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Genetic Characterization of Selected Buffalypso (*Bubalus bubalis*) from Trinidad and Tobago for Potential Use in a Conservation Genetics Programme

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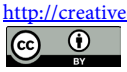

Abstract

The water buffalo (*Bos Bubalus bubalis*) species has been bred in many countries for its incredible production value and benefits to humans. The Buffalypso population in Trinidad and Tobago was an isolated, cross-breed selected for meat production and utilized as well for milk and draft animals. Microsatellite loci are highly polymorphic and are used as ideal markers of genetic variation, commonly used for marker-assisted selection, determination of genetic diversity and genome mapping in Bos species. Limited microsatellite markers have been characterized in water buffalo for use as Quantitative Trait Loci (QTLs). Since the water buffalo and cattle are closely related, cattle derived microsatellite markers were used in this study to genetically characterize 33 individual animals in an isolated Buffalypso population. Ten microsatellite markers were used, but markers BOLADRB1 and MAF45 failed to amplify in any of the 33 samples tested. Unique alleles were observed for all markers that were successfully amplified in the study. The CSSM36 marker was detected in highest proportion of alleles in the Buffalypso population. This pilot project was done to determine proof of principle for expansion to a nationwide Buffalypso genetic characterization study. We propose using comparative genomic tools in a conservation genetics programme in establishment of select breeding groups from the descendants of the Bennett-bred animals, to conserve and optimize traits of production and disease resistance.

Subject Areas

Agricultural Science

Keywords

Water Buffalo, Buffalypso, Microsatellites, Marker-Assisted Selection, Comparative Genomics, Conservation Genetics

1. Introduction

The water buffalo (*Bos Bubalus bubalis*) is a stellar animal in terms of animal production used traditionally in Asia, but now is extensively used in tropical countries worldwide for dairy and meat products. Other countries such as Brazil have also developed breeding centers to create large herds across the Americas. The water buffalo and cattle belong to the subfamily Bovinae whereas sheep and goat belong to the subfamily Caprinae. The use of various genome sequencing tools has provided scientists with the ability to detect micro rearrangements in the marker order of the two due to evolutionary divergence [1]. Microsatellite DNA is the term given to regions within eukaryotic genomes of repeat sequences (usually 1 to 5 nucleotides long) arranged as short tandem repeats; and accounts for approximately 10% - 15% of the mammalian genome and 30% of all repeat sequences within the genome. Microsatellites are utilized as remarkable genetic markers for population studies and as such, can be used to characterize populations of species and have been used to detect within population genetic variation in water buffalo populations [2].

Improving traits which are economically important in livestock species is generally done by genetic selection, largely relying upon applying statistical tools to analyze phenotypic and pedigree data to predict the overall genetic merit of individual animals. Quantitative trait loci (QTL), as defined by Biochard (2003), is a chromosomal segment displaying a Mendelian pattern of transmission and which has an effect on the trait of interest and has been utilized in Bos species extensively to produce excellent breeding stock in species of economic importance throughout the world. This information can be used to develop a utile marker-assisted selection (MAS) process which combines genetic marker information with phenotypic and pedigree information to increase the efficiency of selection for production and health-related traits in bovid animals [3]. These breeding programmes have been quite successful in dairy and beef animals.

Investigating DNA polymorphism using molecular markers can assist scientists in developing better livestock. These polymorphic markers can also show species evolution over time; although microsatellite primer pair sequences are typically conserved across related species. Comparative genomic studies were used to develop a panel that was tested against a total of 108 microsatellite primer pairs [4] showing that cattle primer pairs can be a practical method in developing a genetic linkage basis for genomic analysis. Certain microsatellite loci regions contain genes that are beneficial economically and when used as markers, these can aid in defining genetic variability in related species such as water buf-

falo [5]. Previous studies utilize marker-assisted selection, BAC sequencing, SNP genotyping, Genome Wide Association (GWA) and a modified approach called Genome Wide Selection (GWS) to localize the specific genomic regions involved in natural genetic variation including genes of immunogenetic importance [4]-[9]. The data obtained can aid in examining the genetics of cattle as these analyses, including the sequencing of the genome, provide tools to help in improving productivity and health of cattle [6] [7]. Through comparative genomics, these tools can also be utilized in other related Bos species, like water buffalo, to elucidate the markers of interest for production and disease resistance [6] [7] [8] [9].

Microsatellite loci are multi-allelic, co-dominant systems which are highly polymorphic and have been found to occur with frequencies as high as one every 6kb, making them ideal markers of molecular variation. These loci can be routinely isolated and characterized making them, at present, the markers of choice for population genetic analysis, genetic diversity, gene flow studies, QTL and genome mapping [4] [5] [6]. There have been over 1000 microsatellite markers characterized for cattle which have been used to map the genomes of riverine and water buffalo [8] [9]. Comparative genomic studies have been carried out which illustrate that repeat flanking sequences of microsatellite markers are often conserved across related species. An important implication of this finding is that previously developed microsatellite primer pairs can be used for the development of markers in related species thereby bypassing the laborious and expensive process of isolating and characterizing microsatellite containing sequences through cloning and/or screening of DNA sequence repositories and then designing primer pairs [5]. Though there are presently 1000 microsatellite markers characterized for cattle [5], efforts have been made in mapping genomes of riverine and water buffalos, where fewer microsatellite markers have been characterized in these species. Given that the bovid family of species is relatively closely related there have efforts to report on the ability of cattle derived microsatellite markers to amplify sequences in buffalo [3]-[9]. The sequencing of the *Bos taurus* genome also gives more information and allows a more thorough comparative genomics approach with *Bubalis Bubalis* [6].

The water buffalo was introduced in Trinidad & Tobago from India in 1905 and utilized mostly as draft animals [10] (**Figure 1**). In 1948, Steve Bennett crossed Bhadwari bulls with Jafarabadi cows to create the Buffalypso breed (Buffalo × Calypso, a form of popular Caribbean music), which is historically the only breed selected exclusively for meat (usually sold as beef), but now also used for milk production as well as draft animals [11]. Importation in buffalo ceased in the year 1949, with no further introduction of imported genetic material in Buffalypso, a crossbreed of the originally imported breeds into the local population [10] [11]. However, many animals were exported and left the original herd, spreading throughout the Americas in the 1950's-1980's [12]. The extensive breeding programme implemented by Dr. Bennett generated animals that were so distinct that the Buffalypso breed was born [11], with unique muscling making them an ideal animal for meat production.

Figure 1. Buffalypso animals from a local herd in Trinidad and Tobago. Image accessed from https://www.instagram.com/nationalarchivestt/.

Very little information exists on the genetics of the Buffalypso first developed in Trinidad and Tobago by Dr. Bennett in the 1960's [10] [11] [12] [13]. Also, genotyping has not been sufficiently utilized effectively as a tool in selection or breeding in livestock in this region, although it dominates in other parts of the world especially with tools available from the sequencing of the bovine genome [6] and comparative genomic research [7] [8].

In this study, we propose the use of ten (10) previously published cattle microsatellite loci [8] [9] [14] for genotyping Buffalypso animals (not known to be related) from the isolated population descended from the founding herd in Trinidad and Tobago. Water buffalo RH maps have been produced using cattle markers [15], therefore we propose that microsatellite loci can be a useful tool in studying Buffalypso. This valuable information can be utilized in breeding and evaluation to optimize selection for production traits and disease resistance and contribute to a genetic conservation programme in this breed of national and cultural importance. This pilot study, if successful, would allow for the further expansion and generation of genotyping and sequencing tools that could form the basis of development of a national programme in conservation genetics for the Buffalypso, a breed of national and cultural importance to Trinidad and Tobago.

2. Materials and Methods

2.1. Samples and Sample Collection

Collection of samples from unrelated Buffalypso were taken from thirty-three (33) animals at the Aripo Livestock Research Center and ECIAF campus of Uni-

versity of Trinidad and Tobago, located in Trinidad and Tobago. Genomic DNA was extracted using QIAGEN-DNeasy™ Blood & Tissue Kit (Cat. No. 69504) as directed by the manufacturer's instruction and previously published [7].

2.2. PCR Amplification

Polymerase chain reaction (PCR) was carried out on 1 µl of extracted genomic DNA (40 - 50 ng/uL) in a 5 µl reaction volume. Primers were selected to amplify ten (10) alleles mapped to Bos species in previously published research [8] [9]. The reaction mixture was as follows: 0.250 µl each of 10 mM dATP, dCTP, dGTP and dTTP, 0.6 µl $MgCl_2$ (25 mM), 0.1 µl each of 10 mM forward and reverse primer, 0.5 µl 10X Master Amp solution, 1 µl 5X Buffer solution and 1.375 µl ddH2O with 0.075 µl 5 U/uL Promega Go *Taq Polymerase*. Products were purified using QIAquick™ PCR Purification Kit (50) (Cat. No. 28104) as per Manufacturer's instructions and previously published [7].

2.3. Genotyping of PCR Products

PCR amplicons were separated using gel electrophoresis followed by being sized by running on a Genetic Analyzer Capillary Array, 50 cm (Applied Biosystems). Approximate sizes and sequence reads were then produced and compared for genotyping. Genotyping was carried out by comparison of alleles generated using Gene Mapper™ software as performed in previously published research [7] [8] [9].

3. Results & Discussion

In this study, DNA isolated was utilized in a comparative genomic analysis using ten (10) microsatellite markers identified in previous Bos species. Of the markers tested, only the microsatellite markers BOLADRB1 and MAF45 (previously identified in Bos taurus sp. [8] [9]) failed to amplify in any of the thirty-three samples tested. The *Bubalus bubalis* population in Trinidad, descended from the original founding herd created by Dr. Bennett in the 1940's [11], were shown to contain both Riverine and Swamp water buffalo alleles, which interestingly, means the Buffalypso is a hybrid species.

Unique alleles were found for markers that were successfully amplified in our study. The ratios found were as follows: CSSM36 was amplified in 30.3%, CSSM43 in 24.2%, ILSTS017 in 18.2%, ILSTS030 in 39.4%, INRA048 in 48.5%, INRA 120 in 36.4% of the Buffalypso water buffalo animals tested (see **Table 1**).

The *Artiodactyla* order's largest family is the bovids, that includes the Bovine subfamily of the Bovidae family and its nine genera: *Bos* (cattle), *Poephagus* (yak), Bison (bison), *Syncerus* (African buffalo), *Boselaphus* (nilgai), *Pseudoryx* (saola), *Tetracerus* (four-horned antelope), *Tragelaphus* (kudu and relatives), and *Bubalus* (domestic buffalo) [16]. The water buffalo is divided into two subspecies: river buffalo (*B. bubalis bubalis*) and swamp buffalo (*B. bubalis kerebau*), which are genetically distinct with different chromosome numbers (50 and 48, respectively), as well as distinct morphology, physiology, and productive and

Table 1. Allelic sizes for the markers tested.

Name of Marker	Cattle Alleles	Swamp Buffalo Alleles	Riverine Buffalo Alleles	Buffalypso Alleles Found	No. of Unique Alleles
BOLADRB2	10	-	-	6	4
CSSM036	10	5	7	7	7
CSSMO43	10	9	9	7	5
ILSTS017	9	-	8	6	6
ILSTS017	9	-	8	6	6
ILSTS030	5	-	4	4	4
INRA048	15	6	-	4	4
INRA121	5	-	2	8	8

reproductive performances. The two subspecies are interfertile, and their offspring have 49 chromosomes. Male crossbred progeny may occasionally have fertility issues, but female progeny has longer calving intervals [17]. The two subspecies have very different morphologies. River buffaloes are larger than swamp buffaloes, weighing between 450 and 1000 kg. The majority of breeds have curled horns and are found primarily in India, Pakistan, and a few European, Western Asian, and American countries. River buffalo are predominantly raised for dairy production, particularly in Asia and Europe, although they are also utilized for meat production or as a dual-purpose animal for draft purposes. The Buffalypso herd was the first at the time to be bred specifically for meat production, to be used potentially as a healthier beef substitute to reduce imports into Trinidad and Tobago [11]. Swamp buffaloes are smaller and lighter than river buffaloes, weighing between 325 and 450 kg on average. Swamp buffaloes are mostly raised for draft purposes, but they can also produce a large amount of milk (up to 600 kilograms per year) [16]. The Buffalypso animals sampled for this study were found to have a mix of alleles from both Swamp and River water buffalo, resulting in the generation of a hybrid breed with varying traits from both subspecies.

Buffalypso animals, like water buffalo in other populations, can be infected with the same diseases and parasites as cattle, but the severity of their infection varies greatly depending on the country, region and animal husbandry and management techniques that are utilized. Water Buffalo breeds have varied degrees of tick resistance; yet, are plagued by the species-specific louse *Haematopinus tuberculatus* [18]. The water buffalo industry is heavily impacted by brucellosis, tuberculosis, leptospirosis, bovine viral diarrhea, fasciolosis, foot-and-mouth disease, and protozoal infections [17] [18] [19]. In particular, in Trinidad and Tobago, brucellosis is a serious problem resulting in the culling of several hundreds of Buffalypso in already, small, isolated herds throughout the country, also

seen as an even issue in Amazonian herds of water buffalo which number in the tens of thousands in certain areas; thus emphasizing the need for genetic selection against this disease for conservation this breed [18] [19].

Initially, information on the arrangement of the water buffalo genome was obtained by comparing it to cattle through cytogenetic and c-banding investigations on the chromosomal number discrepancy between the subspecies, as well as the arm-by-arm matching of water buffalo and cow chromosomes [15] [20]. Fluorescent *in situ* hybridisation was utilized to map cattle-derived genes and microsatellite markers to water buffalo chromosomes, whilst early radiation hybrid (RH) panels enabled the production of a first-generation whole-genome RH map, including 2621 cattle-derived loci across all river buffalo chromosomes [21].

Scientists have therefore been successful in generating useful information using comparative genomics of these species to generate data utilized as tools in mapping as well as applied to breeding programmes across Bovid sp. Microsatellite loci are multi allelic, co-dominant systems which are highly polymorphic and characteristically stably inherited; along with high genomic abundance and relatively random distribution throughout the genome, make them ideal markers of molecular variation. Microsatellite loci are the markers of choice for population genetic analysis, genetic diversity, gene flow studies, QTL and RH or genome mapping because these can be routinely isolated and characterized [14] [15] [20] [21] [22]. Information derived from this study adds to the tools that can be utilized from the BOVMAP and genome sequencing consortiums in breeding and selection programmes [6] [21]. In addition, through further comparative genomics studies such as this one, we would be able to generate specific markers for use in breeding programmes for genetic resistance to diseases such as Brucellosis, which has decimated the Buffalypso population in Trinidad and Tobago.

4. Conclusions

We were able to successfully use cattle SNPs to genotype a population of water buffalo in Trinidad and Tobago. Our results show a mixture of Riverine alleles and Swamp alleles in the Buffalypso water buffalo population. We were able to find unique alleles derived from Riverine and Swamp buffalo in the Buffalypso that were previously unknown. Although the initial herds and breeding populations were isolated in Trinidad and Tobago originally, the Buffalypso created by Dr. Bennett was also crossed with the Murrah, Surti, Nili, and Nagpuri breeds, with the largest populations found now in Cuba as well as Trinidad and Tobago [10] [11] [12] [13]. Since animals from the original populations have been exported throughout the world and can be found in Venezuela, Costa Rica, Guatemala, the Republic of Honduras, Nicaragua, Brazil, Panama, Mexico, Colombia, USA, and even Taiwan (China) [13]; genotyping information is important in characterizing the founding populations as well as the subsequent crosses. Bennett *et al.* [13] laments the loss of Buffalypso animals, with only a few thousand

left with very few of the original breeding stock genetics, and very little effort at genetic conservation and management of livestock genetic resources being practiced to date in Trinidad and Tobago.

The recent pandemic and the long-term closed borders in Trinidad and Tobago also revealed that availability of locally derived, healthy food sources is critical in maintaining health and reducing sudden death and disease susceptibility in acute and long-Covid infections [23]. Therefore, it will be of national interest to use genotyping as a tool in a nationally implemented conservation genetics programme, to expand the Buffalypso population via marker-assisted selection to maximize breeding programmes using QTL data. This will allow Buffalypso to be introduced as a more viable source of healthy protein alternatives that can contribute to reducing our dependency on high imports of non-traditional, processed meat and dairy products as Dr. Bennett intended. Additionally, genome mapping resources have been utilized successfully using comparative genomics in cattle [20] [21] [22], even including feral bovid sp. [24], specifically for characterization of immunogenetic genes that are important for disease resistance. This can easily be utilized in other populations of Bovid sp., especially those bred in isolation and more susceptible to diseases such as Brucellosis. Recently, through extensive culling and loss from Brucellosis of animals across the country, the estimated Buffalypso population is less than 2000 animals. Therefore, any attempts at developing a dedicated conservation genetics programme in this breed of national and cultural importance must be done with haste, and is of dire importance to preserve this species and the dwindling population in Trinidad and Tobago, its country of origin.

Here, we have successfully shown that cattle markers can be utilized to genotype this hybrid population of Buffalypso in Trinidad and Tobago. Thus, marker-assisted selection and other genotyping-based techniques can be used in breeding herds to maximize disease resistance and production traits as well as for identity and sib-relationships investigations amongst the hybrid herds and local population. This study shows that genotyping tools can be utilized to characterize the biodiversity of local species, preserve traits of the Buffalypso population of Trinidad and Tobago and secure this hybrid breed by establishing a conservation genetics programme as an important part of our cultural heritage.

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Conflicts of Interest

The author declares no conflicts of interest.

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