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Identification of markers for functional traits in Murrah buffaloes

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ABSTRACT

Twelve half sib families were created and two functional traits, viz. age at first heat and age at first calving were recorded for 4,851 and 946 daughters belonging to 12 sires. Partial genome scan was carried out using Interval Mapping with different algorithms. The QTLs obtained for each half sib family were further subjected to meta analysis to identify chromosomal regions associated with age at first heat and age at first calving on 8 chromosomes of buffalo. We identified 4 metaQTL regions on chromosomes BBU1q, BBU6, BBU7, BBU8 and BBU10; 3 metaQTL regions on BBU2q; 2 metaQTL regions on BBU9 and one on BBU15 of buffalo. Comparative genomics was used for finding out genes underlying the meta-QTL regions. 1019 genes were found to be underlying the metaQTL regions in buffaloes assuming buffalo–cattle–human synteny. Several of these genes are supposedly important candidate genes for further analysis as several of them revealed functions related to fertility. The QTL markers identified in the present analysis can be used in the breeding programs of buffalo to select the bulls which affect the functional traits and thus reduce the cost of production.

Keywords: Age at first heat, Age at first calving, Buffalo, Functional traits, Interval mapping, QTLs

The functional traits are those traits of large ruminants which are quantitative but which do not increase the efficiency by higher output but by reducing the cost of inputs. All the traits are influenced by large number of genes and have large environmental effects and lower heritability values. The reproductive performance of large ruminants include both fertility and calving traits. The fertility traits are based on different measures that reflect the ruminants cows' ability to get pregnant. Thus the information on age at first heat exhibits the ability to reveal its resistance to fertility disorders. The functional traits are important since pregnancy is a prerequisite of milk production and directly impacts on production and management costs. However the relative recent interests in these traits is due to the fact that more emphasis on production has adverse impact on functional traits. These traits have very low heritability values and thus the selection for genetic improvement was considered as futile or at least considered extremely problematic. The genetic variation among these traits is quite large (Philipson and Lindhe 2003). However present day evaluations have functional traits as the criterion. There is at present a widespread interest in international genetic evaluation for fertility (Jorjani 2005). These traits can be very well addressed through the use of molecular markers and thus this attempt was made on identification of QTLs for functional traits in buffaloes.

In this paper we present the chromosomal regions associated with the functional traits, i.e. age at first heat and age at first calving using a well-defined buffalo population consisting of 12 half sib families. We present partial genome scans on 8 chromosomes (of cattle, BTA) equivalent in buffaloes to identify and map the quantitative trait loci affecting the age at first heat and age at first calving in buffaloes.

MATERIALS AND METHODS

The reference family of buffaloes (Animals of known pedigree and having both genotype and phenotype information recorded) was created for the work (Vijh 2013, Vijh *et al.* 2018). The accuracy of the paternity records was authenticated using a set of DNA markers and only the daughters with confirmed paternity were recorded for phenotypes (Vijh *et al.* 2014). The daughters belonging to 12 half sib families were recorded for age at first heat and age at first calving. The genotype data on 8 chromosomes with 79 microsatellites on these animals was generated and has been reported (Vijh 2013, Vijh *et al.* 2018).

A total of 12 data set created in the experiment were utilised for the study, one for each sire family. For a single QTL model, we utilised the standard interval mapping and Haley-Knott regression algorithm (Haley and Knott 1992).

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The values of LOD score were utilized as an evidence of the existence of QTL. The statistical significance of LOD was tested using permutation test (Churchill and Doerge 1994) with 1000 replicates. The multiple QTL models as implemented in R/qtl were used as they have increased power to detect QTL, better separation of linked QTLs and defining epistatic interactions. We fitted the Full QTL model using the *fitqtl* function of R/qtl package and this also included the interaction among the QTLs. The ANOVA table indicates the overall fit of the model; the LOD score obtained is relative to the null model (with no QTL). The drop one QTL model was utilized to see the effect of each identified QTL and its related interactions.

QTL meta-analysis was carried out to synthesize QTL information from 12 independent half sib family analysis results and also to refine the chromosomal region involved using Biomercator software v4.2. The QTL meta-analysis algorithm developed by Goffinet and Gerber (2000) was used. We fitted five models using Gaussian distribution and the best fit was determined by means of the maximum likelihood method and Akaike information content. Using the select model consensus QTL positions were determined as the mean of QTL distribution maximizing the likelihood and confidence interval.

Once the metaQTL regions for reproduction traits, viz age at first heat and age at first calving with their confidence interval known. We utilised web-server AnnotQTL (annotqtl.genouest.org) for the identification of genes underlying the QTL region (Lecerf *et al.* 2011). Each metaQTL region was taken as an input. We utilised the buffalo–cattle synteny as reported based on radiation hybrid panel (Amaral *et al.* 2008).

RESULTS AND DISCUSSION

A total of 4,851 animals data were recorded for the age at first heat with an average of 404.25 daughters per sire. The sire wise distribution of daughters is given in Table 1. The number of daughters which were recorded for the age at first calving were 946 with an average of 79 daughters per sire. The details of number of daughters per sire are presented in Table 2.

The interval mapping as implemented in R/qtl was carried out using the Haley-Knott regression and extended Haley-Knott regression method to fit the multiple QTL model. The chromosome scans of 8 chromosomes studied in the experiments revealed large number of QTLs for age at first heat and age at first calving in buffaloes. The details of the sire families, detected QTLs, their LOD score, percentage of the phenotypic variance explained and level of significance is depicted in the Tables 3, 4. The interaction among the QTLs wherever significant have also been mentioned (shown as @). In all, there are three interactions in age at first calving and three interactions in age of first heat among the QTLs located on different buffalo chromosomes. Only statistically significant interactions have been depicted in tables. In case of age at first calving, out of three statistically significant interactions, two involve chromosome BTA1 and BTA2 while third interaction involved BTA3 and BTA4 chromosome. In case of age at first heat, two interactions involved chromosome 1, i.e. BTA 1 with BTA 6 and BTA 14 and third interaction involved BTA3 and BTA9. The representative chromosomal scans of chromosome 1 to chromosome 9 have been depicted in Fig 1(a) to (d) for age at first calving and Fig 2(a) to (d) for age at first heat. In all, 24 statistically significant QTLs were found to be associated with age at first calving and 30 QTLs were found to be associated with age at first heat in the 12 half sib families analyzed. The QTLs identified in each of the half sibs families were identified independent of one another. QTLs' identification is dependent on the heterozygosity of the sire on particular marker loci (segregation) and thus the different QTLs may be identified in different sire families.

The analysis revealed a total of 24 QTLs for age at first calving and 30 QTLs for age at first heat in buffaloes. There have been no reports of QTL identification for any of the economic traits in buffaloes. These QTL markers are now a part of breeding programs in all the developed countries of the world. In this present experiment we identified QTL

Table 1. Sire wise distribution of daughters for mean of age at first heat

Sire	No. of daughters	Mean±SE (AFH)	Min AFH	Max AFH
S1	742	37.68±0.31	20	70
S2	361	35.87±0.43	20	68
S3	187	37.48±0.52	22	62
S4	424	38.67±0.39	20	68
S5	565	36.63±0.34	21	69
S6	167	37.49±0.65	21	59
S7	717	38.39±0.32	20	65
S8	600	38.11±0.36	20	68
S9	445	36.87±0.37	20	62
S10	283	37.20±0.46	20	53
S11	239	36.97±0.46	20	53
S12	121	38.43±0.75	24	58

Table 2. Sire wise distribution of daughters for mean of age at first calving

Sire	No. of daughters	Mean±SE (AFC)	Min AFC	Max AFC
S1	146	46.95±0.66	29.90	72.27
S2	77	46.54±0.81	32.30	61.58
S3	56	47.17±1.00	32.66	64.57
S4	95	49.08±0.77	30.79	70.46
S5	107	46.24±0.73	29.14	70.56
S6	38	47.99±1.67	29.28	78.49
S7	146	49.25±0.69	31.22	78.98
S8	122	46.88±0.68	29.21	70.07
S9	77	45.36±0.79	29.47	68.78
S10	56	46.82±0.79	34.31	65.59
S11	10	43.33±2.24	34.38	54.14
S12	16	46.16±1.89	31.35	61.05

Table 3. List of significant QTL locations for age at first calving for 12 half sib families using Interval Mapping (Haley-Knott regression and extended Haley-Knott regression) as implemented in R/qtl

Sire	Chromosome (BTA)	Chromosome (BBU)	Position	LOD	% Var	F value	P value (F)	Sign
Sire1	6	BBU7	90	2.569	7.811	1.926	0.0811	P
Sire4	2	BBU2q	20.8	1.7181	7.806	3.7297	0.0281	*
Sire5	1	BBU1q	9.1	1.3381	4.24	2.589	0.081	P
	7	BBU9	34	3.0704	10.12	2.06	0.0666	P
	4	BBU8	80	1.6312	4.7647	2.9506	0.0582	P
	7	BBU9	34	2.0277	5.9762	3.7009	0.0291	*
Sire6	2	BBU2q	94.8	5.356	25.772	3.159	0.024	*
	6	BBU7	64	2.986	12.239	4.5	0.0243	*
	1	BBU1q	115.1	1.392	10.362	2.649	0.0884	P
	2	BBU2q	94.8	1.824	13.954	3.567	0.0417	*
Sire7	3	BBU6	69.3	2.2999	6.912	5.227	0.00651	**
	3	BBU6	19.3	1.246	3.5399	2.7423	0.06807	P
	3	BBU6	69.3	2.5892	7.5194	5.8251	0.00376	**
Sire8	1	BBU1q	85.1	2.636	9.165	1.97	0.0759	P
	2	BBU2q	20.8	3.161	11.102	2.386	0.0331	*
	1@85.1:2@20.8			1.972	6.768	2.182	0.0755	P
	1	BBU1q	45.1	2.9784	10.364	2.201	0.0482	*
	2	BBU2q	94.8	3.2397	11.33	2.406	0.0319	*
	1@45.1:2@94.8			2.7805	9.638	3.07	0.0194	*
Sire9	3	BBU6	23.3	1.2956	6.947	2.8198	0.0664	P
	3	BBU6	29.3	1.3754	5.505	2.744	0.0719	P
	6	BBU7	40	1.5284	6.146	3.063	0.0537	P
	4	BBU8	44.5	2.3206	9.56	4.764	0.0118	*
Sire10	1	BBU1q	45.1	1.5172	6.188	2.4584	0.0994	P
Sire12	3	BBU6	64.9	4.772	47.63	3.44	0.0654	P
	4	BBU8	14	6.106	77.49	5.597	0.0198	*
	3@64.9:4@14.0			4.627	45.03	4.879	0.0338	*

Significance codes: 0; ***, 0.001; **, 0.01; *, 0.05; P, Probable QTL.

markers on 8 chromosomes for age at first calving and age at first heat utilizing 12 half sib families. We identified the QTLs for age at first calving and age at first heat on all the 8 chromosomes although in different sire families.

The positions of the QTL regions may differ from family to family and need to be further analysed to find consensus regions. The meta analysis of the identified QTL regions for age at first calving and age at first heat in buffaloes was carried out on QTLs on 8 chromosomes of buffaloes. The selection of the model from the 5 models tested for the analysis was based on Akaike Information Content. The meta analysis of the QTLs across 12 families of buffaloes are depicted in Table 5. The table depicts the chromosome number, metaQTL location and their confidence interval.

A total of 26 meta-QTL regions were identified as given in the Table 5. The chromosome BBU1q, BBU6, BBU7, BBU8 and BBU10 had four metaQTL regions each while BBU2q had three metaQTL regions on chromosome. Similarly, BBU9 revealed two and BBU15 revealed one metaQTL region. Since no information is available on the QTLs for age at first calving and age at first heat in buffaloes, we are comparing the chromosomal positions of buffalo QTLs with those of cattle QTLs for the functional traits assuming a high degree of synteny between cattle and buffalo chromosomes as reported by Amaral *et al.* (2008). We identified 4 meta-QTL regions on chromosome BBU1q (BTA1) and out of these four locations, the meta-QTL position at 45.25 with a span of 41.41 to 49.09 cM is reported for the trait by Hawken *et al.* (2012). The meta-QTL position on chromosome arm of BBU2q is on 97.21 with confidence interval of 94.76 to 99.67 and has also been reported by Fortes *et al.* (2013) for cattle. We identified 4 meta-QTL regions on chromosome BBU6 (BTA3), the first mean position was at 29.31 and had a confidence interval of 26.2 to 32.43, the second mean position at 65.49 with confidence interval of 63.87 to 67.11, the third mean position was at 101.34 with confidence interval of 96.34 to 108.34 and the fourth mean position at 114.92 with confidence interval of 110.34 to 116.34 have been reported by Hawken *et al.* (2012) for cattle.

The third mean position of 80.0 with confidence interval of 78.0 to 85.0 on chromosome BTA4 (BBU8) has been reported by Trakovick *et al.* (2013). We identified 4 metaQTL regions on chromosome BTA6 (BBU7) and out of these four locations, the metaQTL position at 35.39 with a confidence interval between 32.0 to 40.0 has been reported by Daetwyler *et al.* (2008) while the location at 84.47 with a confidence interval between 83.11 to 88.11 has been reported by Hawken *et al.* (2012). The mean position at 57.2 with a confidence interval of 50.2 to 62.2 on

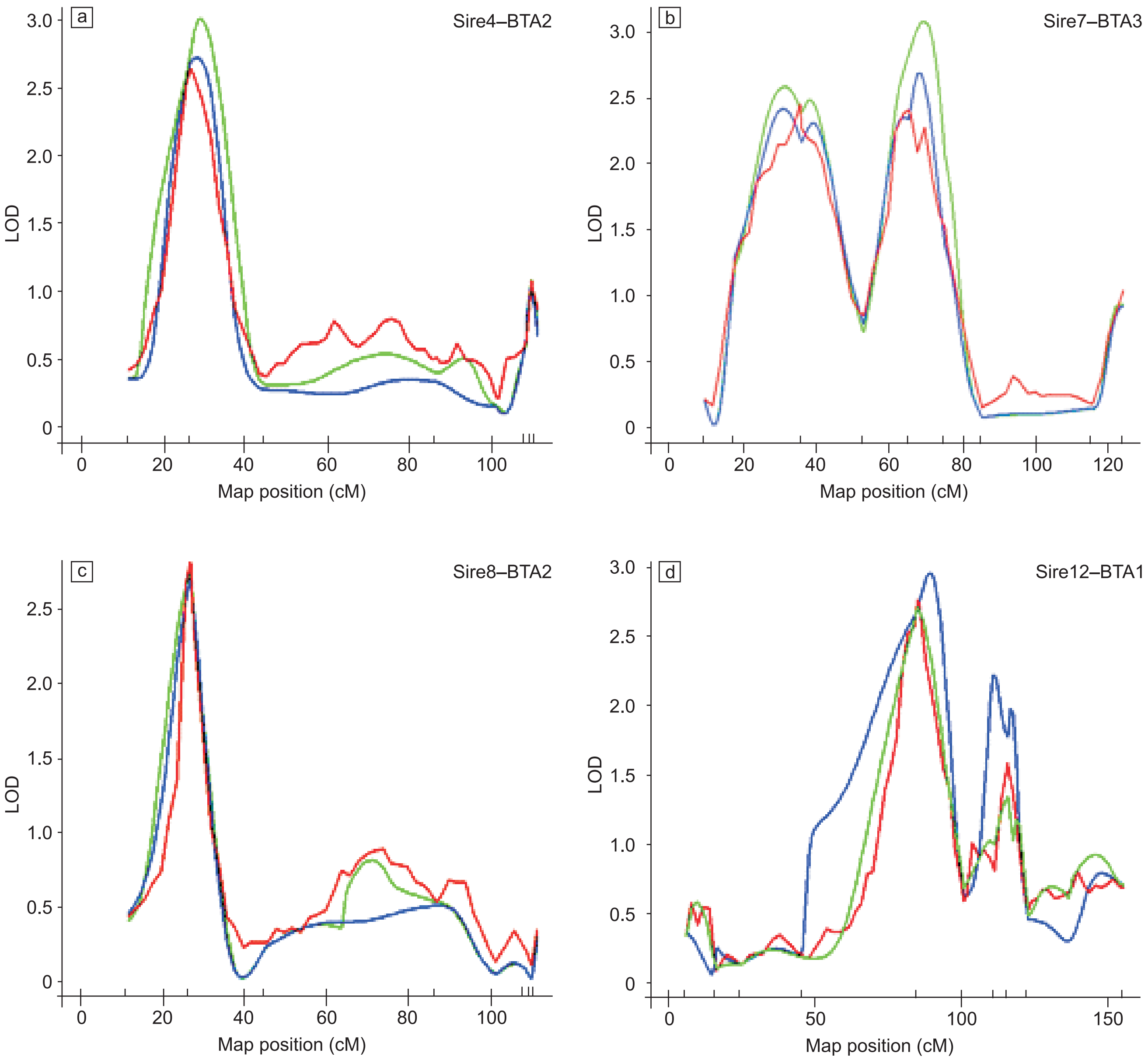

Fig. 1. Representative chromosome scans showing QTLs for age at first calving identified on the basis of high LOD scores for different half sib families.

chromosome BTA9 (BBU10) has been reported by Hyeong *et al.* (2014) and on the same chromosome fourth metaQTL was at mean position 92.8 with the confidence interval of 89.26 to 96.33 which was reported by Costa *et al.* (2015) for cattle chromosomes. On chromosome BTA14 (BBU15), first metaQTL is present on the mean position 35.12 with the confidence interval of 30.12 to 40.12. Similar results have been reported by Costa *et al.* (2015).

Thus most of the identified metaQTL regions had similar locations as reported for QTL regions in cattle for which extensive work has been carried out over the past two decades especially for Holstein and Jersey breeds of cattle.

There has been a dramatic reduction in the number of daughters from age at first heat to age at first calving even though the gestation period in buffaloes had very less variation. The reduction in the number of animals recorded for age at first heat and age at first calving is because there is usually heavy sale of buffaloes once heifers are pregnant. In fact, pregnant buffaloes fetch better price. The birth of male or a female calf born to the animal is a matter of speculation for the farmers and thus the prices are higher for pregnant animals.

Once the QTLs have been identified, it is desirable to know the genes underlying the chromosomal region of interest. We identified the gene underlying the QTLs using a web server and assuming a very close synteny of cattle and buffalo chromosomes. A total of 1,019 genes were identified underlying the QTL regions on various chromosomes. These were 74, 129, 330, 127, 101, 105, 103, 51 respectively for BBU1q, BBU2q, BBU6, BBU8, BBU7, BBU9, BBU10 and BBU15 respectively. All the genes were mapped to gene ontology terms and a total of

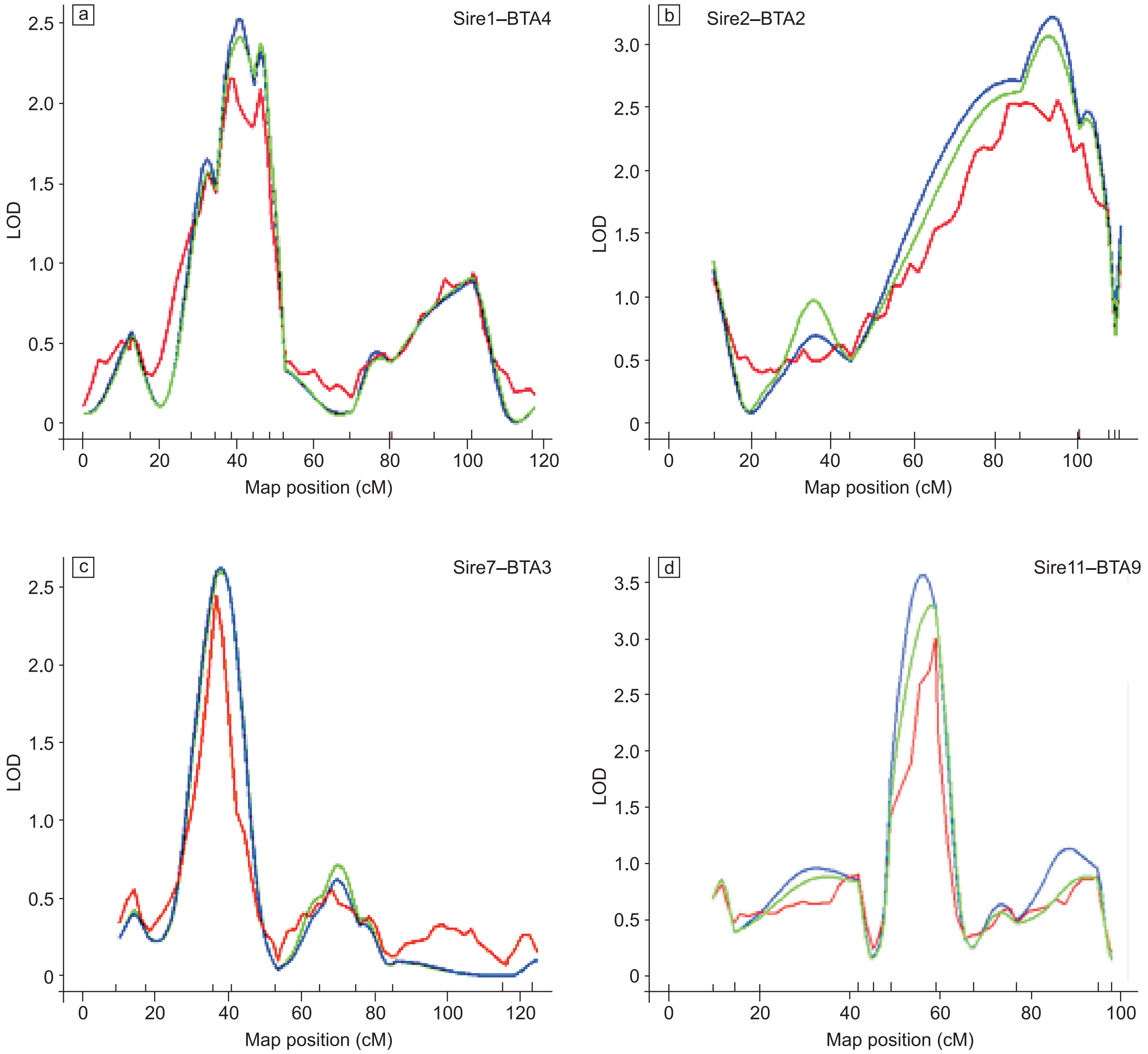

Fig. 2. Representative chromosome scans showing QTLs for age at first heat identified on the basis of high LOD scores for different half sib families.

890 genes mapped the gene ontology and yielded 11357 GO terms. About 5051 of the terms were from biological processes, 3235 terms were for cellular components and 2883 belonged to molecular functions. The analysis of GO terms revealed a large number of GO terms associated with fertility and reproduction including several receptors of various hormones related to these functional traits. The GO terms revealed zona pellucida binding protein, Zyg-11 family members A and B, cell cycle regulator, Ubiquitin protein ligases and peptidases. Thyroid hormone receptor associated protein 3, spermatogenesis associated gene 6, serine rich z like solute carrier families, olfactory receptors, nitric acid synthase, several members of NADH, ubiquinone, insulin like growth factor, gonadotropin releasing hormone, BMP2, ATP binding casette genes to be underlying identified QTLs.

Not much work is available for genes associated with the functional traits of interest even in human beings. The age at first heat is considered a highly polygenic trait and depends upon several other factors which all are multifactorial.

It is however known that the detection of QTLs is an inexact science and thus require the verification in different populations. Taking into consideration the above statements, we not only identified the QTLs in 12 half sib families but also carried out the metaQTL analysis to find the consensus regions affecting the age at first heat and age at first calving trait in buffaloes. Several strategies have been put forward for the confirmation of suggestive QTLs for the traits of interest (Spelman and Bovenhuis1998, Georges 1999). One of the most common ones are to check for the genes underlying QTL regions in the species.

Table 4. List of significant QTL locations for age at first heat for 12 half sib families using Interval Mapping (Haley-Knott regression and extended Haley-Knott regression) as implemented in R/qtl

Sire	Chromosome (BTA)	Chromosome (BBU)	Pos	LOD	% Var	F value	P value (F)	Significance
Sire1	4	BBU8	44.5	2.9988	1.855	2.289	0.0339	*
Sire2	2	BBU2q	90.8	1.3333	1.578	2.9306	0.0547	P
	9	BBU10	95.4	1.58474	1.8786	3.489	0.0316	*
	2	BBU2q	94.8	1.1109	1.2814	2.438	0.0889	P
	4	BBU8	114	1.1199	1.2919	2.458	0.0871	P
	9	BBU10	90.2	2.749	3.2051	2.033	0.0608	P
Sire3	1	BBU1q	9.1	2.528	6.22	1.904	0.083	P
	1	BBU1q	115.1	1.292	2.964	2.769	0.0657	P
	6	BBU7	50	1.163	2.664	2.488	0.0862	P
Sire4	3	BBU6	64.9	3.7316	3.8269	2.79	0.0114	*
	9	BBU10	15.1	3.676	3.7687	2.748	0.0126	*
	3@64.9:9@15.1			2.7021	2.7551	3.013	0.0181	*
	2	BBU2q	26	1.0671	1.1155	2.382	0.0937	P
	6	BBU7	94	2.4319	2.5616	1.824	0.0932	P
Sire5	4	BBU8	4	1.3597	1.0669	3.0403	0.0486	*
	9	BBU10	45.7	1.4774	1.1598	3.305	0.0375	*
Sire6	14	BBU15	35.1	3.194	8.518	2.424	0.0288	*
	1@115.1:14@35.1			1.889	4.943	2.11	0.0822	P
	1	BBU1q	15.1	3.6261	8.479	2.625	0.01905	*
	3	BBU6	114.9	1.1486	2.592	2.408	0.09359	P
	6	BBU7	30	3.3962	7.915	2.451	0.0275	*
	1@15.1:6@30.0			3.2664	7.598	3.529	0.00882	**
Sire7	3	BBU6	39.3	1.6417	1.061	3.7517	0.024	*
	1	BBU1q	100.1	3.2019	2.0574	2.4304	0.0248	*
Sire8	4	BBU8	114	1.9367	1.4297	4.364	0.0131	*
Sire9	1	BBU1q	39.1	2.5101	2.5137	1.886	0.0818	P
Sire10	1	BBU1q	119.1	1.3126	2.035	2.9358	0.0548	P
Sire11	3	BBU6	99.3	1.3645	2.0135	2.7943	0.0635	P
	6	BBU7	94	1.9782	2.9367	4.0755	0.0184	*
	7	BBU9	60	1.7419	2.5799	3.5804	0.0296	*
	1	BBU1q	115.1	1.706	2.6333	3.6065	0.0288	*
	4	BBU8	50	1.5398	2.3729	3.2499	0.0407	*
	9	BBU10	56.2	2.8876	4.5089	2.0585	0.0594	P

Significance codes: 0; ***, 0.001; **, 0.01; *, 0.05; P, Probable QTL.

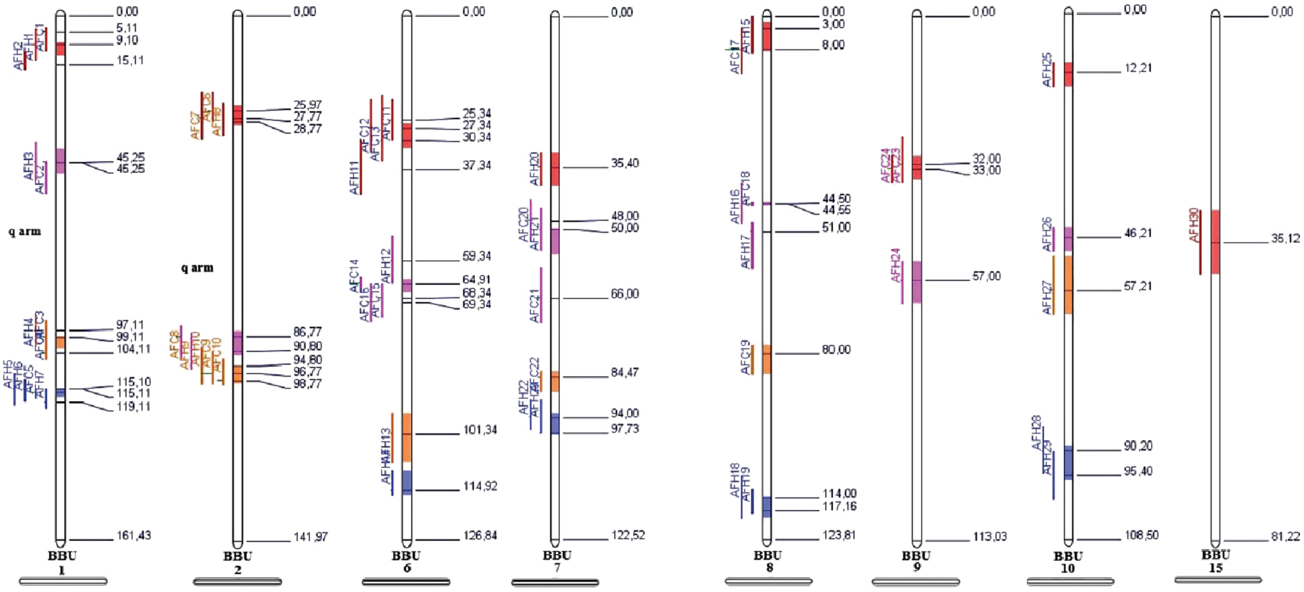

Fig. 3. Meta-QTL positions for AFC and AFH on 8 chromosomes for buffalo.

Table 5. Locations of metaQTL regions (chromosome wise) for reproduction along with their confidence interval

BTA Chr	BBU Chr	AIC value	Mean position 0 (CI)	Mean position 1 (CI)	Mean position 2 (CI)	Mean position 3 (CI)
1	BBU1q	101.75	10.63 (8.54–12.71)	45.25 (41.41–49.09)	100.88 (98.94–102.82)	116.42 (115.19–117.65)
2	BBU2q	50.12	27.3 (24.59–30.02)	88.57 (85.22–91.91)	97.21 (94.76–99.67)	–
3	BBU6	82.93	29.31 (26.2–32.43)	65.49 (63.87–67.11)	101.34 (96.34–108.34)	114.92 (110.34–116.34)
4	BBU8	54.83	5.0 (1.52–8.48)	44.6 (44.1–45.09)	80.0 (78.0–85.0)	116.32 (113.75–118.89)
6	BBU7	66.14	35.39 (32.0–40.0)	52.88 (49.77–55.98)	84.47 (83.11–88.11)	95.61 (92.98–98.25)
7	BBU9	14.21	32.73 (30.16–35.3)	56.99 (53.0–62.0)	–	–
9	BBU10	32.62	12.2 (10.2–15.2)	46.2 (44.2–49.2)	57.2 (50.2–62.2)	92.8 (89.26–96.33)
14	BBU15	5.71	35.12 (30.12–40.12)	–	–	–

Values in ( ) represent the confidence interval of the metaQTL. BTA implies *Bos taurus* chromosomes and BBU implies *Bubalus bubalis* chromosomes.

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