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GENETIC DIFFERENTIATION AND ESTIMATION OF GENE FLOW FROM SEVERAL SUBPOPULATION OF JAVANESE THIN TAIL SHEEP IN WEST JAVA

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ABSTRACT

Predominantly breed of sheep in West Java are Javanese Thin Tail (JTT), and in some areas have been developed several strain, for example Priangan or Garut sheep that have developed for fighting ram, Priangan or Garut sheep for slaughtered and local thin tail. Gene flow among sub-population of JTT tail sheep are probably through genetic improvement using ram from one subpopulation to others or by purchasing sheep from one subpopulation to others. In order to find out gene flow among subpopulation JTT in West Java, five subpopulation were sampled from four sub-districts (kecamatan) in Garut regency, which was two kecamatans representing Garut fighting ram subpopulation and other two kecamatans for Garut meat sheep subpopulation. The local sheep were sampled from one kecamatan in Purwakarta regency. Allele and genotypic frequencies were estimated from four DNA microsatellite ILSTS005, ETH225, CSSM66, and INRA023. Genetic diferentiation or heterogeneity were estimated using Wright's fixation indices (F-statistics) according to Weir and Cockerham (1984) by using program GENEPOP version 3.2, May 2000, and gene flow were estimated by $Nm = [(1/F_{ST}) - 1]/4$. Results of observation shows that F_{ST} from ILSTS005, ETH225, CSSM225, and INRA023 loci were 0.0034, 0.0069, 0.0693, and 0.0382, respectively. F_{ST} from all four loci were 0.0329, and significantly different from zero. F_{ST} from CSSM225, and INRA023 loci were significantly different from zero, but F_{ST} from ILSTS005 and ETH225 loci were not significantly different from zero. The results indicated that there were genetic differentiation among population, and an indication of increasing inbreeding as shown by pooled F_{ST} from four loci were significantly different from zero. Estimated gene flow from four loci is about 7.3. This indicated that the gene flow is relatively high, probably due to no isolation in sub-population.

Key Words: sheep, javanese thin tail, gene, differentiation, flow

INTRODUCTION

The predominant type of sheep breed in West Java which is the province with the largest sheep population in Indonesia are Javanese Thin Tail (JTT) (Merkens and Soemirat, 1926; Iniguez et al., 1991). In West Java, JTT are called as Priangan or Garut sheep and known as fighting ram. Priangan or Garut sheep according to Merkens and Soemirat (1926) are developed from crossing among local sheep, fat tailed sheep from South West Africa and Merino sheep. However proportion of the genotype among three breeds are not known, therefore in West Java have developed several strain of JTT

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sheep, such as Priangan or Garut sheep for fighting ram, JTT sheep for slaughtered and local JTT thin-tail sheep. Gene flow among sub-population of Javanese thin tail sheep probably through genetic improvement using ram from one subpopulation to others or by purchasing sheep from one subpopulation to others. Therefore genetic differentiation among subpopulation probably present in the population of JTT sheep.

In a study on genetic diversity, molecular or genetic markers are a tool usually to be used. The most widespread use of genetic markers in this context is the assessment of diversity either within and between breeds or within and within lines or strain at one breed. Although in the principle all types of markers would be suitable for this purpose, microsatellite are used in 90 percent of all diversity studies (Simianer, 2006). The reasons, because the autosomal microsatellite loci could be used successfully for individual genetic identification and parentage analysis, for monitoring of ex-situ conservation program, population diversity estimation, differentiation population, calculation of genetic distances, genetic relationship and population genetic admixture estimation, as well as used for inbreeding estimation (Hanotte and Jianlin, 2006). In addition microsatellite loci are often highly polymorphic and relatively easy to survey and hence offer the hope of greater understanding of population structure (Slatkin, 1995).

The simplest parameters for assessing the distribution of diversity between breeds using genetic markers are the genetic differentiation or Wright's fixation indices (F_{ST}, G_{ST}, Θ). The most widely used is Wright's fixation indices or F-statistic, because it is well suited for population that are subdivided in smaller unit, such as demes, clans or villages (Crow and Kimura, 1970, Workman and Niswander, 1970, Neel and Ward, 1972, Long, 1986, Wang, 1997, Nagylaki, 1998).

The model's parameters relate the departure from panmictic which measures panmictic expectation in the total population in the Wahlund effect between subdivisions and the average departure from panmixia within subdivisions. The F-statistic model is hierarchical model with genes stratified at three levels: individual (I), within subdivisions (S) and within the total population (T). It has three main parameters which are: (1) F_{IT} is the correlation of uniting gametes relatives to those of the total population; (2) F_{IS} is the average over all subdivisions of the correlations between uniting gametes relative to those of their own subdivision. Thus F_{IS} gives the average deviation of the subdivisions or subpopulations genotypic proportions from Hardy-Weinberg expectation; and (3) F_{ST} is the correlation of the random gametes within subdivisions relative to the total population. Thus F_{ST} measure the degree of heterogeneity among subdivisions or subpopulations. The list can be extended if there are further subdivisions (Crow and Kimura, 1970, Workman and Niswander, 1970, Neel and Ward, 1972, Long, 1986, Wang, 1997, Nagylaki, 1998).

The F-statistics can be thought as inbreeding coefficient as well, in which F_{IS} is related to inbreeding in individuals relative to subpopulations to which they belong, F_{ST} is related to inbreeding in subpopulations relative to the total population of which they are part, and F_{IT} is related to inbreeding in individuals relative to the total population (Wang, 1997). Because F_{IS} is a function of non random mating in the subpopulation therefore the estimate value being negative, zero, and positive. However, the value of FST is always greater or equal to zero because of the Wahlund effect (Yasuda and Morton, 1967, Crow and Kimura, 1970, Wang, 1997). The three F-statistics are not independent and interrelated as $(1-F_{IT}) = (1-F_{ST})(1-F_{IS})$ (Crow and Kimura, 1970, Workman and Niswander, 1970, Neel and Ward, 1972, Long, 1986).

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The application presented here are to identify genetic differentiation using Wright's fixation indices or F-statistics and gene flow among five subpopulations of JTT sheep in West Java using four microsatellite genetic markers.

MATERIALS AND METHODS

To study genetic differentiation and gene flow among sub-population of JTT sheep in West Java, five subpopulations were sampled from four subdistricts (kecamatan) in Garut regency (kabupaten), which were kecamatan Tarogong and Cisurupan representing Priangan or Garut fighting ram subpopulations and kecamatan Wanaraja and Sukawening for Priangan or Garut sheep for meat subpopulations. The local JTT sheep were sampled from one kecamatan in Purwakarta regency. The Garut fighting ram type blood samples as source of DNA were collected from 19 head of ram and 24 head of ewes at Tarogong subpopulations, and 26 head of ram and 10 head of ewes at Cisurupan subpopulations, respectively. The blood samples of Garut meat type were collected from 13 head of rams and 34 head of ewes at Wanaraja subpopulations, and 12 head of rams and 16 head of ewes at Sukawening subpopulations. While for local JTT sheep blood samples were collected from 8 head of rams and 25 head of ewes.

Eleven pairs of microsatellite primers (ETH3, INRA032, ETH 152, HEL1, HEL5, ILSTS005, ETH225, CSSM66, ETH10, INRA023, HAUT12) were used on Polymerase Chain Reaction (PCR), in order to identify individual genotype of the animals. Source of information of the DNA sequences of 11 primers were from Muladno et al. (2001) based on the web sites of http://www.ri.bbsrc.ac.uk. However, only four microsatellite primers (ILSTS005, ETH225, CSSM66 and INRA023) were successfully amplified. This indicates that five subpopulations of JTT did not have microsatellite loci of ETH3, INRA032, ETH152, HEL1, HEL5, ETH10 and HAUT24. Therefore based on these results all data analysis was based on genotypic and allele frequencies from ILSTS005, ETH225, CSSM66 and INRA023 microsatellite loci.

Genetic diferentiation or heterogeneity were estimated using Wright's fixation indices (F-Statistics), by methods of intraclass correlation using allele frequency according to Weir and Cockerham (1984), followed by chi-square test according to Workman and Niswander (1970). Gene flow were estimated by $Nm = [(1/F_{ST}) - 1]/4$ according to Wolf and Soltis (1992). All data were analysed using program GENEPOP version 3.2, May 2000.

RESULTS AND DISCUSSION

Number and Genotypic frequency

There were detected 6 genotypes from ILSTS005, ETH225, CSSM66 and INRA023 loci for the Garut fighting ram type at Cisurupan and Tarogong subpopulations. The genotypes were AA, AB, AC, BB, BD and CE, respectively. The BD genotype was the highest frequency from ILSTS005, ETH225 and CSSM66 loci. The frequency of BD genotype from ILSTS005, ETH225 and CSSM66 loci were 89.66%, 100%, and 82.14%, respectively, at Cisurupan subpopulation.

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Table 1. The number and allelic frequency of five JTT subpopulations according to ILSTS005, ETH225, CSSM225, and INRA023 loci

Microsatellite Locus/ subpopulation	Number of Genes	Allelic frequency (%)						Number of allele
		A	B	C	D	E	F	
ILSTS005								
Cisurupan	58	-	55.2	-	44.8	-	-	2
Tarogong	50	2.00	56.0	-	42.0	-	-	3
Wanaraja	72	-	61.1	-	38.9	-	-	2
Sukawening	48	-	60.4	-	39.6	-	-	2
Purwakarta	60	-	50.0	1.7	48.3	-	-	3
ETH225								
Cisurupan	62	-	50.0	-	50.0	-	-	2
Tarogong	60	-	56.7	-	43.3	-	-	2
Wanaraja	60	-	50.0	-	50.0	-	-	2
Sukawening	46	6.5	43.5	6.5	43.5	-	-	4
Purwakarta	60	-	50.0	-	50.0	-	-	2
CSSM225								
Cisurupan	56	-	41.1	8.9	41.1	8.9	-	4
Tarogong	54	-	46.3	9.3	35.2	9.3	-	4
Wanaraja	74	1.4	47.3	2.7	45.9	2.7	-	5
Sukawening	52	1.9	63.5	-	34.6	-	-	3
Purwakarta	56	-	14.3	21.4	28.6	21.4	14.3	5
INRA023								
Cisurupan	50	44.0	22.0	26.0	8.0	-	-	4
Tarogong	58	46.6	22.4	25.9	5.2	-	-	4
Wanaraja	60	56.7	16.7	16.7	10.0	-	-	4
Sukawening	54	38.9	40.7	7.4	13.0	-	-	4
Purwakarta	38	47.4	7.9	42.1	2.6	-	-	4

While the BD genotype frequency at Tarogong subpopulations were 84.00%, 86.67% and 70.37%, respectively. The BD genotype frequency from INRA023 locus at Cisurupan and Tarogong subpopulations were only 16.00% and 10.34%, respectively. The highest genotype frequency from INRA023 was AC. The AC frequency at Cisurupan and Tarogong subpopulations were 52.00% and 51.72%, respectively.

From four microsatellite studied, there were detected 8 genotypes for Garut meat types, which were AA, AB, AC, AD, BB, BC, BD and CE, respectively. The BD genotype frequency was the highest from ILSTS005, ETH225 and CSSM66 loci.

The BD genotype frequency from those loci were 77.78%, 100%, and 91.90%, respectively, at Wanaraja subpopulations. Meanwhile, the genotypic frequency from those loci were 79.17%, 99.86%, and 65.38%, respectively, at Sukawening subpopulations. However, from the INRA023 locus, the AA and AC genotypes had the same frequency at Wanaraja subpopulations which were 33.33%. The BD genotype frequency from INRA023 was only 20% at Wanaraja subpopulation. The highest genotypic frequency from INRA023 locus was AB (48.15%) at Sukawening subpopulation. The BD genotypic frequency for INRA023 was 25.93% at Sukawening subpopulation.

The same microsatellite loci detected 6 genotypes which were AB, AC, BC, BD, CE and DF for local JTT sheep. The highest genotypic frequency was BD from ILSTS005, and ETH225 loci, and CE and AC genotypes for CSSM66 and INRA023

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loci, respectively. The BD genotype frequency from ILSTS005 and ETH225 loci were 96.67% and 100%, respectively. While the genotypic frequency of CE and AC from CSSM66 and INRA023 loci were 42.86% and 84.21, respectively. The BD genotype frequency from CSSM66 and INRA023 were 28.57% and 5.26%, respectively.

Number and allelic frequency

The ILSTS005 locus were detected 4 different alleles. From this locus there were detected 2 alleles (B and D), and 3 alleles (A, B, and C), respectively, for Garut fighting ram types at Cisurupan and at Sukawening subpopulations. For the Garut meat type at Wanaraja, and Sukawening both were detected 2 alleles (B and D). While local JTT sheep at Purwakarta subpopulation, there was detected 3 alleles (B, C, D).

From ETH225 locus was detected 4 different alleles, where subpopulation of Garut fighting ram type at Cisurupan as well as at Tarogong were detected 2 alleles (B and D). Meanwhile, for the Garut meat type there were detected 2 alleles (B, D) and 4 alleles (A, B, C, D), respectively, at Wanaraja and Sukawening subpopulations. This locus detected 2 alleles (B and D) for local JTT at Purwakarta subpopulation.

Analysis on CSSM225 locus, there were detected 6 different alleles. The Cisurupan, Tarogong, Wanaraja, Sukawening, and Purwakarta subpopulations were detected 4 alleles (B, C, D, and E), 4 alleles (B, C, D, and E), 5 alleles (A, B, C, D, and E), and 5 alleles (B, C, D, and E), respectively. Meanwhile analysis on INRA023 locus, showed that all five subpopulations were detected to have 4 alleles (A, B, C, and D).

Allelic frequency of ISLTS005, ETH225, CSSM225, and INRA023 loci from five subpopulations of JTT sheep are presented in Table 1. Analysis from the allelic frequency from the four microsatellite loci (Table 1) there indicate that two different allele have high frequency as compared to the other allele. The pattern are shown almost in all locus studied and all subpopulation. In ILSTS005, ETH225, and CSSM225 loci, the B and D alleles mostly possess high frequency as compared to other alleles. Even the total frequency of B and D allele from ILSTS005 locus reach 100% (monomorphic) for Garut fighting ram type at Cisurupan, and both subpopulation of Garut meat type at Wanaraja and Sukawening. Similarly the total frequency of B and D allele from ETH225 locus are reach 100% (monomorphic) in both Garut fighting ram type at Cisurupan and Tarogong subpopulations, Garut meat type at Wanaraja subpopulation, and local JTT sheep subpopulation at Purwakarta. Table 1 also showed that the F allele is only detected in CSSM225 locus at local JTT sheep Purwakarta subpopulation, although the allelic frequency is only 14.3%.

Genetic differentiation

Genetic differentiation estimating by Wright's fixation indices or F-satistics are presented in Table 2, and there show that the F_{ST} range from 0.0034 to 0693 for 4 loci with pooled value of 0.0329. Test of significant using chi-square according to Workman and Niswander (1970) for ILSTS005 and ETH225 locus, the F_{ST} do not significantly different from zero ($P > 0.05$), however for CSSM225 and INRA023 loci are significantly differ from zero ($P < 0.05$). Test for all loci indicate that pooled F_{ST} are significantly differ from zero ($P < 0.001$).

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Table 2. F-statistics (F_{IS}, F_{ST}, and F_{IT}) for ILSTS005, ETH225, CSSM225, and INRA023 loci in five JTT subpopulation

Locus	Number of samples	Number of genotype	Number of allele	F_{IS}	F_{ST}	F_{IT}
ILSTS005	144	4	4	-0.7490	0.0034	-0.7430
ETH225	144	3	4	-0.8792	0.0069	-0.8662
CSSM225	146	6	6	-0.4739	0.0693	-0.3717
INRA023	130	4	4	-0.3309	0.0382	-0.2801
All				-0.5841	0.0329	-0.5321

Results indicate that there are genetic differentiation among subpopulation. Two loci are significantly different from zero (CSSM225 and INRA023), while two other loci (ILSTS005 and ETH225) are not significantly different from zero. However, there is indication of increasing inbreeding as shown by estimation of pooled F_{ST} from all four loci is significantly different from zero. The results supported by the results of F_{IS} and F_{IT} that all are negative. The negative results of F_{IS} and F_{IT} indicate that inbreeding present due to non random mating within subpopulations and the effects of population subdivisions (Wang, 1977).

Gene flow among subpopulation that are estimated by $Nm = [(1/F_{ST}) - 1]/4$ according to Wolf and Soltis (1992) for ILSTS005, ETH225, CSSM225 and INRA023 loci are 73.2, 35.98, 3.36, and 6.29, respectively. Meanwhile, gene flow among subpopulations studied for all loci are 7.3. The results indicate that the highest gene flow are from ILSTS005 locus, followed by ETH225, INRA023 and CSSM225.

CONCLUSION

1. The ILST005, ETH225, CSSM66 and INRA023 loci are detected 6, 8 and 6 genotypes for Garut fighting ram types, Garut meat types and local JTT. The BD genotype is the highest genotypic frequency from ILSTS005, ETH225 and CSSM66 loci for Garut fighting ram type and Garut meat types, and from ILSTS005 and ETH225 loci for local JTT.
2. The ILSTS005, ETH225 loci are detected that both had 4 different alleles, while CSSM225, and INRA023 loci are identified to have 6 and 4 different alleles, respectively. Two alleles tend to have high frequency as compared to others. The B and D alleles are monomorphic in ILSTS005 and ETH225 for most of Garut fighting ram types and Garut meat types.
3. Genetic differentiation is present among subpopulation as indicating by FST differences among four loci. However, there is indication of increasing inbreeding due to population subdivision.
4. Gene flow among subpopulations are relatively high, probably due to no isolation in subpopulation.

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