Simultaneous mapping of epistatic QTL in chickens reveals clusters of QTL pairs with similar genetic effects on growth

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Summary

We used simultaneous mapping of interacting quantitative trait locus (QTL) pairs to study various growth traits in a chicken F_2 intercross. The method was shown to increase the number of detected QTLs by 30% compared with a traditional method detecting QTLs by their marginal genetic effects. Epistasis was shown to be an important contributor to the genetic variance of growth, with the largest impact on early growth (before 6 weeks of age). There is also evidence for a discrete set of interacting loci involved in early growth, supporting the previous findings of different genetic regulation of early and late growth in chicken. The genotype–phenotype relationship was evaluated for all interacting QTL pairs and 17 of the 21 evaluated QTL pairs could be assigned to one of four clusters in which the pairs in a cluster have very similar genetic effects on growth. The genetic effects of the pairs indicate commonly occurring dominance-by-dominance, heterosis and multiplicative interactions. The results from this study clearly illustrate the increase in power obtained by using this novel method for simultaneous detection of epistatic QTL, and also how visualization of genotype–phenotype relationships for epistatic QTL pairs provides new insights to biological mechanisms underlying complex traits.

1. Introduction

The desire to dissect the underlying mechanisms of complex traits has led to detection of major genes and quantitative trait loci (QTLs) for many traits in various species. The traditional way to detect major genes and QTLs is by looking for marginal (additive and dominance) effects of the individual loci. Larger sample sizes in QTL mapping studies have increased the opportunity to study the importance of more complex genetic mechanisms such as epistasis. Epistasis has been sought by estimation of the epistatic effects of combinations of QTLs detected by their marginal effects (e.g. Chase *et al.*, 1997) or by using one-dimensional searches with an epistatic model, while including markers to control background genetic effects (e.g. Fijneman *et al.*, 1996). Some attempts have also been made to develop methods that assess the physiological importance of epistasis (Cheverud & Routman, 1995). More recently, several new methods

and technologies have been proposed to increase the power to map epistatic QTLs by performing genome-wide mapping of epistatic QTLs (e.g. Boer *et al.*, 2002; Carlborg *et al.*, 2000; Carlborg & Andersson, 2002; Kao *et al.*, 1999; Jannink & Jansen, 2001; Sen & Churchill, 2001). Several of the methods have also been evaluated by simulation and several have also been applied to map interacting QTLs in various experimental populations (e.g. Carlborg *et al.*, 2003; Leamy *et al.*, 2002; Peripato *et al.*, 2002; Shimomura *et al.*, 2001; Zeng *et al.*, 2000). The application of newly developed methods to experimental datasets is an important part of the process of developing improved method, because it gives new insights into various properties of the analytical method. It also gives an indication of the potential of the new method for revealing previously unnoticed phenomena in experimental data.

Conventional genetic selection has resulted in lines of laying fowl that are small and lean, and produce many eggs in the course of a laying year. Selection of fowl for high growth rates, high muscle yields and

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improved feed efficiency has led to the creation of very large, heavily muscled broiler lines with relatively poor reproductive fitness. Several recent studies have reported associations between genetic markers and quantitative traits of economic importance in chickens (e.g. Dunnington *et al.*, 1992; van Kaam *et al.*, 1998, 1999; Ikeobi *et al.*, 2002; Sewalem *et al.*, 2002). The current study is based on a cross between a layer line with a small body size and a sire line of broiler parent stock with a very large body size that were crossed to produce an F_2 in which many traits were characterized. This cross has previously been analysed using a variety of traditional QTL mapping techniques (Ikeobi *et al.*, 2002; Sewalem *et al.*, 2002). Here, we use the method described by Carlborg *et al.* (2003) to map epistatic QTLs and to evaluate the relative contribution of epistasis to live weight at 3, 6 and 9 weeks of age and for the growth in the age intervals 3–6 weeks and 6–9 weeks of age.

2. Animal material

The mapping population consisted of a three generation F_2 cross between a White Leghorn line and a commercial broiler sire line. The layer was derived from a commercial pure line and the broiler sire line had been selected for high growth rates and breast muscle yields as part of a commercial breeding program. Three females and three males from both lines were used to generate six F_1 families. Subsequently, four of these families (two each of broiler male × layer female and layer male × broiler female) were used to create the F_1 population. Each F_1 family contained 10–16 birds. Eight male and 32 female F_1 were selected to produce an F_2 generation of 546 chickens. The recorded traits were body weight at 3, 6 and 9 weeks of age, and, from these, growth rates at 3–6 and 6–9 weeks of age were calculated. For the total genome scan, 134 microsatellite markers covering 30 autosomal linkage groups and the sex chromosomes were typed on eight F_0 grandparents, 40 F_1 parents and 510 F_2 chickens. After parentage checking and genotyping edits in the F_2, data from 466 F_2 chicks in 30 full-sib families with genotypes on 101 microsatellites covering 27 linkage groups were available for analysis. The total map length was 2499 cM. The average marker spacing was 40 cM and the average polymorphic information content was 0·61 (ranging from 0·19 to 0·98). The sex chromosomes were excluded in the search for epistatic QTLs. A more thorough description of the mapping population can be found in Sewalem *et al.* (2002).

3. QTL mapping methods

This report uses two QTL mapping methods based on two genetic models (without and with epistasis) and two genomic search strategies, forward selection (FS) and simultaneous search (SIM) to map QTLs in an outbred F_2 chicken cross. The methods are compared based on the differences in the number of significant QTLs detected and the amount of genetic variance explained by the detected QTLs. Method I (FS) is a traditional strategy for QTL mapping based on a linear model with marginal (additive and dominance) effects for multiple QTLs. The final genetic model is built by forward selection of significant marginal effects of individual QTLs. Method II (SIM) is a method for simultaneous mapping of epistatic QTLs (Carlborg & Andersson, 2002; Carlborg *et al.*, 2003), which is based on a linear model with marginal effects for a pair of QTLs and their four possible pairwise interactions. The locations for the two QTLs in the model are selected simultaneously using either an exhaustive search (in the real data) or a genetic algorithm (during randomization testing). The contribution of epistasis to the genetic variance explained by the pair was evaluated for all the pairs. The procedures outlined here are described in more detail in the following sections.

(i) Linear models for single and multiple QTLs

In the marginal effects genetic model, used for forward selection of non-epistatic QTL (method I, FS), each QTL is modelled by its marginal (additive and dominance) effects

$$y = \beta_0 + FZ + \beta_{1j} a_j + \beta_{2j} d_j + \epsilon_j \tag{1}$$

where y_i is a vector of phenotypes, β_0 is the mean, F is a vector of regression coefficients for full-sib family, sex, rearing pen and earlier detected QTLs, Z is a matrix of regression variables for full-sib family, sex, rearing pen and earlier detected QTLs, β_{1j}, β_{2j} are regression coefficients for additive and dominance effects at genomic location j, and a_j and d_j are regression indicator variables for additive and dominance effects at genomic location j.

For simultaneous mapping of QTL pairs (method II, SIM), the linear model is a non-orthogonal expansion of model I to include also the marginal genetic effects of a second QTL and the four pairwise interaction terms for a QTL pair

$$\begin{aligned} y = \beta_0 + FZ &+ \beta_{1jk} a_j + \beta_{2jk} d_j + \beta_{3jk} a_k + \beta_{4jk} d_k \\ &+ \beta_{5jk} aa_{jk} + \beta_{6jk} ad_{jk} + \beta_{7jk} da_{jk} + \beta_{8jk} dd_{jk} + \epsilon_{jk} \end{aligned} \tag{2}$$

where y, β_0, F and Z are the same as in model I, β_{1jk}, β_{2jk}, β_{3jk} and β_{4jk} are regression coefficients for additive and dominance effects for QTLs at locations j and k cM, β_{5jk}, β_{6jk}, β_{7jk} and β_{8jk} are regression coefficients for epistatic effects between QTLs at locations j and k cM, a_j, d_j, a_k and d_k are regression indicator variables for additive and dominance effects for QTLs

at locations j and k cM, and aa_{jk}, ad_{jk}, da_{jk} and dd_{jk} are regression indicator variables for epistatic effects for QTLs at locations j and k cM.

(ii) *Parameter estimation*

Estimation of the genetic effects for QTLs was performed using variations of the commonly used least squares framework for QTL mapping in inbred and outbred crosses (Haley & Knott, 1992; Haley *et al.*, 1994). This framework involves two independent tasks. First, QTL genotype probabilities are estimated throughout the genome conditional on the measured marker genotypes. Second, the QTL genotype probabilities are used to calculate regression indicator variables for the genetic effects of QTL, which are then used to estimate the genetic effects using least squares. In this F_2 population, the marker genotypes were used to estimate the probability of an F_2 offspring being each of the four QTL genotypes (QQ, Qq, qQ and qq) at 1 cM intervals throughout the genome. The marginal QTL effects considered are additive (allele substitution) and dominance (heterozygote deviation) effects (model I above). Haley & Knott (1992) describe how to form additive (a_i) and dominance (d_i) indicator regression variables as

$$a_i = P(QQ)_i - P(qq)_i$$
$$d_i = P(Qq)_i + P(qQ)_i,$$

where i is the genome location of QTL$\subset[1\cdots$ genome size cM], and $P(XX)_i$ is the conditional probability of the individual having QTL genotype XX at location i given the flanking marker genotypes. We did not consider parental origin effects because there was no evidence of imprinting in this population (Sewalem *et al.*, 2002).

Method II involves a search for pairwise interactions between QTLs, and the genetic model to evaluate these effects includes four interaction effects in addition to the marginal effects of the two QTLs in the pair. To estimate these effects (additive by additive, additive by dominance, dominance by additive and dominance by dominance interactions), a new set of indicator regression variables needs to be calculated. Haley & Knott (1992) indicated that the indicator regression variables could be calculated by multiplying the respective additive and dominance regression indicator variables for the QTL in the pair

$$aa_{ij12} = a_{i1} \times a_{j2},$$
$$ad_{ij12} = a_{i1} \times d_{j2},$$
$$da_{ij21} = d_{i2} \times a_{j1},$$
$$dd_{ij22} = d_{i1} \times d_{j2},$$

where i and j are the genome locations in cM of QTLs 1 and 2$\subset(1\cdots$ genome size cM).

Using these indicator regression variables, the genetic parameters for single QTL (model I) and epistatic QTL pairs (model II) can be estimated using ordinary least squares.

(iii) *Forward selection interval mapping*

A simple way to map multiple QTLs is by forward selection of non-interacting QTLs. This was the first analysis we performed to detect significant marginal (additive and dominance) effects of QTLs (Fig. 1, step I). QTL genotype probabilities were calculated at 1 cM intervals and QTLs were fitted using model I at 1 cM intervals using ordinary least squares (Haley *et al.*, 1994). The additive and dominance regression indicator variables for the most significant single QTL in this scan were added as cofactors to model I and a new genome scan was performed using the updated model. This procedure was repeated until no additional significant QTLs were detected. Statistical significance was assessed by randomization testing (Churchill & Doerge, 1994) in each step of the procedure using a 5% genome-wide threshold for significant and a 20% genome-wide significance threshold for nearly significant QTLs. All randomization tests are based on analyses of 1000 permuted datasets.

(iv) *Simultaneous interval mapping*

Simultaneous mapping of epistatic QTLs increases the power to detect interacting QTL. The principle of the SIM method performed here is as follows (Fig. 1, step II). First, QTL genotype probabilities were calculated at 1 cM intervals according to Haley *et al.* (1994). An exhaustive simultaneous search for interacting QTL pairs in the real data was performed using model II. For all fitted pairs, the parameters of the model were estimated using least squares and the model fit (residual sum of squares) was retained. Significance of fitted QTL pairs was assessed in three ways depending on the number of QTLs in the pair that had significant marginal effects (for further detail on the randomization procedures see Carlborg & Andersson, 2002). (i) When both QTLs in the pair had significant marginal effects in the FS procedure described above, the QTL pair was declared significant without further significance testing. (ii) Where one of the QTLs in the pair had significant marginal effects, a randomization test was used to test for the combined effects of the marginal effects of the second QTL and the interaction parameters for the pair, conditional on the significant marginal effects of the first QTL. (iii) Where neither of the QTLs had significant marginal effects, the significance of the pair is assessed using a randomization test for a QTL pair without significant marginal effects. For all these tests, a 5% genome-wide threshold was used to declare significant
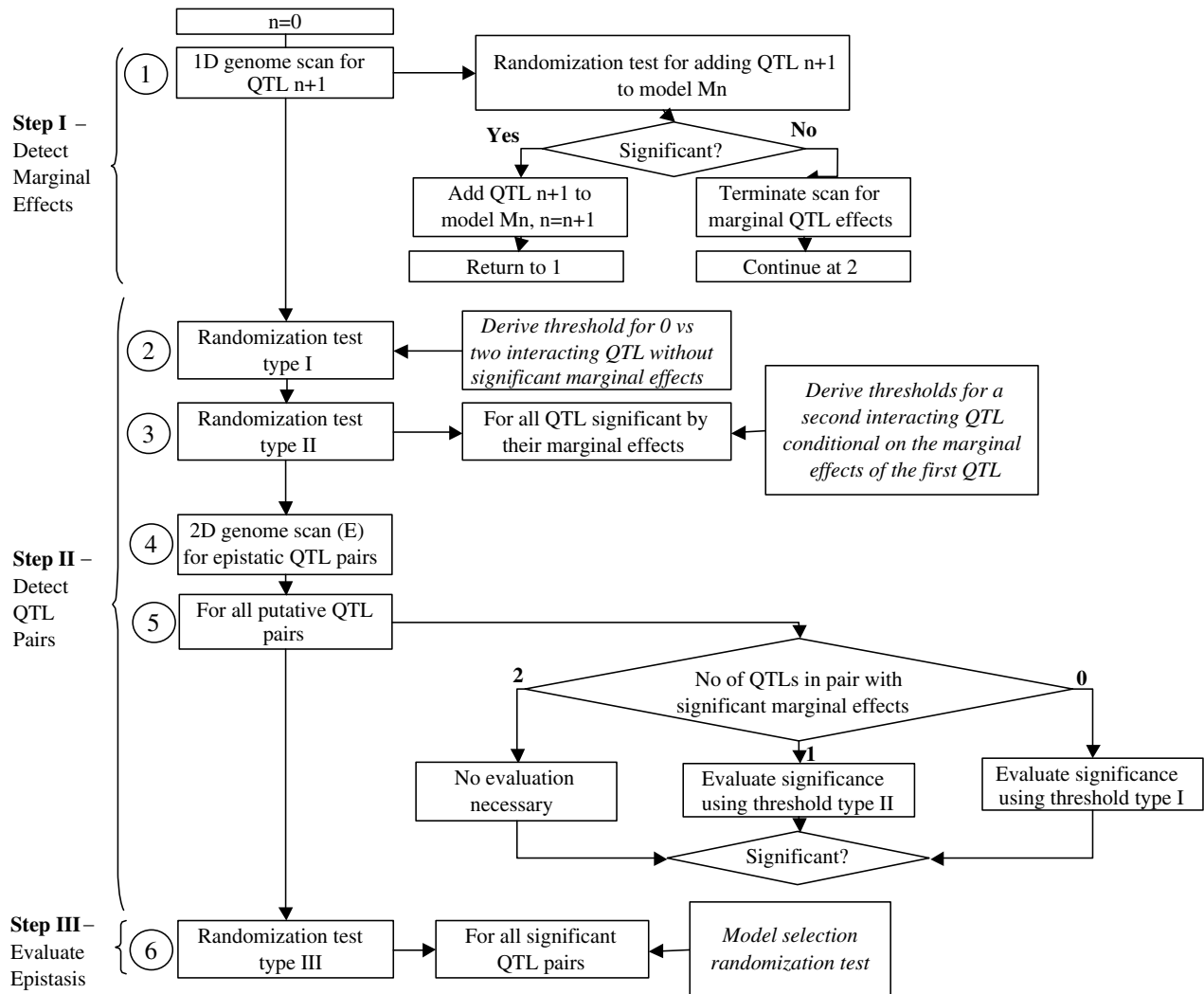
Fig. 1. The analysis procedure used for detection of QTL. Further explanation of the figure can be found in the text. Abbreviations: 1D, one-dimensional; 2D, two-dimensional; E, exhaustive search.

and a 20% genome-wide significance threshold to declare nearly significant QTL pairs.

(v) Model selection for significant QTL pairs

To evaluate whether epistasis contributed significantly to the genetic variance explained by significant and nearly significant QTL pairs, a randomization test was used to test whether a model including both marginal (additive and dominance) and epistatic QTL parameters significantly improved the fit over a model including only marginal QTL effects (Carlborg & Andersson, 2002). A nominal 5% significance threshold was used for each of these tests.

(vi) Multiple regression modelling

To compare the explanatory power of the QTL detected by the SIM and FS procedures, we used multiple regression modelling to fit simultaneously all QTL detected using the FS and SIM procedures. For all traits in turn, we fitted model I with (i) all QTLs detected by FS and (ii) all QTLs detected by SIM. The fits of the models were compared by the reduction of the residual sums of squares of these two models by including the genetic effects of the QTLs. The relative importance of epistasis for the analysed traits was assessed by comparing the fit of model I, including the marginal effects for all QTL detected by the SIM procedure, and model II, including the same marginal effects together with interaction effects for pairs where an epistatic QTL model was significantly better than a marginal effects model. The variances contributed by the marginal effects (model I) and by the marginal and epistatic effects (model II) were compared using the reduction of the residual sum of squares by the respective models by including the genetic effects of the QTLs.

(vii) *Interpretation of epistasis*

If the genetic mechanism behind the observed pairwise QTL interactions could be understood, the information would be valuable for identifying candidate genes for detected QTLs. We have therefore plotted the nine genotype class means for all the significant and nearly significant QTL pairs to identify similarities among the interaction patterns and those of classic mendelian patterns of epistasis. The genotype class means were estimated using the SAS software, by regressing phenotypes on fixed effects and the two-locus genotype probabilities (calculated by multiplying the single locus genotype probabilities described above) of the QTL pair.

4. Computational methods

In QTL mapping, the genome is modelled as a grid based on genetic markers (where each marker is a node in the grid) or on genetic map locations (where each node in the grid is a genomic location in cM). The grid is one-dimensional during a search for a single QTL and multidimensional when multiple QTLs are sought. A genome scan involves fitting a statistical model at multiple locations in the genomic grid with the objective of finding the location(s) in the genome with significant statistical support for a QTL or multiple QTLs. We use a genetic-map-based grid with a genetic distance of 1 cM (Kosambi) between the nodes.

We have used three different algorithms to select the QTLs to be evaluated among all the possible combinations of QTLs that exist in the grid. Below, we give a short introduction to these methods but, for a more thorough discussion of methods to search for QTLs in genetic grids, we refer to Carlborg (2002).

(i) *Exhaustive search*

An exhaustive search involves fitting the statistical model at all nodes in the one- or multidimensional grid. The method guarantees that the best location in the grid, at the given resolution, is found, but at the price of a high computational demand. The computational demand for using an exhaustive search in a one-dimensional grid (i.e. a search for a single QTL), randomization testing in one-dimensional grids or isolated scans in two-dimensional grids (i.e. fitting two QTLs simultaneously in real data) is not prohibitively high, especially when parallel computers are used for the analysis (Carlborg, 2002). However, randomization testing based on two-dimensional grids and scans in grids of dimensions higher than two is computationally intractable using an exhaustive search and, for this, alternative search methods are needed.

(ii) *Forward selection*

Forward selection is a method to reduce a scan of a multidimensional grid to a series of one-dimensional scans. In QTL mapping, the method has been used to map multiple non-interacting QTLs, where the most significant QTL from a series of successive exhaustive one-dimensional genome scans are sequentially added to a multiple-QTL model. The method is expected to perform well when the QTLs are independent, which is the case for non-interacting and non-linked QTLs, and has been widely used for this purpose. We have selected this method to represent a traditional method to search for multiple non-interacting QTLs.

(iii) *Genetic algorithm*

Genetic algorithms are search algorithms based on the mechanisms of genetics and natural selection, and can be used to perform a multidimensional search in a more computationally efficient way than using an exhaustive search. The advantage of using a true multidimensional search instead of a search based on repetitive one-dimensional searches is expected to be greater for interacting QTLs than for non-interacting QTLs, because pairs of QTLs with non-significant marginal effects will not be found in a series of one-dimensional searches. The importance of using true multidimensional searches when mapping interacting QTLs was first shown by Carlborg *et al.* (2000), where a genetic algorithm was shown to be more efficient in detecting interacting QTL than an FS-based method. Here, a genetic algorithm has been used to reduce the computational demand during randomization testing for interacting pairs of QTLs without significant marginal genetic effects. We used a genetic algorithm (GA) from a library named PGAPack (Levine, 1996). Ten independent GA populations of 20 individuals with 1000 iterations per population were used for two-dimensional genome scan. For each independent GA population, a local exhaustive search of ± 5 cM was performed around the found optimum after the GA had converged. More information on specific parameters settings for PGAPack can be found in Carlborg *et al.* (2000).

5. Results

(i) *Detection of non-interacting and interacting QTLs*

For the five analysed bodyweight and growth traits, a total of nine QTL regions were detected as significant using a 5% genome-wide significance threshold (Table 1). Three of the regions (chromosome 1, 150 cM; chromosome 1, 470 cM; chromosome 27, 0 cM) were only detected by their marginal effects and one region was only detected using simultaneous mapping of epistatic QTL pairs (chromosome 2,

Table 1. *Genomic regions with a significant or nearly significant QTL affecting at least one growth trait. The information content at the location of the QTL and the markers flanking the QTL peak are also given*

QTL		LM		RM		BW3		BW6		BW9		GR36		GR69		Sum			IC	Int	
GGA	Pos	Name	Pos	Name	Pos	FS	SIM	FS	SIM	FS	SIM	FS	SIM	FS	SIM	FS	SIM	T		Y/N	Pairs
1	70	MCW010	48	ADL188	109	c	c	a	a	a	–					a	a	a	L	Y	1
1	150	LEI146	145	MCW007	178	–	c	a	c			–	c			a	c	a	H	Y	5(4)
1	390	MCW036	362	LEI106	394	–	c	–	b	a	a	a	c	c	–	a	a	a	M	Y	4(3)
1	470	LEI079	422	ROS025	503	a	c	a	b							a	b	a	L	Y	1
2	240	ADL196	225	LEI127	270			–	a			–	b			–	a	a	M	Y	3(2)
2	290	LEI127	270	ROS074	302			–	b	c	–	c	–			c	b	b	H	N	
3	50	MCW083	51	MCW083	51			–	b					–	c	–	b	b	M	Y	1
4	165	ADL266	126	LEI073	231			a	a	a	c	a	b	a		a	a	a	L	N	
5	127	ROS084	57	ADL298	166									–	c	–	c	c	L	Y	1
6	35	ROS003	33	ADL142	51			a	a			a	b			a	a	a	H	Y	4
7	105	ROS019	101	ADL180	109							–	c			–	c	c	H	Y	1
8	15	ADL179	11	ROS075	80					c	c	c	c			c	c	c	L	Y	1
13	55	ADL147	32	ADL255	70	a	c	a	a	c	a	a	b			a	a	a	M	Y	5(3)
18	15	ROS022	0	ROS027	23			–	c			–	c			–	c	c	H	Y	2(1)
27	0	ROS071	0	ROS071	0			c	–			a	c			a	c	a	H	Y	2

Abbreviations: GGA = *Gallus gallus* chromosome, Pos = Estimated chromosomal position (cM) based on the results from all traits, No = QTL ID number, LM/RM = Left/Right Marker flanking QTL interval (LM = RM if the QTL peak is located at a marker), BW3/6/9 = Body weight at 3/6/9 weeks of age, GR36/69 = Growth from 3 to 6 and 6 to 9 weeks of age, Sum = Summary of QTL mapping results, FS = Significance of QTL mapped using forward selection, SIM = Significance of QTL mapped using simultaneous mapping, T = Significance of QTL mapped by the entire SIM procedure, a/b/c = QTL significant at 5/10/20 % genome-wide significance threshold, Int = QTL involved in interactions, Y/N = Yes/No, Pairs = No. of epistatic pairs (No. of *unique* epistatic pairs) in which QTL is involved, IC = combined information content for QTL location classified as 0 < Low (L) < 0·30, 0·31 < Medium (M) < 0·60, 0·61 < High (H) < 1·00.

240 cM). When a 20% genome-wide significance threshold was used, 15 QTL regions were detected, and five of these (chromosome 2, 240 cM; chromosome 3, 50 cM; chromosome 5, 127 cM; chromosome 7, 105 cM; chromosome 18, 15 cM) were only detected using simultaneous mapping of epistatic QTL pairs. A summary of all QTL pairs that were detected for the five analysed traits and the model that was selected for each of the pairs are given as supplementary information on the publisher's website. Two QTL regions (chromosome 2, 290 cM; chromosome 4, 165 cM) were never included in a significant epistatic QTL pair, and seven regions were significant on more than one occasion and two of these were only detected using SIM (chromosome 2, 240 cM; chromosome 18, 15 cM). The least squares estimates for the two-locus genotypes for all detected QTL pairs are given in Table 2.

(ii) *Variation explained by epistasis*

The additional residual phenotypic variance explained by adding significant epistatic parameters to the genetic model varied from 0% to 34% using a 5% genome-wide threshold and from 20% to 103% using a 20% genome-wide threshold. The largest contribution of epistasis when using the 5% threshold was for bodyweight at 6 weeks and 9 weeks. For the QTLs detected using the 20% threshold, the largest contribution of epistasis was found for bodyweight at 6 weeks and the growth rates at 3–6 and 6–9 weeks. Fig. 2 shows the amount of residual phenotypic variation explained by the QTLs detected by their marginal effects using FS and by the SIM procedure for the five analysed traits.

(iii) *Interpretation of epistasis*

In total, 30 QTL pairs were detected for the five analysed traits (Table 3) and, for 16 of these, an epistatic model was selected. Among the 30 pairs, there were 21 unique combinations of loci that had significant genetic effects on at least one trait. The patterns among the genotypic effects for the locus pairs that had significant effects on multiple traits were very similar and so only the unique combinations were evaluated further. Four clusters of QTL pairs with similar genetic effect patterns were identified by visual inspection (representative pairs are given in Fig. 3).

The first cluster consists of five pairs, in which several of the homozygote–heterozygote genotypes have lower phenotypes than expected under a two-locus additive model. An epistatic model was significant for four of the pairs in this group. An example of a pair from this cluster is shown in Fig. 3A.

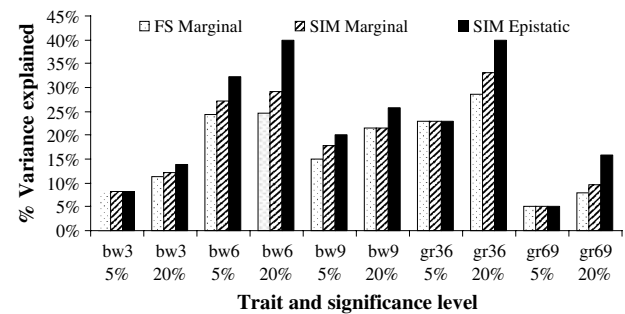The second cluster contains six pairs, in which the broiler double homozygote has a lower phenotype

Fig. 2. The variance explained by the QTLs detected by their marginal effects using forward selection and by the SIM procedure for the five analysed traits, calculated as the reduction of the residual sums of squares by adding marginal and epistatic genetic effects to the model. Abbreviations: FS, QTL mapped using forward selection; SIM, QTL mapped using simultaneous mapping; BWX, bodyweight at X weeks of age; GRXY, growth from X to Y weeks of age; Marginal, model used included additive and dominance effects; Epistatic, model used included additive, dominance and epistatic effects.

than expected given the other genotypic-effects for the pair. The QTL pair in Fig. 3B is an example from this cluster. An epistatic model was selected for the three most significant of the pairs in this group. Three of the six pairs include a QTL on chromosome 1, closely linked to marker LEI106 at 393 cM, and two more pairs include a close, but unlinked, QTL located at 455 cM.

The third cluster includes four pairs that show a continuous increase in the phenotype from the low phenotype Leghorn double homozygote to the high phenotype broiler double homozygote. The transition of the phenotype between the genotypes varies from near linear ('additive') to non-linear. Fig. 3C shows a pair from this cluster with a non-linear phenotype transition between the genotype classes. An epistatic model was selected for the two most non-linear of the pairs in this group.

The last identified cluster includes two pairs that have their genotypic effects divided into three distinct classes, in which the high-effect group contain broiler homozygotes or the double heterozygote, the intermediary-effect group only contains the Leghorn double homozygote and the low-effect group contain the rest of the genotype classes. Both of the pairs are significantly epistatic. Fig. 3D shows one of these pairs.

There are no striking similarities with a mendelian pattern of digenic epistasis or other similarities among the remaining five pairs and they have not been classified further. The plots of the genotypic effects for all the 22 genotype combinations are given as supplementary information on the publishers website.

QTLs with significant pairwise interactions or non-significant interactions for QTL clustering into groups 1 and 2 above were joined by connecting arrows to

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Table 2. *Estimates of the genotypic effects (as deviations from the LLLL genotype) and the respective standard errors for the QTL pairs detected in the study*

QTL information			Genotypes															
			BBBB		BBBL		BBLL		BLBB		BLBL		BLLL		LLBB		LLBL		LLLL	
Pair no	Trait	Location	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	bw3	1-147|1-373	72·0	19·6	82·4	18·8	39·2	20·1	35·2	19·1	47·2	17·7	50·4	19·4	50·9	19·6	60·3	21·8	0·0	–
2	bw3	1-147|1-471	18·1	34·4	36·7	32·7	−53·8	34·8	2·3	31·9	−7·5	29·5	−37·6	31·2	35·4	27·9	−43·7	43·6	0·0	–
3	bw3	1-471|13-59	141·1	51·3	167·8	46·3	70·3	38·0	116·5	50·0	93·7	35·7	121·9	56·3	114·5	50·2	106·6	51·7	0·0	–
4	bw6	1-71|6-34	123·6	100·9	84·3	82·3	96·2	82·1	126·9	85·3	118·2	75·4	−47·5	116·6	−51·9	92·0	−72·9	83·8	0·0	–
2	bw6	1-150|1-455	126·0	107·4	96·8	101·0	−109·0	106·5	35·9	100·4	4·8	88·7	−73·5	102·8	112·9	87·6	−127·4	134·1	0·0	–
5	bw6	1-150|4-168	381·6	144·8	160·2	134·1	−12·5	149·6	165·7	135·1	136·7	114·0	3·5	143·8	339·8	113·1	4·3	191·3	0·0	–
6	bw6	1-150|18-13	61·7	64·3	73·2	56·5	−83·9	62·9	39·2	59·1	−54·3	53·8	−50·7	61·1	−65·3	64·8	−67·4	65·7	0·0	–
7	bw6	1-383|6-34	87·3	59·7	198·2	49·5	125·7	52·6	210·5	48·7	141·4	44·3	112·1	58·8	131·4	55·6	91·7	55·1	0·0	–
8	bw6	1-383|13-56	173·4	86·9	391·0	75·0	120·5	74·9	398·5	77·9	233·9	63·7	243·0	79·7	218·1	83·4	265·2	91·4	0·0	–
9	bw6	1-455|4-168	81·6	324·8	566·4	352·7	−105·7	259·5	495·3	328·5	74·6	191·1	152·2	390·3	194·0	216·6	140·6	391·8	0·0	–
3	bw6	1-455|13-56	387·8	147·1	512·9	132·5	219·6	108·9	365·3	143·2	325·0	102·3	346·8	161·2	366·0	143·9	296·9	148·2	0·0	–
10	bw6	2-239|6-34	178·8	59·7	160·8	53·5	136·2	56·2	159·7	53·5	155·8	49·1	85·6	63·2	123·0	64·4	86·6	54·6	0·0	–
11	bw6	2-239|13-56	360·5	93·8	208·9	69·6	77·9	70·2	88·2	72·3	246·1	58·1	117·7	75·9	344·1	79·7	62·6	85·2	0·0	–
12	bw6	3-34|6-34	148·5	52·3	55·6	47·4	−61·2	51·3	1·0	43·4	59·2	40·4	−30·5	47·9	67·2	53·6	−52·4	48·4	0·0	–
13	bw6	4-168|6-34	476·6	144·1	317·8	124·0	323·5	103·2	210·9	129·2	259·6	115·8	165·3	180·8	118·6	144·1	89·7	127·5	0·0	–
14	bw6	4-168|13-56	481·1	186·9	286·5	171·7	250·1	132·6	59·5	181·7	316·4	122·2	112·0	209·2	364·0	188·0	−13·6	180·5	0·0	–
15	bw9	1-397|4-168	495·3	308·8	456·8	263·0	−60·8	271·4	607·4	256·0	303·5	219·5	72·5	266·7	363·9	202·2	184·1	352·3	0·0	–
8	bw9	1-397|13-55	301·9	129·6	508·8	110·1	113·4	113·4	447·4	112·3	307·6	97·7	318·9	108·6	352·6	127·1	239·8	131·7	0·0	–
16	bw9	8-13|13-55	911·4	270·9	−83·1	216·6	99·0	188·7	−328·7	230·3	334·0	147·4	−131·0	259·9	347·0	241·9	−270·2	230·5	0·0	–
6	gr36	1-145|18-16	46·9	43·8	44·7	38·1	−67·5	42·6	34·8	39·7	−39·7	36·6	−28·7	38·8	−22·7	44·5	−54·6	43·8	0·0	–
17	gr36	1-145|27-0	182·3	41·9	100·4	35·5	34·5	39·4	102·6	37·4	57·0	34·6	47·1	35·9	60·6	44·8	54·1	37·8	0·0	–
15	gr36	1-400|4-170	134·3	149·1	255·1	127·4	−53·3	129·9	306·1	122·7	107·2	104·6	49·2	130·5	170·5	95·6	73·4	168·1	0·0	–
7	gr36	1-400|6-33	92·5	37·8	145·3	32·2	90·1	36·4	129·4	31·9	116·5	28·8	79·1	36·1	117·0	36·1	48·8	34·7	0·0	–
11	gr36	2-245|13-52	269·4	79·3	152·5	60·6	94·5	60·5	65·3	65·2	205·8	50·0	90·4	67·4	275·7	68·8	42·3	75·7	0·0	–
13	gr36	4-170|6-33	294·9	109·2	243·4	93·2	258·3	77·6	180·0	97·5	167·5	88·3	75·8	136·9	22·3	109·3	50·9	94·9	0·0	–
14	gr36	4-170|13-52	375·6	153·7	208·2	141·4	254·1	106·3	60·7	151·4	247·5	96·6	71·7	168·2	251·2	153·6	2·1	148·0	0·0	–
18	gr36	6-33|27-0	146·4	38·9	104·8	30·9	72·5	35·1	154·1	32·5	90·7	28·9	65·2	32·2	64·7	39·0	58·6	32·4	0·0	–
19	gr36	7-105|27-0	77·8	40·9	121·0	33·3	52·5	40·9	142·3	35·1	44·6	31·6	44·2	35·4	92·5	40·9	53·3	34·9	0·0	–
20	gr36	8-25|27-0	333·5	135·3	181·1	86·8	239·1	78·6	120·5	93·7	206·5	73·6	141·1	117·5	271·2	108·2	69·6	83·8	0·0	–
21	gr69	3-63|5-127	720·3	205·0	−386·1	202·2	260·2	193·1	−331·9	203·2	213·6	160·5	−98·4	219·5	240·2	152·3	−162·2	270·9	0·0	–

Abbreviations: Pair no, number of unique QTL pairs if the same pair has significant effects for more traits the pair has the same number; L, Leghorn allele; B, broiler allele; XXYY, genotype XX at locus 1 and genotype YY at locus 2.
Locations: a-b\|c-d, first QTL at chromosome a in location b and second QTL at chromosome c in location d.

Table 3. *Number of QTL pairs identified by a simultaneous mapping strategy for epistatic QTL pairs (SIM) and the number of pairs detected with a marginal effects model including additive and dominance effects (A + D) and an epistatic QTL model (E) were selected. Also, the number of times two, one or none of the QTLs in the detected pair were also detected using forward selection (FS) and a marginal effects model*

| | 5% genome-wide significance | | | | | | 20% genome-wide significance | | | | | |
| | No of pairs by SIM | Selected model | | Detected by FS | | | No of pairs by SIM | Selected model | | Detected by FS | | |
		A + D	E	2	1	0		A + D	E	2	1	0
BW3	0	0	0	0	0	0	3	1	2	2	1	0
BW6	4	1	3	2	2	0	13	6	7	8	5	0
BW9	1	0	1	0	1	0	3	1	2	3	0	0
GR36	0	0	0	0	0	0	10	6	4	6	3	1
GR69	0	0	0	0	0	0	1	0	1	0	0	1
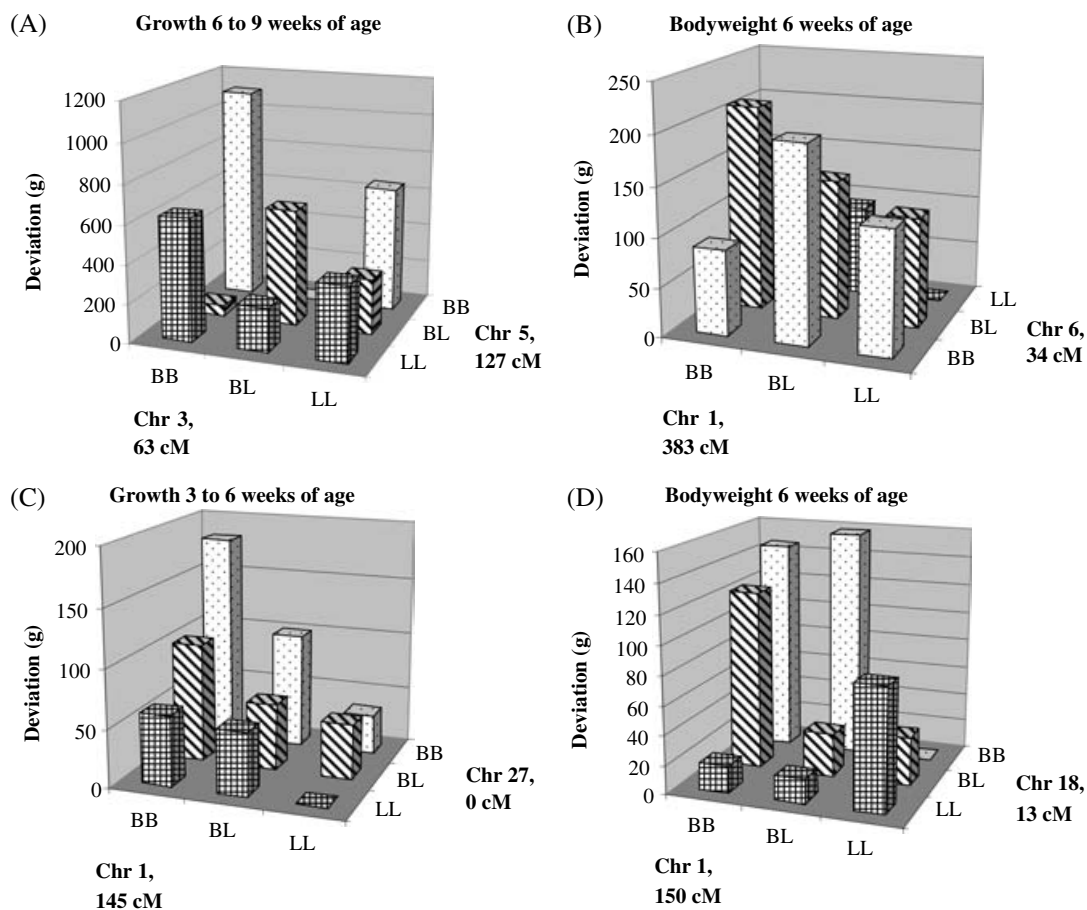
Fig. 3. Phenotypic expression in the nine genotype classes for representative epistatic QTL pairs from the four clusters of QTL pairs with similar genetic effects on growth in a broiler layer cross. Abbreviations: Deviation, phenotype expressed as the deviation (in grams) of the phenotype from the genotype class with the lowest mean; Chr, chromosome; Pos, position; BB, genotype is homozygote broiler; BL, genotype is heterozygote; LL, genotype is homozygote layer.

understand further the genetic architecture of the traits (Fig. 4). The figure shows a chain of eight QTLs linked by pairwise interactions, two branches with a single QTL and three loops on the chain (two of which are created by non-linear type interactions). Two QTLs (chromosome 2, 290 cM, and chromosome 4, 165 cM) are not included in the figure because they were not involved in any significant pairwise interactions.

6. Discussion

The use of efficient computational algorithms in QTL mapping allows researchers to move from approximate
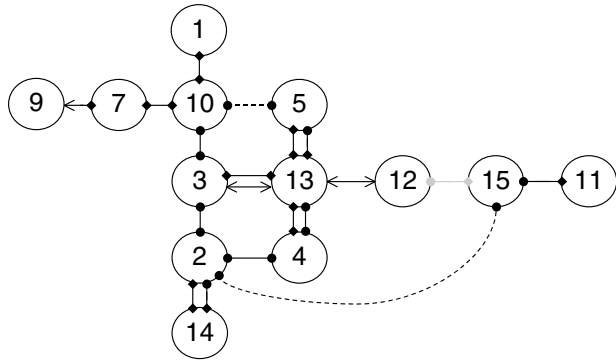
Fig. 4. Graphical representation of the interactions detected between the QTLs affecting growth in a broiler × layer cross. Circles indicate QTLs and the number in the circle is the relevant QTL number given in Table 1. The connectors between the circles indicate two-locus interactions, where black connectors indicate significant epistatic interactions and grey connectors indicate interactions where the significance threshold is not reached but where inspection of the genotype means indicate that the QTL pair belongs to one of the four interaction clusters identified. Dashed connectors indicate additive-like interactions. The style of the ends of the connectors indicate the trait for which the interaction was significant: circular, body weight at 3 weeks; square, body weight at 6 weeks; arrow, body weight at 9 weeks; circular + square, growth between 3 weeks and 6 weeks; square + arrow, growth between 6 weeks and 9 weeks.

methods to screen for epistasis to true multi-dimensional searches (Carlborg *et al.*, 2001; Carlborg, 2002; Ljungberg *et al.*, 2002). We have previously shown by simulations that simultaneous mapping of multiple epistatic QTLs has the potential to increase the power to map interacting QTLs (Carlborg *et al.*, 2000; Carlborg & Andersson, 2002). The benefits of using this method are, however, not universal. Because the true genetic architecture of complex traits, and therefore the impact of epistasis, is unknown, the potential benefit of using these methods can only be assessed when they are applied in analyses of experimental data. High power to detect epistasis can only be expected in reasonably large datasets with high-quality phenotypic measurements and highly informative markers. Owing to limited practical experience from applying these methods to experimental data, the practical usefulness and limitations of the method in experimental datasets are still largely unknown. The analysis of this dataset serves as an exploration of potential use of the method in a reasonably sized experimental dataset, which was not initially designed for detection of epistasis. The model selection procedures used in this study were based on stringent, population-based genome-wide thresholds in order to control the rate of false-positive epistatic QTL pairs. We felt that this was justified, because this study is one of the first aiming to detect genome-wide epistatic QTLs and it is important to avoid making

inferences and recommendations for future studies based on false-positive QTLs. This does limit the power of the study but, when the method has been more thoroughly evaluated, other thresholds can be used to obtain a balance between type I and type II errors that is suitable for each individual experiment.

The first application of the QTL mapping method used in this study was for analyses of growth traits from an exotic cross between the Red Jungle Fowl and a White Leghorn layer (Carlborg *et al.*, 2003). The use of the method increased the number of QTLs detected dramatically, and epistasis was shown to be a large contributor to the genetic variance of early growth in that cross. The study described in this report serves two purposes. First, we analysed a set of similar growth traits in a different chicken cross between broiler and layer chickens. By doing this, we hoped to evaluate further the importance of epistasis in chicken growth and to identify genetic mechanisms underlying detected epistasis. Second, this dataset is significantly smaller (466 rather than 752 F_2 individuals) and the results from this study will indicate the potential of the method in the more moderately sized experiments that are common used.

The previous analyses of the growth traits in this dataset (Sewalem *et al.*, 2002) were based on an older version of the dataset and different combinations of background QTLs and fixed effects in the models used for analyses. Despite this, a brief comparison of the results from these studies shows that the two studies together report 16 QTLs as significant using a 20% genome-wide significance threshold. Sewalem *et al.* (2003) detected 12 QTLs, one of which was unique to that study, and we here report 15 QTLs, four of which could only be detected using SIM and were unique to this study. Two of these unique QTLs were, however, detected for growth rate at 3–6 and 6–9 weeks of age, which have not previously been analysed.

Both the number and the significance of epistatic QTLs were lower in this cross than in the exotic cross between the Red Jungle Fowl and a White Leghorn. This is expected because this cross has about 300 fewer F_2 individuals and a sparser genetic map (average marker spacing is more than 15 cM greater). There are also fewer unique QTLs detected by the SIM procedure and this could be due to the decrease in power caused by the above reasons, but also to the considerably shorter time since the divergence of the broiler and layer than of the domesticated chicken and its wild ancestor (a few hundred compared with several thousand years). This could influence the opportunities for co-adaptation of genes within the lines that might be one cause of the large amount of epistasis detected in the more exotic cross. However, a significant amount of epistasis was still detected in this study, which implies that epistasis is a rather important mechanism for generation of poultry lines in

general, and that certain favourable allelic combinations occur at high frequencies within the lines. The creation of an experimental cross creates new allelic combinations, which in turn increases the power to detect epistasis. Further studies are needed to evaluate how much epistasis that is segregating within natural chicken populations.

In the Red Jungle Fowl × White Leghorn cross, epistasis was found to be very influential on early growth (8–46 days of age), whereas the importance of epistasis on later growth was low. In the broiler × layer cross, the largest total genetic and epistatic contribution to growth is to the bodyweight at 6 weeks of age (42 days of age) and to growth between 3 weeks and 6 weeks of age (21–42 days). There also seems to be a discrete set of epistatic QTLs involved in earlier growth. This study is therefore consistent with the previous finding that there could be different genetic regulation of early and late growth in chickens, and that epistasis is more important for early than for late growth.

There were 101 unique QTL pairs detected in the Red Jungle Fowl × White Leghorn cross, and of the 21 unique pairs detected in the broiler × layer cross, ten mapped to the same chromosome pairs and six mapped to closely linked marker intervals in the two crosses. When the genotype–phenotype relationships were compared between the crosses for these pairs, the Leghorn alleles for one pair (chromosome 1, 417 cM, and chromosome 13, 7 cM) appeared to have a very similar phenotypic effect in both a broiler and a Red Jungle Fowl background.

The marker spacing in this cross is on average 40 cM and, owing to this, there are relatively large proportions of the genome where the genetic information for detecting a QTL is low. Several QTLs have been located in low information regions both as single QTLs and as part of epistatic QTL pairs. The method used for mapping epistatic QTL pairs is designed to detect significant additional variation explained by an epistatic QTL model. There is no evidence here that would suggest that the additional variation explained for the pairs is due to low information content. On the contrary, there is an indication that the additional QTLs found are in most cases located in more informative regions in the genome than the QTLs detected by their marginal effects. There is furthermore no evidence that segregation distortion is more common in the regions detected using the simultaneous mapping procedure. There is also no evidence that there was a deviation from normality within the QTL genotype classes of the detected epistatic QTL pairs. This observation strengthens the evidence that the method is robust when applied to real data.

Close linkage causes some genotype combinations to be rare, which could cause problems in estimating genetic interactions. Several QTLs were detected on chromosome 1 but only in one pair did the epistatic model fit significantly better than the marginal effects model. In that specific case, the QTLs were located 226 cM apart (chromosome 1, 147 cM, and chromosome 1, 373 cM), which means that they are virtually unlinked. For the QTLs that were located closer than that, no interactions were detected, which could be because either there are no interactions or there is a lack of recombination and hence limited information means that the power is too low to detect interactions.

The genotypic patterns for means of the detected QTL pairs suggested four clusters of pairs with similar patterns of genotype–phenotype expression. The first group contain pairs where several (and in some instances all) homozygote–heterozygote genotype combinations have inferior phenotypes. In the estimates for the two-locus interaction model, this type of genotype pattern becomes apparent by large estimates of the two single-locus dominance and the dominance-by-dominance interaction terms. For example, almost all of the variation for the single epistatic QTL pair for growth at 6–9 weeks of age (chromosome 3, 63 cM, and chromosome 5, 127 cM) is due to the dominance and dominance-by-dominance components. The underlying genetic mechanism for this is unclear but the relatively frequent occurrence of the pattern indicates that there could be some general mechanism that causes this phenomenon.

A second commonly occurring interaction pattern is where the hybrid genotypes (i.e. genotypes that contain at least one heterozygote genotype) have higher phenotypes than both double homozygotes. The broiler genotype has a higher phenotypic effect on growth than the layer genotype in all genotypic combinations. This pattern indicates a pair of QTLs with a heterosis-type interaction. One possible explanation for this could be that the broiler line is fixed for an allele with deleterious effect on growth in homozygous form and that the layer allele is able to complement this allele in the hybrid individuals. Five of the six pairs that exhibit this pattern contain QTLs located on the distal end of chromosome 1 (three pairs with one of the QTLs located around 400 cM and two pairs with one of the QTLs located around 455 cM). This similarity could indicate that these QTLs are the same, even though their estimated locations are more than 50 cM apart.

A third group reflect QTL pairs with a genotype–phenotype pattern with a smooth transition from low phenotypes for Leghorn double homozygotes to high phenotypes for broiler homozygotes. The deviation from additivity and dominance for these pairs is generally due to a non-linear ('multiplicative') rather than a linear ('additive') increase in the phenotypic values with genotype. This pattern indicates a co-adaptation between the alleles at the two loci, where the broiler double homozygote was associated with the highest phenotypic values.

The fourth identified group involves two pairs where there are three levels of phenotypes – high, medium and low. One of the pairs (chromosome 1, 150 cM, and chromosome 18, 13 cM) showed a pattern where a high phenotype is expressed when either or both of the loci contain the broiler homozygote and the other loci contain one broiler allele (BBB– or B–BB). The intermediary phenotype was expressed only by the layer double homozygotes (LLLL), and the rest of the genotypes express a low phenotype. Biologically, this could indicate an inhibitory action on growth by the layer alleles at these loci, unless they are present in the double homozygote (where the inhibition is lower) or it is overridden by homozygote broiler alleles from either locus. The second pair in this group has a similar appearance but is more difficult to interpret genetically.

For some of the QTL pairs it is, however, not possible to cluster or find immediate biological explanations for the patterns of the genotypic means. This could be due to our limited knowledge about the relationships between gene interactions and phenotype. It could also be due to violations of assumptions made in the QTL mapping procedure (e.g. segregation of multiple QTL alleles within the original lines, existence of multiple linked genes in the QTL region or simply poor estimates of the genotypic effects owing to chance or low information content at the genomic location of interest). The results for most QTL pairs will therefore only be an estimate of the importance of epistasis for the combined effects of the two genomic regions and aid in the selection of genotypes for further genetic characterisation of these regions.

By creating a figure joining pairwise interacting QTLs, we obtained a visual representation of the complexity of the genetic network behind the analysed traits. The pairs that were detected or assigned to be epistatic in this study are connected as shown in Fig. 4. The interpretation of this figure is speculation until the true genetic components of each QTL have been identified, but it is possible to suggest alternative interpretations of the figure. It could be viewed as an enzymatic chain (the eight connected horizontal QTLs) in which each step is affected by the result of the enzymatic processes that precede and proceed from that step. The branches represent modulators of the enzymatic chain or provide alternative substrates for the chain. The loops (and especially the loops involving non-linear additive type of epistasis) indicate feedback inhibitors or accelerators of the enzymatic activity. An alternative interpretation is that the QTLs in the centre of the chain involved in most interactions are central to the process of growth (e.g. for deposition of protein or fat). There are then several branches (enzymatic chains) leading to these QTLs and providing substrates for the growth process. The loops could also in this scenario indicate feedback regulation.

A QTL study can be used to find the chromosomal locations that contribute to the variation of the F_2 individuals. It can also be used to predict the genetic effects of individual QTL genotypes. The latter is more difficult because many individuals are needed to draw strong conclusions about the magnitude of the effects. In the Jungle Fowl $\times$ Leghorn cross described by Carlborg *et al.* (2003), about half of the detected QTL pairs had epistasis patterns that conformed to previously described mendelian patterns of epistasis (Ö. Carlborg, unpublished results). The evaluations of the effects of the genotypes of the individual QTL pairs in this cross shows that 17 of the 21 unique QTL pairs can be classified into four clusters of similar types of interactions. From this, we conclude that, even though this study was based on a population with a rather low-resolution genetic map and relatively few individuals in each genotype class, the extra effort to map epistatic QTL pairs and inspection of the genotype class made a valuable contribution to interpreting the results.

The method used for mapping of interacting QTLs is based on detection and estimation of epistatic QTL pairs one at the time. Owing to a high computational demand and the small number of individuals in the cross relative to the number of parameters that would need to be estimated, it is not possible simultaneously to fit all QTLs and to estimate their joint effects. Therefore, some of the QTL pairs that are proposed in this article might not be significant if all parameters were fitted jointly. The major aim of this study is, however, not to describe an optimal method for detection of epistatic QTL but rather to highlight genetically interesting findings that deserve to be further evaluated in future generations in this pedigree (e.g. in an advanced intercross line) as well as to indicate the potential benefits of considering epistasis in genome scans for QTLs. If an experiment was designed with the aim of exploring further the importance of epistasis, we recommend that many individuals and a more informative genetic map should be used. Nonetheless, the results from this study show that this method for mapping epistatic QTLs can be valuable for experimental datasets of limited size that are initially not designed for detection of epistasis.

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