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Mapping of QTLs Controlling Egg Quality on Chromosomes 6-8, Z and Three Linkage Groups in Chickens

Research Article

S.J. Rosochacki[1,2*], R. Olszewski[1], B. Wardecka[1], K. Jaszczak[1], G. Zieba[3], E. Juszczuk-Kubiak[1] and J. Poloszynowicz[1]

[1] Institute of Genetics and Animal Breeding, PAS, Jastrzębiec, 05-552, Magdalenka, Poland
[2] Bialystok Technical University, 15-351, Bialystok, Poland
[3] Agriculture Academy, 20-950, Lublin, Poland

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*Correspondence E-mail: s.rosochacki@ighz.pl

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ABSTRACT

Two breeds of chickens were used to develop reference family and detect QTLs affecting egg quality traits: cocks of a native Polish breed Green-Legged Partidgenous (GlP) and hens of Rhode Island Red (RIR). During the first 100 days of the laying period, the GlP hens lay about 40 eggs weighting on average 50 g, whilst the RIR flock laid 83 eggs with a mean weight of 60 g. QTL analysis of egg quality traits was performed on 552 birds of the F_2 generation with the aid of 19 microsatellite loci on chromosomes 6, 7, 8, Z and linkage groups 9 (E 36), 19 (E 52) and 26 (E 46). Linkage analysis was performed for 19 microsatellite markers within the mapped population and ten QTLs were found to affect seven egg quality traits: at week 53-shell thickness, shell strength, albumen weight, egg specific gravity and percent of yolk in egg; and at week 33-percent of yolk in egg and Hugh's units.

KEY WORDS chickens, egg quality traits, microsatellites, QTL mapping.

INTRODUCTION

The practical aim of gene mapping in different organisms is to discover genetic markers and their linkage with quantitative traits loci (QTLs) that can increase the selection response in breeding programs and help to minimize the limitation in using QTL information for marker-assisted selection (MAS), especially for traits that are difficult to improve when using traditional selection (Korstanje and Paigen, 2002; Fadiel *et al*. 2005).

QTL analysis always involves linkage of genetic markers with genome regions influencing the investigated trait (or traits) (Soller, 1991). Prior work using genetic markers has identified the Boroola gene affecting ovulation rate in sheep (fecB), the halothane sensitivity gene in pigs (RYRT), the double muscling gene of cattle (Montgomery *at al*. 1993; Geldermann *et al*. 1999; Grobet *at al*. 1997). Microsatellite markers or simple sequence repeats can satisfy the investigations of quantitative trait loci in animal breeding (Malek *et al*. 2001; Diez-Tascon *et al*. 2001). These markers must be characterized by high heterozygosity and polymorphism and should be evenly distributed throughout the genome and broadly dispersed.

The effect of QTL mapping depends on: the number of genes underlying a polygenic trait, even markers distribution throughout the genome, interactions between genes, traits hereditary and the size of animal population (Soller, 1991; Hillel, 1997; Malek *et al*. 2001).

The reference population can be obtained by crossing genetically distant breeds, families or inbred lines to obtain the mapping populations with the high level of heterozygosity.

For example, the East Lansing reference population was created by crossing of Wild Red Jungle cock with highly inbred of Leghorn lines (Crittenden *et al*. 1993). To identify QTL affecting susceptibility to tumours induced by Marek's disease virus, a second reference population has been created (Vallejo *et al*. 1998). The population created in Wageningen was based on two lines of broiler chicken (White Plymouth Rock), substantially differing in slaughter traits (Groenen *et al*. 1997), whilst the Compton population was obtained by crossing two highly inbred lines of Leghorn chicken differing in their resistance to salmonelosis and Marek's disease (Bumstead and Palyga, 1992).

Some reference populations used for QTLs mapping can be based on a cross between inbred lines (Bumstead and Palyga, 1992; Crittenden *et al*. 1993; Zhou and Lamont, 1999). The advantage of such a situation is that each region of each chromosome homologue can be uniquely identified by a specific marker allele or marker haplotype. The progeny is analyzed in the way of genotype and performance data, which have to be collected on many animals to achieve sufficient power. Availability of powerful statistical methods have permitted QTL mapping of a variety of traits in chickens and allowed researchers to obtain the high-resolution genetic maps. In some laboratories such investigations were performed and dealt with the traits affecting: growth, body weight, carcass production traits, egg quality traits, resistance to disease or feed efficiency (Groenen *et al*. 1997; Kuhilen *et al*. 1997; Van Kaam *et al*. 1998; Van Kaam *et al*. 1999a; Van Kaam *et al*. 1999b; Vallejo *et al*. 1998; Honkatukia *et al*. 1999; Yonash *et al*. 1999; Yonash *et al*. 2001; Tuiskula-Haavisto *et al*. 1999; Tuiskula-Haavisto *et al*. 2002; Tatsuda and Fujinaka 2001; Wardęcka *et al*. 2003; Siwek *et al*. 2004; Sasaki *et al*. 2004; Schreiweis *et al*. 2005; Hansen *et al*. 2005; Abasht *et al*. 2009). Loci affecting egg quality traits have been based firstly on medium-density microsatellite maps and QTL were estimated for various eggshell characteristics, but with much less emphasis on eggshell colour or egg white quality. Zhi-Liang *et al*. (2010) reported only 1863 chicken QTL's with 208 traits (QTL database, December 31, 2009). But in December 28 of 2011, 2451 chicken QTL were reported in the database, only 223 for egg quality traits (plus 66 for egg production). In order to detect QTL affecting egg quality traits, a reference family was created in Poland by crossing distantly related individuals, to obtain highly informative offspring. Green-Legged Partigenous (GIP) cocks a native Polish breed were crossed with a highly productive Rhode Island Red (RIR) hens (Hillel, 1997; Rosochacki *et al*. 1997; Jaszczak *et al*. 2001). The chickens were chosen on the basis of considerable differences in egg laying, body conformation, plumage and behavior. Genetic distance in this population based on 41 DNA microsatellite markers was 0.43 according to Reynolds *et al*. (1983), but according to Nei (1978) -0.69 (Jaszczak *et al*. 2001). In the Wageningen population, the genetic distance was based on 16 microsatellites, being 0.37 (Nei 1978), but in Finnish population-based on 9 microsatellie loci was 1 (Vanhala *et al*. 1998).

QTL analysis concerning laying and egg quality traits, based on population of 519 birds of F_2 generation and with the use of 23 microsatellite loci on chromosomes 1, 2, 3, 4 and 5 were already performed (Wardęcka *et al*. 2003). This report, therefore, extends earlier mapping data on the progeny of second generation for QTLs search on the chromosomes 6, 7, 8, Z and microchromosomes (E 36, E 46 and E 52.

MATERIALS AND METHODS

Experimental population

Parental generation F_0 was established by crossing 10 Green-Legged Partridgenous (GlP) cocks, a native Polish breed maintained as a conservative flock, laying in first 100 days 40 eggs weighting about 50 g in average, and Rhode Island Red (RIR) hens-producing about 81 eggs weighting 60 g in the first 100 days. According to DNA fingerprinting analysis with R18.1 probe (Hillel 1997), 10 birds having the lowest band sharing (RIR-0.619 and GlP-0.431) were chosen to build a reference family (Rosochacki *et al*. 1997). F_0 couples were mated to generate 10 families of 160 birds of F_1 progeny. The F_2 generation was created by crossing one brother and one sister from each of the F_1 families and 552 chickens were obtained. All together, 732 birds were used as the experimental population. The birds were maintained as described previously by Jaszczak *et al*. (2001) and Wardęcka *et al*. (2003) and 26 egg quality traits were measured from F_2 individuals at week 33 and 53 of life (Table 1, Wardęcka *et al*. 2003).

Marker data

Genotypes for microsatellite markers were determined using DNA derived from blood samples. The blood (10 mL) was collected into vacuum tubes containing EDTA and stored at -20 ˚C until extracting DNA by standard methods. The quality of extracted DNA was checked spectrophotometrically and by agar electrophoresis; the final concentration of DNA was adjusted to 0.1µg/µL. The microsatellite markers were chosen from the Roslin Institute database (http://www.therkdb.org) which covers chromosomes 6, 7, 8 and Z and three linkage groups: E 36, E 46 and E 52. Markers were chosen from MCW (Crooijmans *et al*. 1996b; Groenen *et al*. 1997), LEI (Gibbs *et al*. 1997) and ADL (Cheng *et al*. 1995) groups, altogether 19 microsatellites (Table 2).

Table 1 Egg quality traits measured

Trait	Symbol	Trait	Symbol
Egg specific gravity at week 33 (g)	ESG33	Egg specific gravity at week 53 (g)	ESG53
Egg weight at week 33 (g)	EW33	Egg weight at week 53 (g)	EW53
Shell weight at week 33 (g)	SW33	Shell weight at week 53 (g)	SW53
Shell colour at week 33	SC33	Shell colour at week 53	SC53
Shell density at week 33	SD33	Shell density at week 53	SD53
Shell thickness at week 33 (µm)	ST33	Shell thickness at week 53 (µm)	ST53
Shell strength at week 33 (kg/cm2)	SS33	Shell strength at week 53 (kg/cm2)	SS53
Yolk weight at week 33 (g)	YW33	Yolk weight at week 53 (g)	YW53
Percent of yolk in egg at week 33 (%)	PY33	Percent of yolk in egg at week 53 (%)	PY53
Percent of white in egg at week 33 (%)	PW33	Percent of white in egg at week 53 (%)	PW53
Percent of shell in egg at week 33 (%)	PS33	Percent of shall in egg at week 53 (%)	PS53
Hugh units at week 33	HU33	Hugh units at week 53	HU53
Albumen weight at week 33 (g)	AW33	Albumen weight at week 53 (g)	AW53

Polymerase chain reactions and product analysis

Polymerase chain reaction conditions were 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 0.1% Triton X-100, 100 µM of each dNTP, 2.5 pmol of each primer, 0.5 U of DNA Taq polymerase and 100 ng of genomic DNA in a final volume of 7.5 µL. From each pair of primers, one was labeled with fluorescein Cy5. Reactions were denatured for 5 min at 94 ˚C, then 25-37 cycles of denaturation at 94 ˚C for 45 s, annealing at 48-55 ˚C for 60 s, and extension at 72 ˚C for 60 s, with a final 5 min elongation step at 72 ˚C in PTC-200 Programmable Thermal Controller. Five microlitres of stop dye (50% formamide solution containing blue dextran) was added, and the samples were heated to 94 ˚C for 1 min before 2 µL was loaded onto a 6% denaturing polyacrylamide sequencing gel, using an Automated Laser Fluorescent (ALF express) DNA Sequencer. The results were genotyped with Allele Links 1.01, and after checking all individual genotypes by manual inspection, the genotypes were exporting to Excel. The typing errors were rechecked and corrected with CRI-MAP program. Such a data were exported from Excel worksheets to the CRI-MAP linkage analysis program version 2.4 (Green *et al*. 1990).

QTL mapping and significance thresholds

QTL cartographer vers. 1.13 programme was used to map QTL (Basten *et al*. 1999) using the composite interval mapping method elaborated by Zeng (1993) and Zeng (1994). In the analysis all markers of linkage groups were taken into account. For each of the position of QTL the hypothetical test was performed of null hypothesis of no QTL against the alternative hypothesis with test statistic. A statistic test was performed every 1 cM between extreme marker loci of linkage group. The permutation test, an empirical method, was used to calculate significance thresholds (Churchil and Doerge, 1994) which accounts for distribution of marker and phenotypic data. The thresholds were accounted for the tests conducted on the chromosomes analysed and were calculated for each trait by 500 times of permutation of all markers simultaneously in order to construct the distribution under the null hypothesis and three levels of significance threshold were estimated: 1%, 5% (significant) and 10% (suggestive).

RESULTS AND DISCUSSION

The reference population was based on two lines of chicken: Polish Green-Legged Partidgenous and Rohde Island Red characterized by big genetic differences (specific allele for GlP 19 and 28 for RIR) and phenotypic traits (laying and egg quality traits) (Hillel, 1997; Rosochacki *et al.* 1997). Only four loci with the same alleles did not occur in RIR and GlP breeds (ADL244, LEI212, LEI075, MCW157). Three alleles specific for GlP were observed in six loci (ADL180, ADL172, LEI074 and LEI121, MCW0134 and MCW0256), but in RIR populations these were found only in three loci (MCW133, MCW256 and ADL326). Alleles with similar high frequencies were found in the parental, F_1 and F_2 generations. Over 50% of markers in F_2 population showed a high heterozygosity (higher than 60%) which was estimated on the basis of the polymorphism of 19 microsatellite loci. The highest heterozygosity was for LEI212 *locus* (0.8028), the lowest for ADL180 *locus* (0.2784).

This population was firstly analyzed with the aid of 23 microsatellie markers in chromosomes 1-5 (Wardęcka *et al.* 2002; Wardęcka *et al.* 2003), the analysis was then performed on chromosomes 6-8, Z and three linkage groups, which were characterized by size, the number of microsatellite sequences available and expected polymorphism. Estimated number of different alleles per *locus*, estimated with 19 microsatellites, were from 2 in four loci (ADL244, LEI075, MCW154 and MCW157) to 10 in ADL279 *locus*, being in average 4.84 per *locus* and confirmed a high level of polymorphism; nineteen alleles were specific for the GlP and twenty eight for the RIR chicken; forty five alleles were found in both strains.

Table 2 Microsatellite markers and polymerase chain reaction primers used in gene mapping

Locus	Linkage group	Map position (cM)	Repeat	Primers	Product (bp)	Annealing temp (°C)	References
ADL244	6	0	(GT) 9	AGGGTCTGAAGAGAGGTGTT GCAAGATGCAAAGAGATTTC	152	48	(Cheng *et al*. 1995)
ADL320	6	36.7	(TG) 10	AGGGGTTATTGCTGCTCTGC GTCCTCAGTGGCCAAATGC	114	55	(Burt, 1996)
LEI212	6	83.7	(GGAAGAAA) 6	TTTGCCAATCCCTATTGAGC TTTTCATATTTGTGGCGTGC	244	50	(Cheng *et al*. 1995)
ADL180	7	0	(TG) 15	ACCAGAGCATCTACTGAAGA AAACCTGGAAATGAAAGCAT	136	50	(Cheng *et al*. 1995)
ADL326	7	100	(CA) 14	GCTCACAAGAAGGGGTCACA CCACCTCTGGTTTCCTCACC	169	48	(Burt, 1996)
MCW133	7	161.5	(TG) 21	GATCTTTCTGTACAATGAATAC TTAGGAGCAACTCAGTTGGAG	130	55	(Crooijmans *et al*. 1996a)
ADL279	7	220	(GT) 23	CATGGCTGTTGCTTTACATA GTGAACCCCAATGCTCTCTG	110	50	(Cheng *et al*. 1995)
ADL171	8	0	(TG) 18	ACAGGATTCTTGAGATTTTT GGTCTTAGCAGTGTTTGTTT	104	50	(Cheng *et al*. 1995)
ADL322	8	54.2	(CA) 15	TGCGTTCTCCCCTTGGTTGC GCAGCAGCTCCCACGACACA	140	50	(Burt, 1996)
ADL172	8	104.9	(AC) 18	CCCTACAACAAAGAGCAGTG CTATGGAATAAAATGGAAAT	154	55	(Cheng, *et al*. 1995)
MCW154	Z	0	(TG) 11	GATCTGTTTTATCACACACAC CCATTTCCTTTGTTATCAGGC	180	55	(Crooijmans *et al*. 1996a)
LEI075	Z	18.7	(CA) 20	TTTCACATCCAGTGCGTGTCTG GGGCAGAGAAAGACGAAATGG	230	48	(Gibbs *et al*. 1997)
LEI121	Z	43.4	(AC) 15	AACACTGCAATTCTGAGAGTGCC CAGCATCTGTCTTTCAAGGAATC	270	50	(Zhou and Lament, 1999)
MCW134	9 (E 36)	0	(TG) 24	GGAGACTTCATTAGTGTAGCAC ACCAAAAGACTGGAGGTCAAC	270	55	(Crooijmans *et al*. 1996a)
MCW135	9 (E 36)	81	(TG) 25	ATATGCTGCAGAGGGCAGTAG CATGTTCTGCATTATTGCTCC	130	55	(Ruyter-Spira *et al*. 1996)
MCW287	19 (E 52)	0	(CA) 20	GCCGTGTGACATCAGTGCTC TTGCACCAGCGCTGCAAACTG	245	55	(Crooijmans *et al*. 1997)
MCW256	19 (E 52)	100	(CA) 10	GATGGGGCACTGTGGGTCCC TGGTTTCCATCAAGCAGTTCC	170	55	(Crooijmans *et al*. 1997)
LEI074	26 (E 46)	0	(CA) 12	GTTTGCTGATTAGCCATCGCG GACCTGGTCCTGACATGGGTG	310	48	(Gibbs *et al*. 1997)
MCW157	26 (E 46)	77.8	(TG) 11	GTGTGATGTAGGCCAGATGTGC GTGCTGCATTCTGCCAATAGG	290	50	(Ruyter-Spira *et al*. 1996)

In chicken, Hansen *et al*. (2005) reported 3.4 alleles detected for each microsatellite *locus*, Crooijmans *et al*. (1996a) 3.6 in meat line and Zhu *et al*. (2001) 2.0 in laying chicken.

The average heterozygosity in our parent population was 0.69 and was higher than those recorded in the Wageningen (0.60) and East Lansing (0.52) populations. The high number of heterozygotic individuals (47.1% of F_2 chickens) and

the number of informative meiosis (all together 6089 for all analyzed loci) in examined generations (Jaszczak *et al.* 2001) supports that the chosen mapping markers were correct and were in agreement with comparative data of chicken mapping (Cheng *et al.* 1995; Crooijmans *et al.* 1996b; Gibbs *et al.* 1997; Groenen *et al.* 1998).

The critical values for test statistics, which may suggest the QTL presence, were calculated with the aid of a permutation test (Churchill and Doerge, 1994) for a single or all markers (Lander and Kruglyak 1995; Paterson, 1995). The three step significance level of 26 egg quality traits values was taken α= 0.01, α= 0.05 and α= 0.1. We used (Zeng's 1993; Zeng's 1994) composite interval mapping method, which takes into consideration the influence of all markers in linkage groups and gives the QTLs position independently from the other quantitative trait *loci*. Composite interval mapping combines interval mapping with multiple regression.

Linkage between 26 egg quality traits and 19 mikrosatellite loci on chromosomes 6-8 and three linkage groups, using CRI-MAP version 2.4 program (Green *et al.* 1990), allowed establish 10 QTL for seven traits. At significance level of 1%, six sites were found (PY33-2 loci, chromosomes 7 and 8; PY53-chromosome 8; SS53-chromosome 8; ST53-chromosome 7 and ESG53-linkage group 26 (E46)), while at 5% two: Hugh's units at 33 week-linkage group 9(E36) and SS53-linkage group 26 (E46).

The QTL for ST53 and PY33 were found on chromosome 7 very close (159.01 and 158.01) and were linked with marker ALD180. A significant linkage with marker ALD171 was estimated on chromosome 8 for three traits (PY33, PY53 and SS53, α=0.01, and additionally for Hugh's units on suggestive level α=0.1). A significant linkage with marker LEI074 was estimated on linkage group 26 (E46) with ESG3 (α=0.01), SS53 (α=0.05) and with AW53 (α=0.1). QTL mapped by Hansen *et al.* (2005) with respect to egg production and quality traits established 11 QTL for eight traits, among them four traits were linked to egg quality.

The QTL relating to shell shape was mapped to chromosome 8 at position 42 cM. Whilst, the QTL associated with number of eggs from 18 to 40 weeks of age was linked to chromosome 8, as reported by Tuiskula-Haavisto *et al.* (2002). Sasaki *et al.* (2004) and Hansen *et al.* (2005) reported QTL of the sum of the egg laid by a bird (chromosome 1). The QTL accounted for albumen weight and Hugh units were found on chromosome 1 and additionally albumen weight QTL was mapped to chromosome 2 (Hansen *et al.* 2005). In our work, the albumen weight at week 53 was linked to linkage group 26 (E46). Tuiskula-Haavisto *et al.* (2002) confirmed the QTL on chromosome 2 for Hugh units at 40 to 60 weeks of age, however we mapped this QTL at the age of 33 weeks to chromosome 8 and linkage group 9 (E36). Sasaki *et al.* (2004) identified several QTL for egg shell strength, thickness, and weight in chromosome 1. Schreiweis *et al.* (2005) showed two QTL regions on chromosomes 2 and 9 QTL on chromosome 4. These QTL were: egg colour, egg and albumen weight, percent of shell, body weight and egg production. Previous work has suggested that chromosome 4 may be a critical region significantly associated with the variety of traits eg. body weight, (Sewalem *et al.* 2002; Tuiskula-Haavisto *et al.* 2002; Sasaki *et al.* 2004) across multiple resource populations. Wright *et al.* (2006) found QTL for shell thickness at 27 week of age on chromosome 5, here we found QTL for shell thickness at 53 weeks mapped to chromosome 7 and shell strength at 53 weeks linked to chromosome 8 and linkage group 26 (E46). Hansen *et al.* (2005) mapped QTL for shell shape on chromosome 8, for specific gravity to chromosome 2, in our work QTL egg specific gravity at 53 week of age was located on linkage group 26 (E46). The analysis was performed between average phenotypic traits of quality of eggs and microsatellite loci. On the basis of the alleles origin, three genotypes were established in generation F_0: both alleles were from RIR chicken-(RIR/RIR) or from GlP cocks (GlP/GlP), or one allele was from female RIR but the second from GlP cocks-(RIR/GlP). Some statistically important differences between genotypes were established on

Table 3 The QTLs most likely position (in cM) on chromosomes or linkage groups per traits. Genomewise significance level of QTLs at these positions, the closest markers to the QTL and the explained variance of QTLs

Chromosome or linkage group	Traits	QTL position (cM)	Significance level	P-value	Marker	Explained variance (%)
7	PY33	158.01	0.01	16.69	ALD180	18.95
7	ST53	159.01	0.01	26.16	ALD180	21.32
8	SS53	8.01	0.01	35.12	ALD171	29.42
8	HU33	54.01	0.1	13.62	ALD171	6.31
8	PY53	60.21	0.01	31.92	ADL171	28.20
8	PY33	68.21	0.01	20.91	ADL171	24.44
9 (E36)	HU33	61.01	0.05	16.10	MCW134	21.93
26 (E46)	SS53	0.01	0.05	16.46	LEI074	6.47
26 (E46)	ESG53	9.01	0.01	32.80	LEI074	31.00
26 (E46)	AW53	71.01	0.1	13.73	LEI074	19.85

the origin basis and the average value of quality traits of egg and shell. The highest numberat loci (7) were dependent for percent of white in egg and egg specific gravity at week 53. The highest number of traits dependent on one *locus* was 16 for ADL320 (chromosome 6), 8 for ADL244, ADL326 and MCW134 (chromosomes 6, 7 and linkeage group 9 (E36), respectively) and 10 for MCW133 (chromosome 7). The explained proportion of the variance in the QTL identified was 0.063-0.31. The same in the work of Wardęcka *et al.* (2003) was 0.017-0.31, but in Hansen *et al.* (2005) 0.07-0.25. Wardęcka *et al.* (2003) showed in the same population of chicken the presence of 12 regions linked with QTL for 9 traits on chromosomes 1-5 with the use of 23 microsatellite markers (7 traits with significance level P<0.01, one with P<0.05 and two with P<0.1). The East Lansing group using 127 microsatellites found eight chromosomal sub-regions linked with QTL (Vallejo *et al.* 1998), whilst a group of Finnish investigators showed all together 20 chromosomal areas affected egg quality (14 at 1% and 6 at 5% genomewise) using 37 microsatellite sequences (Tuiskula-Haavisto *et al.* 2002), the Wageningen group reported 8 QTL with 420 markers (Van Kaam *et al.* 1999a; Van Kaam *et al.* 1999b), a Japanese group 2 QTL with 78 markers (Tatsuda and Fujinaka, 2001) but used 380 informative microsatellite markers. In our experiments 38 traits were measured (Wardęcka *et al.* 2003) concerning laying traits (12) and quality of eggs traits (26). Finnish group controlled body and egg weight, feed intake, sexual maturity, Hugh's units, thickness and strength of shell (Tuiskula-Haavisto *et al.* 2002). Schreiweis *et al.* (2005) found out eleven QTL concerning egg production and egg quality traits with 120 microsatellite markers. There are 2451 chicken QTLs in the database from 125 publications, representing 248 different chicken traits, and there have been rapid progress in QTL studies during the years beginning from 2008. To the end of 2004, 330 QTLs have been found out 2008-2010, 1753 chicken QTL's were added to the chicken QTL Database 2010 (Zhi-Liang *et al.* 2010), mostly on chromosome 1 (403 QTL), chromosomes 4, 2, 3 and 5-172, 165, 161 and 153, respectively and on chromosome Z-97 QTL's. The top 20 traits in terms of number of QTL's have changed between 2009 and 2010, and only five traits were on both lists (abdominal fat weight, breast muscle weight, heart weight, Marek's disease related traits and carcass weight).

The number of QTL by chicken trait type were mostly connected with growth (1645 QTL), disease resistance (248 QTL), egg quality (223 QTL) and egg production (66 QTL). In 2008 only egg weight was on this list, as a representative of egg production traits, and disappeared in 2010. According to chicken consensus map published in the review paper of Abasht *et al.* (2006) and extended in the paper in 2009, only 8 papers focused on egg production traits on chicken chromosomes, and on chromosome Z eight egg traits were located, on chromosomes 6, 7, 8-seven, five and three, respectively; but on linkage groups 9, 19 and 26-one, one and two, respectively. Gene mapping allowed to identify 10 hypothetical QTL on chromosomes 7, 8 and in linkage groups 9 (E36) and 26 (E46) and was based on 26 egg quality traits recorded for generation F_2 (Table 1) with the use of 19 mikrosatellite markers. The explained variance within the analyzed traits varied between 6.31-31.00%. Two QTLs were fund on chromosome 7 for shell thickness at week 53 (in 2.5 cM distance from marker MCW133, Figure 1) and percent of yolk in egg at week 33 (3.5 cm from marker MCW133). For both traits the linkage were on significance level P= 0.01. Four QTLs were fund on chromosome 8: three with P= 0.01 for percent of yolk in egg at week 33 (14 cM from marker ADL322, Figure 2) and at week 53 (6 cM from marker ADL322, Figure 3) and shell strength at week 53 (8 cM from ADL171); one suggestive linkage (with P=0.1) of Hugh's units at 33 week (0.2 cM from marker ADL322). Hugh's units at week 33 were mapped in a distance of 20 cM from marker MCW135 in linkage group 9 (E36) (P=0.05). With P= 0.01 of probability in linkage group 26 (E46) were found out QTL at week 53 for: egg specific gravity (9.01 cM from marker LEI074, Figure 4), with P= 0.05 shell strength (0.01 cM from LEI074) and with P= 0.1 yolk weight at (6.8 cM from marker MCW157). Identification of quantitative trait loci provides an opportunity to link genetic markers with the genes influencing phenotypic effects. Genetic markers for QTL might be very useful in animal selection leading to exclusion of undesirable alleles in commercial populations (eg. susceptibility for disease) and should be confirmed by linkage of loci and genetic traits (Lynch and Walsh, 1998). Vikki (2009) analyzed production traits for epistatic QTL effects, and did not found any for egg quality traits and concluded that epistatic effects between genes do not play a significant role in the genetics of egg shall quality despite an epistatic effect being found for early growth. Investigations leading to QTL mapping with the aid of genetic markers are the preliminary stage to identify the genes influencing on the traits with the big economical impact. The mapping populations and measured traits for egg characterizations have been different among studies what may reflect the action of some loci. So far, there are not fine-mapping studies for these traits. Due to the microsatellites available at the time of the experiment, we covered firstly chromosome 1-5 (Wardęcka *et al.* 2002; Wardęcka *et al.* 2003) and in the present study chromosome 6-8, Z and some linkage groups, but to lack of informative markers, gaps in genome coverage occurred, as in the others QTL experiments performed on livestock.

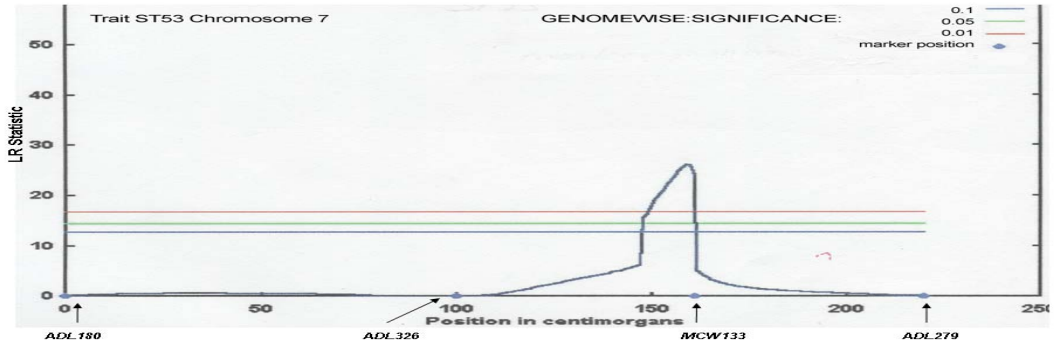

Figure 1 Test statistic values from the analysis of shell thickness at week 53 of life for quantitative trait loci on chromosome 7

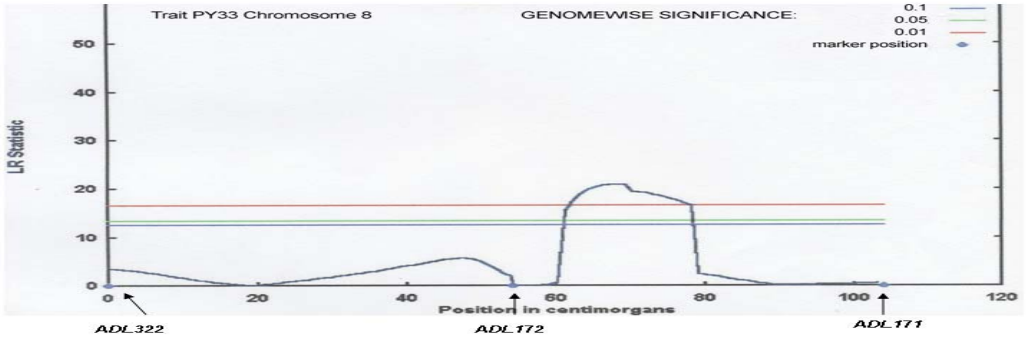

Figure 2 Test statistic values from the analysis of percent of yolk in egg at week 33 of life for quantitative trait loci on chromosome 8

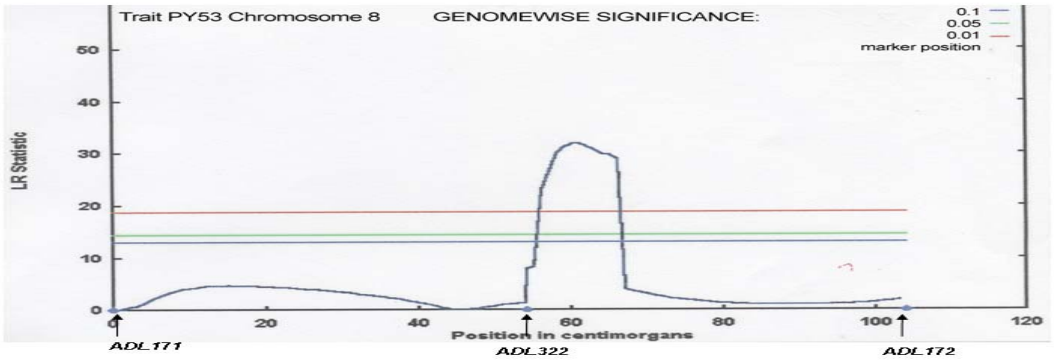

Figure 3 Test statistic values from the analysis of percent of yolk in egg at week 53 of life for quantitative trait loci on chromosome 8

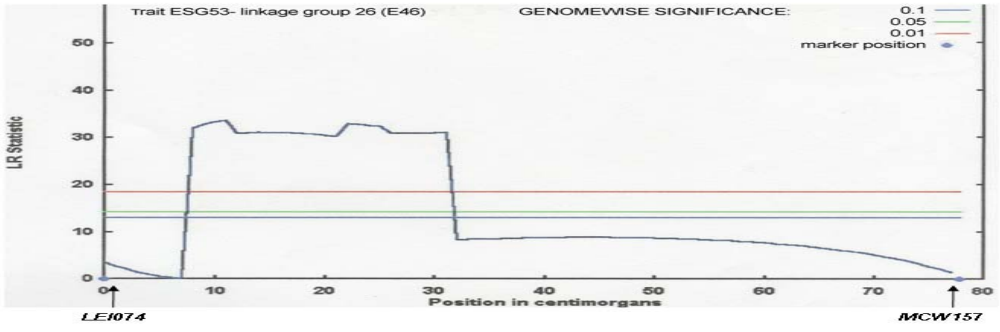

Figure 4 Test statistic values from the analysis of egg specific gravity at week 53 of life for quantitative trait loci in linkage group 26 (E46)

It is thought, that use of dense chicken SNP panels in genome wide associations analyses will be tested in commercial population for egg quality.

CONCLUSION

Based on obtained data, the conclusion is, that chromosomes 6-8, Z and three linkage groups 9(E36), 19(E52), 26(E36) could contain in regions of investigated *loci* the genes controlling chicken egg quality traits. In this work, 10 regions were probably responsible for the variability of some genetic traits which might be the goal for the next investigations to find out (isolate) the genes influencing on egg quality traits. Further investigations should lead to saturate with a large number of markers the examined chromosome regions in which linkage with QTLs was observed in the order to more precise their localizations for finding candidate genes.

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