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

S.J. Rosochacki1,2*, R. Olszewski1, B. Wardecka1, K. Jaszczak1, G. Zieba3, E. Juszczuk-Kubiak1 and J. Poloszynowicz1

1 Institute of Genetics and Animal Breeding, PAS, Jastrzębiec, 05-552, Magdalenka, Poland
2 Białystok Technical University, 15-351, Białystok, 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 (GIP) and hens of Rhode Island Red (RIR). During the first 100 days of the laying period, the GIP 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 F2 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 et al. 1993; Geldermann et al. 1999; Grobet et 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 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; Schreweis 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 Partridge (GIP) cocks a native Polish breed were crossed with a highly productive Rhode Island Red (RIR) hens (Hillel 1997; Rosochacki et al. 1997; Jaszczyk 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 microsatellite 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 F0 was established by crossing 10 Green-Legged Partridgeous (GIP) 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 GIP-0.431) were chosen to build a reference family (Rosochacki et al. 1997). F0 couples were mated to generate 10 families of 160 birds of F1 progeny. The F2 generation was created by crossing one brother and one sister from each of the F1 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 F2 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 0.1 μg/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).
Polymerase chain reactions and product analysis
Polymerase chain reaction conditions were 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl₂, 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 in a water bath. 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 a water bath.

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 GIP 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 GIP breeds (ADL244, LEI212, LEI075, MCW157). Three alleles specific for GIP 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, F₁ and F₂ generations. Over 50% of markers in F₂ 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 microsatellite 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 GIP and twenty eight for the RIR chicken; forty five alleles were found in both strains.
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 F2 chickens) and

![Image of a table with microsatellite markers and polymerase chain reaction primers used in gene mapping.](https://example.com/table.png)
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

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

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. Schrieweis 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 F0: both alleles were from RIR chicken-(RIR/RIR) or from GIP cocks (GIP/GIP), or one allele was from female RIR but the second from GIP cocks-(RIR/GIP). Some statistically important differences between genotypes were established on...
the origin basis and the highest number of value traits of egg and shell. The highest number of loci 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 linkage 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 Wardcęka et al. (2003) was 0.017-0.31, but in Hansen et al. (2005) 0.07-0.25. Wardcęka 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 (Wardcęka et al. 2003) concerning laying traits (12) and quality of egg 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 F2 (Table 1) with the use of 19 microsatellite markers. The explained variance within the analyzed traits varied between 63.1-31.00%. Two QTLs were found 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 (Wardcęka et al. 2002; Wardcęka 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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