also been introduced to many tropical regions of the world including Southeast Asia where they have been extensively crossed with the local goats. At present the local goat populations of Southeast Asia are essentially unknown from the research and development viewpoints. These animals are mainly kept for meat and are of particular importance to subsistence farmers and landless households. As with buffalo, the problem is to identify whether genetically different strains of goats exist in Southeast Asia and if so, which are the best for existing and future production systems. The indigenous meat goats of Southeast Asia are the Kambing

Katjang (pea goat in the Malay language). They can also be found in Taiwan and in the south-west islands of Japan. Their morphology and body size resemble the south Chinese and the Black Bengal goats. The coat colour is black or brown, sometimes with white markings.

The introduction of tilapia fish into Malaysia was made during the Japanese occupation of the country during World War II. Since then its aquaculture has become an important component of the Malaysian freshwater fish production industry which is becoming increasingly important because of the depleting supply of marine fish. Many species and strains of tilapia had been introduced into Malaysia over the years both by the government and private sectors. As tilapia is a hardy fish and interspecific hydridization occurs readily it is imperative to characterize genetically strains that are to be used in any breeding programme such as the one that we are involved in (Tan et al., 1996). Among the species and strains of tilapia known to have been introduced into Malaysia are *Oreochromis mossambicus*, the Chitralada, Israel and Philippine strains of *O. niloticus, O. aureus* and the red tilapia. Since interspecific hybridization commonly occurs during pond culture of tilapia, the genetic integrity of the local tilapia strains is in doubt. Moreover, morphometric identification is often difficult. We have done a study using 35 electrophoretic loci on eight strains of tilapia available in Malaysia.

GENETIC STUDIES ON BUFFALO AND GOAT POPULATIONS

Buffalo blood samples were collected from 15 Swamp buffalo populations between 1988 and 1991 in Malaysia (three localities: Terengganu, Sabah and Sarawak), Indonesia (Bogor, Medan and Sulawesi), the Philippines (Mindanao), Thailand (Chengmai, Haadyai, Surin and Kam Paeng Seng), Sri Lanka (South, North-central and Kandy) and Australia (Northern Territory) and from two populations of the Murrah breed of River buffalo (Universiti Pertanian Malaysia, Serdang, Malaysia and Kotaliya in Sri Lanka). The samples were collected, processed and separated into plasma, red cells and buffy coat, transported in liquid nitrogen to Kuala Lumpur and stored at -70°C (Tan et al., 1991) until used. The plasma and red cells were used for analysing nuclear biochemical markers by cellulose acetate, polyacrylamide and starch gel electrophoresis (Tan et al., 1991) and polyacrylamide gel isoelectric focusing (Tan et al., 1993). The BIOSYS-1 computer package (Swofford and Selander, 1989) was used to calculate Nei's D based on the allelic frequencies and to generate an UPGMA dendrogram. DNA extraction, polymerase chain reaction (PCR) and cycle sequencing for a 303 base fragment of the mitochondrial DNA cytochrome b gene were done following a user-friendly system (Lau et al., 1995). This PCR and cycle sequencing system was also used to sequence a 260 base pair segment of the mitochondrial D-loop region using primers derived from the known sequence of bovine mtDNA. However, due to unequal reading of the sequences at the terminii, only a common 158 base sequence which was obtainable for all the 80 animals typed were compared and 33 D-loop genotypes were observed. The 33 D-loop genotypes observed were individually clustered using the proportion of nucleotide differences and neighbour joining with the corresponding bovine sequence as the outgroup. Twenty one microsatellite loci were typed for using the method of Moore et al (1995) from eight Southeast Asian Swamp populations namely Surin, Trengganu, Sabah, Sarawak, Bogor, Sulawesi, Musuan and Australia; from the South Lankan population and from the Murah populations of Sri Lanka and Malaysia. The microsatellite data were analysed in a manner similar to that used for the allozyme data.

Katjang goat blood samples were collected from almost the same localities as buffalo except that for Peninsular Malaysia the collection site was the Malaysian Agricultural Research and Development Institute (MARDI) and the University of Malaya (UM) farms, both in the Klang Valley, no goat sample was collected from Surin and Kam Paeng Seng in Thailand and Kandy in Sri Lanka while the Australian feral samples were obtained from New South Wales. The 42 loci from the red blood cells and plasma were analysed using cellulose acetate, agarose and polyacrylamide gel electrophoresis (Tan et al., 1991).

Data are presently avaliable for 53 biochemical genetic loci in 17 buffalo populations of which two are Murrah populations. In addition, the three phenotypically Swamp Lankan populations were shown electrophoretically to be of River type since they clustered with the two Murrah populations on the UPGMA dendrogram. Hence there are 12 genetically Swamp populations, three from Indonesia, one from the Philippines, four from Thailand, three from Malaysia and one from Australia. In most populations, about 50 animals were typed for the polymorphic loci and 25 for the monomorphic ones. Of the 53 loci analysed, 25 (47%) were polymorphic. The mean observed heterozygosity was the lowest in the Northern Territory population (0.102) suggesting that this population probably underwent a genetic bottleneck on introduction to Australia. Of the Southeast Asian populations, the mean heterozygosity ranged from 0.115 to 0.166 while those of the Murrah and Lankan populations ranged from 0.130 to 0.143. The matrix of genetic distances based on 53 loci among each pair of Swamp populations showed values ranging from 0.007 to 0.066 and with a mean value of 0.030. Our estimates of D among the Swamp buffalo populations were of the same order of magnitude as distances among recognized livestock breeds in developed countries for example those for European breeds of cattle

(Gonzalez et al., 1987) and also for those among different geographic populations of Asian cattle (Namikawa et al., 1986). The mean genetic distance (D) among the 5 River populations (Murrah and Lankan) was 0.018 with a range from 0.014 to 0.036. The River populations were distinct (mean D=0.054) from the Swamp populations with a range from 0.033 to 0.074.

About 20 animals from each of the 8 Swamp populations, the South Lankan population and the 2 Murrah populations were analysed for 21 nuclear microsatellite loci (Barker et al., 1995). All 21 loci were polymorphic with the number of alleles detected per locus ranging from 2 to 14 in Swamp buffalo and from 2 to 18 in River buffalo, with one or more alleles at each locus shared between the two buffalo types. The mean number of alleles per locus ranged from 2.6 (Sarawak) to 5.3 (Lankan buffalo). The mean observed heterozygosity among the Swamp ranged from 0.589 (Surin) to 0.400 (Sabah) while among the River group the Sri Lanka Murrah had the highest value of 0.613 while the values for the Lankan buffalo and the Malaysian Murrah were the same (0.531). The Australian population had a mean heterozygosity value of 0.409 and 3.0 alleles per locus. Nei's D among the Swamp populations ranged from 0.026 between the Surin and Trengganu populations to 0.492 between the Australian and Sarawak populations. Among the River group the lowest D value (0.041) was between the two Murrah populations while the highest D value of 0.067 was between the Lankan and the Murrah population from Sri Lanka. The highest D value among the 11 Asian buffalo populations was between the Sabah Swamp population and the Murrah population from Malaysia (1.125). In the UPGMA dendrogram based on the 21 microsatellite loci and D the Lankan and the 2 Murrah populations form one cluster while the 8 Swamp populations form another cluster.

When D values based on protein data for the 25 polymorphic loci only were calculated for the same 11 populations with microsatellite data available, it was observed that the mean D among the 8 genetically Swamp populations was 0.080, among the 3 genetically River (which included the Lankan) populations it was 0.048 and for the genetically Swamp versus River populations it was 0.146. In comparision, the values based on the 21 microsatellite loci were 0.228, 0.056 and 0.897 respectively (Barker et al., 1995). Thus, microsatellite loci showed much greater discrimination among populations in terms of larger genetic distance values than protein loci. MacHugh et al. (1994) typed 11 microsatellite loci in 6 European breeds of dairy and beef cattle namely Aberdeen Angus, Hereford, Simmental, Charolais, Jersey and Friesian. The average of all pairwise genetic distances (D) among these 6 breeds was 0.083. Therefore, the magnitude of genetic differentiation (D=0.228) based on 21 microsatellite loci among the 8 genetically Swamp Southeast Asian buffalo populations is much greater than that among well recognised and long-established cattle breeds.

Our sequencing of a 303 base fragment of the mitochondrial cytochrome b gene of Swamp buffalo from the three populations from Malaysia, four populations from Thailand, two from Indonesia (Bogor in Java island and Sulawesi), one from the Philippines and one from Australia and the two Murrah populations revealed that the Swamp and the Murrah River buffaloes differed diagnostically at two sites. The South Lankan population posessed the River genotype. Among the Southeast Asian Swamp four genotypes were observed in the mainland populations whereas only the most frequent one of these was present in the archipelago populations (Sabah, Sarawak, Mindanao, Bogor and Sulawesi). This indicates that the archipelago buffaloes originated from mainland Southeast Asia. All the nucleotide substitutions for buffalo cytochrome b we observed were transitions and silent.

Comparision of the sequences obtained from 80 animals for a 158 base fragment of the mitochondrial D-loop region revealed the presence of 33 genotypes. Clustering of these 33 genotypes by the neighbour joining method (Saitou and Nei, 1987) resulted in two major clusters. One major cluster contained genotypes found in the Swamp only and the other cluster contained genotypes that were found in the Swamp only, the River (including the Lankan) only and in both the Swamp and River types. Hence, our results showed the presence of a substantial amount of variation in the D-loop and seemed to indicate that the Swamp is the ancestral type (Barker et al., 1995).

Of the 42 biochemical loci we typed in goats, 10 (24%) were polymorphic (Barker et al., 1991). In their study of Okinawa goats using 27 loci, Nozawa et al. (1978) found 9 loci (33%) to be polymorphic. Thus the level of polymorphism in Asian goats is lower than in buffaloes. The D values among the goat populations ranged from 0.000 for Chengmai-Sulawesi to 0.033 for North-central Sri Lanka-Haadyai. These values are much lower than those among the Swamp or the River buffalo populations but similar to those reported by Nozawa et al (1978) for eight populations of Okinawa native meat goats, those for three populations of Indonesian native goats (Katsumata et al., 1981) and for five Sri Lankan goat populations (Shotake et al., 1986). The D values for Spanish native goats however was higher and ranged from 0.003 to 0.097 (Tunon et al., 1989). On the dendrogram of genetic relationships, the Southeast Asian Katjang populations from Malaysia, Indonesia, Thailand and the Philippines formed one cluster while the two Sri Lankan populations clustered together with the Australian feral population which originated from goats imported by European settlers over the past 200 years. Sri Lankan goats would be expected to be more closely related to the improved breeds of the Indian subcontinent. Thus, Indian breeds would be most useful in crossbreeding programmes to improve the productivity of

Southeast Asian goats. The observed mean heterozygosity values in the goat populations that we typed ranged from 0.054 (Australia) to 0.084 (Philippines and Haadyai).

Both our biochemical and molecular results revealed that the phenotypically Swamp Lankan buffalo is actually of River type genetically. This is in accord with the findings of Amano et al. (1992). The geographically separated Swamp buffalo populations of Southeast Asia are generally phenotypically similar but there is sufficient genetic variation present among them for us to suggest to buffalo breeders to consider crossing between animals from genetically distant Swamp populations as an alternative to relying on crosses between Swamp and River buffaloes in order to obtain hybrid vigour (Tan et al., 1994). Moreover, crossing among the Swamp would avoid any future fertility problem that may arise at the village level as a result of the current widespread practice in Southeast Asian countries like the Philippines (Steane et al., 1994) of crossing between the River and the Swamp which differ in their chromosome numbers. Our results emphasize the importance of conserving Swamp buffalo populations from different geographical areas of Asia in order to preserve and utilize the genetic variations and biodiversities present in them so as to improve the Swamp buffalo herds at the village and farm levels. In addition our data support the hypothesis of Amano et al. (1992) that the Swamp and the River buffaloes have been domesticated from different origins and that mitochondrial DNA polymorphisms could be used to distinguish between them (Amano et al., 1994, Tanaka et al., 1995). It should be emphasized that although in our studies DNA level markers gave higher estimates of genetic distances and heterozygosities than biochemical loci and may therefore be the method of choice for future global programmes to characterize livestock species and populations, overall the genetic relationships obtained among the populations that we studied are similar irrespective of whether biochemical or molecular markers were used to obtain them. This similarity greatly strenghtens the confidence that can be placed on the measures of genetic relationships among the buffalo populations which we have studied.

The low values for genetic distances among the 9 Southeast Asian Katjang goat populations that we studied (from Malaysia, Thailand, the Philippines and Indonesia) which ranged from 0.000 for Chengmai-Sulawesi to 0.012 for MARDI/UM-Haadyai may reflect the widespread practice of hybridization with imported exotic breeds or the use of imported semen in artificial insemination programmes throughout the region or it may be that since goats are smaller and therefore easier to handle and transport than buffaloes, there is more movement of goats between various geographical regions than for buffaloes. Hence, there is closer genetic relationship among different geographical populations of Asian goats than among Asian buffalo populations.

Such information about the different populations and breeds of indigenous Asian livestock species is essential for the continued development of the Asian livestock industry which should conserve, upgrade and utilize the rich and varied animal genetic resources present within Asia instead of relying mainly on imported breeds from the developed countries. The indigenous livestock populations are already well adapted to the local ecosystem and this is especially of relevance to the humid tropical regions of Southeast Asia where imported breeds of cattle and sheep especially from temperate countries face serious disease, parasite and insect pest problems. All these problems could be alleviated if we could select, upgrade and improve the productivity of already existing populations and breeds of livestock animals as well as farm and popularize the utilization of currently little used animals such as deer.

GENETIC STUDIES ON STRAINS OF TILAPIA (*OREOCHROMIS* SPECIES) FOUND IN MALAYSIA

The strains of tilapia studied were the Thai, Israel, Philippines and Malaysian strains of *O. niloticus*, the Singapore strain of *O. aureus*, the Malaysian and Thai strains of *O. mossambicus* and a hybrid red tilapia strain from Taiwan. The 35 electrophoteric loci were typed using cellulose acetate electrophoresis following the methods of Macaranas et al (1992) and Selvaraj et al (1994). Data analysis were done using the BIOSYS-1 computer package of Swofford and Selander (1989) and genetic variation in each strain was measured by the percentage of polymorphic loci and the mean heterozygosity. Genetic distances and similarities among strains were estimated using Nei's genetic distances and similarities (1978) and a dendrogram of genetic relationships among the strains was constructed using the unweighted pair group method with arithematic averaging (Sneath and Sokal, 1973).

The following enzymes were typed electrophoretically: aconitase (Acon), aspartate aminotransferase (Aat), acid phosphatase (Acp), adenosine deaminase (Ada), alcohol dehydrogenase (Adh), adenylate kinase (Ak), carbonic anhydrase (Ca), creatinine kinase (Ck), esterase (Est), fumarate hydratase (Fum), fructose biphosphatase (Fbp), glyceraldehyde-3-phosphate dehydrogenase (Gapdh), glycerol-3-phosphate dehydrogenase (Gpd), glucose-6-phosphate dehydrogenase (G6pd). glucose phosphate isomerase (Gpi), isocitrate dehydrogenase (Idh), iditol dehydrogenase (Iddh), lactate dehydrogenase (Ldh), malate dehydrogenase (Mdh), malic enzyme (Me), mannose phosphate isomerase (Mpi), 6-phosphogluconate dehydrogenase (Pgdh) phosphoglucomutase (Pgm) and superoxide dismutase (Sod). Thirty five loci were found to code for these 24 enzymes. Of these, 18 loci namely Acon, Aat-2, Acp, Ada, Adh, Ca, Est-1, Est-2, Fbp-2, Fum-1, Gapd, Gpi-1, G6pd, Iddh, Idh, Me, Mpi and Sod were polymorphic.

A preliminary analysis involving 32 loci, 19 of which were polymorphic in four tilapia strains namely the Malaysian strain of *O. mossambicus* and the Israel, Thai (Chitralada) and the Philippines strains of *O. niloticus* showed very close genetic relationships among themselves (Selvaraj et al., 1994). The similarity values based on the 19 polymorphic loci between the Malaysian mossambicus strain and the Israel, Chitralada and Philippines niloticus strains were 0.974, 0.983 and 0.985 respectively, values that were higher than those among the niloticus strains among themselves which ranged from 0.958 to 0.962, thus indicating that a high level of introgression of niloticus genes had occurred into this Malaysian strain of mossambicus. Hence, use of this mossambicus strain in our breeding programme was discontinued and it was replaced by the Thai strain of *O. mossambicus*.

The genetic distance and similarity values, percentage of polymorphic loci, and heterozygosity values based on 35 loci and the dendrogram of genetic relationships are presented in Tan et al. (1996). Both in terms of percentage of polymorphic loci and heterozygosity value, the Thai mossambicus strain was the least variable while the Singapore aureus strain was the most variable. In their genetic relationships, the Thai *O. mossambicus* was distinct from both *O. niloticus* and *O.aureus*. The four *O. niloticus* strains clustered together and the Taiwan red hybrid strain clustered with them forming a subcluster together with the red Israel and Chitralada strains while the black local niloticus and Philippine strains formed another subcluster within the niloticus cluster. *O. aureus* was more closely related to *O. niloticus* than *O. mossambicus* was. A similar result regarding the relationships among *O. mossambicus*, *O. aureus* and *O. niloticus* was obtained by McAndrew and Majumdar (1984). It should be noted that the Israel and Chitralada Thai tilapia strains from Fiji are black in colour (Macaranas et. al., 1992) unlike those found in Malaysia which are red. Thus, this emphasizes the fact that for tilapia, similar strain names used in different geographical areas need not necessarily mean that the fishes have the same genetic constitution and that it is essential to do a molecular genetic characterization of each strain to be used in any proposed tiliaia breeding programme.

The genetic distance and similarity values and the dendrogram of genetic relationships are of importance because they reveal the relationships among the tilapia strains especially among different strains of *O. niloticus*. It is expected that crosses between strains that are genetically distant as measured through the use of genetic distance estimates would exhibit maximum hybrid vigour. This was found to be the case in pigs by Cepica et al. (1995). Although diagnostic biochemical markers were available between *O. mossambicus* and *O. niloticus* (Macaranas et al., 1992) which enabled us to conclude, together with the genetic similarity and distance values, that introgression of *O. niloticus* genes had occurred into one of our *O. mossambicus* strains none was observed between the different strains of *O. niloticus* or between *O. niloticus* and *O. aureus*. Since the best performing hybrids in our breeding experiments were the result of crosses between different strains of *O. niloticus* or between them and *O. aureus* it is essential to develop DNA level markers that could distinguish diagnostically between them in order to monitor every generation of our breeding programme. We are currently doing research toward achieving this goal.

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