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Age-dependent quantitative trait loci affecting growth traits in Scottish Blackface sheep

G. Hadjipavlou*,† and S. C. Bishop*

*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, Midlothian EH25 9PS, UK. †Institute of Evolutionary Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK

Summary

To dissect age-dependent quantitative trait loci (QTL) associated with growth and to examine changes in QTL effects over time, the Gompertz growth model was fitted to longitudinal live weight data on 788 Scottish Blackface lambs from nine half-sib families. QTL were mapped for model parameters and weekly live weights and growth rates using microsatellite markers on chromosomes 1, 2, 3, 5, 14, 18, 20 and 21. QTL significance (using $\alpha = 0.05$ chromosome-wide significance thresholds, unless otherwise stated) varied with age, and those for growth rate occurred earlier than equivalent QTL for live weight. A chromosome 20 QTL for growth rate was significant from 4 to 9 weeks (maximum significance at 6 weeks) and for maximum growth rate. For live weight, this QTL was significant from 8 to 16 weeks (maximum significance at 12 weeks). A nominally significant chromosome 14 QTL was detected for growth rates from birth to week 2 in the same families and location as an 8-week weight QTL. In addition, at the same position on chromosome 14, a QTL was significant for growth rate for 17–28 weeks (maximum significance at 24 weeks). A chromosome 3 QTL was significant for weights at early ages (birth to week 4) and a growth rate QTL on chromosome 18 was significant from 8 to 12 weeks. Fitting growth curves allowed the combination of information from multiple measurements into a few biologically meaningful variables, and the detection of growth QTL that were not observed from analyses of raw weight data. These QTL describe distinct parts of an animal's growth curve trajectory, possibly enabling manipulation of this trajectory.

Keywords growth, growth model, growth parameter, growth rate, longitudinal trait, marker assisted selection, quantitative trait loci, sheep.

Introduction

Growth is an economically important trait for the sheep industry as it is directly related to meat production. Production of faster-growing lambs would be highly beneficial for producers because, apart from the fact that higher lamb weight would mean greater revenues, it would result in enhanced feed conversion efficiency. This would lead to various benefits including lower production costs, higher product yields, less nitrogenous-waste excretion into the environment and decreased grazing pressure (Cockett *et al.* 2005). Genetic selection is a valuable approach for achieving improved lamb growth. For more effective genetic selection on growth, it is advantageous to identify the genetic loci that influence growth in terms of body weight and weight gain of each animal.

Several studies in livestock (although few in sheep) have reported quantitative trait loci (QTL) associated with growth traits in terms of average daily gain, weight at a specific age, and days to reach a particular weight (e.g. Stone *et al.* 1999 for cattle; Nagamine *et al.* 2003 and Stearns *et al.* 2005 for pigs). The majority of QTL mapping studies have used univariate approaches to detect QTL, treating weights recorded at a particular growth point as separate traits. This is despite the fact that live weights across time comprise a longitudinal trait that is a function of several physiological processes and a composite of phenotypes recorded over time. Thus, strong genetic correlations exist among live weights at different ages (Corva & Medrano 2001; Riggio *et al.* 2008), although patterns of correlations often suggest additional complexity. For example, using sheep data, Riggio *et al.*

Address for correspondence

G. Hadjipavlou, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, Midlothian EH25 9PS, UK.
E-mail: georgia.hadjipavlou@roslin.ed.ac.uk

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(2008) showed that inter-age genetic correlations for live weight, whilst strongly positive, are often different from unity, with the correlation decreasing as the time between the weight measurements increases. Thus, it is likely that distinct loci act on live weights at different growth stages. For the detection of QTL that are associated with growth or live weight, it would be beneficial to simultaneously analyse multiple measurements and take account of the correlation structure of measurements across time.

Fitting growth models on body weight data from different time points and extracting the relevant growth parameters provides a way to combine phenotypic information from multiple measurements into a few variables in a biologically meaningful manner. This approach has been previously applied in livestock (Lopez *et al.* 2000, several species; Schinckel *et al.* 2004, pigs; Lambe *et al.* 2006, sheep). Flexible sigmoid curves, such as the logistic, the Brody, Bertalanffy, Gompertz and Richards curves, often represent the best fitting growth models and are extensively described in the literature for livestock (e.g. Pittroff *et al.* 2008). Many growth curve variables relevant to genetic studies may be derived from such models, describing growth rates and live weights, maximum growth rate and the age at which it is predicted to occur, and mature weight.

In sheep, there is little published information on the genetic control of growth curve variables and the different stages of growth. A few studies have investigated the polygenic components of growth curve parameters using growth models describing weight and growth rate (as the derivative of the weight function) as a function of time, and random regression methodology (Lewis & Brotherstone 2002; Lambe *et al.* 2006). These indicated that growth variables are indeed heritable (Lewis & Brotherstone 2002) and that genetic differences seem to exist among growth parameters of various breeds (Lambe *et al.* 2006). Moreover, the study conducted by Lambe *et al.* (2006) suggested that early growth rate is a different genetic trait to later growth rate. All these postulations support a hypothesis that growth curve variables are under genetic regulation and that they may constitute separate aspects of the complex, longitudinal trait of growth. Validation of this assertion requires a more detailed description of the genetic control of growth. To this end, it would be informative to dissect the genetic loci that underlie growth curve predictors.

To date, no QTL study on growth curve parameters has been performed in sheep or any other livestock species, although a Bayesian procedure for QTL detection using prior information obtained from a growth model was applied in pigs (Varona *et al.* 2005). The main objective of this study was to identify and describe QTL in Scottish Blackface sheep that directly influence longitudinal live weights and growth as a process, using descriptors of growth derived from fitted growth curves. We also aimed to examine whether, for particular growth traits, the effects of different QTL were constant over time or changed as the animals grew. If the latter were the case, we were interested in quantifying how the QTL effects changed over time.

Materials and methods

Animals and traits measured

The population studied has been previously described in detail (Davies *et al.* 2006; Karamichou *et al.* 2006). In brief, the population consisted of 830 Scottish Blackface lambs from nine half-sib families, with progeny per family ranging from 34 to 154 individuals. The animals were bred over a 3-year period (2001–2003). Standard records (such as parentage, day of birth, sex, etc.) and weight measurements at birth and at 4-week intervals after birth (up to 24 weeks) were collected, ranging from 830 to 691 records per time point. The distribution of live weights across age is given in Fig. 1. The complete pedigree for this population contained 4866 animals, with records dating back to 1986.

Genotyping and linkage map construction

Lambs were genotyped for informative microsatellite markers, i.e. markers that were heterozygous in their sire, on chromosomes 1, 2, 3, 5, 14, 18, 20 and 21, as detailed in Davies *et al.* (2006) and Karamichou *et al.* (2006). A linkage map was constructed for the markers on each chromosome using the 'build', 'all' and 'flips' options of CRIMAP version 2.4 (Green *et al.* 1990). The marker order with the highest likelihood was selected in order to construct the consensus linkage map for each chromosome that was subsequently used in all QTL analyses (Tables S1–S8). The linkage maps constructed were in close agreement with those from other mapping studies (Maddox *et al.* 2001; Australian sheep gene mapping website: http://rubens.its.unimelb.edu.au/~jillm/jill.htm).

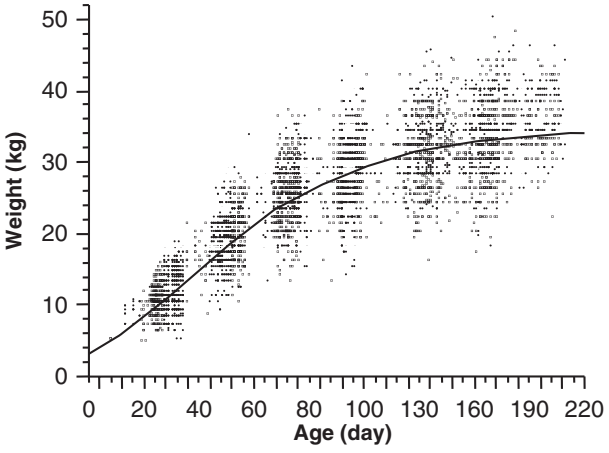

Figure 1 Distribution of live weights of Scottish Blackface lambs across age (dots) and the average growth curve obtained after fitting the Gompertz model to the weight data (solid line).

Phenotypic data treatment

Initially, live weights measured at birth and 4-week intervals were treated as separate traits. Multiple regression analyses were performed on each phenotype (using the R statistical package) in order to identify significant fixed effects. Fixed effects significant for all live weights were sex (two levels), litter size (two levels), age of dam (four levels) and year by management group (i.e. field) (six levels). The day of birth or the age at the time of measurement was fitted as a covariate for each trait. These fixed effects and covariates were fitted in all subsequent regression analyses.

Growth model choice

Five growth functions were fitted to live weight measurements from all 788 animals for which five or more data points were available, using non-linear regression in SAS (release 9.1). The non-linear growth functions fitted were the generalized Michaelis–Menten (GMM) (Lopez *et al.* 2000), the Gompertz, the logistic, the Richards and the exponential models. The Gompertz, logistic and Richards functions have been described and applied by Renne *et al.* (2003) and Lambe *et al.* (2006) and are special cases of a more general model (Turner *et al.* 1976). The reparameterized version of the exponential model has been explained by Bünger & Herrendörfer (1994) and employed by Lambe *et al.* (2006). The formulas and parameter details for the above growth functions are given in Table 1. These parameterizations of growth models were chosen in order to study parameters with direct biological interpretation as explained previously in Renne *et al.* (2003).

Growth model choice followed a similar procedure as described in detail by Pittroff *et al.* (2008). Specifically, each model was first fitted to the dataset as a whole, i.e. one curve fitted to all the data as shown in Fig. 1, and three models were immediately rejected: (i) the logistic model provided a poor fit to the live weight data and it generally would not converge unless one of the model parameters was fixed *a priori*; (ii) the exponential model fitted the live weight data but no estimate for parameter C_E was obtainable; (iii) the Richards model converged for the live weight data, and provided an overall good fit, as assessed by the residual mean square (RMSQ), but resulted in a high correlation between parameter estimates of C and D ($r^2 = 0.98$). The GMM and Gompertz models for growth provided a good overall fit to the data, low correlations were observed between the estimated parameters and the RMSQ were comparable for the two models. Thus, the Gompertz and GMM models were used to model live weights for each animal separately, using both the NLIN procedure of SAS (release 9.1) and the non-linear modelling procedure of JMP (release 7; SAS Inst.). The JMP procedure provided graphical representation of the fit whilst the model fitting iterations were running, and thus allowed immediate examination of prediction bias, i.e. systematic deviations between the observed and fitted values.

Although the GMM model fitted the whole dataset well, it provided a poorer fit when applied to individual animals, resulting in negative predicted birth weights for some animals and implausible values for mature weight for 80 out of 788 animals, even when convergence was achieved. This characteristic was also observed by Pittroff *et al.* (2008). Therefore, the Gompertz growth model was chosen as it converged for each of the 788 animals in the dataset and had low apparent prediction bias, i.e. few systematic deviations between the observed and fitted values.

The Gompertz model was then used to predict live weights and growth rates at weekly intervals from birth up to 24 weeks of age for all 788 lambs. In addition, maximum growth rate and time at maximum growth was estimated for each lamb. The following equations were used:

$$y(t) = Ae^{\{-e^{[Be(C-t)/A]}\}} \text{ and } dy/dt = [(B/A)e]y(t)\ln[A/y(t)],$$

where $y(t)$ is the live weight at time t; A is the estimated final body weight, kg; B is the maximum growth rate (average daily gain), kg/day; C is the age at maximum growth rate, days; and A/e is the live weight at maximum growth, kg.

Table 1 Growth model equations.

Growth function	Parameters	$y(t)$[1]
Generalized Michaelis–Menten[2]	W_o, W_f, K, c	$(W_oK^c + W_ft^c)/(K^c + t^c)$
Gompertz[3]	A, B, C	$Ae^{\{-e^{[Be(C-t)/A]}\}}$
Logistic[3]	A, B, C	$A\{1 + e^{[4B(C-t/A)]}\}^{-1}$
Richards[4]	A, B, C, D	$A\{1 + De^{\{[B(C-t)(D+1)^{1+1/D}]/A\}}\}^{1/D}$
Exponential[5]	A, B_E, C_E	$A - (A - C_E)e^{[B_Et/(A-C_E)]}$

[1] $y(t)$ is the live weight at time t.
[2] Described by Lopez *et al.* (2000).
[3] Reparameterizations described by Renne *et al.* (2003).
[4] Reparameterized by E. Schönfelder, Institut für angewandte Tierhygiene, Eberswalde, Germany (Lambe *et al.* 2006).
[5] Reparameterized as suggested by Bünger & Herrendörfer (1994).

Half-sib QTL regression model

QTL analyses were conducted using a univariate multimarker approach for interval mapping in half-sib families, as described by Knott *et al.* (1996) and applied by the web-based software package QTL EXPRESS (Seaton *et al.* 2002). The probability of inheriting a particular sire allele was calculated at 1-cM intervals for each offspring, conditional on the marker genotypes of the individual and its sire and on the sire's linkage phase. Subsequently, the trait phenotype was regressed on the conditional probability of the offspring genotypic inheritance for a given position. For each regression, an F-ratio of the full model including the significant fixed effects (sex, litter size, age of dam and year by group),

covariate (day of birth/age at measurement) and the inheritance probabilities vs. the same model without the inheritance probabilities was calculated. The chromosomal location with the largest *F*-ratio was taken to be the best estimated position for a QTL for each trait. In addition, the within-sire substitution effects for each sire family were obtained from the analysis. An estimate of the overall QTL effect was obtained by calculating the average of the absolute values of the QTL allelic substitution effects across families for which the QTL was significant. The proportion of phenotypic variance (V_p), corrected for all fitted fixed effects and covariates, that was explained by the QTL for each trait, was estimated as $4 \times (1 - \mathrm{RMSQ}_{full}/\mathrm{RMSQ}_{reduced})$ (Knott *et al.* 1996), where 'full' is the model with the QTL effect fitted and 'reduced' is the model without the QTL effect.

Significance thresholds

Chromosome-wide empirical threshold values were determined for the test statistics obtained from the regression analysis at $\alpha = 0.05$ and 0.01 by applying 1000 chromosome-wide permutations for each trait (Churchill & Doerge 1994). Threshold values varied between chromosomes depending on their length and marker content. For all chromosomes, the nominal threshold for significance was determined for a single test for QTL detection, using the *F*-ratio ($P < 0.05$) for the model including the QTL as a fixed effect [(9, 711–750) d.f.]. Thus the nominal *F*-ratio was set to 1.89.

Traits analysed

Prior to QTL analysis, the distribution of each of the extracted model variables (*A*, *B*, *C*) was examined and extreme outliers deviating more than three standard deviations from the mean were removed. The distribution of the *C*-variable was skewed, and, therefore, the *C* estimates were transformed using a natural log transformation. All other parameters were analysed without transformation as they appeared normally distributed.

Live weight at birth and each of 4-week intervals was subjected to univariate interval QTL mapping for each chromosome using data and marker genotype information at each chromosome, as described above. Moreover, each of the Gompertz model parameters [*A*, *B*, ln *C*], predicted growth rates and live weights at weekly intervals and at maximum growth (point of inflection), were analysed for QTL detection.

Results

Gompertz growth model description

The growth curve resulting from fitting the Gompertz model to the combined live weight data from all animals is shown in Fig. 1. For each model fitted and converged, the RMSQ are shown in Table 2 along with *F*-ratios for the fit of the model. The means for Gompertz model parameter estimates (averaged across the predicted values for each of the 788 animals), their standard errors and asymptotic (i.e approximate) 95% confidence intervals are given in Table 3. Live weights were predicted at each 4-week time point for the 788 animals for which the Gompertz model was fitted, and no significant differences were found between the predicted and observed values at any age, using a *t*-test (results not shown).

QTL results

All QTL for observed live weight whose significance exceeded the 5% chromosome-wide threshold are reported in Table 4, along with significant QTL for the Gompertz function parameters. The live weight QTL were detected on the complete dataset and no changes in the location or significance of these QTL were observed when the dataset of observed live weights was reduced to the 788 animals included in the Gompertz model procedure (results not shown). Genetic linkage map positions of genetic markers for the ovine chromosomes studied are shown in Tables S1–S8.

Table 2 Non-linear regression least square statistics for each model that fitted the entire live weight dataset[1].

Model	RMSQ	*F*-ratio	Numerator d.f.[2]	*P*-value
Gompertz	1569	68 769	3	<0.0001
Generalized Michaelis–Menten	1538	52 648	4	<0.0001
Richards	1551	52 173	4	<0.0001
Exponential	12 085	10 975	3	<0.0001

[1]Model equations and the parameters fitted for each model are described in Table 1.

[2]Numerator degrees of freedom (d.f.) corresponded to the number of parameters fitted for each model. Denominator d.f. corresponded to the residual d.f. and ranged from 5549–5551 across models.

Table 3 Estimated means and standard errors for Gompertz model parameters and weight at point of inflection[1].

Parameter	Sample mean	Approx. SEM	Approx. 95% confidence interval
A (kg)	35.1	0.19	34.72–35.47
B (kg/day)	0.284	0.002	0.280–0.288
C (days)	36.9	0.32	36.27–37.54
ln *C* (ln days)	3.58	0.009	3.559–3.594
Weight at point of inflection (kg)	12.9	0.08	12.77–13.07

SEM, standard error of the mean.

[1]The average of individual lamb means, predicted after fitting the Gompertz model to live weight measurements for each animal, was estimated for each parameter.

Table 4 Summary of the significant QTL for observed live weights and growth curve functions from across-family univariate QTL analyses.

Trait	Chromosome	QTL position (cM)[1]	Marker interval	*F*-ratio[2]	Families significant[3]	% V_p explained by QTL[4]	QTL effect[5]
Birth weight (kg)	14	82	*BMS833*	4.13 (2.76, 3.38)	1, 6	14	0.912
8-week weight (kg)	14	110	*ILSTS002–LSCV30*	2.8 (2.47, 3.00)	3, 8	9	2.51
16-week weight (kg)	20	61	*DRB1–TGLA387*	2.53 (2.47, 3.03)	4, 6	8	4.63
Growth rate at point of inflection (*B*) (kg/day)	20	60	*DRB1–TGLA387*	2.92 (2.41, 2.92)	5, 6	9	0.054
Age at point of inflection (*C* or ln *C*)	14	98	*ILSTS002–LSCV30*	3.18 (2.37, 2.81)	8	11	ln *C* = 0.1657 *C* = 1.18
(days or ln days)	5	123	*MCM527–CSRD2134*	2.41 (2.33, 2.78)	4, 8	7	ln *C* = 0.1661 *C* = 1.18

[1]QTL position is defined relative to the first marker present in the genetic map for each chromosome; first marker positioned at 0 cM.
[2]Chromosome-wide *F*-statistics for $P < 0.05$ and <0.01 (as determined by permutation testing) are given in parentheses.
[3]Families within which a QTL effect was deemed significant using a *t*-test, when the half-sib QTL regression model was fitted across families.
[4]V_p refers to the phenotypic variance for each trait, after correction for all fitted fixed effects and covariates. The proportion of V_p due to the QTL was estimated as $4 \times (1 - \text{RMSQ}_{\text{full}}/\text{RMSQ}_{\text{reduced}})$, where 'full' is the model with the QTL effect fitted and 'reduced' is the model without the QTL effect (Knott *et al*. 1996).
[5]QTL allelic substitution effect, determined as the average of the estimated absolute values across families for which the QTL was significant. It corresponds to the difference in trait values between the two QTL alleles that can be inherited from a sire heterozygous for the QTL.

For predicted live weight and growth rates, the detected QTL tended to be significant over a period of 2 weeks or more; *F*-ratio trajectories for these QTL are shown in Figs 2–5, for chromosomes 20, 14, 3 and 18 respectively. A summary of these QTL is provided in Table 5, giving results for the time points at which the QTL were of maximum significance. Chromosome-wide significance thresholds varied marginally over time, and the maximum observed threshold on each chromosome across age for the relevant traits is plotted in each figure. The *F*-statistic presented in the trajectories in Figs 2–5 is for the best estimated position at each time point. Along each growth trajectory, this position was consistent to within 1–3 cM. More importantly, the QTL resided in the same marker interval and was detected in the same families for all plots (connected points) for predicted growth rate or weight.

The significance trajectories for a QTL on chromosome 20 for predicted growth rates and weights across age are given in Fig. 2. The chromosome 20 QTL for growth rate became significant (at the chromosome-wide level) at 3 weeks, its significance maximized at 6 weeks and it retained at least nominal significance up to 9 weeks (Table 5). A QTL located in the same marker interval on chromosome 20 and in the same families (5 and 6) was also highly significant for maximum growth rate (parameter *B* of the Gompertz model; Table 4). This QTL was apparent for predicted weight at a later age, being nominally significant from 8 to 17 weeks, with the highest *F*-ratio at 12 weeks (segregating in families

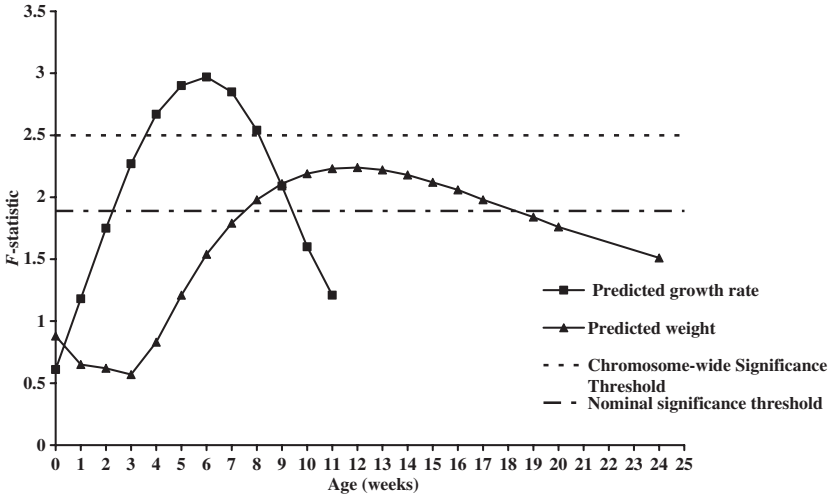

Figure 2 Across-age significance of QTL on chromosome 20 for live weights and growth rates, predicted using the Gompertz curve for weekly intervals. The chromosome-wide significance threshold ($P < 0.05$) was determined by permutation testing. The nominal threshold for significance ($P < 0.05$) was estimated for a single test. The estimated QTL position and the QTL-segregating families for each trait are given in Table 5.

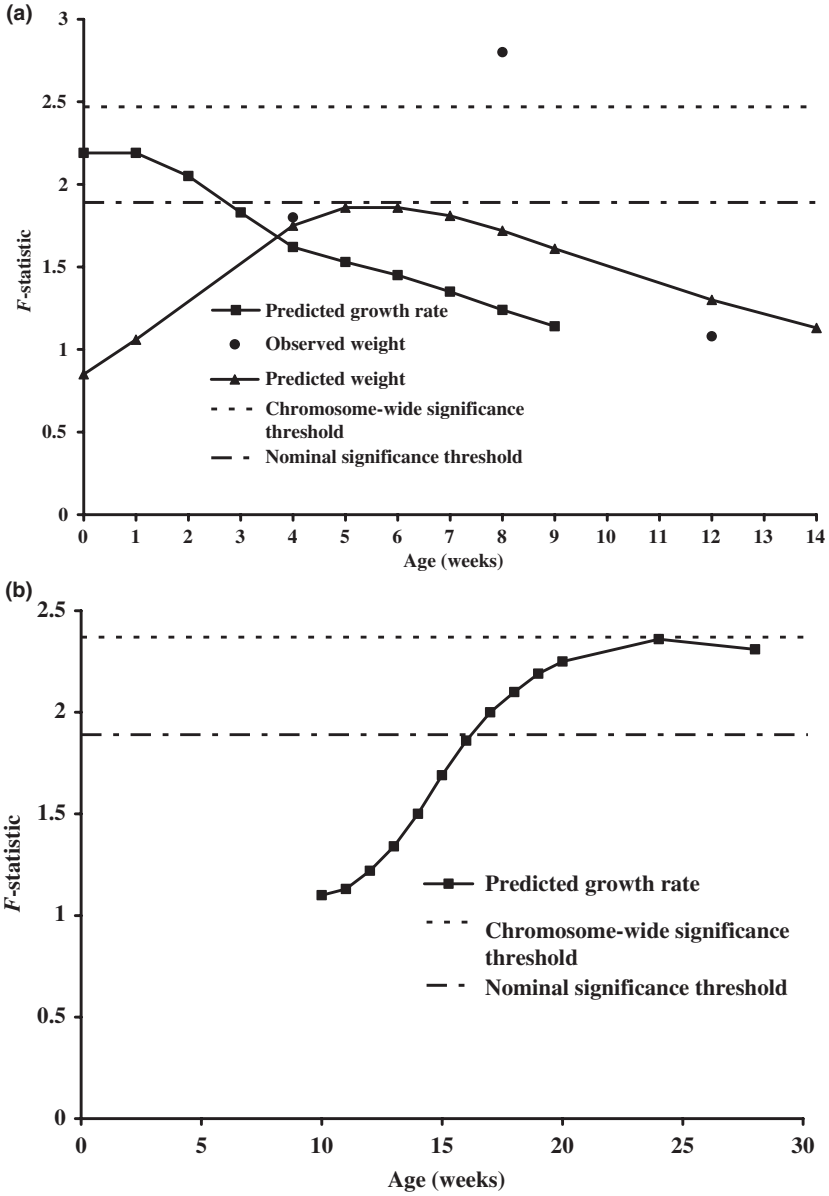

Figure 3 Across-age significance of (a) early and (b) late growth QTL on chromosome 14. Live weights and growth rates were predicted using the Gompertz curve for weekly intervals. The chromosome-wide significance threshold ($P < 0.05$) was determined by permutation testing. The nominal threshold for significance ($P < 0.05$) was estimated for a single test. The estimated QTL position and the QTL-segregating families for each trait are given in Tables 4 & 5.

4 and 6). For observed live weight, this QTL (i.e. same position and segregating in the same two families; 4 and 6) was observed at 16 weeks (Table 4). For observed weight at 12 weeks, QTL segregation was significant in one of the two families (family 6; *t*-test = 2.85) but did not reach nominal significance across all nine families (*F*-ratio = 1.79). In summary, a growth rate QTL on chromosome 20 was observed at and around the estimated age of maximum growth, which subsequently manifested itself as a live weight QTL.

Significant QTL were detected on chromosome 14 for observed birth weight and 8-week weight, although the families in which these QTL segregated differed. Although QTL for predicted weight at birth and 8 weeks were not deemed significant across all families, a birth weight QTL segregated in family 6 and a QTL at 8 weeks was significant in the same families (3 and 8) as for the observed 8-week QTL. Further, a nominal QTL on chromosome 14 was detected for growth rates at birth and weeks 1 and 2 in families 3 and 8; these being the same families and chromosomal location as seen for the 8-week weight QTL. Thus, the trend of *F*-ratio trajectories (Fig. 3a) is the same as seen on chromosome 20, i.e. the QTL significance varies with time and statistical significance for growth rate is seen at an earlier age than that for live weight. A further chromosome 14 QTL became nominally significant in families 8 and 9 for growth rate at 17 weeks with maximum significance at 24 weeks (Fig. 3b). This QTL may represent a separate locus associated with late growth; however, it is possible that different allelic effects for the same QTL are

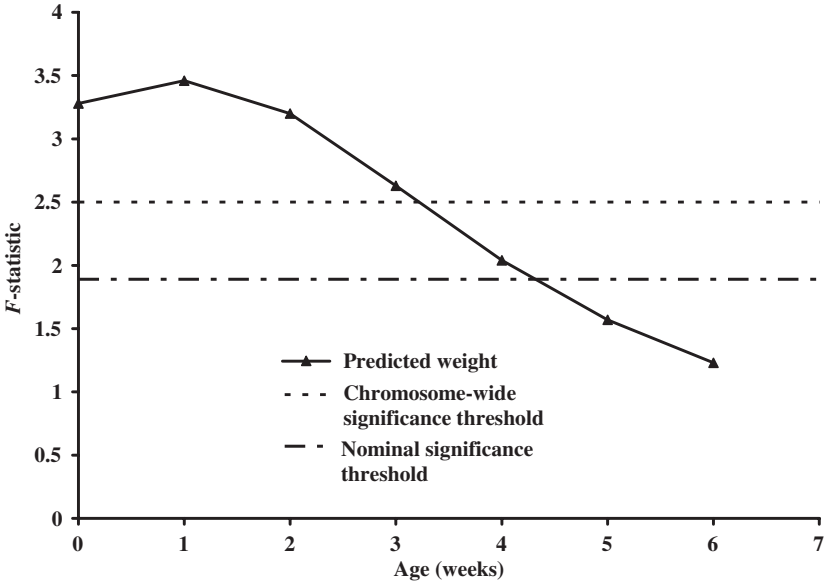

Figure 4 Across-age significance of QTL for predicted weight on chromosome 3. Live weight was predicted using the Gompertz curve for weekly intervals. The chromosome-wide significance threshold ($P < 0.05$) was determined by permutation testing. The nominal threshold for significance ($P < 0.05$) was estimated for a single test. The estimated QTL position and the QTL-segregating families are given in Table 5.

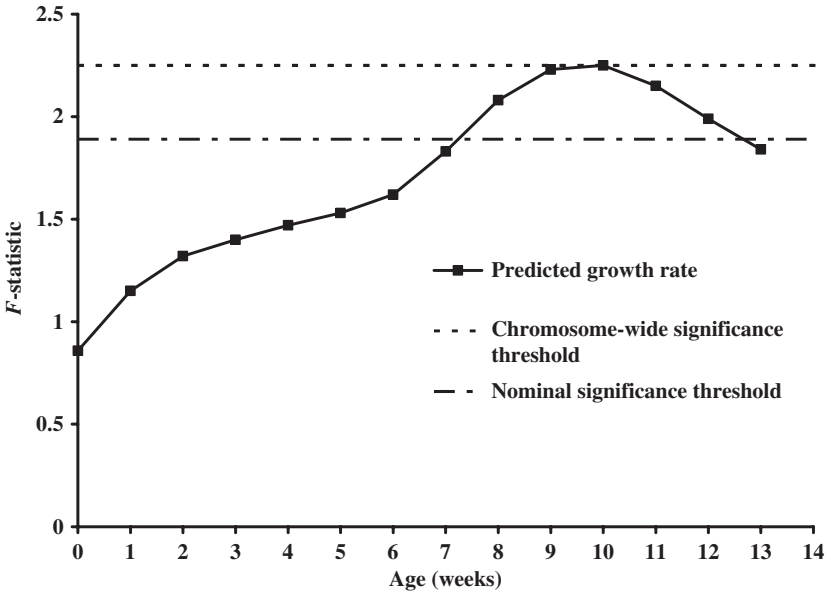

Figure 5 Across-age significance of QTL for growth rate on chromosome 18. Growth rates were predicted using the Gompertz curve for weekly intervals. The chromosome-wide significance threshold ($P < 0.05$) was determined by permutation testing. The nominal threshold for significance ($P < 0.05$) was estimated for a single test. The estimated QTL position and the QTL-segregating families are given in Table 5.

being detected for early and late growth rate. This hypothesis is supported by the fact that in family 8, segregation for the QTL was statistically significant in both growth stages, with the estimated QTL effects for the two occasions being of opposite sign.

Two additional chromosomes yielded significant QTL for growth rate or live weight. The *F*-ratio trajectory across age of a QTL for live weight on chromosome 3 is shown in Fig. 4. This QTL was highly significant for estimated weights at early ages (birth up to week 4) and significant within (but not across) the same three families (2, 3, 8) for observed weights at these ages. Yet, this QTL was not detected for predicted growth rates at any age point. A growth rate QTL on chromosome 18 was of nominal significance at 8 weeks (Fig. 5). The *F*-ratio for this QTL increased with age and became significant chromosome-wise for growth rate at 10 weeks. The QTL significance remained nominal up to 12 weeks. No QTL was detected on this linkage group for observed or predicted live weights within the age range of our dataset.

Finally, two QTL were detected for the log-transformed age at point of inflection (population mean parameter *C* is 36.9 ± 3.02 days; Table 3). These QTL were seen on chromosomes 14 and 5 (Table 4). No significant QTL were found for estimated final weight (parameter *A*).

Inspection of the estimated QTL effects for each observed or predicted growth trait (Tables 4 & 5) suggests that the size of the detected QTL effects, in terms of the proportion of

Table 5 Summary of the significant QTL from across-family QTL analyses of predicted live weights and growth rates, at the time point when the significance was maximum.

Trait	Chromosome	QTL position (cM)[1]	Marker interval	*F*-ratio[2]	Age range of significance (weeks)[3]	Families significant[4]	% V_p explained by QTL[5]	QTL effect[6]
1-week weight (kg)	3	213	*AGLA293–BL4/LYZ*	3.46 (2.49, 2.86)	Birth to 4	2, 3, 8	12	0.696
12-week weight (kg)	20	59	*DRB1–TGLA387*	2.24 (2.47, 2.93)	8–17	4, 6	6	3.19
1-week growth rate (kg/day)	14	105	*ILSTS002–LSCV30*	2.19 (2.32, 2.98)	1–2	3, 8	6	0.034
6-week growth rate (kg/day)	20	60	*DRB1–TGLA387*	2.97 (2.45, 2.96)	3–9	5, 6	10	0.051
10-week growth rate (kg/day)	18	59	*TGLA337–TGLA122*	2.25 (2.23, 2.77)	8–12	3, 7	6	0.025
24-week growth rate (kg/day)	14	99	*ILSTS002–LSCV30*	2.36 (2.37, 2.90)	17–28	8, 9	7	0.017

[1]QTL position is defined relative to the first marker present in the genetic map for each chromosome; first marker positioned at 0 cM.
[2]Chromosome-wide *F*-statistics for $P < 0.05$ and <0.01 (determined by permutation testing) are given in parentheses.
[3]Refers to chromosome-wide significance for all traits apart from 12-week weight and 1-week growth rate for which the age range of nominal significance is given.
[4]Families within which a QTL effect was deemed significant using a *t*-test, when the half-sib QTL regression model was fitted across families.
[5]V_p refers to the phenotypic variance for each trait, after correction for all fitted fixed effects and covariate. The proportion of V_p due to the QTL was estimated as $4 \times (1 - RMSQ_{full}/RMSQ_{reduced})$, where 'full' is the model with the QTL effect fitted and 'reduced' is the model without the QTL effect (Knott *et al.* 1996).
[6]QTL allelic substitution effect, determined as the average of the estimated absolute values across families for which the QTL was significant. It corresponds to the difference in trait values between the two QTL alleles that can be inherited from a sire heterozygous for the QTL.

phenotypic variance explained by the QTL at its maximum significance, remained roughly constant across QTL detected on different linkage groups. However, the absolute size of distinct QTL effects varied according to the trait means, i.e. live weight QTL effects increased with age, whereas the growth rate effects initially increased, then declined as growth rate slowed.

Discussion

Growth and its manifestation, live weight, are quantitative traits of major importance for livestock species. Yet, although it is an easily recorded phenotype, live weight is a complex trait because it is the integral of all growth rates prior to that age point, which in turn are a function of the animal's health, physiological state, strength of immune system and even its ability to compete for sometimes limited nutritional resources. Therefore, it would be beneficial to determine aspects of growth governed by genetic effects, and to utilize this information for genetic improvement. In order to dissect the genetic components of growth, we: (i) utilized longitudinal live weight information available for growing lambs and (ii) defined informative descriptors of growth and its components.

Univariate QTL analyses of sheep live weight phenotypes at a particular point in time have been performed previously (e.g. Walling *et al.* 2004; McRae *et al.* 2005). However, isolation of live weights as single traits fails to capture the correlations between the components underlying growth. As a result, univariate studies have reduced power to detect QTL compared with techniques that combine information from multiple or longitudinal phenotypes.

Multivariate QTL models have previously been developed for longitudinal traits. These approaches have either transformed multivariate data into a single summary or composite measure (e.g. Weller *et al.* 1996; Gilbert & Le Roy 2003), or have modelled the time dependent QTL effects. As an example of the latter, Ma *et al.* (2002) and Wu & Hou (2006) described a maximum likelihood method for simple genetic structures (backcross, F_2, full-sibs). In this method, the QTL was assumed to be a fixed effect with a specified number of alleles. This approach was applied to study growth QTL in mice and forest trees (Wu *et al.* 2004a,b, 2005). Alternatively, a longitudinal method using random regression was described by Lund *et al.* (2002) for animals and Macgregor *et al.* (2005) for human populations. Both modelled a multi-allelic QTL as a random effect using random regression. This method enabled the analysis of more general pedigrees. Recently, Lund *et al.* (2008) extended their model to allow for a genome scan for QTL, testing their methodology against univariate QTL analysis. Overall, these approaches allowed for a substantial increase in power for QTL detection in longitudinal data. Yet a number of issues arise when applying these techniques, mainly regarding computational time and statistical difficulties in estimating many model parameters simultaneously. These issues are affected by the modelling choice for the QTL effect (fixed or random) and the need in random regression models to fit polynomials of different order for random QTL effects in order to capture fluctuations in the QTL variance over time. In addition, it is usually difficult to assign biological meaning to the polynomials chosen to describe the QTL effect fitted in the model.

We chose an alternative approach for overcoming the limitations of univariate QTL studies of longitudinal traits.

We sought to apply a multivariate method for the extraction of all phenotypic information present in longitudinal data and to then decompose the data in simpler but more informative variables for QTL analyses. For this purpose, we first modelled live weight measurements over time using growth curve functions, of which the Gompertz curve was found to be the most appropriate. By fitting the Gompertz model, we used information on weight measurements over time to estimate three parameters: *A* (weight at maturity); *B* (maximum growth rate); *C* (age at maximum growth rate). Thus, the use of the growth model allowed a reduction in the number of independent traits. Subsequently, predictions for weekly weights and growth rates were combinations (non-linear and linear respectively) of the three estimated model parameters. In this respect, a multiple testing issue was largely avoided when performing the QTL analyses.

Growth curve equations had previously been employed to describe growth patterns, estimate growth parameters and dissect polygenic (and maternal genetic) components of growth variables in sheep (Lewis & Brotherstone 2002; Lambe *et al.* 2006; Molina *et al.* 2007) and other species (Wang & Zuidhof 2004). We extended the application of growth models by treating growth curve variables or predictions as traits for QTL studies. An analogous approach was employed by Rodriguez-Zas *et al.* (2002) to detect QTL for dairy traits. In their study, they used a non-linear lactation curve to model individual production curves and to extract curve parameters. They then detected QTL for the parameters for milk yield, protein and fat percentage, and somatic cell score, within each of (but not across) three grandsire families. Moreover, for some of these QTL, Rodriguez-Zas *et al.* (2002) utilized the lactation equation to estimate the trait values (e.g. milk yield, protein percentage) over time for alternative QTL allelic effects. However, no time-dependent predictions of the studied traits were made from the lactation model and, thus, trends in the overall QTL significance across time were not determined.

The procedure used in this study made no assumptions about the distribution of the QTL effects or their changes across time, and it allowed the detection of various QTL with different expression (significance and variance) patterns across time. QTL on chromosomes 3 and 14 had significant effects during early growth, whereas a QTL on chromosome 20 had significant effects on growth variables around intermediate/maximum growth. A growth rate QTL on chromosome 18 was only significant for later growth points (10 weeks). Further, the QTL on chromosome 14 seemed to have contrasting allelic effects for early and late growth (around 24 weeks of age). Although the possibility that two distinct growth QTL were detected on chromosome 14 cannot be excluded based solely on results from these analyses, our approach would have the ability to detect QTL with alleles that have opposing action on growth in different stages of the animal's development.

The phenotypes analysed in this dataset were a subset of those subjected to a heritability analysis by Riggio *et al.* (2008). Our results, in which QTL effects differ with age, are consistent with the results of Riggio *et al.* (2008) in which inter-age genetic correlations declined as the time period between weight measurements increased.

Another conclusion from the QTL analyses is that growth rate phenotypes may allow more effective detection of growth QTL effects than live weight phenotypes (either actual or predicted). This may well be the result of live weight being the more complex trait, i.e. the integral of all previous growth rates to that point in time. Furthermore, our analyses indicated that the same QTL have significant effects earlier for growth rate than for live weight, explicable by the fact that live weight is completely dependent on previous growth rates. This is apparent in the QTL significance trajectories for growth rate or predicted weight across age on chromosomes 14 and 20 (Figs 2 & 3). This shift to an earlier age for growth rate QTL on chromosomes 14 and 20 is observable because it lies within the age range of live weights present in our dataset and described by the growth curve. Failure to detect growth rate QTL on chromosome 3 and live weight QTL on chromosome 18 actually fits the observed pattern. A growth rate QTL on chromosome 3 QTL would be expected to be significant prior to birth. In an analogous manner, the live weight QTL on chromosome 18 would manifest itself at a later age point than the maximum age accurately covered by our data.

In conclusion, QTL analysis of growth parameters estimated from the Gompertz function provided important insight into growth as a multi-stage process in sheep. Distinct loci seem to be active in at least three stages: early growth, intermediate/maximum and late growth. In addition, our studies revealed a trend by which loci associated with growth are apparent at a younger age for growth rate than for live weight. Finally, as distinct loci govern different growth stages, manipulation of the genetic factors underlying the different parts of an animal's growth curve to achieve distinct growth objectives may indeed be feasible.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Genetic linkage map positions of genetic markers for ovine chromosome 1.

Table S2 Genetic linkage map positions of genetic markers for ovine chromosome 2.

Table S3 Genetic linkage map positions of genetic markers for ovine chromosome 3.

Table S4 Genetic linkage map positions of genetic markers for ovine chromosome 5.

Table S5 Genetic linkage map positions of genetic markers for ovine chromosome 14.

Table S6 Genetic linkage map positions of genetic markers for ovine chromosome 18.

Table S7 Genetic linkage map positions of genetic markers for ovine chromosome 20.

Table S8 Genetic linkage map positions of genetic markers for ovine chromosome 21.

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