

QTL in chicken: a look back and forward – a review*

Alexei Sazanov^{1,**}, Anna Sazanova¹, Olga Barkova¹, Kazimierz Jaszczak²

¹ Laboratory of Molecular Genome Organization,
Institute of Farm Animal Genetics and Breeding,
Russian Academy of Agricultural Sciences,
Moskovskoye Sh. 55A, St Petersburg-Pushkin 196601, Russia

² Department of Molecular Cytogenetics, Institute of Genetics and Animal Breeding,
Polish Academy of Sciences, Jastrzebiec, 05-552 Wolka Kosowska, Poland

(Received July 13, 2010; accepted October 1, 2010)

Identification of genes determining the expression of economically important traits of plants and animals is a main research focus in agricultural genomics. Most of these traits are characterized by a wide variability of the expression of genes at certain *loci* called quantitative trait loci (QTL). Characterization of the chromosomal regions carrying QTL can be applied in marker-assisted selection (MAS) to improve breeding efficiency. Molecular linkage maps in combination with powerful statistical methods facilitate the genetic dissection of complex traits, and the chicken is ideally suited for this task due to a relatively short life cycle and large number of progeny. Two major approaches are employed in understanding the genomic architecture of economically important traits: QTL mapping and, more recently, functional genomics.

KEY WORDS: behaviour / disease resistance / egg production / egg quality / growth / hatchability / quantitative trait loci / microsatellites / shell thickness

QTL: growth, meat quality, meat productivity

To elucidate QTL that affect growth, the genome-wide scans with microsatellite markers have been employed. E.g. van Kaam *et al.* [1999a] performed a whole-genome scan for QTL affecting growth and feed efficiency in chickens and detected four QTL

*Supported by the MSHE grant 311 32 34 36

**Corresponding author: alexei_sazanov@mail.ru

on *Gallus gallus* autosomes (GGA1, GGA2, GGA4, GGA23) that exceeded the thresholds of significance. The same research group has carried out a whole-genome scan in chicken for QTL affecting carcass traits [van Kaam *et al.* 1999b]. Two QTL were shown to be located on GGA1 and GGA2. These results were confirmed and refined using Bayesian analysis [van Kaam *et al.* 2002]. Tatsuda and Fujinaka [2002] detected a QTL affecting body weight closely aligned with those reported using a reference population derived from a cross of a Satsumadori (slow-growing, light-weight Japanese native breed used for meat production) sire and a White Plymouth Rock (early maturing, heavy weight broiler) dam. Two QTL affecting body weight at 13 and 16 weeks were mapped at 220 cM on GGA1 and 60 cM on GGA2. The closest QTL markers were LEI0071 on GGA1 and LMU0013 and MCW0184 on GGA2. QTL for body weight at 3, 6 and 9 weeks of age were investigated by Sewalem *et al.* [2002] using a broiler \times layer crossbred. A QTL on GGA13 affected body weight at all three ages and QTL significant at the genome-wide level that affected body weight at two ages were found on chromosomes 1, 2, 4, 7 and 8. QTL for meat production and quality in a commercial population of broilers were identified by de Koning *et al.* [2004]. Using genotypes for 52 microsatellite *loci* spanning regions of nine chicken chromosomes and a half-sib analyses with a multiple QTL model, linkage between these nine regions and growth, carcass traits and feed intake was established. QTL affecting fatness in chicken were investigated and mapped by Ikeobi *et al.* [2002] in an F2 population developed by crossing a broiler line with a layer line. Using within-family regression analyses of 102 microsatellite *loci* in 27 linkage groups the QTL for abdominal fat weight were identified on chromosomes 3, 7, 15 and 28, for abdominal fat weight adjusted for carcass weight on chromosomes 1, 5, 7, and 28, for skin and subcutaneous fat on chromosomes 3, 7, and 13, for skin fat weight adjusted for carcass weight on chromosomes 3 and 28 and for skin fat weight adjusted for abdominal fat weight on chromosomes 5, 7 and 15. Significant positive and negative QTL alleles occurred in both lines.

Several QTLs affecting fatness in broilers were detected by Jennen *et al.* [2004] using two genetically different outcross broiler dam lines originating from the White Plymouth Rock breed. Genetic architecture of growth and body composition was investigated in reference chicken populations obtained by crossing one modern broiler male from a commercial broiler breeder male line with females from two unrelated highly inbred lines [Deeb and Lamont 2003]. Traditionally selected phenotypic traits in broilers were suggested to be controlled by a large number of genes with small epistatic effects, while fitness-related traits could be determined by a lower number of genes with major effects.

After simultaneous mapping epistatic QTL in a chicken F2 intercross, clusters of QTL pairs with similar genetic effects on growth were found by Carlborg *et al.* [2004]. They used simultaneous mapping of interacting QTL pairs to study growth traits. Such an approach increased the number of detected QTL by 30%. The genetic variance of growth was significantly influenced by epistasis, the largest impact being

on early growth (before week 6 of age). Because early growth was shown to be associated with a discrete set of interacting *loci* involved in it, these results provided further insight into different genetic regulations in early and late growth of chicken found in other studies.

QTL: productivity and quality of eggs

Genome-wide scans of QTL for egg quality and productivity have been achieved using reference populations, while a border line between two egg layer lines was used in the study by Tuiskula-Haavisto *et al.* [2002]. The authors determined 14 genome-wide significant and six suggestive QTL located in chromosomes 2, 3, 4, 5, 8, and Z. The most interesting area was found on GGA4, with QTL for body weight, egg weight and feed intake. A related investigation was conducted by Wardęcka *et al.* [2002] to determine the influence of genotypes of the Rhode Island Red (RIR) and Green-legged Partridge (GLP) breeds on egg production and egg quality traits based on analysis of 23 microsatellite markers. Significant effects were demonstrated for 16 traits.

Marker *loci* detected by QTL mapping can serve as multiple entry points into the physical BAC-contig map and sequence of the chicken genome. E.g. two QTL from the mentioned study were selected for FISH mapping using microsatellite-specific large-insert clones [Sazanov *et al.* 2005]. This strategy helps to specify genes that might underlie QTL and is known as QTL positional cloning. Genetic mapping of QTL affecting egg production, egg traits and body weight in F2 White Leghorn × RIR intercross chickens was done by Sasaki *et al.* [2004] using 123 microsatellite markers. They assigned 96 markers to 25 autosomal linkage groups and 13 markers to the Z chromosome, including eight previously unmapped markers. Significant QTL were discovered for body weight on chromosomes 4 and 27, egg weight on GGA4, the egg short length on GGA4 and egg shell redness on GGA11. A significant QTL for age at first egg was found on GGAZ. Overall, 6-19% of the phenotypic variation in the F2 population may be explained by these QTL.

QTL: shell thickness

The results of the whole genome scan for detection and localization of QTL affecting egg quality traits were described by Tuiskula-Haavisto *et al.* [2002]. At 1% genome-wise significance level 14 chromosomal areas affecting egg quality, while at 5% level only 6 suggestive QTL were found. Another whole genome scan was done in Green-legged Partridge (GLP) chickens, a native Polish breed maintained as a conservative flock, and in a highly productive stock of Rhode Island Red (RIR) – Wardęcka *et al.* [2002, 2003]. The significant effect of the genotype (GLP-GLP, RIR-RIR, and GLP-RIR) was found for 16 traits: age at sexual maturity, body weight on week 20 and 33, feed intake on week 33, total individual egg production, egg weight on week 53, egg specific gravity on week 33, Haugh units on week 53, yolk

weight on week 33, albumen weight on week 33 and 53, shell weight on week 33 and 53, shell thickness on week 33 and 53 and shell colour on week 33 of life [Wardęcka *et al.* 2003].

The eggshell is a highly ordered structure resulting from the deposition of calcium carbonate concomitantly with an organic matrix upon the eggshell membranes. Mineralization takes place in an acellular uterine fluid, which contains the ionic and matrix precursors of the eggshell [Gautron *et al.* 2001]. It is formed in the uterine (shell gland) region of the oviduct in an acellular milieu that is supersaturated with respect to Ca and bicarbonate and contains a variety of proteins that vary in concentration during the sequential process of shell formation [Gautron *et al.* 2001]. Formation of eggshell microstructure underlay complex regulations imposed by the resident egg [Lavelin *et al.* 2000]. Significant chicken age and environment effects were found for shell thickness [Edmond *et al.* 2005]. Shell thickness at week 53 of lay age (ST53) was mapped on GGA4 very close to MCW0114 [Wardęcka *et al.* 2002, 2003].

Nine chicken genomic bacterial artificial chromosome clones containing the MCW0114 were mapped to GGA4q11-12 using fluorescence *in situ* hybridization [Sazanov *et al.* 2005]. Below the expression profiling of 12 positional candidates for QTL affecting ST53 investigated by realtime PCR in the lower part of chicken oviducts with a forming eggshell in GLP and RIR is reported.

The PCR with 20 target sequence primers using cDNA prepared from the oviduct tissue samples as templates resulted in getting 12 fragments of expected size. The mRNA expression of these 12 target sequences was measured by real-time quantitative reverse transcription PCR (Tab. 1). No significant expression differences between the group with high shell thickness (STH) and low shell thickness (STL) and no significant correlation of expression level with shell thickness on week 53 of a layer life (ST53) were detected in RIR (Tab. 1). Thus, genetic heterogeneity of factors affecting shell thickness is expected to be less in RIR than in the conserved GLP chickens, which has been kept without selection for many generations. In GLP, the CR523443 was downregulated with ratio of means 0.49 ($P \leq 0.01$) in STL relative to STH (Tab. 1). Expression of the gene in question occurred to be significantly correlated (0.85, $P \leq 0.01$) with shell thickness. These data suggested CR523443 as a potential candidate gene for QTL ST53 in chicken. The 2,102 bp CR523443 sequence (*Gallus gallus* finished cDNA, clone ChEST985k21) was primarily found in mRNA extracted from adult muscle and then was identified in brain, cartilage, female genital, and head (<http://www.ncbi.nlm.nih.gov/UniGene>). A BLAST search did not show any significant homology to other vertebrate sequences. Recapitulating, relatively little is known about the genes that are involved in the formation of eggshell in birds.

In the present study, real-time PCR was used to identify genes affecting ST53 based on their position close to the microsatellite *loci* linked to the QTL. Positional approach was successfully applied in genetic dissection and searching for candidate genes for QTL in different species [Glazier *et al.* 2002]. Optimization of shell thickness has economical importance because it reduces transportation losses. Finding

QTL in chicken: a look back and forward

Table 1. Expression profiling of candidate EST and correlation between shell thickness on week 53 of age and expression level of positional candidates

GeneBank accession no.	Gene/homology	RIR		GIP	
		ratio of means	correlation of ST and EST expression	ratio of means	correlation of ST and EST expression
U10548	Chicken <i>lysosome-associated membrane glycoprotein LAMP-2c (LAMP-2)</i>	1.12	-0.04	0.80	0.26
CR390814	Chicken finished cDNA, clone ChEST974b18	1.31	-0.14	0.75	0.26
CR523443	Chicken finished cDNA, clone ChEST985k21	1.34	-0.25	0.49**	0.85**
NM_001031126	Chicken <i>mitogen-activated protein kinase 4 (MAP4K4)</i>	1.49	-0.18	0.85	0.28
XM_420350	Homology to hypothetical protein FLJ10178	1.10	-0.47	1.20	0.31
XM_420354	Homology to 2610030H06Rik protein	0.74	0.38	0.70	0.41
NM_205361	Chicken <i>Mel-1c melatonin receptor</i>	1.10	-0.13	1.42	-0.07
NM_205295	Chicken <i>HMG2a</i>	1.35	-0.40	1.39	-0.11
XM_420356	Homology to <i>myotubularin-related protein 1 isoform 1</i>	0.76	0.14	0.96	0.35
XM_420357	Homology to <i>myotubularin</i>	0.70	0.17	0.99	0.06
XM_420359	Homology to hypothetical protein LOC91966	1.34	0.36	1.24	-0.05
XM_420360	Homology to <i>family with sequence similarity 11, member A (FAM11A)</i>	1.30	-0.26	0.72	0.28

**P≤0.01.

RIR – Rhode Island Red; GIP – Green-legged Partridge; STL – low shell thickness; STH – high shell thickness.

the candidate gene for ST53 provides a tool for searching for QTL, which could be applied in marker-assisted selection. A skeptical opinion about utilizing microsatellite information for selection on egg quality and shell features was, however, expressed by Boruszewska *et al.* [2009].

QTL: disease resistance

Immune response and disease resistance can be improved by selection. Heritability of these quantitative traits is low to moderate, and thus they may respond more efficiently to marker-assisted than to conventional selection [Yonash *et al.* 2001]. As an alternative to vaccination control, increased genetic resistance to Marek's disease (MD) represents an attractive solution for lowering disease outbreaks. Genetic mapping of QTL affecting susceptibility to MD virus-induced tumors was conducted by Vallejo *et al.* [1998] who for the first time reported the mapping of non-major histocompatibility complex QTL involved in MD susceptibility in chickens. Two

significant and two suggestive MD QTL were detected in four chromosomal regions, explaining 11-23% of the phenotypic, or 32-68% of the genetic MD variation.

A genome-wide scan using 119 microsatellite *loci* allowed Zhu *et al.* [2003] to map QTLs associated with resistance to avian coccidiosis to GGA1. QTL associated with immune response to SRBC, Newcastle disease virus and *E. coli*, and with survival were investigated by Yonash *et al.* [2001]. Three markers were shown to be significantly related to these traits. Besides its own economic importance, the chicken can be considered as a model object for human diseases, e.g. for genetic susceptibility to form-deprivation myopia [Dodgson and Romanov 2004].

QTL: behaviour

Several QTL affecting feather pecking (a major problem in large group housing systems) and stress response in laying hens were detected by Buitenhuis *et al.* [2003]. Using genotypes at 180 microsatellite *loci*, one significant QTL for severe feather pecking was detected in GGA2, and suggestive QTL for gentle feather pecking in GGA1, GGA2, and GGA10. A genome-wide scan using 104 microsatellite markers was conducted to identify QTL affecting foraging behaviour and social motivation in F2 birds from a White Leghorn × Red Junglefowl intercross [Schutz *et al.* 2002]. Significant QTL were found for preference of free food without social stimuli and low contra-freeloading in GGA27 and GGA7, respectively. Interestingly, the location of QTL coincided with known QTL for growth rate and body weight. QTL studies in the chicken have rapidly expanded, and a specialized chicken QTL database has been established (NAGRP, <http://www.animalgenome.org/QTLdb/chicken.html>). With the availability of dense genetic linkage maps, QTL studies are becoming more feasible in other poultry species.

REFERENCES

1. BORUSZEWSKA K., ŁUKASZEWICZ M., ZIĘBA G., WITKOWSKI A., HORBAŃCZUK J., JASZCZAK K., 2009 – Microsatellite markers may be ineffective in selection of laying hens for polygenic production traits. *Poultry Science* 88, 932-937.
2. BUITENHUIS A.J., RODENBURG T.B., SIWEK M., CORNELISSEN S.J.B., NIEUWLAND M.G.B., CROOIJMANS R.P.M.A., GROENEN M.A.M., KOENE P., BOVENHUIS H., VAN DER POEL J.J., 2003 – Identification of quantitative trait loci for receiving pecks in young and adult laying hens. *Poultry Science* 1661-1667.
3. CARLBORG O., HOCKING P.M., BURT D.W., HALEY C.S., 2004 – Simultaneous mapping of epistatic QTL in chickens reveals clusters of QTL pairs with similar genetic effects on growth. *Genetics Research* 83, 197-209.
4. DEEB N., LAMONT S.J., 2003 – Use of a novel outbred by inbred F1 cross to detect genetic markers for growth *Animal Genetics* 34, 205-212.
5. DODGSON J.B., ROMANOV M.N., 2004 – Use of chicken models for the analysis of human disease. In: DRACOPOLI N.C., HAINES J.L., KORF B.R., MOIR D.T., MORTON C.C., SEIDMAN C.E., SEIDMAN J.G., SMITH D.R., (eds). *Current Protocols in Human Genetics*. Wiley, Hoboken, USA. Unit 15.5, 15.5.1–15.5.11.

6. EDMOND A., KING L.A., SOLOMON S.E., BAIN M.M., 2005 – Effect of environmental enrichment during the rearing phase on subsequent eggshell quality in broiler breeders. *Poultry Science* 46, 182-189.
7. GAUTRON J., HINCKE M.T., PANHELEUX M., GARCIA-RUIZ J.M., BOLDICKE T., NYS Y., 2001 – Ovotransferrin is a matrix protein of the hen eggshell membranes and basal calcified layer. *Connective Tissue Research* 42, 255-267.
8. GLAZIER A.M., NADEAU J.H., AITMAN T.J., 2002 – Finding genes that underlie complex traits. *Science* 298, 2345-2349.
9. IKEOBI C.O., WOOLLIAMS J.A., MORRICE D.R., LAW A., WINDSOR D., BURT D.W., HOCKING P.M., 2002 – Quantitative trait loci affecting fatness in the chicken. *Animal Genetics* 33, 428-435.
10. JENNEN D.G.J., VEREIJKEN A.L.J., BOVENHUIS H., CROOIJMANS R.P.M.A., VEENENDAAL A., VAN DER POEL J.J., GROENEN M.A.M., 2004 – Detection and localization of quantitative trait loci affecting fatness in broilers. *Poultry Science* 83, 295-301.
11. LAVELIN I., MEIRI N., PINES M., 2000 – New insight in eggshell formation. *Poultry Science* 79, 1014-1017.
12. SASAKI O., ODAWARA S., TAKAHASHI H., NIRASAWA K., OYAMADA Y., YAMAMOTO R., ISHII K., NAGAMINE Y., TAKEDA H., KOBAYASHI E., FURUKAWA T., 2004 – Genetic mapping of quantitative trait loci affecting body weight, egg character and egg production in F2 intercross chickens. *Animal Genetics* 35, 188-194.
13. SAZANOV A.A., ROMANOV M.N., WARDECKA B., SAZANOVA A.L., KORCZAK M., STEKOL'NIKOVA V.A., KOZYREVA A.A., SMIRNOV A.F., JASZCZAK K., DODGSON J.B., 2005 – Chromosomal localization of fifteen large insert BAC clones containing three microsatellites on chicken chromosome 4 (GGA4) which refine its centromere position. *Animal Genetics* 36, 161-163.
14. SCHMID M., NANDA I., HOEHN H., SCHARTL M., HAAF T., BUERSTEDDE J.M., ARAKAWA H., CALDWELL R.B., WEIGEND S., BURT D.W., SMITH J., GRIFFIN D.K., MASABANDA J.S., GROENEN M.A.M., CROOIJMANS R.P.M.A., VIGNAL A., FILLON V., MORISSON M., PITEL F., VIGNOLES M., GARRIGUES A., GELLIN J., RODIONOV A.V., GALKINA S.A., LUKINA N.A., BEN-ARI G., BLUM S., HILLEL J., TWITO T., LAVI U., DAVID L., FELDMAN M.W., DELANY M.E., CONLEY C.A., FOWLER V.M., HEDGES S.B., GODBOUT R., KATYAL S., SMITH C., HUDSON Q., SINCLAIR A., MIZUNO S., 2005 – Second report on chicken genes and chromosomes. *Cytogenetic and Genome Research* 109, 415-479.
15. SCHUTZ K., KERJE S., CARLBORG O., JACOBSON L., ANDERSSON L., JENEN P., 2002 – QTL analysis of a Red Junglefowl x White Leghorn intercross reveals trade-off in resource allocation between behavior and production traits. *Behaviour Genetics* 32, 423-433.
16. TATSUDA K., FUJINAKA K., 2001 – Genetic mapping of QTL affecting body weight in chickens using a F2 family. *Poultry Science* 42, 333-337.
17. TUISKULA-HAAVISTO M., HONKATUKIA M., VIKKI J., DE KONING D.J., SCHULMAN N.F., MÄKI-TANILA A., 2002 – Mapping of quantitative trait loci affecting quality and production traits in eggs layers. *Poultry Science* 81, 919-927.
18. VALLEJO R.L., BACON L.D., LIU H.C., WITTER R.L., GROENEN M.A.M., HILLEL J., CHENG H.H., 1998 - Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced tumors in F2 intercross chickens. *Genetics* 148, 349-360.
19. VAN KAAM J.B.C.H.M., VAN ARENDONK J.A.M., GROENEN M.A.M., BOVENHUIS H., VEREIJKEN A.L.J., CROOIJMANS R.P.M.A., VAN DER POEL J.J., VEENENDAAL A., 1998 – Whole genome scan in chickens for quantitative trait loci affecting body weight in chickens using a three generation design. *Livestock Production Science* 54, 133-150.

20. VAN KAAM J.B.C.H.M., GROENEN M.A.M., BOVENHUIS H., VEENENDAALA., VEREIJKEN A.L.J., VAN ARENDONK J.A.M., 1999a – Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency. *Poultry Science* 78, 15-23.
21. VAN KAAM J.B.C.H.M., GROENEN M.A.M., BOVENHUIS H., VEENENDAALA., VEREIJKEN A.L.J., VAN ARENDONK J.A.M., 1999b – Whole genome scan in chickens for quantitative trait loci affecting carcass traits. *Poultry Science* 78, 1091-1099.
22. VAN KAAM J.B., BINK M.C., BOVENHUIS H., QUAAS R.L., 2002 - Scaling to account for heterogeneous variances in a Bayesian analysis of broiler quantitative trait loci. *Journal of Animal Science*, 80, 45-56.
23. WARDECKA B., OLSZEWSKI R., JASZCZAK K., ZIĘBA G., PIERZCHAŁA M., WICIŃSKA K., 2002 – Relationship between microsatellite marker alleles on chromosome 1–5 originating from the Rhode Island Red and Green-legged Partridge breeds and egg production and quality traits in F2 mapping population. *Journal of Applied Genetics* 43, 319-329.
24. WARDECKA B., OLSZEWSKI R., JASZCZAK K., ZIĘBA G., PIERZCHAŁA M., 2003 – Preliminary mapping of QTLs affecting egg quality on chromosomes 1–5 in chickens. *Czech Journal of Animal Science* 48, 97-105.
25. XU G., GOODRIDGE A.G., 1998 – A CT repeat in the promoter of the chicken malic enzyme gene is essential for function at an alternative transcription start site. *Archives of Biochemistry and Biophysics* 358, 83-91.
26. YONASHN., CHENG H.H., HILLEL J., HELLER D.E., CAHANERA., 2001 – DNA microsatellites linked to quantitative trait loci affecting antibody response and survival rate in meat-type chickens. *Poultry Science* 80, 22-28.
27. ZHU J.J., LILLEHOJ H.S., ALLEN P.C., VAN TASSELL C.P., SONSTEGARD T.S., CHENG H.H., POLLOCK D., SADJADI M., MIN W., EMARA M.G., 2003 – Mapping quantitative trait loci associated with resistance to coccidiosis and growth. *Poultry Science* 82, 9-16.