The twofold difference in adult size between the red junglefowl and White Leghorn chickens is largely explained by a limited number of QTLs

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Summary

A large intercross between the domestic White Leghorn chicken and the wild ancestor, the red junglefowl, has been used in a Quantitative Trait Loci (QTL) study of growth and egg production. The linkage map based on 105 marker loci was in good agreement with the chicken consensus map. The growth of the 851 F_2 individuals was lower than both parental lines prior to 46 days of age and intermediate to the two parental lines thereafter. The QTL analysis of growth traits revealed 13 loci that showed genome-wide significance. The four major growth QTLs explained 50 and 80% of the difference in adult body weight between the founder populations for females and males, respectively. A major QTL for growth, located on chromosome 1 appears to have pleiotropic effects on feed consumption, egg production and behaviour. There was a strong positive correlation between adult body weight and average egg weight. However, three QTLs affecting average egg weight but not body weight were identified. An interesting observation was that the estimated effects for the four major growth QTLs all indicated a codominant inheritance.

Keywords additive effects, chicken, egg production, growth, Quantitative Trait Locus.

Introduction

Domestic animals provide unique opportunities to study the genetic basis for phenotypic diversity and are excellent models for evolution by natural selection (Andersson 2001). We have generated a resource pedigree for mapping Quantitative Trait Loci (QTLs) by crossing the red junglefowl (*Gallus gallus* spp.) with White Leghorn chickens. The red junglefowl is the wild ancestor of the domestic chicken and the process of chicken domestication is believed to have started well over 8000 years ago in South-east Asia (Yamada 1988; Fumihito *et al.* 1994). Initially the chicken was used as a sacrificial or religious bird, or for cockfighting. It was the Romans who developed its potential as an agricultural animal, creating specialized breeds, including dual-purpose breeds and productive layers. With the decline of the Roman Empire the poultry industry collapsed and very little systematic selection was practiced for many centuries, with the exception of birds for cockfighting. The Leghorn type chicken is derived from the mediterranean type of chicken and was developed during the nineteenth century. The White Leghorn is a light, egg-laying breed that has been selected for efficiency – maximum output of eggs for minimum food intake. Despite this, the White Leghorn is about twice as large as the red junglefowl, and this marked phenotypic difference was utilized in the present study. The red junglefowl and White Leghorn chickens also differ markedly for a number of other traits including plumage colour, egg weight, egg production, age of sexual maturity and, as recently demonstrated, behaviour (Schütz *et al.* 2001, 2002; Schütz & Jensen 2001). A red junglefowl by White Leghorn backcross, established by others, has been widely used for chicken genome mapping but not for QTL mapping (Crittenden *et al.* 1993). Thus, although there are a number of previous QTL studies in the chicken (Dunnington *et al.* 1992; Plotsky *et al.* 1993; Vallejo *et al.* 1998; Van Kaam *et al.* 1999a, b; Yonash *et al.* 1999, 2001; Tatsuda & Fujinaka 2001a, b; Ikeobi *et al.* 2002; Sewalem *et al.* 2002) this is the first study testing for QTL differences between the red junglefowl and a domestic breed.

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In this paper we report a genome scan for QTLs affecting growth, body size and egg production based on data on more than 800 F_2 animals scored for more than 100 genetic markers.

Material and Methods

Animals

A three-generation resource pedigree was generated after mating one red junglefowl male with three White Leghorn females (Schütz *et al.* 2002). The red junglefowl male was obtained from a Swedish zoo, and originated from a relatively closed European zoo population, originally obtained from Thailand. The particular line of White Leghorn used in this cross (SLU13) has been developed at the Swedish University of Agricultural Sciences (Liljedahl *et al.* 1979). Four F_1 males and 37 F_1 females were intercrossed and 851 F_2 animals have been used for the QTL study. Animals were kept at the research facilities, Swedish University of Agricultural Sciences, Skara where all phenotype recordings were performed. The F_2 animals were raised in six batches comprising about 150 birds each.

Phenotypic traits

All F_2 birds were weighed at 1, 8, 46, 112 and 200 days to obtain growth rates. Average egg weight and total egg production were measured individually at 29 weeks of age by collecting eggs for 1 week.

DNA isolation, marker selection and genotyping

Blood samples were collected from all F_2 individuals, their parents (F_1) and grandparents (F_0). Seven microlitres of blood were used for DNA isolation using the DNeasy^{TM}96 Tissue Kit for mouse tails (Qiagen, Valencia, CA, USA) with some minor modifications.

A total of 189 previously described microsatellite markers were initially tested on a limited number of animals to select the most informative ones to be used in this study. A set of 105 markers was selected for the genome scan. The information content for each marker was calculated using the web based QTL Express software (Seaton *et al.* 2002; http://qtl.cap.edu.ac.uk/). Primer details for microsatellite markers can be found at http://poultry.mph.msu.edu or http://www.thearkdb.org/.

Polymerase chain reaction (PCR) amplifications of the microsatellite markers were carried out using fluorescently labelled primers. Polymerase chain reactions were performed in a total volume of 5 μl containing 1× PCR Buffer II (Applied Biosystems, Foster City, CA, USA), 2.5 mM $MgCl_2$, 200 μM of each dNTP, 1–5 pmol of each primer, 0.25 U of AmpliTaq Gold^{TM} DNA polymerase (Applied Biosystems) and 20–50 ng of genomic DNA. The PCR reaction was initiated with an incubation for 5 min at 95 °C to activate the polymerase, followed by a touchdown PCR cycle starting with annealing for 30 s at 65 °C and decreasing by 1 °C per cycle to 52 °C. Forty cycles of PCR were performed with annealing at 52 °C, denaturation for 45 s at 95 °C and extension for 30 s at 72 °C. The last cycle included an extension step for 5 min at 72 °C. The PCR products were denatured 2 min before electrophoresis in 4% polyacrylamide gels using an ABI377 sequencer (Applied Biosystems) or a MegaBACE capillary instrument (Amersham Biosciences, Uppsala, Sweden). The results were analysed with the Genescan and Genotyper software (Applied Biosystems) or Genetic Profiler (Amersham Biosciences).

Five additional loci were included in the genome scan. The dominant white locus (*I*) for plumage colour was scored as a single dominant trait, for which the White Leghorn (*I*/*I*) and the red junglefowl (*i*/*i*) are fixed for different alleles. The two populations are also fixed for alternate alleles at the melanocortin-1 receptor (*MC1R*) locus controlling black or wild type plumage colour; the presumed causative mutation was scored using pyrosequencing (Kerje *et al.* 2003). Highly informative PCR-RFLPs representing the loci for the melanocortin-3 receptor (*MC3R*; S. Jiang, S. Kerje & L. Andersson, unpublished data), melanocortin-4 receptor (*MC4R*; S. Jiang, S. Kerje & L. Andersson, unpublished data) and the KIT receptor (*KIT*; described below) were also used.

A 570 bp fragment from the *KIT* gene was amplified using the chKITfwd (5′-TTACATAGACCCAACGCAACT–3′) and chKITrev (5′-TAGTGCAAGCTCCAAGTAGAT– 3′) primers designed from the cDNA sequence in GenBank (D13225). The PCR contained 1× PCR buffer II (Applied Biosystems), 1.87 mM $MgCl_2$, 300 μM of each dNTP, 20 pmol of each primer, 1 U of AmpliTaq Gold^{TM} DNA polymerase (Applied Biosystems) and about 100 ng DNA in a total volume of 20 μl. The following PCR profile was used in a PTC-200 thermal cycler (MJ Research, Inc., Waltham, MA, USA), 5 min at 94 °C, 35 cycles with 45 s at 94 °C, 30 s at 53 °C, 1 min at 72 °C and finally 5 min at 72 °C. The PCR product was purified using the QIAquick® PCR Purification Kit (Qiagen) and sequenced from both ends with BigDye Terminator Cycle Sequencing chemistry (Applied Biosystems). A sequence comparison revealed a single nucleotide polymorphism, where the White Leghorn sequence had a *Taq*I recognition site, which was utilized for genotyping using a PCR-RFLP assay. For the restriction enzyme reaction, 15 μl of the PCR reaction (generated as described above) was digested with 1.5 U *Taq*I enzyme (New England BioLabs, Inc., Beverly, MA, USA) for 1 h at 65 °C in 1× *Taq*I buffer (New England BioLabs). The alleles were scored after electrophoresis in an 1.5% agarose gel (Nusieve:Seakem, 1:1).

Statistical analysis

Linkage maps for 25 autosomal linkage groups were generated using the CRI-MAP software (Green *et al.* 1990). The

functions FLIPS and FIXED were used to evaluate the order of markers along the chromosomes and to estimate the map distance between markers. The sex-specific recombination rates were estimated using CRI-MAP and the statistical evaluation was done with a likelihood ratio test (Ott 1985); this test statistic is expected to follow a χ^2 distribution with degrees of freedom equal to the number of marker intervals tested.

The software used for QTL mapping was developed for improved computational efficiency. This has been achieved by utilization of parallel computing, supercomputers and the application of new efficient numerical algorithms (Carlborg *et al.* 2001; Carlborg 2002; Ljungberg *et al.* 2003). The method used for QTL mapping is based on the ordinary least-squares based method for mapping QTL in outbred line crosses described by Haley *et al.* (1994). Marker genotypes were used to estimate the probabilities of breed origin of each gamete at 1 cM intervals throughout the genome for each F_2 individual. These probabilities were used to calculate additive and dominance coefficients for a putative QTL at each position under the assumption that the QTL was fixed for alternative alleles in the two breeds. The trait values were then regressed onto these coefficients in intervals of 1 cM. The additive and dominance regression indicator variables for the most significant single QTL in this scan were added as cofactors to the model used for the scan and a new genome scan was performed using the updated model. Adding the previously detected QTL to the model decreases the error variance, which will increase the power to detect QTLs with smaller genetic effects. This procedure was repeated until no additional significant QTL was detected.

The least-squares regression model used for QTL analysis included the fixed effects of sex and batch along with additive and dominance coefficients for the putative QTL for all traits. There was a highly significant correlation between body weight at 200 days and egg weight. Therefore, body weight at 200 days was included as a covariate in the QTL analysis to allow us to detect differences in egg weight at a fixed weight.

Statistical significance was assessed in each successive step in the QTL mapping procedure by randomization testing using 1000 permutations of data (Churchill & Doerge 1994). Genome-wide thresholds (1 and 5%) for significant QTLs and a 20% genome-wide significance threshold for suggestive QTLs were applied. Thus, we have used a more stringent threshold for suggestive QTLs compared with the commonly used threshold that is expected to give one false positive QTL per genome scan (Lander & Kruglyak 1995). The two randomization testing thresholds were fairly constant throughout the study.

Correlation coefficients and the proportion of residual variances explained by the detected QTLs were calculated using the SAS software (SAS 1990).

Results

Descriptive statistics

We analyzed body weights, growth and egg production of 767-814 F_2 chickens. Corresponding estimates were measured for the parental lines in the same environment and with the same feeding regime but not in parallel during the same time period. The body weight for the parental red junglefowl and White Leghorn populations and for the F_2 chickens were measured at 1, 8, 46, 112 and 200 days of age, and between these ages growth rates were calculated. The phenotypic means, standard errors of the means and standard deviations for weight at hatch, the growth traits and egg production traits are given in Table 1. The growth of the F_2 chickens was lower than the parental lines prior to 46 days of age, whereas it was intermediate to the two parental lines for growth after 46 days of age.

Linkage map

The linkage analysis was based on 105 loci, including 100 microsatellites, four SNPs and one phenotypic trait, representing 25 of 39 chromosomes in the chicken genome leaving 14 microchromosomes uncovered. The average information content for all markers was 0.77 (Table 2). The sex-averaged map spanned 2552 cM and the average marker spacing was 24.3 cM. The marker order

	Red junglefowl $n = 20$		White Leghorn $n = 31$		F_2 progeny $n = 767$–814	
Trait	Mean ± SEM	SD	Mean ± SEM	SD	Mean ± SEM	SD
Body weight 1 day (g)	26.5 ± 0.6	2.7	37.6 ± 0.9	4.8	36.9 ± 0.1	3.9
Growth 1–8 days (g)	38.5 ± 2.5	11.0	46.1 ± 1.6	9.0	10.4 ± 0.2	4.9
Growth 8–46 days (g)	316.4 ± 15.9	71.1	505.4 ± 12.2	67.7	269.6 ± 1.9	52.8
Growth 46–112 days (g)	414.6 ± 30.5	136.5	758.7 ± 43.6	242.8	607.9 ± 5.4	153.5
Growth 112–200 days (g)	147.3 ± 14.8	66.0	426.3 ± 18.5	102.8	353.8 ± 4.3	121.9
Egg weight (g)	23.0 ± 6.2	19.8	57.5 ± 3.8	15.2	43.2 ± 0.6	11.1
Total egg weight (g)[1]	97.3 ± 30.5	96.6	367.1 ± 27.4	109.6	221.9 ± 3.9	77.8

[1]Produced during 1 week.

Table 1 Weight at hatch, four growth rates and egg production measured in red junglefowl, White Leghorn and red junglefowl × White Leghorn F_2 chickens. Mean, standard errors of the mean (SEM) and standard deviations (SD) are provided.

Table 2 Genetic markers used for QTL mapping in a red junglefowl × White Leghorn intercross and information content (IC) for each marker. Distances are in Kosambi cM relative to the position of the first marker on each chromosome.

Marker	Chromosome/linkage group	Position sex average map	IC
ADL160	1	0	0.88
LEI209	1	27.7	0.71
MCW010	1	35.3	0.73
ADL019	1	91.3	0.87
LEI146	1	124.3	0.87
MCW018	1	154.2	0.91
LEI071	1	189.7	0.97
LEI101	1	209.3	0.89
MCW068	1	233	0.92
LEI088	1	258.8	0.83
LEI139	1	337.4	0.89
LEI107	1	372.3	0.91
LEI246	1	407.9	0.50
ADL328	1	425.9	0.87
LEI134	1	475.4	0.30
ADL228	2	0	0.87
MCW247	2	77.8	0.87
MCW063	2	125.6	0.77
ADL257	2	157.9	0.82
MCW062	2	168.4	0.89
MCW042	2	229.3	0.95
MC4R	2	242.6	0.89
MCW087	2	259.2	0.87
LEI147	2	280.3	0.85
MCW264	2	316.2	0.66
MCW166	2	335.5	0.94
LEI070	2	358.2	0.95
MCW176	2	362.9	0.78
MCW073	2	448.7	0.77
MCW157	2	467.6	0.83
MCW261	3	0	0.81
MCW169	3	30.3	0.40
HUJ006	3	101.9	0.80
LEI161	3	131.1	0.90
LEI115	3	161.8	0.94
ADL371	3	167.4	0.95
MCW126	3	233.9	0.66
LEI265	3	254.1	0.90
ADL237	3	273.4	0.87
ADL255	4	0	0.06
ADL145	4	70	0.93
MCW005	4	81.7	0.92
ADL266	4	113.2	0.92
LEI094	4	128.3	0.89
KIT	4	164.1	0.43
MCW122	4	183	0.78
LEI073	4	208.8	0.69
LEI082	5	0	0.83
MCW038	5	38.9	0.38
MCW029	5	82.5	0.92
MCW081	5	97.4	0.87
ADL323	6	0	0.86
ADL036	6	49.8	0.66
LEI097	6	71.1	0.93
MCW250	6	81.6	0.87
LEI192	6	117.2	0.93
ADL169	7	0	0.91
MCW236	7	34.9	0.80
MCW133	7	65.3	0.91
LEI064	7	165.3	0.52
ADL278	8	0	0.87
ADL154	8	60.8	0.88
ADL258	8	75.7	0.83
ADL191	9	0	0.63
MCW135	9	16.8	0.84
ADL136	9	42.9	0.39
MCW228	10	0	0.83
ADL209	10	27.5	0.47
ADL038	10	45.3	0.64
ADL158	10	99.9	0.53
LEI110	11	0	0.19
ADL210	11	47.6	0.93
ADL308	11	70.2	0.88
MC1R	11	93.2	0.91
ADL044	12	0	0.90
ADL372	12	66.1	0.51
MCW322	13	0	0.91
MCW213	13	26	0.85
ADL118	14	0	0.86
LEI098	14	38.1	0.86
MCW211	15	0	0.71
LEI120	15	52	0.90
ADL293	17	0	0.49
ADL290	18	0	0.84
ADL304	18	28.4	0.39
MCW256	19	0	0.55
MCW287	19	22.5	0.83
LEI090	23	0	0.96
MCW165	23	81.2	0.91
MCW069	26	0	0.91
MCW300	27	0	0.37
MCW328	27	27.3	0.89
ADL284	28	0	0.27
ADL299	28	34.8	0.90
I	E22C19W28	0	0.44
MCW317	E22C19W28	22.9	0.21
LEI080	E47W24	0	0.89
GCT004	E50C23	0	0.69
MC3R	UN		0.95
MCW055	Z	0	0.88
ADL273	Z	40.3	0.91
MCW241	Z	50.5	0.94
LEI229	Z	56.7	0.93
LEI121	Z	77.4	0.91
LEI075	Z	106.3	0.89

UN, unassigned.

Table 3 Summary statistics of the chicken linkage map based on a red junglefowl/White Leghorn intercross.

Chromosome/linkage group	Number of loci	Map length (Kosambi cM)			Sex differences χ^2_{df}
		Average	Female	Male	
1	15	475.4	471.5	489.4	35.9_{14}**
2	15	467.6	520.4	434.5	39.2_{14}***
3	9	273.4	269.9	277.7	4.0_{8}
4	8	138.8	154.2	125.6	43.6_{7}***
5	4	97.4	103.9	90.5	7.0_{3}
6	5	117.2	112.9	121.9	13.0_{4}*
7	4	165.3	163.9	168.0	8.9_{3}*
8	3	75.7	98.1	63.1	4.8_{2}
9	3	42.9	43.1	45.9	4.7_{2}
10	4	99.9	105.2	89.5	5.0_{3}
11	4	93.2	80.3	104.8	11.9_{3}**
12	2	66.1	100.0	52.8	3.1_{1}
13	2	26.0	31.3	21.3	8.2_{1}**
14	2	38.1	36.7	39.6	0.2_{1}
15	2	52.0	55.2	48.8	0.7_{1}
18	2	28.4	28.8	27.8	0_{1}
19	2	22.5	25.6	19.5	1.8_{1}
23	2	81.2	82.7	79.7	0_{1}
27	2	27.3	19.6	36.7	2.1_{1}
28	2	34.8	34.8	34.8	0_{1}
E22C19W28	2	22.9	22.9	0	0_{1}
Z	6	–	–	106.3	
Total	94	2446.1[1]	2561.0[1]	2371.9[1]	194.1_{73}***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

[1]Includes only autosomes.

corresponds very well with the chicken consensus map (Groenen *et al.* 2000; Schmid *et al.* 2000) but with one exception. *MCW176* is found on chromosome 6 in the consensus map but we mapped it to chromosome 2. This assignment was supported by highly significant lod scores to several markers (e.g. lod score = 51.3, recombination fraction = 0.05 against *LEI070*).

The intercross design and the large size of this pedigree allowed us to test for sex differences in recombination rates. We found significant sex differences for seven linkage groups (Table 3). However, there was no clear overall trend because the male map was longer in four cases and the female map in the other three. The total map length for autosomes was marginally longer (+8%) in females (2561 cM) than in males (2372 cM).

QTL analysis of growth and body weight

Nine measurements of body weight and growth were tested. We did not find any QTL for weight at hatch and this was not unexpected as this trait has a very strong maternal component. Among the other eight traits, 38 QTL tests were declared significant at least at the 20% suggestive level (Table 4); QTL graphs for the four major loci affecting adult body weight are shown in Fig. 1. These represented a minimum of 14 QTLs, designated *Growth1* to *Growth14*, when adopting a conservative interpretation of the number of QTLs. This means that we did not infer more than a single QTL for a given trait in a chromosome region unless the two estimated QTL positions differed by a considerable recombination distance, >30 cM. As many as 13 of these QTLs were significant at the 5% level for at least one growth or body weight trait. There was also a very clear trend that QTL alleles inherited from the red junglefowl were associated with a lower growth rate and smaller body size as expected from the difference between populations (Table 4). There were three exceptions to this rule. *Growth9* on chromosome 7 was significant for only one trait, body weight 112 days, and the red junglefowl allele was associated with slightly higher body weight but the major effect of this locus appears to be overdominance i.e. a superior growth of the heterozygote. *Growth10* on chromosome 8 affected early growth between day 1 and 8, and the red junglefowl allele increased the growth rate slightly. Finally, the red junglefowl allele at *Growth14* on the Z chromosome was associated with higher growth but the effect was restricted to female growth (data not shown). This may reflect a sex-specific effect of this QTL or a recessive inheritance of the low growth allele from White Leghorn as the F_2 males in this cross were Z^{rjf}/Z^{wl} or Z^{rjf}/Z^{rjf} whereas F_2

Table 4 Quantitative Trait Loci (QTL) for growth (GR), body weight (BW) and egg weight (EW) detected in a red junglefowl/White Leghorn intercross. Test statistics, estimated QTL effects, % of residual F_2 variance explained by each QTL and covariates used in the QTL analysis are given.

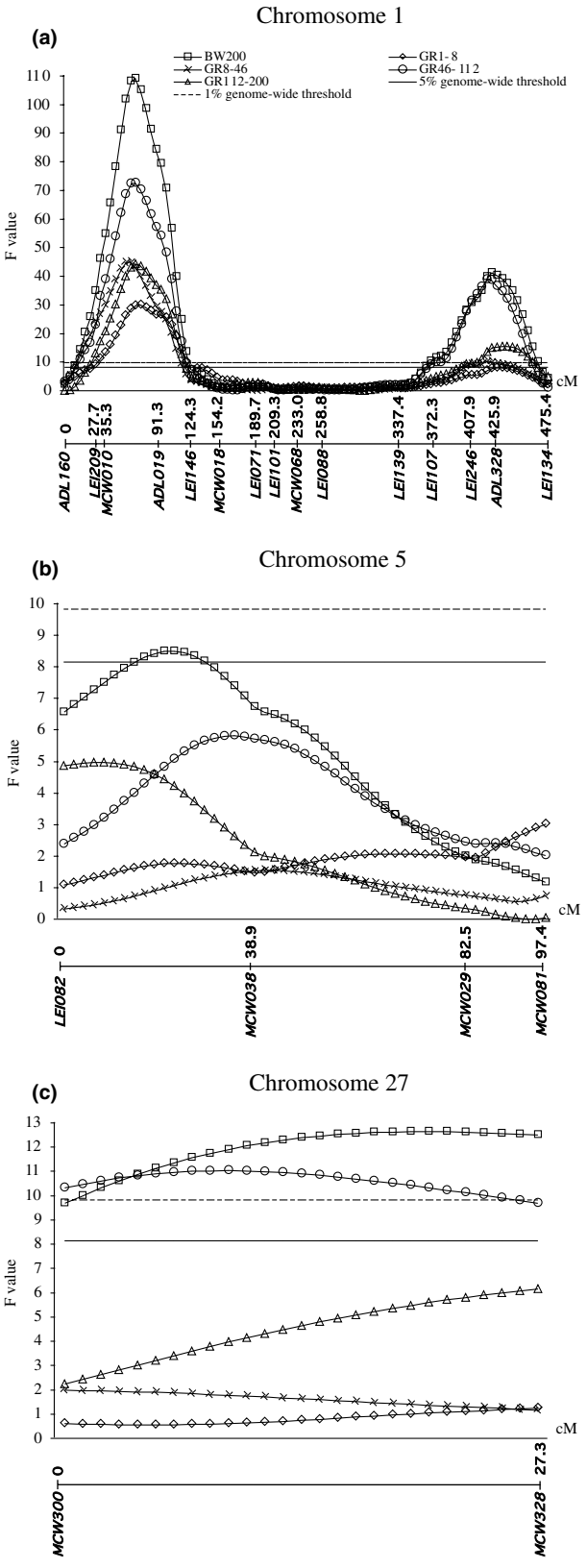
QTL	Chr.	Position, cM	Trait (g)	F-value[1]	Additive effect ± SE[2]	Dominance effect ± SE[2]	% variance[3]	Covariates
G1	1	71	BW8	22.4**	−2.6 ± 0.4	1.1 ± 0.8	5.5	
		64	BW46	50.0**	−35.9 ± 3.6	5.9 ± 7.9	11.1	
		67	BW112	81.3**	−114.7 ± 8.9	−1.0 ± 19.4	16.8	
		68	BW200	109.4**	−173.2 ± 11.6	9.6 ± 25.0	21.6	
		73	GR1-8	30.0**	−2.5 ± 0.3	0.3 ± 64.0	7.3	
		63	GR8-46	45.0**	−32.4 ± 3.4	7.7 ± 7.5	10.6	
		68	GR46-112	72.9**	−78.3 ± 6.4	−0.3 ± 13.9	15.4	
		70	GR112-200	44.2**	−59.7 ± 6.3	0.3 ± 13.3	10.0	
		58	Total EW	15.3**	−26.4 ± 5.6	41.5 ± 12.4	7.8	
		61	Aver. EW	57.8**	−5.1 ± 0.5	2.8 ± 1.1	24.1	
		59	Aver. EW	19.4**	−2.8 ± 0.4	1.6 ± 0.9	9.6	BW200
G2	1	399	BW8	6.6†	−1.1 ± 0.4	1.4 ± 0.7	1.7	
		419	BW46	14.8**	−14.8 ± 2.7	4.1 ± 4.2	3.6	G1
		418	BW112	39.7**	−60.9 ± 6.8	−1.3 ± 10.7	9.0	G1
		420	BW200	50.6**	−86.7 ± 8.6	7.9 ± 13.1	11.3	G1
		426	GR1-8	7.1†	−0.9 ± 0.2	−0.2 ± 0.3	1.9	
		417	GR8-46	11.1**	−12.4 ± 2.7	6.0 ± 4.3	2.8	G1
		416	GR46-112	44.4**	−47.6 ± 5.1	−4.4 ± 8.1	10.0	G1
		431	GR112-200	15.9**	−27.5 ± 4.9	6.1 ± 7.4	3.9	G1
G3	2	411	BW200	8.4*	−49.4 ± 13.2	71.9 ± 38.3	2.1	G1, G2, G6, G8, G13
G4	3	50	BW8	8.8*	−0.7 ± 0.5	−5.1 ± 1.3	2.3	G1
G5	3	117	BW46	6.7†	−8.3 ± 2.8	−12.2 ± 4.7	1.7	
		112	GR1-8	9.4*	−1.0 ± 0.3	−1.2 ± 0.5	2.4	G1, G10
E1	3	162	Aver. EW	7.7†	0.9 ± 0.2	0.5 ± 0.4	4.1	BW200
G6	3	208	BW112	7.1†	−29.7 ± 10.3	84.9 ± 29.0	1.7	
		201	BW200	8.3*	−37.9 ± 12.5	107.5 ± 33.6	2.1	G1, G2, G8, G13
G7	4	122	BW112	7.9†	−20.0 ± 5.9	22.6 ± 9.2	1.9	
		150	GR46-112	6.8†	−21.3 ± 5.7	4.8 ± 11.2	1.7	
		137	Total EW	7.2†	−17.3 ± 4.5	−1.2 ± 7.4	3.8	
G8	5	21	BW200	8.5*	−44.1 ± 10.6	13.1 ± 21.6	2.1	G1, G2, G13
G9	7	145	BW112	8.4*	28.8 ± 11.0	104.2 ± 31.6	2.1	G1, G2, G6, G13
G10	8	64	BW8	8.0*	0.8 ± 0.3	1.3 ± 0.4	2.1	G1, G4
		69	GR1-8	8.6*	0.9 ± 0.2	−0.8 ± 0.4	2.5	G1
G11	11	92	BW8	7.5†	−1.1 ± 0.3	0.2 ± 0.4	1.9	
		84	BW46	11.7**	−12.8 ± 2.6	3.5 ± 4.2	2.8	G1, G2
		60	GR8-46	9.8**	−10.8 ± 2.4	−2.6 ± 4.1	2.5	G1, G2
G12	12	59	BW46	6.5†	−11.9 ± 3.2	−2.9 ± 6.1	1.6	
		65	BW112	8.8*	−30.6 ± 7.1	−3.4 ± 11.9	2.2	G1, G2, G13
E2	14	14	Aver. EW	11.7**	1.6 ± 0.3	−0.1 ± 0.6	6.1	G1, E3, BW200
E3	23	72	Aver. EW	11.4**	−1.5 ± 0.3	1.4 ± 0.6	5.9	G1, BW200
G13	27	7	BW112	11.0**	−37.0 ± 7.8	−3.6 ± 14.3	2.7	G1, G2
		20	BW200	12.6**	−41.8 ± 8.3	−5.7 ± 13.6	3.1	G1, G2
		9	GR46-112	11.0**	−25.7 ± 5.6	−7.8 ± 10.2	2.7	G1, G2
G14	Z	22	BW200	9.3*	31.5 ± 7.2	−3.6 ± 7.1	2.3	G1, G2, G3, G6, G8, G13
		22	GR112-200	8.9*	18.3 ± 4.3	2.1 ± 4.3	2.2	G1, G2

[1]*F* statistic for the QTL at this genomic location and significance level; **F*-value above the empirical 5% genome-wide significance threshold, varying between 7.9 and 8.6 for different traits; ***F*-value above the empirical 1% genome-wide significance threshold, varying between 9.5 and 10.8 for different traits; †*F*-value above the empirical 20% genome-wide significance threshold at 6.5.

[2]The additive effect (a) and the dominance effect (d) were defined as deviation of animals homozygous for the red junglefowl allele or heterozygous, respectively, from the mean of the two homozygotes. Standard errors (SE) are also given.

[3]Percentage residual variance explained by the QTL.

G1–G14, *Growth1* to *Growth14*; E1–E3, *Eggweight1* to *Eggweight3*; Aver. EW, average weight of eggs produced during 1 week; Total EW, total egg weight during 1 week.



females had the genotype Z^{rjf}/W^{wl} or Z^{wl}/W^{wl} as a result of the design of this intercross (rjf = red junglefowl; wl = White Leghorn).

The two major QTLs for growth were both located on chromosome 1 at around positions 68 and 416 cM (Table 4 and Fig. 1a). *Growth1* (at 68 cM) did not affect weight at hatch but had a large effect on growth from the first week of age and during the entire growth period. This locus on its own explained more than 20% of the residual phenotypic variance for adult body weight and explained about 35% of the difference in adult size between the two populations. Does this very large QTL effect represent a single QTL or a cluster of linked QTLs in this part of the chromosome? To assess this important question we included *Growth1* as a cofactor with the estimated additive effect as given in Table 4. The QTL graph for this region became completely flat showing that recombination is not able to break apart this QTL peak (data not shown). Thus, we conclude that *Growth1* behaves as a single locus that may contain one or several linked causative genes.

The QTLs detected in this study explain a large proportion of the difference in adult body size between the two founder populations. We estimated the individual effects as well as the combined effects of the four major QTLs by including all four loci simultaneously in a least-square analysis (Table 5). The four major QTLs for this trait explain 31% of the residual variance in the F_2 generation and two thirds of the difference between populations in adult body weight (sex-average). An interesting finding was that these four QTLs all show a codominant inheritance as no dominance effect was observed. There is a marked sex-difference in growth in chickens so we also estimated the sex-specific effects of these QTLs. Although we observed the same trend in both sexes it is clear that these QTLs have a more pronounced effect on male growth. There was in fact a significant interaction between the action of *Growth1* and sex ($F_{1,796} = 22.7$, $P < 0.0001$), and between *Growth2* and sex ($F_{1,796} = 7.3$, $P < 0.007$) The four QTLs explained about 80% of the difference between the founder populations for male growth but only about 50% for female growth. The lack of dominance is less clear in the sex-specific estimates but these are also more uncertain as each estimate is based on only 50% of the material.

The QTL analysis has been carried out with a model assuming that the founder populations are fixed for different QTL alleles. The power of QTL detection is reduced and the estimated QTL effects are biased downwards if this assumption is not met. Therefore, we decided to investigate

Figure 1 Test statistic curves for the four major QTLs affecting adult body weight in a red junglefowl/White Leghorn intercross. (a) *Growth1* and *2* on chromosome 1. (b) *Growth8* on chromosome 5. (c) *Growth13* on chromosome 27. The graph represents the test for a single QTL at a given position along the chromosome and the marker map (with the distances between markers in Kosambi cM) is given on the X-axis. The horizontal line shows the 1 and 5% threshold for genome-wide significance.

Table 5 Estimated additive (a) and dominance (d) effects on adult body weight of four major growth Quantitative Trait Loci (QTLs) in comparison with body weight in the parental red junglefowl and White Leghorn populations.

	Sex-average		Males		Females	
Body weight in parentals (in grams)						
Red junglefowl	960		1120		800	
White Leghorn	1870		2110		1630	
Difference	−910		−990		−830	
Effects of QTLs (in grams)						
Locus	2a[1]	d	2a[1]	d	2a[1]	d
Growth1	−306	5	−400	−22	−198	45
Growth2	−166	11	−210	6	−128	6
Growth13	−92	6	−98	43	−74	−29
Growth8	−54	−1	−76	20	−24	−42
Sum	−618		−784		−424	
Percentage residual variance	31.0		38.6		17.1	
Percentage population difference	67.9		79.2		51.1	

[1]The additive effect represents by definition half the estimated phenotypic difference between the two homozygotes and therefore we provide here the estimate for 2a.

this assumption for the four major QTLs for adult body weight by a heterogeneity test among the four large F_1 half-sib families present in this material. There was no significant heterogeneity for *Growth1*, *Growth8* or *Growth13*, but there was a highly significant heterogeneity for *Growth2* ($F_{3,785} = 5.74$, $P = 0.0007$). The results showed that the estimated additive effect of *Growth2* was only -31.6 ± 15.8 for sire 1008 whereas the corresponding estimates for the other three sires were in the range -94.5 ± 20.0 to -119.5 ± 18.1. Thus, sire 1008 may be homozygous at *Growth2* but heterozygous for a linked minor QTL or there may be three alleles segregating at this QTL. The information about this heterogeneity among sires is very important for future attempts to identify the causative gene(s) for this major QTL.

QTL analysis of egg production

The average egg weight showed a strong positive correlation with adult body weight ($r = 0.62$, $P < 0.0001$). The following linear regression between average egg weight (EW in grams) and body weight at 200 days (BW200 in grams) were estimated in the F_2 population: EW = 21.9 + (0.02 × BW200). This means that the larger adult body size in White Leghorn females (+∼800 g) should explain about 50% of the difference in average egg weight between the two populations (Table 1).

As shown in Table 4, the *Growth1* QTL has a huge effect also on the average egg weight. About half of the effect can be explained due to the effect on adult body size but the QTL analysis including body weight as a covariate shows that *Growth1* also has a direct effect on the size of the eggs. The additive effect of this QTL explains about 30% of the difference in average egg weight between the two populations.

Three additional QTLs for average egg weight were detected using a model including body weight as a covariate. These are located on chromosomes 3, 11 and 14, and they were designated *Eggweight1–3* as they were not colocalized with any growth QTL (Table 4). Two of these QTLs showed the expected trend of an association between the red junglefowl allele and smaller eggs whereas *Eggweight2* showed the opposite effect.

Only two QTLs for total egg weight during 1 week were detected and they were both colocalized with two growth QTLs, *Growth1* and *7*. The QTL effect on total egg weight disappeared when body weight was included in the model.

Discussion

A common problem in genetic studies of multifactorial traits is a low statistical power, caused by the combination of limited sample sizes and the rather small effect of each locus. The consequence of this is that reproducibility is poor and the estimated effects of detected QTLs are uncertain and often inflated (Mackinnon & Georges 1992; Goring *et al.* 2001). We generated a large F_2 generation of more than 800 progeny in an attempt to obtain a high statistical power for QTL detection. The results imply that we in fact have achieved this for growth, in particular late growth, because many QTLs segregating in this cross appear to have a sufficiently large effect to be detected in a QTL experiment of this size. This is evident from the fact that 13 of 14 QTLs that were significant at the 20% genome-wide level also were significant at the 5% level, and that the QTLs explain a large part of the difference in adult body weight between the parental populations. This high statistical power in QTL detection allows us to get some insight into the genetic background of growth and to the effects of the QTLs. The classical infinitesimal model for inheritance of multifactorial trait involves an infinite number of loci each with an infinitesimal small effect (Lynch & Walsh 1998). This is obviously an unrealistic theoretical model that has been useful for the development of quantitative genetics theory and its practical application. The ancestor of the domesticated

White Leghorn diverged from the red junglefowl thousands of years ago and our results demonstrate that the twofold difference in adult body weight between these populations is largely explained by a limited number of QTLs with large and moderate effects. We can refute the possibility that the difference is explained by hundreds of QTL each with a very small effect. The QTLs detected in this study do not explain the entire difference in growth but it should be noted that our genome scan is not complete as we are lacking markers on several microchromosomes and there are also some regions on macrochromosomes with poor coverage that may harbour additional QTLs with large or moderate effects. Furthermore, in another paper based on the same material we report that epistasis between QTLs plays a significant role for early growth (Carlborg *et al.* 2003).

An interesting observation was that several of the major QTLs show large additive effects but no significant dominance effects, which means that the heterozygotes have an intermediate phenotype. This is in contrast to the great majority of trait loci with a monogenic inheritance that so far have been studied at the molecular level. A search of the Mouse Genome Informatics database (http://www.informatics.jax.org/; June 2002) with the inheritance mode 'dominant', 'recessive', and 'codominant' gave 367, 1508 and 22 hits, respectively. The codominant hits reflected 12 loci and ten of these were in fact QTLs. Since the early history of genetics there has been much debate on the genetic and physiological basis for dominance (Lynch & Walsh 1998). Kacser & Burns (1981) provided an elegant molecular explanation for dominance based on the flux in a biochemical pathway composed of many interacting enzymes. They showed that dominance is expected as a reduction to 50% activity of an individual enzyme in a loss-of-function heterozygote will often have a negligible effect on the total flux in the system and thus on the phenotype. Dominance may also occur because of dominant negative mutations, inactivating a certain biochemical function, or gain-of-function mutations, such as a mutation that leads to constitutive activation of a strictly regulated molecule. So, for which type of genes and mutations is the heterozygote expected to give an intermediate phenotype? The Kacser & Burns theory also predicts that alleles with small differences in enzyme activity are likely to give intermediate heterozygotes because of the hyperbolic relationship between enzyme activity and flux. Genes encoding molecules that are rate-limiting in a biochemical pathway are also expected to be associated with intermediate heterozygotes. Thus the molecular characterization of some of the major codominant QTLs detected in this study is of major general interest.

The rather low early growth (up to 46 days of age) of the F_2 chickens was unexpected and has no obvious explanation. It could have a biological basis and represent a mild form of hybrid dysgenesis. It is well known that a reduced fitness may be observed in the F_2 generation of wide crosses and it has been attributed to possible epistatic interactions (Falconer 1981). Interestingly, this possible explanation is in fact supported by our study of epistasis in the same cross as we observed a considerable amount of epistasis for early growth but not for late growth (Carlborg *et al.* 2003). Another possibility is that the single outbred, red junglefowl founder male was not representative of the red junglefowl population as regards early growth. Furthermore, we cannot exclude that the low early growth was caused by an unknown environmental factor as the growth of the F_2 and parental populations were measured under the same environmental conditions but not in the same time period for practical reasons.

There is some overlap between the QTL positions detected in the present study and those detected in previous studies. Van Kaam *et al.* (1999a, b) performed a genome scan for growth and carcass composition using a cross between two broiler lines. Only one QTL reached genome-wise significance. This was a growth QTL located at chromosome 1 at 235 cM (Van Kaam *et al.* 1999a), thus far away from the two growth QTLs detected at chromosome 1 in the present study. However, a suggestive QTL affecting carcass percentage was detected in the vicinity of our *Growth2* QTL on chromosome 1 (Van Kaam *et al.* 1999b). Tatsuda & Fujinaka (2001a, b) identified three highly significant QTLs affecting body weight or fat deposition using an intercross between a Japanese native breed (Satsumadori) and White Plymouth Rock broilers but none of these overlapped with the QTL regions identified in our study. There is more overlap between the results of our QTL study and a recently published QTL study involving an intercross between a White Leghorn line and a commercial broiler sire line (Sewalem *et al.* 2002). Our *Growth1* on chromosome 1 maps to approximately the same region as a suggestive QTL for body weight at 9 weeks in the Leghorn × broiler intercross. However, the small effect excludes the possibility that this locus reflects the segregation of the same alleles at *Growth1* as detected in this study. Furthermore, our *Growth2, 7, 9, 10* and *13* on chromosomes 1, 4, 7, 8 and 27, respectively, maps to approximately the same region as QTLs for body weight at 9 weeks in the Broiler intercross. However, the poor precision in map positions in both studies excludes any firm conclusions about the possible identity of segregating QTLs in the two studies.

The major QTL for growth located around position 68 cM on chromosome 1 explains a large proportion of the difference in adult body size as well as in the size of eggs between the two founder populations in this study. In our previous study we observed that this chromosomal region also shows a highly significant effect on one behavioural trait, tonic immobility considered as a measure of the fear response (Schütz *et al.* 2002); the White Leghorn allele (associated with faster growth and larger eggs) was associated with a longer period of tonic immobility. Future studies will show whether the colocalization of QTLs for growth and

behaviour is a coincidence or because of a single pleiotropic QTL. It is obvious that *Growth1* must have been one of the major loci responding to selection for growth and/or improved egg production in the domestic chicken. It is an open question whether the favourable QTL allele was selected in modern time (during the 20th century) or early during the domestication of chickens. The fact that Sewalem *et al.* (2002) did not observe the segregation of a major QTL in this region in their Leghorn/broiler intercross suggests that the divergence of the *Growth1* alleles predates the development of specialized layer and broiler lines during the last century. A molecular characterization of this QTL will make it possible to trace its evolutionary history.

For most QTLs reported in this study any obvious positional candidate genes using the current, rather sparse, chicken genetic map (Schmid *et al.* 2000) were not identified. However, *Growth13* maps to the same region on chromosome 27 as the growth hormone (*GH*) gene and *Growth14* maps to the same region on the Z chromosome as the growth hormone receptor (*GHR*) gene and the prolactin receptor (*PRLR*). It has been previously reported that mutations in *GHR* cause sex-linked dwarfism in the chicken (Burnside *et al.* 1991) and *GHR* is thus an interesting positional candidate gene for this growth QTL.

Haldane's (1926) prediction of a higher recombination rate in the homogametic sex is supported by empirical data in various species. Accordingly, there is a general trend towards a higher female recombination rate in mammals. In pigs there is on average 40% excess of female recombination (Marklund *et al.* 1996; Bidanel *et al.* 2001) and the corresponding female excess in humans is about 70% (Morton 1991). Chicken appears to be an exception to this rule and shows no clear overall trend as regards sex differences. We observed an 8% higher recombination rate in the heterogametic sex (females) whereas Groenen *et al.* (1998) reported a very weak trend (+1%) in the opposite direction. This study shows that there exist highly significant sex differences in the recombination rate in certain chromosome regions but the direction varies from region to region.

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