Overlap of quantitative trait loci for early growth rate, and for body weight and age at onset of sexual maturity in chickens

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Abstract

Critical age, weight and body composition have been suggested as necessary correlates of sexual maturity. A genome scan to identify quantitative trait loci (QTL) for age and body weight at first egg (AFE and WFE) was conducted on 912 birds from an F₂ broiler–layer cross using 106 microsatellite markers. Without a covariate, QTL for body WFE were detected on chromosomes 2, 4, 8, 27 and Z and a single QTL for AFE was detected on chromosome 2. With AFE as a covariate, additional QTL for body WFE were found on chromosomes 1 and 13, with abdominal fat pad as covariate a QTL for body WFE was found on chromosome 1. With body WFE as covariate, additional QTL for AFE were found on chromosomes 1, 3, 4, 13 and 27. The QTL generally acted additively and there was no evidence for epistasis. Consistent with the original line differences, broiler alleles had positive effects on body WFE and negative effects on AFE, whereas the phenotypic correlation between the two traits was positive. The mapped QTL for body WFE cumulatively accounted for almost half the body weight difference between the chicken lines at puberty. Overlapping QTL for body WFE and body weight to 9 weeks of age indicate that most QTL affecting growth rate also affect body WFE. The co-localisation of QTL for body weight, growth and sexual maturity suggests that body weight and growth rate are closely related to the attainment of sexual maturity and that the genetic determination of growth rate has correlated effects on puberty.

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Introduction

Understanding the genetic mechanism between growth rate and the onset of puberty is of significant biological and agricultural interest. Identification of quantitative trait loci (QTL) and their related genetic relationships is important in understanding the genetic factors controlling reproductive traits associated with sexual maturity, such as age and weight at puberty. Several studies across species have shown that the attainment of puberty (sexual maturity) is dependent on a number of factors such as age, minimum weight and body composition (Frisch 1994, Yannakopoulos *et al.* 1995, Eitan & Soller 2001).

In humans, the attainment of a critical body weight of 48 kg and a fat percentage of 22% for the onset of puberty in girls was proposed in the early 1970s (Frisch & Revelle 1970). Recent interest in this issue has been reignited by the observed early puberty in girls associated with increasing levels of obesity (Kaplowitz 2008, Aksglaede *et al.* 2009). Similarly, agricultural species that have been intensely selected for early growth, such as broiler chickens, have also become heavier and higher in body fat content, with negative

effects on their reproductive performance as adults (Hocking *et al.* 2002, Brewer & Balen 2010). Early maturity has also been associated with reproductive problems such as abnormal ovarian hierarchies in chickens (Lacassag & Jacquet 1965, Hocking *et al.* 1987, Hocking 2004), and understanding the genetic mechanisms influencing these conditions could shed light on reproductive dysfunction in other species (Onagbesan *et al.* 2009).

The existence of a threshold level of weight or fatness that is critical for menarche has been disputed (Garn *et al.* 1983). The hypothesis that puberty depends on a critical amount of body fat has been rejected repeatedly by experimentalists (Bronson 2001). The linkage between body fat and the reproductive axis in girls is thought to be the result of an evolutionary mechanism in mammals for ensuring that pregnancy will not occur unless there are adequate fat stores to sustain both the mother and the growing foetus (Kaplowitz 2008). According to Kaplowitz (2008), published evidence suggests that obesity may be causally related to earlier puberty in girls. Rodent and human studies suggest that leptin is the critical link between body fat and early puberty but the question of whether earlier puberty is

the cause or the result of increased body fat has not been resolved (Kaplowitz 2008).

An alternative view based on chicken studies is that the fat deposition is a result of processes associated with steroidogenesis driven by the development of the ovary (Hocking & Robertson 2000). Some authors have suggested that there is a minimum fat requirement and that body weight is not a limiting factor for achievement of sexual maturity (Robinson *et al.* 2001) whereas Soller *et al.* (1984) showed that a critical body fat content was not required for the attainment of puberty. While some data point to a possible minimum fat requirement to attain sexual maturity, a cautious approach is needed to identify the actual mechanisms involved (Chen *et al.* 2007) and that there is possible distortion due to effects of selection (Reddish *et al.* 2003).

The chicken (*Gallus gallus domesticus*) is a model organism used in genetic studies with implications for agriculture and biology (Griffin & Goddard 1994, Burt 2007). The relative ease of using chickens to generate DNA-based genetic data and the similarities in the sexual maturity phenomena across species makes the chicken a relevant model to gain more understanding of the genetic relationship between growth rate and the onset of sexual maturity across species. Interactions of several loci have been reported to influence early growth in chickens, for example (Carlborg *et al.* 2003), but genetic mechanisms involved in the interplay of other factors impacted by early growth remain to be elucidated.

This study adopted a QTL approach to address four issues. First, to investigate the relationship between growth rate and sexual maturity by identifying the QTL for age at first egg (AFE) and weight at first egg (WFE) in the Roslin broiler-layer cross in which the White Leghorn layer was much later maturing than the male broiler line. Secondly, to identify the relative importance of age and body weight for the attainment of puberty in chickens. Thirdly, reproductive traits are known to exhibit high heterosis (Williams *et al.* 2002), which may be explained partly by epistasis, the non-additive interaction of genes with one another (Williams *et al.* 2002, Carlborg *et al.* 2004, Melchinger *et al.* 2007). For this reason, the effects of epistasis on AFE and WFE were investigated using recently developed software (Wei *et al.* 2009). Finally, we investigated the effect of adiposity on puberty by using abdominal fat weight (ABF) as a covariate due to its high correlation with WFE and total body fat.

Results

Broiler and layer phenotypes

The mean AFE and WFE of the broiler male line and the White Leghorn layer line are presented in Table 1. At first egg, the broiler line was heavier (5.4 vs 1.5 kg) and earlier maturing than the layer (130 vs 177 days).

Table 1 Means and s.d.s for age, abdominal fat and weight at the onset of lay in male line broiler and White Leghorn layer chicken females.

Trait	Male line broiler (n=10)		White Leghorn layer (n=12)	
	Mean	s.d.	Mean	s.d.
Weight at first egg, WFE (kg)	5.4	0.6	1.5	0.1
Age at first egg, AFE (days)	130	8	177	10
Abdominal fat, ABF (g)	255	71	44	9

F₂ phenotypes

Trait means, s.d.s, ranges and phenotypic correlations between the phenotypic traits in the F₂ population are given in Table 2. AFE for the F₂ population ranged from 99 to 226 days. The mean AFE for the broiler male line stock and F₂ were similar (130 and 134 days respectively) and lower than that of the White Leghorn layer line (177 days). The Pearson correlation between WFE and the natural logarithm of AFE minus 94 days (lnAFE94) in the F₂ population was low (0.31; Table 2). The transformation of AFE was done to normalise residual errors.

Location of QTL and genetic effects

Without lnAFE94 as a covariate, significant effects were detected on chromosomes 2, 4, 8, 27 and Z (Table 3) for WFE. Two peaks of significance were found on the Z chromosome and their confidence intervals (CI) barely overlap. This suggests that two QTL affecting WFE may be located in this chromosome. When including lnABF as a covariate in the analysis of WFE, an additional QTL on chromosome 1 became significant but evidence was lost for the Z chromosome QTL. Including lnAFE94 as a covariate for WFE detected a further three QTL on chromosomes 3, 13 and 28, with only one of the original Z chromosome QTL being significant. The mapped QTL varied widely in their effects explaining from 1.0 to 9.5% of trait variance in WFE, with half of the QTL

Table 2 Number of records (N), means, s.d.s, range and phenotypic correlations for age, weight, natural logarithm of age-94 days and abdominal fat at the onset of lay in an F₂ broiler-layer chicken population.

Variable	N	Mean	s.d.	Min	Max	Correlation	
						AFE	WFE
Weight at first egg, WFE (kg)	912	2.9	0.4	1.4	4.2	0.32	–
Age at first egg, AFE (days)	912	134.2	21.1	99	226.0	–	0.32
ln(AFE94), ln(days)	912	3.56	0.54	1.61	4.88	–	0.31
Abdominal fat, ABF (g)	455	134.5	48.0	17.4	409.2	0.27	0.71
lnABF, ln(g)	455	4.84	0.37	2.86	6.01	0.18	0.69

Table 3 Chromosome, *F* ratio, quantitative trait loci (QTL) position, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation explained for weight at first egg (WFE) with and without adjustment for covariates in a broiler-layer F_2 chicken population.

Chrom	<i>F</i> ratio ^a	Position (cM)	CI	Flanking markers	Additive effect (S.E.M.)	Dominance effect (S.E.M.)	VP (%)
WFE (with no covariate) (g)							
2	10.29 [†]	326	246–422	ADL0114–MCW0056	85 (18.6)	2 (31.2)	1.9
4	36.40 [†]	157	144–184	ADL0241–MCW0180	381 (45.1)	170 (172.3)	7.2
8	10.10 [†]	60	0–74	MCW0100–ROS0075	110 (25.0)	38 (48.2)	1.9
27	19.00 [†]	0	0	ROS0071	107 (17.3)	2 (25.1)	3.7
Z	9.23*	103	63–127	LEI0111–LEI0075	96 (31.6)	–	1.0
Z	10.64 [†]	0	0–65	ROS0072–ADLO201	82 (25.0)	–	1.0
Total					861	212	16.7
WFE (lnABF fitted as covariate) (g)							
1	11.25 [†]	134	118–494	LEI0068–LEI146	89 (18.2)	12 (27.7)	4.1
2	10.17*	325	246–340	ADL0114–MCW0056	82 (17.9)	52 (31.1)	4.1
4	15.79 [†]	175	147–195	ADL0241–MCW0180	201 (33.8)	–39 (93.1)	6.3
8	7.87*	23	0–63	ADL0179–MCW0095	71 (18.5)	–6 (26.1)	2.4
27	23.30 [†]	0	0	ROS0071	124 (17.0)	–6.8 (26.0)	9.5
Total					567	21.2	26.4
WFE (lnAFE94 fitted as covariate) (g)							
1	8.78*	141	90–501	LEI0068–LEI0146	64 (16.5)	42 (24.3)	1.5
2	16.65 [†]	307	285–333	ROS0074–ADL0114	95 (17.2)	–50 (29.7)	3.0
3	7.42 [†]	136	11–250	MCW0127–LEI0118	43.8 (16.1)	63 (22.4)	1.2
4	45.05 [†]	155	144–172	ADL00241–MCW0180	401 (41.8)	–94 (164.7)	8.3
8	9.93 [†]	60	12–78	MCW0100–ROS0075	100 (22.6)	39 (43.4)	1.7
13	7.43 [†]	54	26–68	MCW0340–ADL0225	101 (27.3)	–85 (57.9)	1.2
27	31.82 [†]	0	0	ROS0071	125 (15.5)	8 (22.6)	5.8
28	6.98 [†]	1	0–39	ROS0095–ROS0085	–62 (16.3)	–1 (22.7)	1.1
Z	14.65 [†]	17	0–113	ROS0072–ADL0201	116 (30.2)	–	1.6
Total					983.8	–78	25.4

Chrom, chromosome; CI, 95% confidence interval; VP%, percentage of phenotypic variation explained by the QTL; lnABF, log_e (abdominal fat weight); lnAFE94, log_e (age at first egg minus 94 days).

^aSignificant at 0.05 (*) and 0.01 (†) levels experiment-wide, and (‡) suggestive.

explaining 2.4% or more of the trait variance. QTL on chromosomes 4 and 27 accounted for the highest proportion of phenotypic variation (8.3 and 5.8% respectively) when including lnAFE94 in the analysis. Without WFE as a covariate, only one significant QTL for lnAFE94 was detected on chromosome 2 with suggestive QTL on chromosomes 1, 3, 13 and 15. With WFE as a covariate, the evidence for a QTL became significant in similar regions of chromosomes 1 and 13. In addition, QTL were found on chromosomes 4, 27 and a different region of chromosome 3. There was also evidence for two QTL on both chromosomes 3 and 4 (Table 4). The QTL explained relatively small proportions of 1.0–3.1% of the phenotypic variation for lnAFE94; only two of the QTL had effects explaining 2.0% or more of trait variation.

A QTL was detected for lnAFE94 and for WFE in similar regions on chromosome 2, indicating that the same QTL may influence both traits. With no covariate in the analysis, this was the only QTL found to affect both WFE and AFE. With the alternative trait as covariate in the analysis, however, there was extensive commonality with QTL significant for the two traits on chromosomes 1, 2, 3, 4, 13 and 27. The effects of including a covariate in the analysis will be considered in detail in the 'Discussion' section.

The additive effects for the WFE QTL were all positive, indicating that the increasing allele was from the broiler line (Table 3), except for the suggestive QTL on chromosome 28, which had a negative estimate. Dominance effects were not significant indicating that dominance was not an important mode of gene action for this trait. The largest (401 g) and the smallest (64 g) additive effects observed for significant WFE QTL were respectively on chromosomes 4 and 1 when lnAFE94 was fitted as a covariate. The mapped QTL accounted for almost half of the difference in trait value between the two parental lines, and 25% of F_2 trait variance. Inclusion of lnAFE94 as a covariate in the analysis for this trait increased the number of significant QTL and slightly increased the magnitude of the effects for each QTL. Fitting lnABF as a covariate had less effect on the results.

The results for the additive and dominance effects for lnAFE94 QTL are presented in Table 4. Here, too, the dominance effects were not significant. Similar to WFE, the additive gene action was also important for lnAFE94. The QTL additive effects for lnAFE94 were small and mainly negative. The mapped QTL accounted for 14% of F_2 trait variance, but only a small proportion of the difference between the two parental lines. There was no support for epistatic interactions between QTL for either trait.

Table 4 Chromosome, *F* ratio, quantitative trait loci (QTL) position, confidence interval, flanking markers, additive and dominance effects and proportion of phenotypic variation explained for the natural logarithm transformed age at first egg (lnAFE94) in a broiler–layer F₂ chicken population.

Chr	<i>F</i> ratio ^a	Position (cM)	CI	Flanking markers	Additive effect ± S.E.M.	Dominance effect ± S.E.M.	VP (%)
lnAFE94 (with no covariate fitted), ln(days)							
1	5.70 [‡]	164	0–380	LEI0146–ADL0319	–0.07 (0.022)	0.06 (0.042)	1.1
2	8.43*	291	115–358	ROS0023–ADL0236	–0.06 (0.017)	0.04 (0.025)	1.7
3	5.50 [‡]	24	16–185	MCW0083–HUJ0006	–0.07 (0.021)	0.01 (0.035)	1.0
13	7.00 [‡]	0	0–44	MCW0340–ADL0225	–0.07 (0.018)	0.02 (0.027)	1.4
15	5.47 [‡]	41	11–49	LEI0083–MCW0080	–0.08 (0.025)	0.02 (0.052)	1.5
Total					–0.35	0.15	6.7
lnAFE94 (with WFE fitted as a covariate), ln(days)							
1	9.0 [‡]	153	30–386	LEI0146–ADL0319	–0.08 (0.019)	0.01 (0.032)	1.7
2	15.4 [‡]	291	229–298	ROS0023–ADL0236	–0.08 (0.015)	0.05 (0.023)	3.1
3	8.2*	139	5–230	MCW0127–LEI0118	–0.06 (0.017)	–0.05 (0.024)	1.5
3	7.7 [‡]	23	9–250	MCW0083–HUJ0006	–0.07 (0.019)	–0.03 (0.031)	1.4
4	8.1*	195	65–195	ADL0241–MCW0180	–0.08 (0.021)	–0.06 (0.037)	1.5
4	6.7 [‡]	3	0–194	ADL0317–MCW0295	–0.07 (0.018)	0.01 (0.028)	1.2
13	8.6*	20	0–46	MCW0340–ADL0225	–0.11 (0.026)	–0.01 (0.057)	1.6
27	10.1 [‡]	0	–	ROS0071	–0.06 (0.016)	–0.04 (0.023)	2.0
Total					–0.61	–0.12	14

Chr, chromosome; CI, 95% confidence interval; VP%, percentage of phenotypic variation explained by the QTL; lnAFE94, log_e (age at first egg minus 94 days); WFE, weight at first egg.

^aSignificant at 0.05 (*) and 0.01 (†) levels experiment-wide, and (‡) suggestive.

Discussion

Effect of including a covariate in the QTL analysis

Including a genetically controlled and phenotypically correlated trait as a covariate in the analysis will affect the evidence for a QTL at a specific location, depending on the direction and magnitude of QTL effects on the two traits (Goddard *et al.* 2001, Neuschl *et al.* 2007, Chiu *et al.* 2010). If the QTL affects the trait, but not the covariate, inclusion of the covariate will increase the evidence for the QTL. This may have happened for the QTL for WFE on chromosome 27, when lnAFE was fitted as covariate. If the QTL affects the covariate but not the trait, inclusion of the covariate will identify a QTL for the covariate rather than a QTL for the trait under investigation. The QTL on chromosomes 4 and 27 originally detected for WFE became significant for lnAFE94 when WFE was included as a covariate, suggesting that these QTL affected WFE rather than lnAFE94. If the QTL affects both the trait and the covariate (i.e. a pleiotropic QTL), the ability to detect it will depend on the QTL and the phenotypic correlations (i.e. the evidence may be lost or enhanced). In this study, the traits in the F₂ were positively correlated phenotypically (i.e. individuals with late AFE tended to have high WFE, since these birds had more time to gain weight before first egg). QTL operating in the same direction in both traits, consistent with the phenotypic correlation, will generally lose evidence when one of the traits is fitted as a covariate in the analysis of the other. Apparently, this is what happened to the QTL on chromosomes 4, 8 and Z when lnABF was fitted as a covariate to WFE (Table 3). The original breed difference,

however, implies that birds with later AFE have low WFE and vice versa. This will tend to enhance the evidence for such a pleiotropic QTL and increase the effect estimates when fitting one of the traits as a covariate in the analysis of the other. The QTL identified for both WFE and lnAFE94 on chromosome 2 could be such a case, as significance for both traits increased when the alternative trait was used as a covariate. The QTL on chromosomes 1 and 13 detected for lnAFE94 became significant when WFE was included as a covariate, and were also identified for WFE when lnAFE94 was included as a covariate. These could be additional examples of pleiotropic QTL, with the QTL affecting both WFE and lnAFE94. Finally, the QTL on chromosome 3 in interval MCW0127–LEI0118 may be another example as it was detected for both traits but only in the presence of the other as a covariate. In each of these instances, as an alternative to pleiotropy, the co-location of QTL affecting each of the two traits could be due to separate tightly clustered loci that individually influence a single trait (Almasy *et al.* 1997). A better way to detect pleiotropic QTL would be to analyse the traits simultaneously (Knott & Haley 2000).

Importance of the identified QTL

The sum of the additive effects for significant WFE QTL was 0.86 kg accounting for 1.7 kg additive effects difference between the lines. This represents 44% of the live weight difference (3.9 kg) between the lines at the onset of lay (Table 1). For all of these QTL, the allele increasing WFE was inherited from the broiler line, which is consistent with the breed difference (Table 1).

The sum of the additive effects for InAFE94 (adjusted for WFE) was ~12 days. This represents an additive effect difference of 24 days or about half the phenotypic difference between the lines (Table 1). In comparison to this analysis, when the covariate was not fitted the sum of the additive effects was only 3.4 days, explaining <15% of the line difference. This is consistent with the conclusion that QTL for weight rather than age explain far more of the observed difference in age at onset of puberty between the lines.

Additive effects for InAFE94 were generally negative (Table 4) indicating that the broiler line was earlier maturing than the layer genotype (consistent with the breed differences, Table 1) and is consistent with the expectation that fast growing (heavy) birds tend to reach sexual maturity earlier. However, it should be noted that the layer line was unusually late maturing (Table 1), possibly due to the lack of photostimulation in this experiment. A constant photoperiod was adopted to avoid confounding differences in maturity with the timing of photostimulation.

QTL affecting early growth rate affect WFE

An earlier study on broiler offspring of the same parents as the present data (Sewalem *et al.* 2002) reported significant QTL for live weight at earlier ages (3–9 weeks of age) than in this study on chromosomes 1, 7, 13 and Z (for 3 weeks of age), chromosomes 1, 2, 4, 7, 8, 13 (for 6 weeks of age) and on chromosomes 1, 2, 4, 8, 13 and 27 (for 9 weeks of age). In that study, covariates were not fitted in the analysis. Even though the earlier study used much younger ages (3–9 weeks) than in our study (19 weeks of age), the QTL detected for live weight (in the earlier study) and for WFE (without a covariate) in our study were at similar positions across the implicated linkage groups.

Similar results were obtained when comparing our results on WFE with those of other studies on early growth rate. The highly significant QTL for body weight on chromosome 4 detected in this study confirm findings from several studies that have reported large effect body weight QTL on this chromosome (Schreiweis *et al.* 2006). Zhou *et al.* (2006) reported significant QTL for body weight at 8 weeks of age in a broiler–White Leghorn cross on chromosomes 1, 2, 4, 7, 9 and 18. Body weight QTL (at 7 weeks of age) were reported on chromosomes 1, 2 and 13 from a broiler population (Atzmon *et al.* 2006). Ruy *et al.* (2005) reported four suggestive QTL on chromosome 3 and three suggestive QTL on chromosome 5 for WFE from a layer–broiler cross. Thus, the growth QTL reported in other studies were also in similar chromosome positions to the ones observed in our study and this suggests that sexual maturity QTL are generally not distinct from those for growth.

In contrast to our results, Carlborg *et al.* (2003) observed a pronounced role of epistatic effects on

growth prior to 46 days of age in a red jungle fowl–White Leghorn cross and reported significant QTL for body weight in the 1–200 days age bracket on chromosomes 1, 2, 3, 4, 7, 8, 11, 12, 13, 14, 27 and E27W24. The important contribution of epistasis to early growth (before 6 weeks of age) was also observed in a White Leghorn layer and broiler sire line cross population from the same parents as the present flock (Carlborg *et al.* 2004).

The WFE QTL on chromosome 27 appears worthy of special attention. When InABF was used as a covariate, this QTL explained almost 10% of the phenotypic variance in WFE. The QTL is located in a region that contains several growth-related genes (e.g. chicken growth hormone (*GH*); Lei *et al.* 2007). The G+1705A in Intron 3 of *GH* could have a direct effect on chicken growth via an influence on *GH* gene expression (Nie *et al.* 2005).

There are specific QTL (on chromosomes 8 and 13) associated with age and weight at sexual maturity, which are not found when analysing weight at younger ages. However, the magnitude of their additive effects for InAFE94 is relatively small, suggesting that there is limited opportunity to genetically manipulate sexual maturity independently of commercial broiler growth traits.

Schreiweis *et al.* (2006) reported a suggestive AFE QTL on chromosome 3, which is similar to the location of a QTL in this study. AFE QTL have also been reported on chromosome 4 (Schreiweis *et al.* 2006). However, a QTL for AFE reported on chromosome Z by Tuiskula-Haavisto *et al.* (2002) and Sasaki *et al.* (2004) was not detected in this study.

Abdominal fat, body weight and puberty

The high correlation (0.71) between ABF and WFE (Table 2) suggests that these traits are controlled by similar factors. Circulating lipids increase at the onset of lay for deposition into the developing yolky follicles (Jaccoby *et al.* 1995). The accumulation of body fat is likely to be important for this reason, as it is a source of circulating lipid as well as *de novo* synthesis in the liver. Therefore, the use of InABF to model QTL affecting WFE may identify regions of the genome, which are associated with lean tissue mass, at the onset of lay. The fact that the majority of the QTL are not affected by fitting body fat as a covariate suggests that the amount of fat does not explain the onset of sexual maturity in poultry (Soller *et al.* 1984) except in the case of QTL on chromosome 1.

In general, the results are consistent with the concept that achieving a minimum body weight is permissive for the attainment of sexual maturity (Brody *et al.* 1984, Eitan & Soller 2001). This is interesting because the estimated WFE QTL effects at these locations are genetically determined and are not the

result of dietary manipulation. Therefore, this may be one of the clearest demonstrations that genetic determination of growth rate results in correlated effects on puberty. The possibility that the converse is true can be eliminated because the effects of the growth QTL take place before the age of puberty is reached. Clear evidence of genetic correlations between growth rate and puberty are not numerous: studies in female pigs suggest that there is a negative phenotypic correlation between growth and puberty (Hutchens *et al.* 1981) and in humans it was estimated that 57% of the additive genetic effects for the age of menarche and body mass index are common (Kaprio *et al.* 1995). The genetic and physiological determinates of sexual maturity underlying the QTL identified in this study remain to be elucidated through a combination of fine mapping and identification of candidate genes for subsequent validation.

In conclusion, the QTL for WFE and lnAFE94 detected in this study generally acted additively and the broiler alleles were associated with heavier body weights and earlier ages at the onset of lay. The indication that the loci for growth and puberty are common provides a clear demonstration of the genetic basis for the phenotypic correlation between growth and puberty and that body weight is an important determinant for the attainment of sexual maturity.

Materials and Methods

Parent lines

One-day-old female chicks were obtained from the Ross 308 male line broiler (Aviagen, Newbridge, UK) and a White Leghorn egg laying line maintained at the Roslin Institute. The chicks were brooded and reared under conventional husbandry practices in floor pens. At 12 weeks of age, 12 birds from each line were randomly allocated to individual cages to record phenotypic data on the age and weight at the onset of lay. However, for the broilers, only ten birds that survived were used.

Animals and genotyping

The F₂ population was created by crossing two males and two females from the broiler male line with two females and two males from the White Leghorn line to produce an F₁ generation (Sewalem *et al.* 2002). Eight males and 32 females of the F₁ generation were selected and mated in a balanced mating scheme to produce over 2000 F₂ birds (one F₁ female died and was replaced making a total of 33 full sib families). The female birds were reared in floor pens and moved to individual cages that measured 40 cm wide × 45 cm deep × 80 cm high at 12 weeks of age. The birds were fed *ad libitum* on a conventional poultry diet. The birds were exposed to a constant photoperiod of 14 h per day from hatch to the end of the experiment. WFE and AFE were recorded at the onset of lay, which was defined as the day of first recorded oviposition. In a subset of the birds

(about half of the hatches), the birds were killed and the weight of abdominal fat was recorded. AFE was transformed to natural logarithms of (AFE – 94 days) to normalise residual errors. The 94 days was chosen as the lowest AFE in the unedited data. The QTL effects on the original scale were calculated from the back-transformed means of the three genotype classes. The additive effects were obtained from the average of the back-transformed means of the two homozygotes.

Blood samples were obtained after caging for extracting DNA by standard methods (Sewalem *et al.* 2002). Genotyping was conducted using 106 microsatellite markers covering 25 autosomal linkage groups and the Z sex chromosome (Table 5) on the 8 F₀ grandparents, 41 F₁ and 912 F₂ offspring with data on age and WFE. All pedigree, marker genotypes and recorded traits were stored in the resSpecies database (Law & Archibald 2000). The 2005 consensus genetic linkage map (ArkDB, 2007) was used to add new markers to a linkage map based on the same population used in the analysis (Navarro *et al.* 2005). The total map length was 2479 cM (Table 5).

QTL analysis

The interval mapping method (Haley *et al.* 1994) for QTL detection was conducted using a newly developed module for analysis of epistasis in GridQTL (Seaton *et al.* 2006, Wei *et al.* 2009). The programme initially conducts the standard processes of QTL searching, testing, permutation and bootstrapping for a single QTL F₂ analysis. Genome scans were conducted iteratively using forward selection of significant QTL for each trait (Carlborg *et al.* 2004). Analyses were performed at 1 cM

Table 5 The number of microsatellite markers, first and last marker and map length on each linkage chromosome in the quantitative trait loci analysis of the chicken broiler–layer cross.

Chromosome	Number of markers	First marker	Last marker	Map length (cM)
1	17	ROS0008	MCW0107	548
2	13	LEI0163	MCW0157	473
3	15	MCW0169	MCW0037	286
4	4	ADL0317	MCW0180	195
5	5	ROS0013	ADL0298	119
6	4	ADL0323	ADL0142	113
7	3	LEI0064	ADL0180	109
8	9	ROS0021	ROS0075	92
9	4	ROS0078	MCW0134	132
10	1	ADL0209.2	ADL0209	–
11	5	LEI0110	ROS00112	71
12	2	ADL0240	ADL0044	34
13	2	MCW0340	ADL0225	68
14	1	MCW0123	MCW0123	–
15	2	LEI0083	MCW0080	49
16	1	LEI0258	LEI0258	–
17	1	ADL0199	ADL0199	–
18	2	ROS0022	ROS0027	24
19	1	MCW0094	MCW0094	–
22	1	ROS0073	ROS0073	–
23	1	MCW0249	MCW0249	–
26	2	ADL0285	LEI0074	–
27	1	ROS0071	ROS0071	–
28	3	ROS0095	ADL0299	39
Z	6	ROS0072	LEI0075	127
Total	106			2479

intervals. Exhaustive QTL searches with an updated model were implemented by fitting the significant QTL as co-factors (Jansen 1994, Zeng 1994) until no additional significant QTL were detected.

Epistatic QTL were mapped simultaneously by extending the method for a single QTL search using a linear model with marginal effects for a pair of QTL and their four possible pairwise interactions in 1 cM genome scans (Wei *et al.* 2009). A significant outcome in the overall test of the marginal effects and all two-way interactions between the additive and dominance effects at two loci and of this test compared to the two-locus model with no inter-locus interactions is indicative of epistasis. The method detects epistatic pairs of QTL by using two complementary search approaches. In the first stage, the algorithm automates a genome scan to identify interactions between pre-identified single QTL with all the other genomic positions. In the second approach, a two-dimensional genome scan involving all combinations of two positions in the genome is conducted to search for epistatic interactions regardless of the locations of the pre-identified marginal effect QTL.

Determination of significance thresholds

In single QTL detection, significance thresholds were determined by conducting 5000 permutations (Churchill & Doerge 1994), and 1000 bootstraps were used to generate 95% CI (Lander & Botstein 1989, Visscher *et al.* 1996). A QTL was considered as being significant if it had an F value greater than the $P \leq 0.05$ experiment-wide threshold value and highly significant if the F value exceeded the $P \leq 0.01$ threshold (Kruglyak & Lander 1995). Alternatively, the QTL was considered to be suggestive if it had an F value exceeding the $P \leq 0.05$ chromosome-wide threshold. All significant and suggestive QTL were used in the first approach for detection of epistatic QTL pairs.

Significance testing for epistatic pairs in both approaches used F ratio tests for model comparisons in a nested test framework. Permutations based on 1000 replications were used to determine genome-wide thresholds. In the first stage, exhaustive genome scans were performed on permuted data to derive thresholds for each pre-identified marginal effect QTL. The DIRECT algorithm (Ljungberg *et al.* 2004) was used to perform fast two-dimensional genome scans in permutations to derive genome-wide thresholds for the second stage (Wei *et al.* 2009).

Models

In a preliminary analysis, different models with additive, dominance and parent-of-origin genetic effects with family and pen as fixed effects were analysed (hatch was confounded with pen). There was no evidence for parent-of-origin effects (detected as a difference in effect between the alternative heterozygous genotypes that differ in which allele has been inherited from each parent), and a simple additive–dominance model was adopted for subsequent analyses. Additive genetic effects were defined as half the difference between the broiler and layer homozygotes and dominance effects as

the difference between the heterozygote and the mean of the two homozygotes (Falconer & Mackay 1996). A positive additive effect indicates that the QTL allele originating from the broiler line increased the trait value relative to that from the layer line. The Z chromosome was analysed with an additive genetic effects model.

For each trait, models with and without a covariate were fitted. Covariates were included in the model of analysis to detect differences in the assessed trait at a fixed level of the covariate trait (Kerje *et al.* 2003, Park *et al.* 2006). A regression analysis on InAFE94 and WFE showed that InAFE94 explained 29% of the variation in WFE where pen and family were included as fixed effects. Conversely, fitting the same effects, WFE explained a moderately high proportion of the variation (64%) in InAFE94. In the QTL analysis for WFE, InAFE94 was added in the model as a covariate. Additionally, for the subset of the individuals with ABF recorded, another model was run for WFE with InABF as a covariate. Similarly, WFE was fitted as a covariate in the model for InAFE94.

The percentage of the F_2 phenotypic variance explained by the QTL was calculated as percentage variance = $((RRMS - FRMS) / RRMS) \times 100$ (Zhou *et al.* 2006), where RRMS is the residual mean square from the reduced model in which all the effects including background QTL effects are fitted but the QTL is omitted. The FRMS is the residual mean square from the model in which all the effects and QTL are fitted.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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