NOVEL GENETIC POPULATIONS FOR BOVINE BREEDING PROGRAMS IDENTIFIED THROUGH MOLECULAR DIVERSITY ANALYSIS



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KNOWLEDGE GAP

Climate change affects animal populations.











KNOWLEDGE GAP

There is a decreasing biodiversity.

- At least one breed of domestic species disappeared each month worldwide (FAO).
- The need to develop strategies how to conserve local genetic groups.









OBJECTIVES

Identify genetic populations that can survive climate related concerns.

- 1. Estimate inbreeding within individuals, subpopulations and populations.
- 2. Evaluate variability within individuals, subpopulations and populations.
- 3. Construct a phylogenetic tree for Batanes, Ilocos, Brahman and Holstein-Sahiwal cattle.



METHODS

Assessment of performance and molecular features.

1. Blood Collection

FTA cards were used to store the blood collected from jugular veins of the samples.





2. DNA Extraction



A micropuncher was used to obtain discs from the cards and a lysis solution was made to extract the DNA.

3. Evaluation of Quality and Quantity

Purity and concentration of the extracts using the novel techniques was evaluated using NanoDrop.



METHODS

4. Polymerase Chain Reaction

Primers were clustered based on sizes to allow running samples efficiently.



1	120	1	130 14	0 150	160	170	180	190	200	210	220	23	30 240	250	260	270	280	290	300	
2																				
3	Group I	120-126		139	-169		179-181				218	-226								
4		ILSTS013		ILST	5033		ILSTS008				ILSTS	6103								
5																				
6																				
7	Group II			128-160								1	70-284							
8				ILSTS028								ILS	TS023							
9																				
10				<	Primer 있음>	•		<prime< td=""><td>r 있음></td><td></td><td></td><td></td><td><</td><td>Primer 있을</td><td>></td><td></td><td></td><td><prime< td=""><td>있음></td><td></td></prime<></td></prime<>	r 있음>				<	Primer 있을	>			<prime< td=""><td>있음></td><td></td></prime<>	있음>	
11	Group III				157-169			181-	185					237-257				281-	300	
12					ETH152 F			ILSTS	6005					AGLA293				ILSTS	006	
13																				
14	Single			148	3-160															
15				ILST	S050															



5. Analysis of PCR Products by ABI Sequencing Machine

6. Data Analysis Using POPGENE

F-statistics analysis and gene flow for each of the 11 microsatellite markers in four cattle populations.

Locus	F _{is}	F _{st}	F _{it}	Nm
AGLA293	0.3878	0.4872	0.1624	1.2895
ETH152	0.6367	0.7492	0.3096	0.5574
ILSTS005	0.4987	0.5238	0.0501	4.7439
ILSTS006	0.5150	0.6281	0.2331	0.8224
ILSTS008	0.4346	0.4629	0.0501	4.7430
ILSTS013	0.3818	0.5297	0.2392	0.7950
ILSTS023	0.3225	0.4566	0.1979	1.0134
ILSTS028	0.4396	0.5441	0.1866	1.0898
ILSTS033	0.4218	0.5412	0.2065	0.9608
ILSTS050	0.5007	0.5554	0.1095	2.0322
ILSTS103	0.3684	0.4914	0.1947	1.0339
Mean	0.44	0.55	0.18	1.09

Inbreeding **Estimate** a) heterozygote deficiency b) inbreeding within subpopulations c) inbreeding bet. populations



Genetic Diversity

a) F_{st} = 25% - 55%, indicates high genetic differences bet. populations based on Sewall and Wrights proposed F_{st} range.

Population	Batanes	llocos	Brahman	HS
Batanes	*			
llocos	0.1443	*		
Brahman	0.3091	0.4423	*	
HS	0.4744	0.6071	0.2366	*

Nei's original measures of genetic identity and genetic distances based on 11 microsatellite markers in four cattle populations



Dendogram showing the genetic relationship among the four genetic groups of cattle populations based on Nei's genetic distance (1000 bootstrap) using the modified neighbor procedure of PHYLIP Version 3.5

Genotype frequencies & fitness to Hardy-Weinberg equilibrium of llocos cattle for *GH1*.

				•	Chi-
LOCATION	GENOTYPE	OBSERVED	EXPECTED	ALLELE	Square
					HWE
	AA	21	17	0.65	x ² =8.63
Banna	AL	10	18	0.35	p<0.003
	LL	9	5		(df=1)
	AA	17	10	0.51 0.49	x ² =17.56
Marcos	AL	7	20		p<0.0003
	LL	16	10		(df=1)
	AA	31	28	0.84 0.16	x ² =12.81
Solsona	AL	5	11		p<0.003
	LL	4	1		(df=1)

1801	cacct cggac	cgtgtctatg	agaagctgaa	g gacctggag	gaaggcatcc	tggccctgat
1861	gcgggtgggg	atggcgttgt	gggtcccttc	catgtggggg	ccatgcccgc	cctctcctgg
1921	cttagccagg	agaatgcacg	tgggcttggg	gagacagatc	cctgctctct	ccctctttct
1981	agcagtccag	ccttgaccca	ggggaaacct	tttccccttt	tgaaacctcc	ttcctcgccc
2041	ttctccaagc	ctgtagggga	gggtggaaa <mark>a</mark>	t ggagcgggc	aggagggagc	tgctcctgag
2101	ggcccttcgg	cctctctgtc	tctccctccc	ttggcaggag	ctggaagatg	gcaccccccg
2161	ggctg g g cag	g atcctcaago	c agacctatga	a caaatttgad	c acaaacatgo	c gcag tga cga
2221	cgcgctgctc	aag aactaco	g gtetgetete	c ctgcttccgg	g aaggacctgo	c ataagacgga

Genotype frequencies & fitness to Hardy-Weinberg equilibrium of llocos cattle for *GH2*.

				•	Chi-
LOCATION	GENOTYPE	OBSERVED	EXPECTED	ALLELE	Square
· · · · · · · · · · · · · · · · · · ·					HWE
	AA	11	3		
	LL	0	1	0.28	x ² =80.38
Banna	LV	29	11	0.36	p<0.003
	AV	0	8	0.36	(df=3)
	VV	0	1		
	AA	6	1		
	$\mathbf{L}\mathbf{L}$	0	7	0.14	x ² =82.09
Marcos	LV	34	15	0.43	p<0.000
	AV	0	5	0.43	(df=3)
	VV	0	7		
	AA	9	2		
	LL	1	6	0.23	x ² =76.05
Solsona	LV	0	7	0.40	p<0.000
	AV	30	12	0.38	(df=3)
	VV	0	6		

2041 ttctccaage ctgtagggga gggtggaaaa tggageggge aggaggga**ge <u>tgetectgag</u>** 2101 **ggccetteg**g cetetetgte tetecetee ttggeaggag etggaagatg geaceeeeg 2161 ggetgggeag atceteaage agaeetatga caaatttgae acaaaeatge geagtgaega 2221 egegetgete aagaaetaeg gtetgetete etgettee**g|g** aaggaeetge ataagaegga 2281 gaegtaeetg **agggteatga agtgeegeeg e|t**teggggag geeagetgtg eettetagtt

Genotype frequencies & fitness to Hardy-Weinberg equilibrium of llocos cattle for *GHR*.

LOCATION	GENOTYPE	OBSERVED	EXPECTED	ALLELE
Location 1	AA	1	1.00	1.00
Location 2	AA	1	1.00	1.00
Location 3	AA	1	1.00	1.00

GENE FIXATION!!!!!

No Opportunity for Phenotypic and Genetic Improvement

IMPLICATIONS

- 1. Batanes and llocos cattle are genetically distant from one another.
- 2. Batanes and Ilocos cattle have a very high degree of introgression from that of the imported breeds.
- The high genetic variation of the two local groups shows the potential of these animals to have diverged in adapting to local ecotypes.
- 4. PHILIPPINES HAS AN EXISTING BOVINE POPULATIONS THAT CAN SUBSIST EXTREME ENVIRONMENTAL DUE TO CLIMATE CHANGE.