Quantitative trait loci with parent-of-origin effects in chicken

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Summary

We investigated potential effects of parent-of-origin specific quantitative trait loci (QTL) in chicken. Two divergent egg-layer lines differing in egg quality were reciprocally crossed to produce 305 F_2 hens. Searching the genome using models with uni-parental expression, we identified four genome-wide significant QTL with parent-of-origin effects and three highly suggestive QTL affecting age at first egg, egg weight, number of eggs, body weight, feed intake, and egg white quality. None of these QTL had been detected previously using Mendelian models. Two genome-wide significant and one highly suggestive QTL show exclusive paternal expression while the others show exclusive maternal expression. Each of the parent-of-origin specific QTL explained 3–5 % of the total phenotypic variance, with the effects ranging from 0·18 to 0·4 phenotypic SD in the F_2. Using simulations and further detailed analyses, it was shown that departure from fixation in the founder lines, grand-maternal effects (i.e. mitochondrial or W-linked) and Z-linked QTL were unlikely to give rise to any spurious parent-of-origin effects. The present results suggest that QTL with parent-of-origin specific expression are a plausible explanation for some reciprocal effects in poultry and deserve more attention. An intriguing hypothesis is whether these effects could be the result of genomic imprinting, which is often assumed to be unique to eutherian mammals.

1. Introduction

Intensive poultry breeding, which began in the 1940s, is based on the development of lines that are each selected for a set of traits. The production animals are hybrids in which all the desired traits are combined with a full exploitation of heterosis. The reciprocal recurrent selection or modifications thereof exploit the entire genetic variance, both additive (general combining ability) and non-additive (specific combining ability due to, e.g. heterosis, dominance, over-dominance and epistasis; Hartmann, 1988). In poultry, reciprocal effects in crosses have been detected for egg production traits, sexual maturity, egg quality traits and viability (Fairfull *et al.*, 1983). However, the

effects vary greatly between crosses, making the outcome of each cross unpredictable. Reciprocal effects in poultry have been hypothesized to originate from sex-linked genes and/or maternal effects (Fairfull, 1990). An alternative explanation would be parent-of-origin specific expression, where the expression of alleles is dependent on the parent from which they are inherited. Parent-of-origin effects are often caused by genomic imprinting, which has been shown to influence several genes and traits in mammals (including humans) (Morison & Reeve, 1998), as well as in plants (Alleman & Doctor, 2000) and insects (Lloyd *et al.*, 1999). Recently, evidence has been presented on the role of parent-of-origin specific expression (imprinting) of genes in economically important traits in livestock such as sheep (Charlier *et al.*, 2001) and pigs (Nezer *et al.*, 1999; de Koning *et al.*, 2000).

In previous genome scans of chicken, quantitative trait loci (QTL) have been identified for egg production

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and egg quality traits (Tuiskula-Haavisto *et al.*, 2002) and for feed consumption, body weight and fatness (Ikeobi *et al.*, 2002; Sewalem *et al.*, 2002; Van Kaam *et al.*, 1999). In the present study, we investigated the role of parent-of-origin effects on QTL affecting production traits in the experimental chicken cross described by Tuiskula-Haavisto *et al.* (2002). Detection of significant parent-of-origin effects in poultry would raise questions whether this could be due to a mechanism resembling genomic imprinting, which is often assumed to be unique to mammals among vertebrates.

2. Materials and methods

(i) *Experimental population*

The mapping population is an F_2 cross between two extreme egg layer lines: Rhode Island Red (RIR) and White Leghorn (WL). These two lines showed the largest pair-wise genetic distance among eight lines studied for genetic diversity (Vanhala *et al.*, 1998). The RIR line is a typical brown egg layer with high feed intake and body weight. The eggshell quality of RIR is good, but the egg white quality is poor. The WL line has been selected for several generations for high egg production and good feed conversion. From each line two hens and two roosters were reciprocally crossed. The population design is added as Fig. S1 of supplementary web information (http://journals.cambridge.org/). From the F_1, 32 hens and 8 roosters were crossed to produce a total of 305 F_2 hens in three different hatches: From each of the four grandparental combinations, two roosters were mated to four hens each that were offspring from the other grandparental combinations, thus avoiding any sib mating (supplementary Fig. S1). All individuals of the F_0, F_1 and F_2 generations were genotyped and phenotypes were recorded on the F_1 (93 from RIR ♂ × WL ♀ and 63 from WL ♂ × RIR ♀) and F_2 hens (Tuiskula-Haavisto *et al.*, 2002). Phenotypic data was also available on pure-line animals (128 RIR hens, 500 WL hens) that were raised simultaneously with the F_1 birds in two hatches under the same circumstances as the F_1.

(ii) *Phenotypic measurements*

We determined sexual maturity as the age at first egg (AFE, in days). Egg quality was measured at 36–39 weeks of age (symbol: __40) and at 57–58 weeks of age (symbol: __60) where one egg per week was collected for every hen. We measured albumen quality in Haugh units (HU40, HU60), and eggshell quality as eggshell strength (ES40, ES60) and specific gravity (SG40, SG60). Egg production was assigned to two periods: early production (between 18 and 40 weeks of age, symbolized by 'a') and late production (between 41 and 60 weeks, symbolized by 'b'. Egg weight (EWa and EWb) represents the mean of weekly measurements (1 egg per week) in the respective production periods. Egg number (ENa and ENb) represents the total number of eggs in the period. We measured body weight at 40 weeks of age and feed consumption (FI40: g/day) and feed efficiency (FE40: kg feed/kg egg) during a 4 week period from 37 to 40 weeks of age.

(iii) *Systematic effects*

Estimates of the purebred means and the reciprocal effects for the F_1 were obtained using least squares analysis (SAS Proc GLM) fitting hatch as a fixed effect. For part of the analyses F_2 records were pre-corrected for any significant effect of hatch number using least squares analysis (SAS Proc GLM). The effect of hatch was significant for nearly all the traits analysed in the present study.

(iv) *Genetic models*

The F_2 data were analysed following a line cross model (Haley *et al.*, 1994), where for every F_2 individual the probabilities that it inherited two RIR alleles (p_{11}), two WL alleles (p_{22}), or one allele from each line (p_{12} or p_{21}; the first subscript indicating the paternally inherited, the second the maternally inherited allele) were inferred at 1 cM intervals across the genome. At every position, the following Mendelian model was fitted:

$$y_j = m + ap_{aj} + dp_{dj} + e_j, \quad [1]$$

where y_j is the trait score of animal j, m is the population mean, a and d are the estimated additive and dominance effect of a putative QTL at the given location, p_{aj} is the probability of animal j carrying two RIR alleles, p_{dj} the conditional probability of animal j being heterozygous, and e_j is the residual error. An outbred line cross design provides the possibility of tracing the parental origin of alleles in F_2 individuals back to F_1 parents. This enables analysis of potential parent-of-origin effects. Knott *et al.* (1998) introduced the contrast between heterozygous individuals with alternative parental origin as a test for parent-of-origin effects ($p_i = p_{12} - p_{21}$):

$$y_j = m + ap_{a_j} + dp_{d_j} + ip_{i_j} + e_j. \quad [2]$$

Variables are as in [1]; with the extension that i is the estimated imprinting effect. The model for parent-of-origin effects by Knott *et al.* (1998) was reparameterized to enable a direct test for the contribution of the paternally and maternally inherited effect (de Koning *et al.*, 2000). Model [2] can be rewritten with a specific maternal and paternal QTL

component:

$$y_j = m + a_{pat}p_{pat_j} + a_{mat}p_{mat_j} + dp_{d_j} + e_j, \qquad [3]$$

where a_{pat} is the paternally inherited QTL effect, a_{mat} is the maternally inherited QTL effect, $p_{pat} = [p_{11} + p_{12}] - [p_{22} + p_{21}]$ and $p_{mat} = [p_{11} + p_{21}] - [p_{22} + p_{12}]$. Models [2] and [3] are identical in terms of total variance explained by the model. This re-parameterization allows additional models to be fitted with exclusive paternal or maternal expression:

$$Y_j = m + a_{pat}p_{pat_j} + e_j,$$
$$Y_j = m + a_{mat}p_{mat_j} + e_j. \qquad [4]$$

(v) *QTL mapping*

We analysed 13 autosomal linkage groups with 92 microsatellite markers for parent-of-origin specific QTL. For details on genotyping and linkage map construction, see Tuiskula-Haavisto *et al.* (2002). Exploratory analyses were performed with QTL Express (Seaton *et al.*, 2002; http://qtl.cap.ed.ac.uk/) searching the most significant QTL using a full model [2] including hatch as a fixed effect. Significance of the parent-of-origin effect was assessed using an *F*-test of whether a full model ([2] or [3]) explains significantly more variation than a Mendelian model [1] (test A). Subsequently, all autosomes were re-analysed using models with exclusive paternal or maternal expression [4]. These models are not available in QTL Express and we used custom-written software and adjusted the phenotypes for hatch effects prior to QTL analysis. To avoid potential effects of pre-adjustment for hatch effects versus inclusion of hatch in the model, which were found to be negligible, models [1] and [2] were re-estimated using pre-adjusted phenotypes for testing parent-of-origin effects. Following the recommendations by de Koning *et al.* (2002), the genetic model for the most significant QTL identified under [4] was inferred using two criteria: (A) An *F*-test of the full imprinting model ([2] or [3]) versus the Mendelian model [1] (H_0: QTL is Mendelian; Knott *et al.*, 1998). (B) An *F*-test of the full imprinting model [3] versus the best model with only a single parental effect [4] (H_0: QTL has only uni-parental effect; de Koning *et al.*, 2000, 2002). Both tests were carried out at the position of the most significant QTL from model [4]. Imposing a nominal threshold of $P < 0.05$, parent-of-origin specific expression was inferred when H_0 was rejected for A but not for B. These tests identify QTL with exclusive uni-parental expression and exclude QTL that also have a significant dominance effect. de Koning *et al.* (2002) showed that applying both tests minimizes the detection of spurious parent-of-origin effects. After derivation of the genetic model, the significance level, the QTL effects, and the confidence intervals were estimated using the inferred genetic model.

(vi) *Significance thresholds*

Significance thresholds for the presence of QTL against the H_0 of no QTL were determined empirically for individual chromosomes by randomization tests (Churchill & Doerge, 1994). The first level of significance was suggestive linkage where one false positive is expected in a genome scan (Lander & Kruglyak, 1995). In order to claim significant linkage, we applied a 5% genome-wide significance level (Lander & Kruglyak, 1995). To derive genome-wide significance levels from the chromosome-wide significance levels, we applied the following Bonferroni correction: $P_{\text{genome-wide}} = 1 - (1 - P_{\text{chromosome-wide}})^{1/r}$, where r is the relative contribution of the chromosome under study to the total genome length (r = chromosome length/genome length).

The empirical genome-wide significance threshold for the presence of a QTL against the H_0 of no QTL effect varied between 7·3 and 8·2 in the autosomes for the Mendelian line-cross analyses and between 11·7 and 13·2 for parent-of-origin analyses fitting only a single parental QTL effect. To facilitate graphical comparisons of different models, the negative logarithm of the comparison-wise *P* values [$-\log_{10}(P)$] of the *F* statistics is presented in the graphs (de Koning *et al.*, 2001). The thresholds in the graphs are averaged over all models that are represented in a graph.

Confidence intervals for QTL positions were obtained by bootstrapping. The sorted *F* ratios from the bootstrap replicates were used to determine the test statistic value corresponding to a desired (i.e. 90%) confidence interval (de Koning *et al.*, 2000). This is an alternative to other bootstrapping strategies in which the QTL positions of the replicates are sorted to determine an empirical confidence interval (Visscher *et al.*, 1996). The method used here allows for non-continuous confidence intervals and is closer to the traditional LOD drop-off methods.

(vii) *Additional simulations and analyses*

The number of founders (8) and F_1 birds (40) was moderate, and it is not known whether any of the QTL were segregating in the parental lines and how this could have resulted in spurious identification of parent-of-origin effects (de Koning *et al.*, 2002). To scrutinize whether the parent-of-origin effects could reflect spurious effects of segregation, we performed additional simulations and analyses. Using the same population structure as the present design, Mendelian QTL of varying magnitude were simulated in an F_2 of 320 animals, following the procedures described by de Koning *et al.* (2002). The QTL was segregating in

the founder lines (0·80 and 0·20) and 1000 replicates were analysed under Mendelian, maternal and paternal models. Permutation tests were carried out on 50 replicates to provide empirical 5% (chromosome-wide) thresholds. Further details on the simulation procedure can be found from de Koning *et al.* (2002).

Additionally, we explored the possibility of segregation as a source of spurious parent-of-origin effects by selectively removing offspring of each of four grandparent pairs in turn: (1) omit offspring of the first F_1 sire, (2) omit offspring of the second F_1 sire, (3) omit offspring of F_1 dams, (4) omit all offspring from these grandparents. The resulting 16 sets of trimmed data were re-analysed, including all the tests for parent-of-origin effects. If results are robust against such subdivision it is unlikely that the parent-of-origin effects are due to segregation in the founders.

Maternal effects and sex-linked genes are often presented as 'plausible explanations' for reciprocal differences and parent-of-origin effects. However, maternal (egg) effects are the same for all full-sib offspring, while maternally expressed QTL show differences between individuals that inherit alternative maternal QTL alleles. Likewise, segregation at Z-linked loci shows phenotypic differences between offspring inheriting alternative paternal Z alleles which should be independent of inheritance of autosomal alleles. Nevertheless, we have investigated the potential effects of these alternative explanations on our QTL mapping results. For ENa, ENb, EWb and AFE, QTL on the Z chromosome have been described in an earlier study (Tuiskula-Haavisto *et al.*, 2002). We re-analysed the parent-of-origin specific QTL for these traits using the Z-linked QTL as cofactors. To reflect the potential effect of mitochondrial DNA or the W chromosome, grand-maternal effects were estimated using Proc GLM in SAS. For traits where we detected a significant grand-maternal effect, we adjusted the phenotypes for this effect and re-analysed the traits. Fitting grand-dam instead of F_1 family prevented the absorption of any within-family genetic effects.

To validate the results (Table 2) that were obtained with custom-written software and with pre-adjusted phenotypes, we re-analysed the QTL positions using publicly available and commercial software. For the best QTL positions (Table 2) we extracted the three probabilities (p_a, p_d, p_i) from QTL Express and derived p_{mat} and p_{pat} as outlined in the 'genetic models' section. Using raw phenotypes, all genetic models were subsequently analysed using GENSTAT, including hatch and, for some traits, grand-maternal origin.

3. Results

Significant phenotypic differences between reciprocal F_1 crosses were found for age at first egg (AFE),

number of eggs (ENa, ENb), egg weight (EWa, EWb), feed efficiency (FE40), feed intake (FI40), and egg white quality (HU40, HU60) (Table 1). The WL male × RIR female cross resulted in lower AFE, FI40, EWa and EWb but in higher ENa and ENb, and in better FE40 than the reciprocal cross (Table 1). The direction of the cross did not affect BW40 or eggshell quality.

Searching the 13 autosomes using models with exclusive uni-parental expression [4] revealed four genome-wide significant QTL ($P = 0.02 - 0.047$) on chromosome 1 (3 QTL) and linkage group 30 (E30C14W10) as well as three strongly suggestive QTL ($P = 0.053 - 0.09$) on GGA1 (*Gallus gallus* autosome 1), GGA3 and linkage group 36 (E36C06W08) (Table 2). Analysing the autosomes with a full model [2] using QTL Express showed five QTL with a significant parent-of-origin component (test A) on GGA1 (affecting body weight, HU40, and FI40), linkage group 30 (affecting ENa) and linkage group 36 (affecting FI40), but they exceeded only the threshold for suggestive significance (see supplementary Table S1). The suggestive QTL on GGA1 (affecting AFE) and GGA3 did not exceed the threshold for suggestive linkage using model [2] in QTL Express, although the parent-of-origin effect was significant (test A; see supplementary Table S1). All the QTL locations are new compared to the genome-wide QTL detected in this population using a Mendelian model (Tuiskula-Haavisto *et al.*, 2002; Fig. 1) although suggestive Mendelian QTL were observed for GGA1 (AFE, BW40, FI40) and linkage group 36 (FI40). For all the QTL in Table 2, H_0 was rejected for test A but not for test B. In other words, a parent-specific model with a maternal, paternal and dominance effect [3] explained significantly more variance than a Mendelian model [1] with an additive and dominance effect (test A, Table 2). For the second criterion to evaluate parent-of-origin effect (test B), a full model [3] did not explain significantly more variance than the inferred reduced model [4] for any of the QTL in Table 2 ($P > 0.40$, data not shown).

Chromosome 1 showed genome-wide significant maternally expressed QTL affecting BW40 (239 cM) and FI40 (338 cM), while a highly suggestive QTL was detected around 120 cM affecting HU40 (Fig. 2A–C). A significant paternally expressed QTL affecting AFE mapped to 204 cM (Fig. 2D). For BW40, the 90% confidence interval extended from 208 to 289 cM. This overlaps with the confidence interval for the paternally expressed QTL affecting AFE, which was from 179 to 232 cM (Fig. 1).

On chromosome 3, there was a strongly suggestive paternally expressed QTL affecting EWb (233 cM; Table 2). A significant paternally expressed QTL affecting ENa mapped to linkage group 30 (E30C14W10) (Fig. 1). Finally, a strongly suggestive maternally

Table 1. *Least square means of performance and standard errors (SE) for pure lines and crossbreeds*

Trait	Acronym	RIR	SE	WL	SE	RIR ♂ × WL♀	SE	WL ♂ × RIR ♀	SE
Number of animals		128♀		500♀		93♀		63♀	
Age at first egg (days)	AFE	141·3[a]	0·8	135·6[b]	0·4	138·1[c]	0·9	130·4[d]	1·3
Body weight at 40 weeks (g)	BW40	2153[a]	16·1	1468[b]	8·0	1837[c]	18·6	1823[c]	23·9
Egg weight 18–40 weeks (g)	EWa	56·4[a]	0·3	57·4[b]	0·2	58·1[b]	0·4	56·1[a]	0·5
Egg weight 41–60 weeks (g)	EWb	61·5[a]	3·7	63·9[b]	1·8	64·3[b]	4·3	62·6[a]	5·5
Number of eggs 18–40 weeks	ENa	140[a]	1·6	139[a]	0·8	136[a]	1·8	148[b]	2·3
Number of eggs 41–60 weeks	ENb	95[a,b]	1·8	96[a]	0·9	91[b]	2·1	100[a]	2·7
Feed intake per day (g)	FI40	137·8[a]	1·1	112·4[b]	0·6	131·5[c]	1·3	125·4[d]	1·7
kg feed/kg eggs	FE40	2·70[a]	0·03	2·23[b]	0·02	2·64[a]	0·04	2·40[c]	0·05
Haugh 40 weeks (HU)	HU40	75·2[a]	0·5	87·1[b]	0·2	80·3[c]	0·6	75·8[a]	0·7
Haugh 60 weeks (HU)	HU60	66·0[a]	0·8	82·0[b]	0·3	71·5[c]	0·9	67·8[a]	1·1
Specific gravity 40 weeks (×1000)	SG40	1081·8[a]	0·4	1080·7[b]	0·2	1082[a]	0·5	1082·5[a]	0·7
Specific gravity 60 weeks (×1000)	SG60	1080·8[a]	0·7	1077·2[b]	0·3	1081·7[a]	0·7	1080·9[a]	1·0
Eggshell strength 40 weeks (kp)	ES40	3·4[a]	0·04	3·4[a]	0·02	3·6[b]	0·05	3·6[b]	0·07
Eggshell strength 60 weeks (kp)	ES60	3·3[a]	0·06	3·1[b]	0·03	3·5[a]	0·07	3·6[a]	0·09

Columns with different superscripts are significantly different from each other.

Table 2. *Genome-wide significant* (P < 0·10) *QTL with parent-of-origin specific expression*

Trait[a]	Chr.	cM	F ratio Imprint vs Mendelian	Maternal	Paternal	Dominance	P[b]	QTL effect[c]
AFE	1	204	4·95*	0·065	**11·98**	0·95	0·046	−2·1 ± 0·6 days
BW40	1	239	6·3*	**13·88**	0·065	0·2	0·02	−66·5 ± 17·9 g
HU40	1	121	7·7**	**11·97**	0·22	0·16	0·053	1·8 ± 0·5 HU
FI40	1	338	8·6**	**11·75**	0·23	0·29	0·046	−4·1 ± 1·2 g/day
EWb	3	233	5·6*	0·04	**10·52**	0·4	0·09	1·4 ± 0·4 g
ENa	E30	1	7·7**	0·07	**13·03**	0·21	0·032	−4·7 ± 1·3 eggs
FI40	E36	13	4·7*	**11·2**	0·06	0·49	0·08	−2·9 ± 0·8 g/day

Uniparentally expressed QTL that are significantly supported by comparison of the full model against a Mendelian model are shown (test A). For all QTL test B was not significant (P > 0·40). F ratios for the individual components of the model at the most likely position of the QTL are shown. The F ratio for the inferred genetic model is shown in bold.
[a] Trait definitions are given in Table 1.
[b] Genome-wide significance for the QTL under the inferred genetic model.
[c] The deviation of the Rhode Island Red (RIR) allele from the White Leghorn (WL) allele under the inferred genetic model (maternal or paternal expression).
* P ≤ 0·05; ** P ≤ 0·01.

expressed QTL affecting FI40 mapped to linkage group 36 (E36C06W08; Fig. 1).

Each of the QTL with parent-of-origin specific expression explain 3–5% of the total phenotypic variance, with the effect size ranging from 0·18 to 0·4 standard deviations of F_2. All the QTL effects were opposite to the phenotypic line differences ('cryptic' alleles or transgressive variation) (Tables 1, 2) except egg number, for which the lines were not phenotypically divergent. For four QTL (affecting FI40, EWb and ENa) the direction of the uni-parental effect (Table 2) is in agreement with the direction of the reciprocal difference (Table 1), while for two others (affecting AFE and HU40) the direction of the QTL effect is opposite to that of the reciprocal difference.

4. Additional simulations and analyses

Using the present population structure we simulated Mendelian QTL of varying size (0·15–1 phenotypic SD) that were segregating in the founder lines. Imposing the same tests as the present study, maternal expression was spuriously detected in 5–8% of the replicates, depending on size of the QTL effect. For
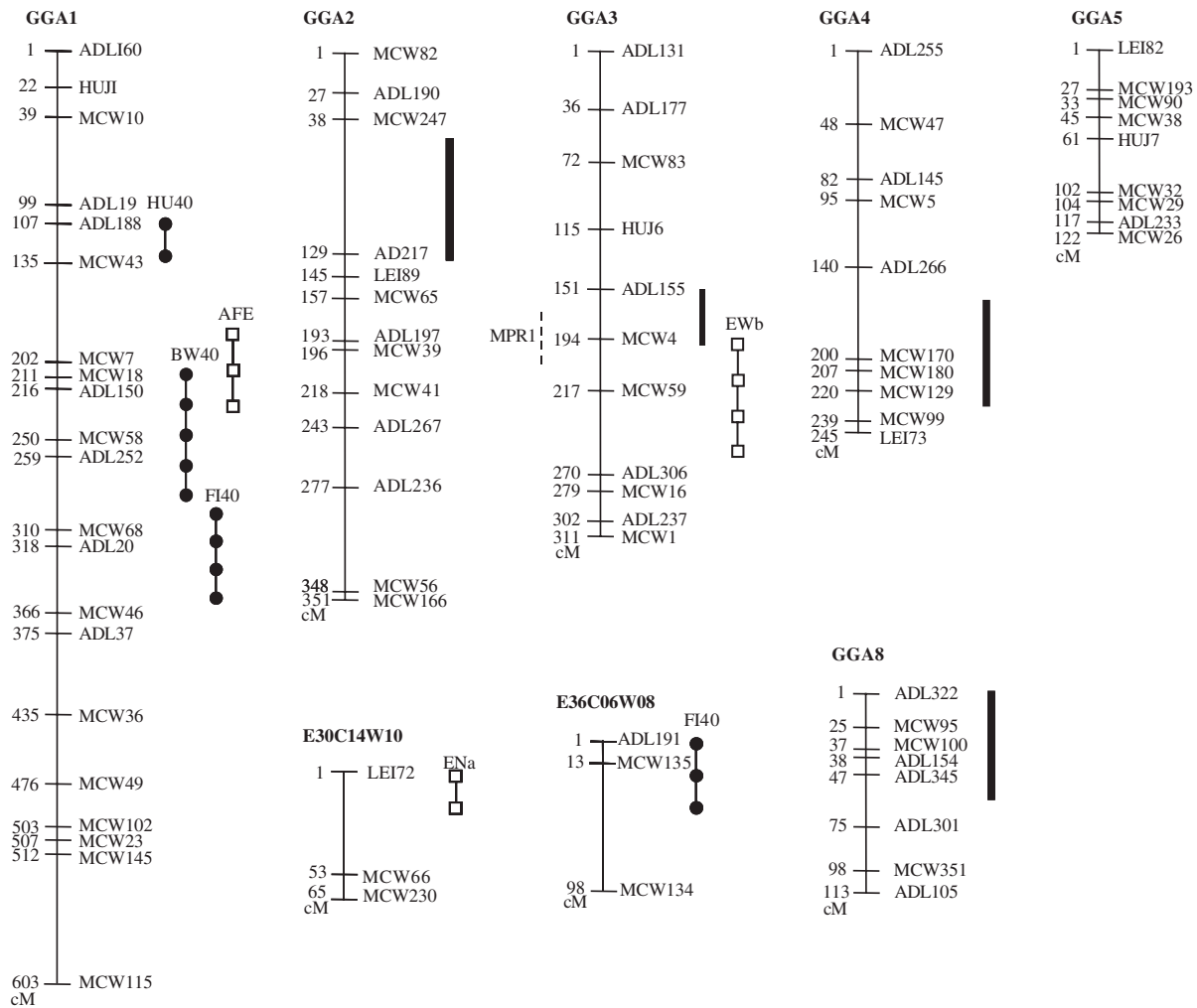
Fig. 1. Autosomal linkage groups showing evidence for Mendelian or uni-parentally expressed QTL affecting production and egg quality traits with 90% confidence intervals for paternally (line with open squares) or maternally (line with filled circles) expressed QTL. The solid bars indicate regions with previously identified Mendelian QTL from the same cross. HU40, Haugh unit at 40 weeks of age; AFE, age at first egg; BW40, bodyweight at 40 weeks of age; EWb, average egg weight at 41–60 weeks of age; ENa, total number of eggs at 18–40 weeks of age; FI40, feed intake per day at 37–40 weeks of age. The localization of the chicken *MPR1/Igf2r* gene (Groenen *et al.*, 2000) is indicated by a dashed line.

smaller effects the spurious detection of a parent-of-origin effect increased but this was counteracted by a decrease in power to detect any QTL (for a more elaborate discussion see de Koning *et al.*, 2002). Spurious paternal expression was detected in 3–6% of the replicates. These rates are only marginally larger than the commonly acceptable 5% false positive rate and therefore spurious detection of parent-of-origin effects should not be a major problem for the present design. Nevertheless, we have explored the possibility of segregation by repeatedly removing offspring of individual sires and/or all dams with the same grandparents and subsequently re-analysing the data. Most QTL proved robust against such subdivision of data, except for probable chance effects when the remaining number of F_2 animals was very small (< 200). However, the maternal QTL affecting BW40 and FI40 on different regions of chromosome 1 each appeared to

be caused exclusively by the females from a single grandparent combination (different females and grandparents for each QTL). Because the offspring of these females make up approximately 25% of the F_2, the actual QTL effect must be very large to be detected across the entire F_2. Two full-sib brothers of these dams were each mated to four dams from different grandparent combinations. These sires carried the same marker genotypes as their full-sib sisters (dams causing the effect) in the QTL region but they showed no QTL effect in their offspring ($n \sim 25\%$ of F_2). This lack of QTL effects in the offspring of these sires is more likely to be due to parent-of-origin specific expression than to segregation in the founder lines.

Table 3 shows the results for parent-of-origin specific QTL when Z-linked QTL were included as cofactors. For one QTL the test statistic increased,
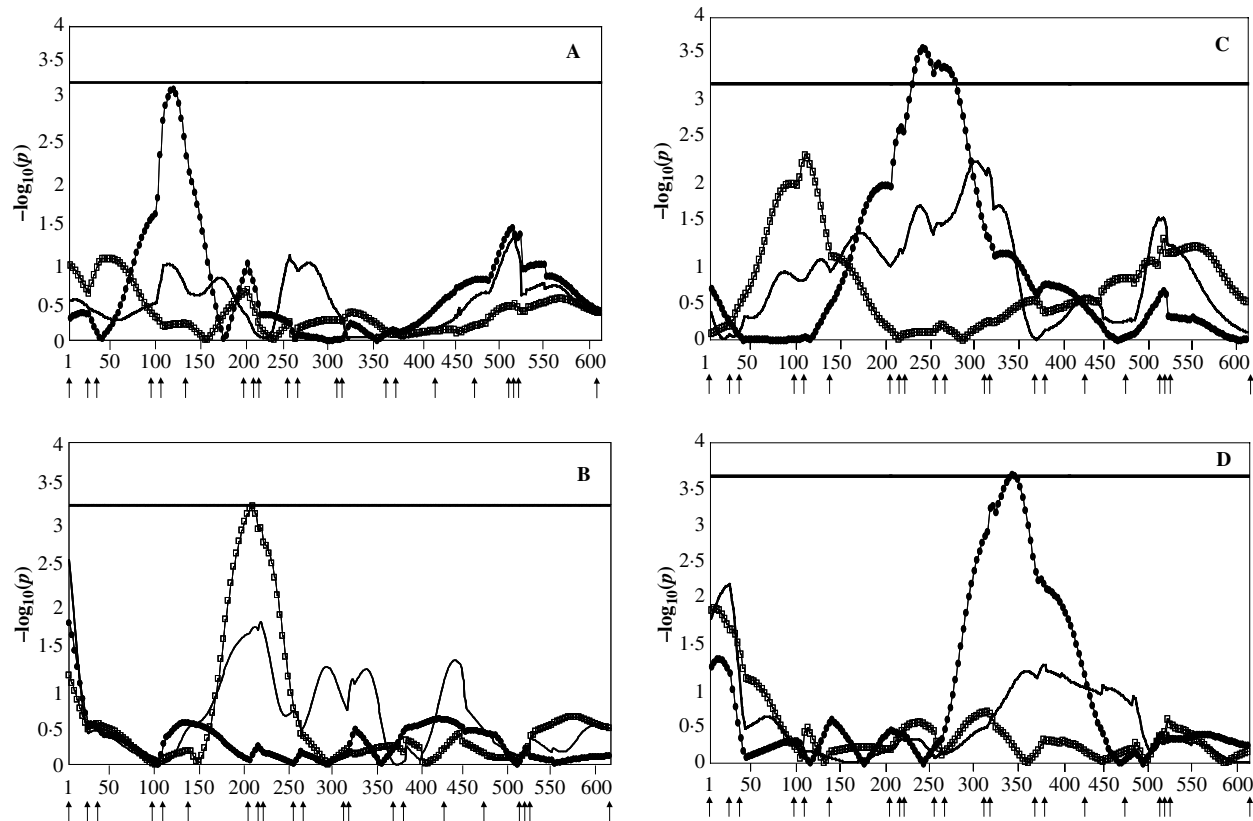
Fig. 2. Test statistic profiles for uni-parentally expressed QTL on chicken chromosome 1 for four traits: Haugh unit at 40 weeks of age (A), age at first egg (day) (B), body weight at 40 weeks of age (C) and average daily feed intake between 37 and 40 weeks of age (D). The black continuous curve represents the test statistics for a Mendelian QTL versus no QTL. The line with squares represents the test statistics for paternally expressed QTL versus no QTL. The line with black circles represents the model with a maternally expressed QTL versus no QTL. The continuous horizontal line denotes the 5% genome-wise threshold for all models. The marker locations are indicated by arrows on the *x*-axis.

while it decreased slightly for two other QTL. The test statistic of four other QTL was not affected (Table 3). The parent-of-origin effect remained significant throughout, proving that there is no confounding between maternal expression and Z-linked QTL. Although for three traits we found significant 'grand-maternal' effects, including these in the analyses gave only small differences in the results and did not change any of the conclusions (Table 3).

For the QTL in Table 2, we did an independent verification using QTL Express and GENSTAT. We have added these additional results as supplementary web information. Supplementary Table S1 compares the best QTL from a parent-of-origin model [2] to the QTL positions from Table 2, both analysed using QTL Express. Supplementary Table S2 shows the results of full [3] and reduced [4] imprinting models for the QTL in Table 2, using GENSTAT with line-origin probabilities from QTL Express. Both supplementary tables (S1 and S2) also include the results that were obtained when including the grand-maternal effect. The main conclusions from these additional analyses are: (1) For all QTL in Table 2, the parent-of-origin

effect is also significant when using QTL Express. (2) Results from the reduced models [4], for which we used custom written software, can be replicated in GENSTAT. (3) Whether phenotypes are pre-adjusted for fixed effects or whether these effects are included in the QTL model, makes little difference for the present results.

5. Discussion

We have detected QTL with parent-of origin effects for age at first egg, egg weight, number of eggs, feed intake, body weight and egg quality. These traits are all very important for poultry breeding and reciprocal effects have been commonly reported for these traits (Fairfull *et al.*, 1983). We also observed these reciprocal effects in our F_1, with the exception of body weight. However, this is in agreement with Wearden *et al.* (1967), who did not detect any reciprocal effect for body weight in their cross between White Leghorn and Rhode Island Red. It is remarkable that all parent-of-origin specific QTL show cryptic effects with regard to the line means. Imprinted genes can escape

Table 3. *Effect of inclusion of Z-linked QTL (F_Z) or grand-dam effects (F_{GD}) on parent-of-origin specific QTL*

Chromosome	Trait	$F_{original}$	F_Z	F_{GD}
1	AFE	12·0	7·8	9·6
1	HU40	12·0	11·9	9·7
1	BW40	13·9	14·3	–
1	FI40	11·8	11·6	–
3	EWb	10·5	8·1	10·1
30	ENa	13·0	12·7	–
36	FI40	11·2	10·9	–

–, Grand-dam effect was not significant and hence not included.

phenotypic selection because they are only expressed when transmitted through a specific parent. It is therefore not unexpected that the proportion of cryptic effects is larger among parent-of-origin specific QTL than among Mendelian QTL (Tuiskula-Haavisto *et al.*, 2002). Whether more intense selection on males compared with females would result in the detection of fewer QTL with paternal expression that show transgressive variation than those with maternal expression cannot be established based on the present results. The (cryptic) reciprocal effects are well known and utilized in chicken breeding, for example by using different reciprocal crosses (of the same lines) for hybrids for product diversification (e.g. smaller eggs for specific markets). The 13 significant and six chromosome-wide significant QTL that were detected previously using a Mendelian model (Tuiskula-Haavisto *et al.*, 2002), did not coincide with any of the parent-of-origin specific QTL in the present study. However, the maternally expressed QTL for body weight on chromosome 1 coincides with a QTL for body weight identified by Van Kaam *et al.* (1998) in a cross between two broiler dam lines, using a Mendelian full-sib model. Buitenhuis *et al.* (2003) describe a chromosome-wide significant QTL affecting cortisol level with a significant parent-of-origin effect on GGA5. They report three other regions with suggestive parent-of-origin effects, but for those regions a full model [3] did not explain significantly more variance than a Mendelian model [1]. Siwek *et al.* (2003) describe the detection of QTL affecting immune response against sheep red blood cells. Using the same criteria to detect parent-of-origin effects as the present paper, they find significant parent-of-origin specific QTL on GGA1, GGA3 and GGA6. The regions on GGA1 and GGA3 correspond very well to the parent-of-origin QTL in the present study although Siwek *et al.* (2003) describe maternal expression for GGA3, where we detected paternal expression (Table 2). Other studies on chicken crosses did not report any significant parent-of-origin effects (Ikeobi *et al.*, 2002; Sewalem *et al.*,

2002) but these analyses were restricted to the full model [2] and did not evaluate reduced models [4]. It seems surprising that the QTL from the present study that are genome-wide significant under the reduced model [4] are only suggestive or even non-significant under the Mendelian [1] or full imprinting model [2]. However, de Koning *et al.* (2002) demonstrated that for many simulated scenarios the correct reduced model [4] was much more powerful than a Mendelian model [1]. To examine the difference between the power of the full parent-of-origin model [3] and the reduced model [4], we have simulated 1000 replicates of an imprinted QTL following the scenarios described by de Koning *et al.* (2002) but added analyses where we also search the genome with a full model [3]. The results are summarized in web supplement Table S3. Although the full model performs better than the Mendelian model, there are still quite a proportion of analyses where the Mendelian and full imprinting models have considerably less power than the correct reduced imprinting model.

Additional power could be added to our analyses by estimating parent-of-origin specific QTL jointly with other QTL (Mendelian, parent-of-origin specific or Z-linked) and systematic effects. An *ad hoc* analysis where we have pre-adjusted the data for the jointly estimated effects of all Mendelian QTL, does not change any of the conclusions with regard to our parent-of-origin specific QTL (data not shown).

The additional simulations and analyses give added confidence that most of the parent-of-origin effects presented here are likely to be true effects. This demonstrates that parent-of-origin specific expression of important production genes is, in some cases, a possible explanation for reciprocal effects in poultry and demonstrates that it deserves closer scrutiny.

The best-known epigenetic phenomenon leading to parent-of-origin-specific expression is genomic imprinting. Several theories have been presented to explain the evolution of imprinting, but the so-called parental conflict hypothesis (Moore & Haig, 1991) has dominated the discussion. The core of this hypothesis is that imprinting evolved as a result of conflicting interests between paternal and maternal genes affecting transfer of nutrients to the fetus from the mother. According to the hypothesis, the maternal genome would tend to save maternal resources and the paternal genome would enhance the growth of individual offspring. Consequently, imprinting should not occur in oviparous taxa.

The genes most intensively studied for imprinting have been the growth-regulating genes in the *IGF2* pathway, and these have shown the best support for the conflict hypothesis. The observation that *IGF2* is imprinted in marsupials and mammals, but not in monotremes, suggests that imprinting of the *IGF2* pathway has evolved in eutherian animals. Studies on

imprinting in avian species are few – and conflicting (Koski *et al.*, 2000; Nolan *et al.*, 2001; O'Neill *et al.*, 2000). These analyses have also concentrated on the *IGF2* pathway. However, imprinting has been reported in a wide variety of organisms such as angiosperms (Alleman & Doctor, 2000), insects (Lloyd *et al.*, 1999) and fish (Martin & McGowan, 1995). In maize and Drosophilae, the imprinted genes currently known are not essential for development (Alleman & Doctor, 2000). There may be other reasons for haploid expression than those put forward by the conflict hypothesis, and therefore other kinds of genes may be affected also in birds.

Several molecular mechanisms have been suggested that could be involved in imprinting, for example promoter methylation, antisense transcripts, silencers and chromatin structure (Reik & Walter, 2001). In eutherians, differential methylation of *cis*-regulatory regions is predominant. However, in different evolutionary lineages different mechanisms may occur. Likewise, the possible mechanism for gene silencing in birds may be different from mammalian imprinting. For example, it had been thought that dosage compensation does not occur in birds until McQueen *et al.* (2001) showed that many Z-linked genes in chicken are dosage-compensated. The process does not involve the sex chromosome inactivation typical of mammals; rather, there is some other unknown mechanism.

Imprinted genes often appear in clusters, and this is thought to reflect their coordinated regulation in a chromosomal domain (Reik & Walter, 2001). The uni-parentally expressed QTL in our study also seem to be concentrated on a few chromosomes (mainly GGA1), although this could also be the result of pleiotropic QTL effects. Comparison of the orthologous locations of the imprinted regions in mice and man with the chicken map (Groenen *et al.*, 2000; www.geneimprint.com/databases/) reveals that none of the uni-parentally expressed QTL appear in regions imprinted in mammals. The only exception is the estimated location of *MPR1* (the chicken orthologue of human *IGF2R*), which lies within the 90 % confidence interval of the paternally expressed QTL for egg weight on chicken chromosome 3. This would suggest that the genes regulated by parent-of-origin-specific expression control in chicken are different from those in mammals. It may be of importance that a region with two of the uni-parentally expressed QTL on chromosome 1 (BW40 and FI40) is orthologous to the human X chromosome, which is dosage-compensated. This indicates that the region includes genes that can adapt to haploid expression. It must be noted that with the availability of full-genome sequence and novel bioinformatics tools, additional imprinted regions will probably be identified in mammals and other organisms in the future. For example, Wang *et al.* (2004) demonstrate a search algorithm for detecting imprinting signatures in a genome sequence.

Our results suggest that the reasons for reciprocal differences between lines are not limited to the effects of sex-linked genes on the Z and W chromosomes or to mitochondrial or other maternal effects. At present, the QTL confidence intervals are too large to pinpoint any candidate genes whose expression status could be analysed to verify uni-parental expression at the transcriptional level. However, we suggest that genomic imprinting or a related mechanism should not be dismissed on the basis of the sparse empirical evidence that some genes that are imprinted in mammals are not imprinted in birds. Further fine-mapping analyses and testing for uni-parental expression in other genes besides those imprinted in mammals may lead to new insights into the evolution of the regulation of gene expression and may also have relevance for hybrid breeding programs.

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