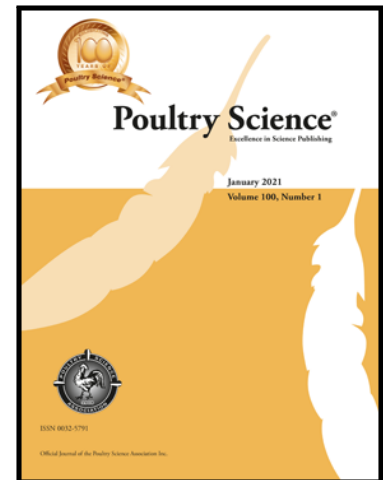


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GENOMIC REGIONS FOR CHICKEN BODY SIZE

High-density genotyping reveals candidate genomic regions for chicken body size in breeds of Asian origin

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ABSTRACT Body size is one of the main selection indices in chicken breeding. Although often investigated, knowledge of the underlying genetic mechanisms is incomplete. The aim of the current study was to identify genomic regions associated with body size differences between Asian Game and Asian Bantam type chickens. In this study, 94 and 107 chickens from four Asian Game and five Asian Bantam type breeds, respectively, were genotyped using the chicken 580K single nucleotide polymorphism (SNP) array. A genome-wide association study (GWAS) and principal component analyses (PCA) were performed to identify genomic regions associated with body size related-traits such as wing length, shank length, shank thickness, keel length, and body weight. Hierarchical clustering of genotype data showed a clear genetic difference between the investigated Asian Game and Asian Bantam chicken types. GWAS identified 16 genomic regions associated with wing length (2."HFT"Ö"2023:), shank thickness (6."HFT"Ö"2022:), keel length (5."HFT"Ö"20245), and body weight (3."HFT"Ö"20263). PCA showed that the first principal component (PC1) separated the two chicken types and significantly correlated with the measured body size related-traits ($p \leq 4.6 \times 10^{-4}$). SNPs contributing significantly to PC1 were subjected to a more detailed investigation. This analysis identified 11 regions potentially associated with differences in body size related-traits. A region on chromosome 4 (GGA4) (17.3 - 21.3 Mb) was detected in both analyses GWAS and PCA. This region harbors 60 genes. Among them are myotubularin 1 (*MTMI*) and secreted frizzled-related protein 2 (*SFPR2*) which can be considered as potential candidate genes for body size related-traits. Our results clearly show that the investigated Asian Game type chicken breeds are genetically different from the Asian Bantam breeds. A region on GGA4 between 17.3 and 21.3 Mb was identified which contributes to the phenotypic difference, though further validation of candidate genes is necessary.

Key words: Asian Game type chicken, Asian Bantam type chicken, growth, GWAS, PCA

INTRODUCTION

The genetic mechanisms underlying variation in chicken body size is still insufficiently understood. Chicken body size reflects body development, especially bone and muscle growth (Gao et al., 2011; Geng et al., 2021). Growth is one of the main selection criteria in chicken breeding. Genetically, chicken body size is affected by many genes on the autosomes and the sex-chromosome, making it a truly complex trait. Hundreds of quantitative trait loci (QTL) have been mapped on autosomes for body size related-traits such as shank length, keel length, and body weight (Gao et al., 2011; Hu et al., 2012; Lyu et al., 2017; Johnsson et al., 2018; Mebratie et al., 2019; Dadousis et al., 2021). On the Z chromosome, for example, a mutation in the growth hormone receptor (*GHR*) gene results in sex-linked dwarfism (Agarwal et al., 1994). Many mapping studies used commercial chicken populations (Hu et al., 2012; Mebratie et al., 2019; Dadousis et al., 2021). In such populations, genetic loci are detected which account significantly to the variance of the examined population. However, natural variation contributing to body size related-traits cannot be detected completely using this approach. For a more comprehensive understanding of the genetic determination of growth, the identification of additional genetic variants that contribute to body size in other wild populations is desirable. Thus, the investigation of genetically more diverse populations could help to identify additional genetic variants with influence on body size.

Besides mapping studies, comparative genomic analyses within and among different chicken breeds have been successfully used to identify signatures of selection and candidate genes for body size (Rubin et al., 2010; Wang et al., 2016, 2017). Since selection results in an increase of the frequency of beneficial alleles within a breed up to fixation the history of selection leaves behind selection signatures in the genome around the gene(s) that contributed to the selection response (Walugembe et al., 2018). However, when comparing breeds, not only the trait of interest (e.g. body weight) may be responsible for these signatures of selection, other traits that characterize a breed (e.g. the comb shape) can also cause such signatures of selection.

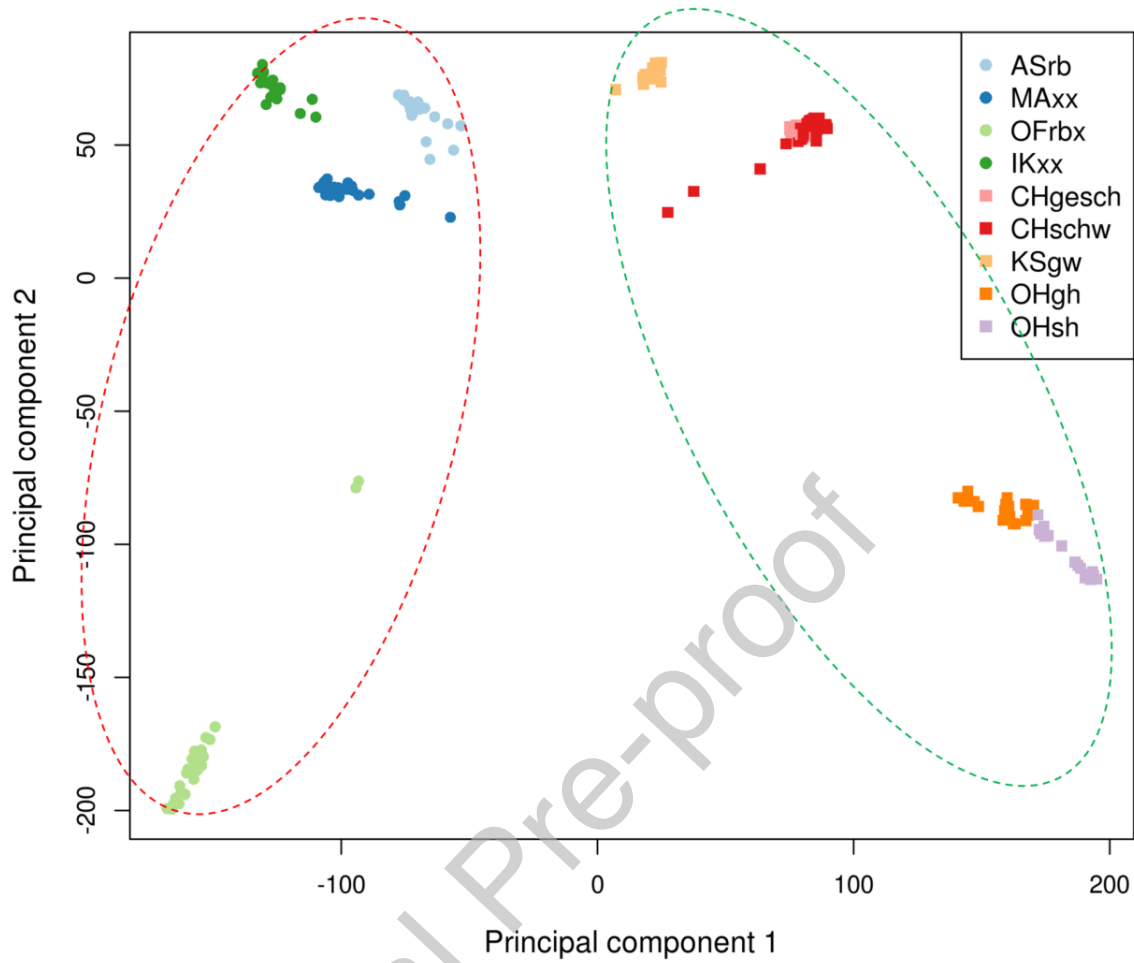


Table 1. Number of chickens in each breed

Type	Breed	Number of individuals	
		Males	Females
Asian Game type	Aseel red mottled (ASrb)	9	10
	Malay black red (MAxx)	15	15
	Orloff red spangled (OFrbx)	12	13
	IKxx (Indian Game dark)	10	10
Asian Bantam type	Japanese Bantam black tailed buff (Chgesch)	10	12
	Japanese Bantam black mottled (CHschw)	13	16
	Ko Shamo black-red (KSgw)	10	10
	Ohiki red duckwing (OHgh)	8	10
	Ohiki silver duckwing (OHsh)	10	8

Table 2. Significant results of the genome-wide association study

Top SNP ¹	Region ²	Genotypes (number of individuals)	Minor allele	MAF ³	Raw <i>p</i> -value ⁴	FDR ⁵	(SE) ⁶	Var % ⁷	Overlap with QTLs publ
s318160403	23: 97236 - 98116	GG (54), AG (79), AA (68)	G	0.47	3.63E-08	0.004	0.232 (0.039)	16.5	-
s316879242	Z: 57658911 - 57666420	GG (11), AA (180)	G	0.06	5.50E-07	0.018	0.316 (0.061)	15.7	-
s314712355	2: 27742242 - 27760815	CC (15), CT (15), TT (171)	C	0.11	1.00E-07	0.003	0.555 (0.134)	15.6	-
s314245637	2: 28143942 - 28240351	GG (78), AG (23), AA (100)	G	0.45	2.09E-07	0.005	-0.412 (0.118)	15.0	-
s316969703	2: 50086804 - 50116697	GG (23), AG (12), AA (166)	G	0.14	6.61E-07	0.008	-0.587 (0.120)	13.9	-
s15997785	2: 55910522 - 55928488	AA (50), AG (26), GG (123)	A	0.32	3.63E-07	0.006	-0.457 (0.095)	14.8	-
s315729689	10: 4534620 - 7754637	TT (25), CT (19), CC (157)	T	0.17	3.89E-08	0.002	-0.157 (0.167)	16.4	Body weight and Blood gluc 71191, broiler x Fayoumi)
s14773049	Z: 63407861 - 63418506	AA (85), AG (12), GG (104)	A	0.45	1.38E-08	0.001	-0.342 (0.110)	17.3	-
s315730958	1: 80330867 - 80373370	AA (47), AG (64), GG (90)	A	0.39	1.95E-07	0.023	-0.304 (0.101)	15.0	-
s14251327	2: 129987598 - 130043402	AA (18), AG (24), GG (159)	A	0.15	3.16E-07	0.023	0.222 (0.112)	14.6	-
s314042510	4: 17284783 - 21270248	CC (15), CT (18), TT (167)	C	0.12	2.95E-08	0.010	-0.918 (0.148)	16.8	-
s315991717	6: 10926588 - 10933626	AA (12), AC (27), CC (162)	A	0.13	1.15E-06	0.023	-0.679 (0.142)	13.4	Feed conversion ratio (13940 indigenous broiler line from mineral content and Tibia 135882, meat-type chicken line
s315851423	10: 9230030 - 9231656	GG (11), AG (11), AA (177)	G	0.08	8.51E-07	0.023	-0.447 (0.218)	15.1	-
s318014118	1: 147653720 - 147680675	GG (17), AG (32), AA (152)	G	0.16	8.32E-07	0.037	0.143 (0.037)	13.7	-
s314732179	4: 18316381 - 21250309	AA (45), AG (25), GG (131)	A	0.29	7.41E-07	0.037	0.227 (0.049)	13.8	-
s312580626	27: 3375301 - 3375436	TT (21), CT (38), CC (141)	T	0.20	1.62E-06	0.041	0.201 (0.043)	13.2	Femur bone mineral conten (130479 and 130480, Chines White Leghorn)

¹ The SNP with the lowest *p*-value in the genome-wide association analysis for the relevant trait in a defined region.

² The defined region associated with the relevant trait. The regions were defined by merging adjacent significant SNPs that were in strong LD ($r^2 > 0.7$). The chromosome locations are based on Galgal5 (Ensembl 91).

³ MAF: minor allele frequency of the top SNP.

⁴ The *p*-value of the top SNPs without correction for multiple testing.

⁵ The *p*-value of the top SNPs were corrected for multiple testing by estimating the FDR using the Benjamini and Hochberg method.

⁶ corresponds to the effect size of the minor allele of the top SNP on phenotype corrected for sex differences per breed. SE: standard error.

⁷ Percentage of variance explained by the top SNP.

⁸ Previously identified QTLs with records in the QTLdb (<https://www.animalgenome.org/cgi-bin/QTLdb/index>). IDs of the QTLs were shown in brackets.

7 D E O H p q o k e "tegrions identified by principal component analysis

Top SNP ¹	Region ²	Minor allele	Major allele	MAF ³	Raw <i>p</i> -value	P _{Bonf} ⁴	Overlap w
rs317999764	1: 64288915 - 65870094	T	C	0.41	1.00E-07	0.017	Body weight (62153, 62163) (63779, 63929), Egg number shell thickness (57889, 57896) (62007), Ovarian follicle weight (62096, 62096)
rs317769208	1: 72486629 - 72576268	G	T	0.49	7.94E-09	0.001	-
rs315645218	2: 45148419 - 46360352	C	T	0.49	1.58E-08	0.003	Body weight (95404, 95412), (127112), Wattles weight (127112)
rs317007003	4: 20113606 - 20379905	T	G	0.43	2.51E-08	0.004	-
rs317717388	7: 6976467 - 8981201	T	C	0.5	7.94E-09	0.001	Feed conversion ratio (1397 weight (127115), Feather peck (127115))
rs313910027	14: 11892340 - 11901609	C	T	0.49	2.00E-09	0.0003	Wattles length (127121)
rs315783766	20: 438802 - 441494	T	G	0.44	1.58E-07	0.027	-
rs317391544	Z: 11370833 - 11685040	A	T	0.49	3.16E-10	0.00005	Body weight (136476, 136866), shell strength (24936)
rs313366229	Z: 18044764 - 19994683	T	A	0.41	1.26E-07	0.022	Body weight (136324, 136905), Intramuscular fat (136993)
rs317812756	Z: 50692623 - 51927985	T	G	0.45	1.00E-08	0.002	Feed intake (64577), Earlobe color (64577)
rs16118569	Z: 63378841 - 63380441	A	G	0.48	1.58E-07	0.027	-

¹ The SNP with the most significant contribution to principal component 1 (PC1).

² The defined region associated with the difference in body size between Asian Game and Bantam chickens. SNPs contributing significantly to PC1 were merged into a region if they are 2 Mb of each other. The Regions were shown in chromosome coordinates (bp) (e.g., 1: 64288915-65870094).

³ MAF: minor allele frequency of the top SNP.

⁴ The adjusted *p*-value using Bonferroni correction for multiple testing.

⁵ Previously identified QTLs with records in the Animal QTL Database (<https://www.animalgenome.org/cgi-bin/QTLdb/index>). If there are more than two QTLs mapped for one trait, only two were listed with the QTL ID.