Genotype and gender effects on sheep limb bone growth and maturation: selection for loin depth causes bone hypotrophy

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Abstract

This study aimed to compare limb bone growth between offspring of typical crosses used in Australian prime lamb production. Limb bones from sheep of five genotypes – Merino (M × M), Border Leicester sire × Merino (BL × M), Poll Dorset sires selected for growth × Merino (PD_g × M), Poll Dorset sires selected for eye muscle depth (PD_m × M) × Merino, or second cross (PD_g × BLM) – at four time points from 4 to 22 months of age (*n* = 593) were dissected, measured and weighed. Growth curves were fitted within genotype groups and used to compare (i) overall limb bone growth in terms of length and weight, (ii) differences in allometric growth coefficients for individual bones, (iii) relative limb bone proportions, and (iv) maturity proportion. Results showed two distinct phenotypes in terms of limb bone

growth: (i) relative bone hypotrophy of lambs from PD_m × M, suggesting that selection for loin depth (PEMD EBV) may be linked with smaller limb size and that their higher muscle : bone ratio may be due to a relative decrease in bone rather than increase muscle weight; and (ii) Merinos were found to have comparable limb length to terminal sire crosses, although distal limb elements were proportionately longer at the expense of the proximal segments that are associated larger muscles. There was a general lack of major differences in bone growth between sheep very different in other production traits, particularly when compared allometrically. Thus, differences in bone growth, proportion or skeletal maturation were greater between ewes and wethers than between these divergent genotypes. PD_m × M and BL × M were found to be earlier maturing in terms of limb length, although the bone mineral profile (magnesium content) of PD_m × M was suggestive of relative physiological immaturity.

Introduction

The increasing genotypic diversity in the Australian sheep meat industry has seen the emergence of breeds with significantly different growth rates and maturity patterns (Donnelly *et al*. 1985; Fogarty *et al*. 2000). The main genotypes used in Australian lamb meat production are a first-cross of Poll Dorset or Border Leicester terminal sires to Merino ewes, second cross of terminal sires to cross-bred ewes (typically Border Leicester × Merino), and purebred Merino strains. These genotypes are known to differ widely in size and conformation and this is likely to reflect different rates and patterns of skeletal growth. However, it is not known to what extent these differences reflect variation in the proportionate (i.e. allometric) growth of certain parts or in the more general rate of ontogenetic development.

Further refinement of genetic selection has seen the increasing application of estimated breeding values (EBVs) to define specific traits such as eye muscle depth (PEMD EBV) or growth rate (e.g. postweaning weight; PWWT EBV) (Banks 1994). Previous experiments have suggested that selection for loin depth may be associated with relative limb bone hypotrophy (Cake *et al*. 2006*a*), consistent with the assertion that shortening of the distal limb bones is a defining characteristic of 'improved' meat breeds (Hammond 1932).

Expanding export markets and recent findings that optimally processed cuts from older animals can maintain acceptable eating quality scores (Pethick *et al*. 2005) have reinvigorated interest in defining the growth patterns of prime lamb genotypes out to older, and potentially more 'mature', animals. Defining maturity using traditional indices referenced to bodyweight or composition (Berg and Butterfield 1966; McClelland *et al*. 1976; Butterfield*et al*. 1983) becomes problematic when assessing divergent genotypes, particularly terminal sires selected for rapid lean growth. However, it is not clear to what extent alternative, qualitative maturity indices such as physeal closure or dental eruption accurately reflect physiological or even skeletal maturation. An alternative index of maturity may be bone mineral profile, in particular magnesium content which steadily declines in a manner which appears to reflect physiological rather than chronological age (Ho *et al*. 1989; Ravaglioli *et al*. 1996; Cake *et al*. 2006*b*).

The aims of this study were to measure limb bone lengths from ewe and wether representatives of the above mentioned genotypes across four age cohorts spanning the lamb–hogget transition, in order to model and compare absolute and allometric bone growth. This

study represents the limb bone data of a larger 5 × 4 × 2 factorial, genotype × age × gender experiment, details of which are fully described by Hopkins *et al*. (2007).

Materials and methods

Progeny (*n* = 593) of 16 sires from five divergent genotypes were serially slaughtered at four ages (~4, 8, 14 and 22 months). Sire selection was based on sire EBVs for growth and muscle development and consisted of five crosses of Merino (M), Border Leicester (BL) and Poll Dorset (PD), including PD sires selected for high postweaning growth (PWWT) or eye muscle depth (PEMD) EBVs (PD_g and PD_m). PWWT EBV references liveweight at 225 days of age, after adjustment for environmental effects and heritability; PEMD EBV references ultrasonic loin muscle depth at the 12th rib, corrected to 45 kg liveweight and adjusted as for PWWT (Anon. 2004). All ewes were artificially inseminated with thawed frozen semen from the 16 sires, by commercial contractors using laparoscopy. Semen from the four Poll Dorset sires selected for growth (PD_g) was used in both BL × M and M ewes, with the other sires used only across M ewes. Genotype groups were therefore: M × M, PD_m × M, PD_g × M, BL × M, and PD_g × BLM (average of 30 progeny per genotype × age group; range 22–39). One of the Poll Dorset sires selected for loin depth (PD_m, sire 5) was known to be homozygous for the Carwell gene (Hopkins *et al*. 2007). Flock management and nutrition pre- and postinsemination was as described by Hopkins *et al*. (2007). At marking the lambs were vaccinated against clostridial diseases, their tails were docked and the males were castrated. After weaning, lambs were grazed on a combination of lucerne and pasture species with some supplementation, as detailed by Hopkins *et al*. (2007).

Bone analysis

Metacarpal and metatarsal bones were collected from the slaughter chain in a commercial abattoir and stored at 4°C overnight. One day after slaughter the forelimb and hindlimb bones were excised from the left side of each carcass, partially cleaned of muscle tissue, sealed in plastic bags and stored frozen at –20°C for transport to Murdoch University. Upon thawing, bones were disjointed and cleaned of all remaining muscle and connective tissue and immediately weighed on digital scales. Radius and ulna were weighed together. Bone length measurements were made using 300-mm precision calipers (Mitutoyo Corporation, Japan) as previously described in the forelimb (Cake *et al*. 2006*a*), and in the hindlimb between defined landmarks of the femur (major trochanter to lateral condyle), tibia (medial tubercle of the intercondylar eminence to medial malleolus), metatarsal (proximal articular surface to sagittal ridge) and proximal phalanx of digit IV (sagittal groove to distal articular surface).

Mineral analysis

Bone ash calcium, phosphorus and magnesium content were determined to assess the 'mineral maturity' of bone (Grynpas 1993; Ravaglioli *et al*. 1996; Cake *et al*. 2006*b*). Cylinders of cortical bone from the midshaft metacarpal were defatted in ethanol and diethyl ether, then dried (100°C overnight) before ashing in a muffle furnace (36 h at 600°C). For mineral analysis, samples were prepared from 200 mg of ash powder by digestion in aqua regia (1 : 3 conc. HNO_3 : HCl), before further dilution in 1% nitric acid. Analysis was conducted by inductively coupled plasma-atomic emission spectrometry (ICP-AES), using a Varian Vista Simultaneous ICP-AES with a nebuliser and glass-cyclonic spray chamber, and appropriate standards for calibration.

Data analysis

As only the terminal, decelerating phase of bone growth was observed, this was found to be best fitted using the time exponent function $L = a - b(r^t)$ where L is bone length at age t in days, a is the mature length, b is the Y intercept, and r, which was always less than 1 (typically 0.993–0.998), reflects the rate of maturation. This function was fitted iteratively within each sex, using the exponential FITCURVE function in Genstat (VSN International, Hemel Hempstead, UK), and the coefficients (a, b and r) tested for significant genotype effects ($P< 0.05$). Bone length and bone proportion (relative to the summed length of all bones for each limb), were tested for genotype and gender differences within each age group using a linear mixed effects model (SAS v.9.1, SAS Institute Inc.), with sire included as a random term within genotype. As genotype × gender interactions were always statistically significant, all presented data are split by gender.

In order to assess allometric relationships in bone growth within each limb, lengthwise growth of each bone was compared with total limb bone length (sum of scapula + humerus + radius + metacarpal + proximal phalanx length, or femur + tibia + metatarsal + proximal phalanx length). This was done using the log form ($\log y = \log a + b.\log x$) of Huxley's equation $y = ax^b$ (Huxley 1932), where y represents the individual bone and x is the total limb bone length, analysed using a general linear model (GLM) procedure (SAS, v.9.1). In this case, genotype and gender were included in the model (where significant; $P < 0.05$) as fixed effects (thus representing adjustments for the a term), and as interactive terms with total limb bone length (representing adjustment for the$b$ term). Similar allometric modelling was performed for bone weight *v*. hot carcass weight, to assess growth relative to all carcass musculoskeletal components.

Maturity proportion was calculated for both total forelimb and hindlimb length (M_{fore} and M_{hind}) for each animal using the formula $M = \text{L} / \text{L}_a$ where L is the measured total limb bone length and L_a is the mean asymptotic (adult) total limb length determined for each genotype and gender group, i.e. the *a* term of the exponential growth curve described above. This describes a proportion of maturity converging on a mean of 1 (fully mature). Data was insufficient to accurately model *a* by sire. Graphical representations of maturity were generated by fitting an expression of Brody's growth equation (Brody 1945), where: $M = a\,(1 - e^{-k(t-t^*)})$ constrained such that $a = 1$ and where k = decay constant, t = time (age in days), and t^* = time at origin (of decelerating growth phase). Results were separated by gender.

Results

All measured limb bones were significantly shorter and matured earlier in ewes compared with wethers, such that, although the length difference was only 2–3% at 8 months (mean age 236 days, Fig. 1), modelled mature bone lengths (*a*) differed in the range of 6–12% between sexes (Fig. 2). Sexual dimorphism in bone length was greatest overall at 8 months and was statistically significant in the $\text{PD}_\text{m} \times \text{M}$ and $\text{PD}_\text{g} \times \text{BLM}$ genotypes at this timepoint, though for mature lengths sexual dimorphism was greatest for the M × M genotype.

Limb bone lengths differed significantly across the five genotype groups. BL × M and PD_g × M progeny had the longest limb bones at 8 months, particularly for the more distal bones (Fig. 1). By contrast, $\text{PD}_\text{m} \times \text{M}$ progeny had significantly shorter lengths for most bones examined at all time points, with this difference greatest in the more distal bones. For modelled mature lengths, $\text{PD}_\text{m} \times \text{M}$ wethers had significantly shorter bones for all bones

measured (except for scapula, humerus and femur, which did not differ from M × M), while PD_m × M ewes showed a similar but less consistent pattern (Fig. 2). At 8 months, genotype differences in individual bone lengths were significant only for female lambs (Fig. 1). Exponential growth functions solved for $y = a - b(r^t)$ showed that, while wethers differed only in mature length (*a* term), ewes also differed in the *b* term, with the PD_g× BLM genotype having a lower *b* coefficient for most bones, suggesting earlier maturation in terms of prenatal or early antenatal growth (data not shown).

The proportions of each limb bone relative to the greater limb length also differed between genotypes (Fig. 3), with the most notable differences at 8 months being the proportionately shorter proximal bones (scapula, humerus, femur) and proportionately longer distal bones (radius, metacarpal, metatarsal) of M × M progeny. Also notable was the proportionately longer proximal bones of PD_m × M progeny (scapula of ewes, humerus of wethers), and the proportionately shorter distal bones (metacarpal, metatarsal) of both PD_m × M and PD_g × BLM lambs.

Genotype significantly affected limb bone weights at 8 months (Table 1) for all bones except the metatarsal of ewes. M × M bones were the lightest for most limb segments, whilst PD_g × BLM were the heaviest, particularly in PD_g × BLM wethers whose bones were significantly heavier than other genotypes for all proximal bones (scapula, humerus, femur, tibia). Gender differences were significant in the model for all bones but were greatest in PD_g × BLM sheep.

Allometric analysis of length *v*. total limb bone length (Table 2) confirmed that, as shown previously (Cake *et al*. 2006*b*), most limb bones obey a strict allometric relationship with the greater limb length, though this relationship is less consistent (i.e. higher residual error and lower r^2 values) for the fore and hind cannon bones. Gender had a significant effect only on allometric *a* terms, principally in the metacarpal and to a lesser extent the scapula and femur. Genotype significantly affected *a* terms for all bones, but affected *b* terms only for the scapula, radius and metacarpal. M × M sheep showed *b* terms which were greater for the scapula and smaller for the radius and metacarpal, suggesting late and early maturation of proximal and distal bones, respectively; PD_g × BLM sheep showed the opposite pattern. Genotypic differences in allometric growth, though statistically significant in both limbs, were quantitatively much smaller for hindlimb compared with forelimb bones. Allometric solutions for bone weight *v*. hot carcass weight (Table 3) showed a significant effect for gender primarily on allometric *a* terms (mostly higher in wethers), with an effect on *b* term only for the metacarpal and metatarsal. Genotype affected *a* terms for all bones and affected *b* terms for the scapula and metatarsal only. M × M sheep consistently showed the greatest allometric coefficients for all bones, suggesting early maturation of limb bones relative to the remainder of the frame; PD_m × M and to a lesser extent PD_g × BLM sheep mostly showed the opposite pattern (late maturation of limb bone weight relative to carcass weight).

Estimates of limb bone length maturity proportion (M_{fore} and M_{hind}) were significantly lower in wethers compared with ewes at each time point (Table 4). *M* values were consistently significantly lower at the first three sampling points in M × M wethers, compared with higher values in PD_m × M and BL × M wethers. However, there were fewer differences in females, the only notable difference being the PD_m × M ewes were less mature than PD_g × BLM ewes

at the first kill (mean age 110 days). Modelling of limb length maturation curves (Fig. 4) demonstrated a similar pattern, with the most rapid maturation in wethers occurring in PD_m × M and BL × M sheep and slowest maturation in M × M sheep (Fig. 4*a*, *b*) while, to some extent, ewes showed the opposite pattern in the forelimb (Fig. 4*c*) and few differences in length-wise maturation in the hindlimb (Fig. 4*d*). From these maturation curves, age differences at the same level of maturity could be interpolated for sexes and genotypes. Wethers took an additional 52 days to reach M_{hind} = 0.80, and an additional 188 days to reach M_{hind}= 0.95. For genotypes, age at M_{hind} = 0.80 was 116, 128, 134, 162 and 168 days for PD_m × M, BL × M, PD_g × M, PD_g × BLM, and M × M, respectively. Age at M_{hind} = 0.95 was 400, 438, 542, 576 and 588 days, respectively. Bone Ca^{2+}-corrected magnesium content (Table 3) was greatest in PD_m × M sheep and lowest in M × M and PD_g × M sheep at 110 days. Gender did not have a statistically significant effect on bone magnesium content.

Discussion

Hammond (1932) considered hypotrophy of distal limb bones, particularly the cannon, to be characteristic of ‘improved’ meat breeds and, thus, indicative of inherent productive ability. Of all the variation in bone growth observed in this study, the most striking phenotype is that of PD_m × M sheep, which showed substantially shorter bones compared with other genotypes, particularly for more distal bones such as the fore and hind cannon. Allometric modelling showed PD_m × M sheep to have lower weights for most bones relative to hot carcass weight. For example, they possessed less than 97% of the combined limb bone weight of PD_g × M sheep. This confirms Hammond’s general association, but specifically suggests that selection pressure for muscling traits (in this case eye muscle depth) rather than general growth is associated with a decrease in limb length. This parallels the known phenomenon of

relative bone hypotrophy in ‘double-muscled’ cattle breeds, which has been attributed to improved support for the increased body mass (Shanin and Berg 1987).

Given that lengthening of bone normally accounts for the majority of increases in volume of dependant muscles (Young and Sykes 1987) and, conversely, that muscle hypertrophy increases dependant bone length (Banu *et al*. 2003), this association is not easily explained, though several possibilities might be advanced. First, there may be skewing of nutrient partitioning and/or sequence of tissue maturation promoting early muscle growth over bone (Cake *et al*. 2006*a*). Second, acceleration of overall maturation rate might be involved given that the*M. longissimus thoracis et lumborum* is an early maturing muscle (Butterfield 1988) and selection is based on eye muscle depth. While PD_m × M wether progeny were found to be earlier maturing in this study, the same could not be shown for ewe lambs, while the early maturing BL × M genotype showed relatively increased growth of distal parts. Third, selection for loin depth may be acting through hormonal drivers of bone growth such as growth hormone (GH), since sheep expressing excess GH show increased bone growth yet relative under-muscling (Adams *et al*. 2006), the opposite to that observed in the PD_m × M genotype in the present study. Alternatively, a more prosaic explanation is that selection for PEMD EBV, which is referenced to bodyweight (Hall *et al*. 2002) may inadvertently produce selection pressure for lighter appendages thus shorter limbs, as has been shown in other production animals such as rabbits (Gondret *et al*. 2005) and broiler chickens (Reddish and Lilburn 2004). However, other data from this study demonstrating shorter carcass length in PD_m × M compared with PD_g × M animals (Ponnampalam *et al*. 2007*a*), suggest that limb shortness is indicative of more generalised skeletal hypotrophy in this genotype. In this instance, selection for eye muscle depth might effectively select for a thicker, but shorter loin, with limited net effect on yield. DXA-derived carcass composition data from this study

(Ponnampalam *et al*. 2007*b*) similarly suggest that, although PD_m × M sheep have a higher lean : ash ratio than PD_g × M sheep, this is primarily the result of the significantly lower ash percentage in the short-legged PD_m × M sheep, rather than a true difference in carcass lean or fat percentage, which were not significantly different between the two groups.

This growth study clearly demonstrated the distinctive proportions of purebred Merinos, whose combined limb length matured to a comparable length to the prime lamb crosses, but with growth impetus favouring comparatively longer distal bones and shorter proximal bones. Bone weights were shown allometrically to be heavier at a given carcass weight, indicative of the higher bone trim of this breed (Hopkins and Fogarty 1998) which may be in part a result of its 'leggy' proportions. As expected, the terminal sire crosses (PD_g × M, BL × M, PD_g × BLM) possessed larger limb bones, in accordance with their larger size. BL × M sheep showed earlier limb bone maturation and thus had the longest limbs at earlier time points, though not at maturity. By contrast, second cross (PD_g × BLM) lambs matured more slowly to a longer mature limb length, though limb bone weights were comparatively low relative to hot carcass weight, due to their higher fatness (Ponnampalam *et al*. 2007*b*).

Allometric *b* terms for scapula and metacarpal length relative to total limb length were both greater in BL × M and lower in PD_g × BLM sheep, suggesting respectively earlier and later maturation of both bones in these genotypes. Thus, PD_g × BLM and PD_m × M sheep shared certain characteristics such as proportionately shorter cannons and longer proximal bones, despite their different skeletal maturation rates; likewise BL × M and M × M sheep. Other than suggesting that Border Leicesters are more Merino-like in their limb proportions, these genotypic differences are not easily reconciled with the crosses involved (for example, why larger second-cross PD_g × BLM sheep should have a lighter limb skeleton than first-cross PD_g × M sheep) and appear to deny any inherent association between early limb proportions

and either productive capacity or skeletal maturity, as suggested by Hammond (1932). By contrast PD_g × M sheep were unexceptional in most aspects of their limb bone growth. This result is contrary to that of Thompson *et al*. (1985), who found that selection of Merino strains for high weaning weight induced proportionately heavier skeletons (total bone weight as a fraction of total mature bodyweight), though it can be argued that the opposite finding for the PD_m × M genotype in this study may have, in part, resulted from the selection of sires with low PWWT EBV.

Gender differences in limb length were larger than expected and were greatest in PD_m × M and PD_g × BLM lambs, in line with the parallel suggestion of Hopkins *et al*. (2007) that genetic improvement may have accentuated differences in size (i.e. bodyweight) in ewes *v*. wethers of modern prime lamb genotypes compared with historical reports. However, gender differences in allometric terms, though statistically significant, were quantitatively minor for most bones. Similarly, while some genotypic differences in allometric growth relationships were observed, these were modest and were mainly confined to the forelimb. The scapula (unique in this series in not being a long bone) showed the greatest genotype difference in allometric growth, probably accounting for other differences in growth relative to the total forelimb length. The absent or minor differences in allometric *b* terms suggest that genotypic differences in bone length are mainly secondary to differences in prenatal or early antenatal growth. These results conform with other studies showing limb bone growth coefficients to be highly conserved genetically, for example between male and female Jersey cattle, or various pig breeds (Richmond *et al*. 1979; Davies *et al*. 1984). Results suggest that, although genotype and gender differences in conformation (for example, the predominance of distal over proximal parts in M × M sheep, and vice versa in PD_g × BLM sheep) are partly

explained by differences in allometric growth gradients, heterochronic alterations in whole limb growth rate play a greater role.

Sheep maturity is usually indexed either as a proportion of bodyweight (McClelland *et al*. 1976; Butterfield *et al*. 1983) or carcass composition (Berg and Butterfield 1966; Oberbauer *et al*. 1994), or using qualitative traits such as the 'breakjoints' of USDA maturity scores (USDA 1982; Ho *et al*. 1989) or eruption of permanent dentition. We have previously advocated the use of maturity proportions based on limb bone lengths indexed to a known or estimated mature endpoint (Cake *et al*. 2006*b*). This index demonstrates that ewes mature skeletally earlier than wethers, and that PD_m × M and BL × M wethers mature earlier compared with wethers of other genotypes. This is only partly consistent with tooth eruption data from the same study (Hopkins *et al*. 2007), showing that BL × M and PD_g × BLM, but not PD_m × M, lambs showed earlier eruption of permanent dentition. In agreement with this discrepancy, bone magnesium content of PD_m × M lambs at earlier timepoints was also consistent with the mineral profile of physiologically less mature animals, despite their more relatively advanced skeletal maturity (Ravaglioli *et al*. 1996; Cake *et al*. 2006*b*). Similarly, although ewes are known to mature earlier in terms of metacarpal growth plate closure (Ho *et al*. 1989; Jeremiah *et al*. 1997) and were clearly demonstrated in this study to be earlier maturing skeletally, the absence of a gender difference in bone magnesium content and the later eruption of permanent dentition in ewes shown by Hopkins *et al*. (2007) suggests this does not necessarily reflect earlier physiological maturation.

In summary, of the five genotypes assessed in this study as representatives of typical crosses used in Australian prime lamb production, two phenotypes were distinct in terms of limb

bone growth. The relative bone hypotrophy of lambs from Poll Dorset sires with high EBVs for eye muscle depth ($PD_m \times M$) provides further evidence that selection for specific muscling traits may be linked with reduced body size. This highlights a potential danger of the muscle : bone ratio as a primary index of productivity; namely, that selection may relatively decrease bone rather than increase muscling. Second, Merinos were found to have comparable limb length to terminal sire crosses, but with this comprising more distal limb elements at the expense of more productive proximal segments, a phenomenon possibly contributing to the greater bone trim and lower yield of some cuts (e.g. silverside, topside) from Merino lambs (Hopkins and Fogarty 1998). However, also notable was the lack of differences in bone growth between sheep very different in other production traits, particularly when compared allometrically. Thus, differences in bone growth, proportion, or skeletal maturation were greater between ewes and wethers than between these divergent genotypes.

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Fig. 1. Limb bone lengths (mm) at 8 months (mean age 236 days) by genotype and sex. Arrows indicate positive (↑) or negative (↓) deviation from gender-specific mean by ± <1% (1 arrow), <2% (2 arrows), or <3% (3 arrows). Values followed by the same letter do not differ ($P > 0.05$); wethers (M) and ewes (F) differ by *, $P < 0.05$; **, $P < 0.005$; ***$P < 0.0005$.

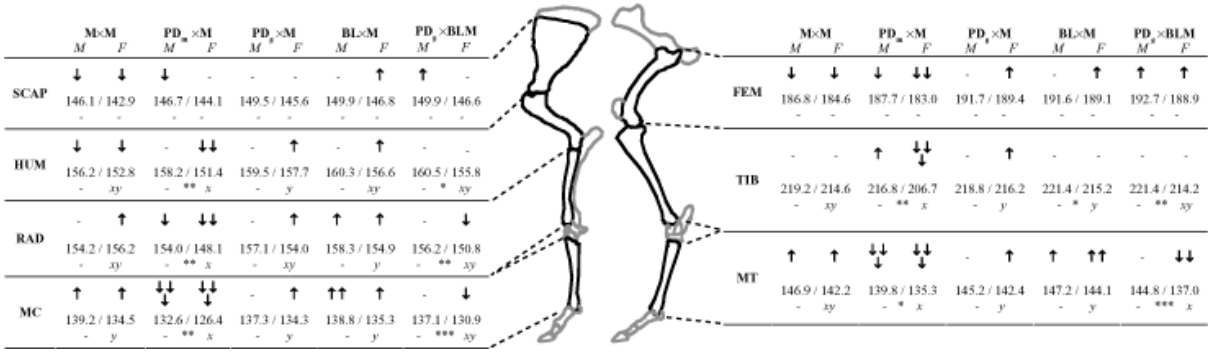
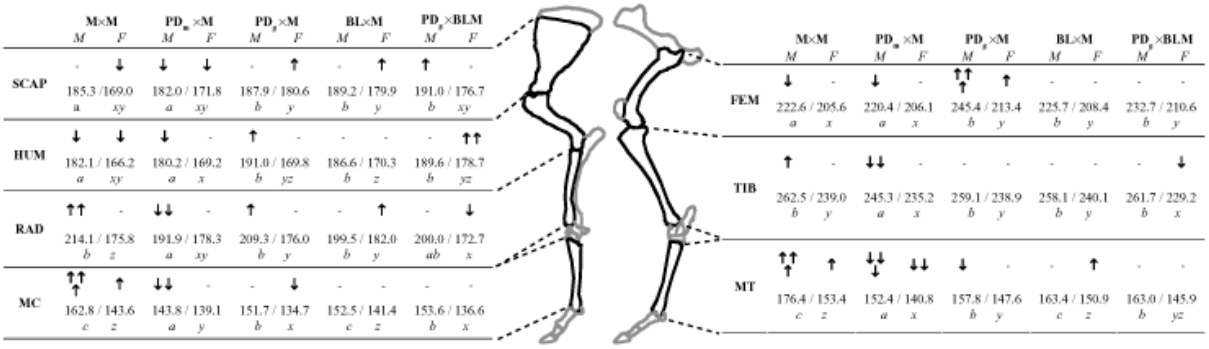


Fig. 2. Predicted limb bone lengths (mm) at maturity [*a* where L = $a - b(r^t)$] by genotype and sex. Arrows indicate positive (↑) or negative (↓) deviation from gender-specific mean by ± <2% (1 arrow), <4% (2 arrows), or <6% (3 arrows). Values followed by the same letter do not differ between genotypes ($P > 0.05$) within wethers (*a*,*b*,*c*) or ewes (*x*,*y*,*z*); wethers (M) and ewes (F) differ within genotype by *, $P < 0.05$; **, $P < 0.005$; ***$P < 0.0005$.



	M×M		PD_m ×M		PD_g ×M		BL×M		PD_g ×BLM	
	M	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
SCAP	-	↓	↓	↓	-	↑	-	↑	↑	-
	185.3 *a*	/ 169.0 *xy*	182.0 *a*	/ 171.8 *xy*	187.9 *b*	/ 180.6 *y*	189.2 *b*	/ 179.9 *y*	191.0 *b*	/ 176.7 *xy*
HUM	↓	↓	↓	-	↑	-	-	-	-	↑↑
	182.1 *a*	/ 166.2 *xy*	180.2 *a*	/ 169.2 *x*	191.0 *b*	/ 169.8 *yz*	186.6 *b*	/ 170.3 *z*	189.6 *b*	/ 178.7 *yz*
RAD	↑↑	-	↓↓	-	↑	-	-	↑	-	↓
	214.1 *b*	/ 175.8 *z*	191.9 *a*	/ 178.3 *xy*	209.3 *b*	/ 176.0 *y*	199.5 *b*	/ 182.0 *y*	200.0 *ab*	/ 172.7 *x*
MC	↑↑ ↑	↑	↓↓	-	-	↓	-	-	-	-
	162.8 *c*	/ 143.6 *z*	143.8 *a*	/ 139.1 *y*	151.7 *b*	/ 134.7 *x*	152.5 *c*	/ 141.4 *z*	153.6 *b*	/ 136.6 *x*

	M×M		PD_m ×M		PD_g ×M		BL×M		PD_g ×BLM	
	M	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
FEM	↓	-	↓	-	↑↑ ↑	↑	-	-	-	-
	222.6 *a*	/ 205.6 *x*	220.4 *a*	/ 206.1 *x*	245.4 *b*	/ 213.4 *y*	225.7 *b*	/ 208.4 *y*	232.7 *b*	/ 210.6 *y*
TIB	↑	-	↓↓	-	-	-	-	-	-	↓
	262.5 *b*	/ 239.0 *y*	245.3 *a*	/ 235.2 *x*	259.1 *b*	/ 238.9 *y*	258.1 *b*	/ 240.1 *y*	261.7 *b*	/ 229.2 *x*
MT	↑↑ ↑	↑	↓↓ ↓	↓↓	↓	-	-	↑	-	-
	176.4 *c*	/ 153.4 *z*	152.4 *a*	/ 140.8 *x*	157.8 *b*	/ 147.6 *y*	163.4 *c*	/ 150.9 *z*	163.0 *b*	/ 145.9 *yz*

Fig. 3. Predicted limb bone proportions (i.e. fraction of total limb length) at 8 months (mean age 236 days), by genotype and sex. Arrows indicate positive (↑) or negative (↓) deviation from gender-specific mean by ± <1% (1 arrow), <2% (2 arrows), or <3% (3 arrows). Values followed by the same letter do not differ between genotypes ($P > 0.05$) within wethers (*a*,*b*,*c*) or ewes (*x*,*y*,*z*); wethers (M) and ewes (F) differ within genotype by *, $P < 0.05$; **, $P < 0.005$; ***$P < 0.0005$.

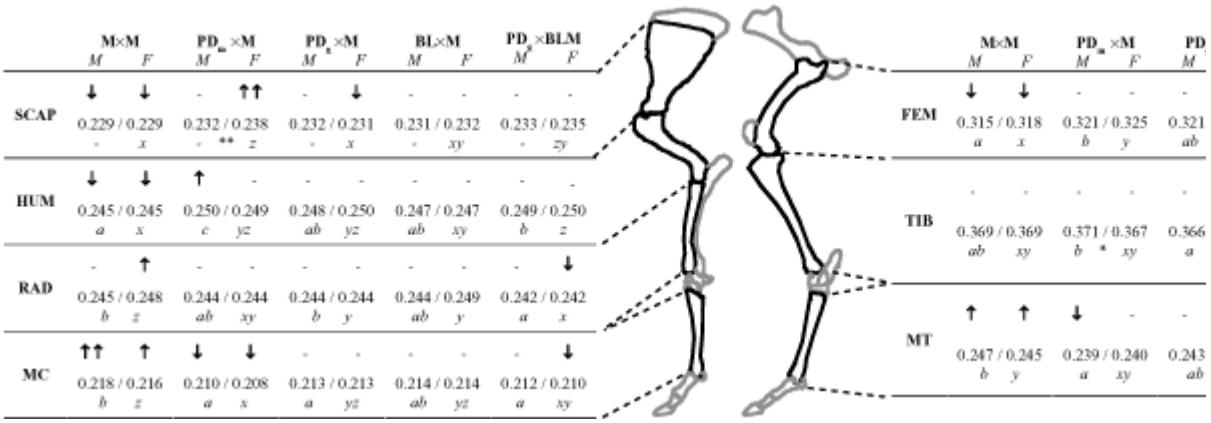


Fig. 4. Modelled plots of maturity proportion (i.e. proportion of mature length *a* where *a* = 1) for forelimb (M_{fore}; *a*, *c*) and hindlimb (M_{hind}; *b*, *d*) length of wethers (*a*, *b*) and ewes (*c*, *d*).

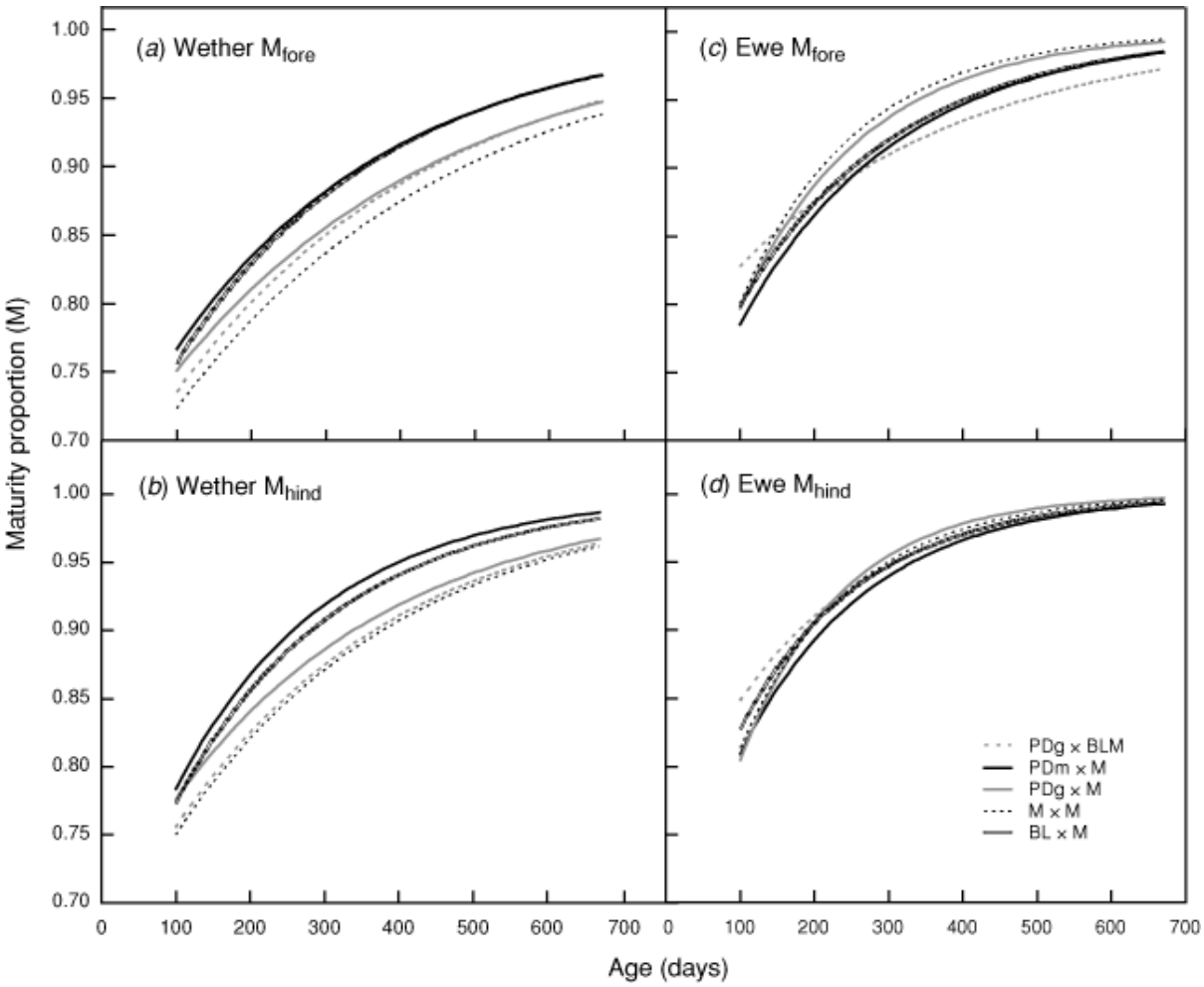


Table 1. Limb bone weights (g) by gender and genotype, at kills 2 (mean age 236 days) and 4 (662 days)

Values followed by the same letter do not differ between genotypes ($P < 0.05$) within wethers (*a*,*b*,*c*) or ewes (*x*,*y*,*z*). Differences between wethers and ewes within genotype are: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; s.e., standard error of mean

Bone	Wethers					Ewes					Av.
	M × M	PD_m × M	PD_g × M	BL × M	PD_g × BLM	M × M	PD_m × M	PD_g × M	BL × M	PD_g × BLM	s.e.
8 months (236 days)											
Scapula	65.1a	76.0b	79.0b	78.9b	87.3c	63.4x	71.6xy	75.2yz	74.7yz	78.5z**	2.40
Humerus	119.1a	132.9b	137.8b	137.2b	150.3c	109.0x	120.4xy	127.9y*	137.3y*	131.6y***	4.55
Radius/ulna	94.1a	101.3ab	106.1b	107.2bc	114.2c	87.2x	90.5x*	96.7xy*	97.9y*	100.0y***	3.46
Metacarpal	54.6a	54.0a	58.5ab	57.7ab	61.3b	49.0xy*	48.1x**	54.8y	53.3xy	54.4y**	1.91
Femur	153.5a	172.5b	180.5b	174.8b	194.6c	144.0x	157.2xy*	168.6y	161.2y	172.3y**	5.68
Tibia	129.3a	139.4ab	146.6b	140.8ab	156.7c	122.1x	127.2xy*	137.4y	133.5xy	142.5y**	4.43
Metatarsal	55.0a	56.3a	61.3ab	60.3ab	62.8b	51.3	51.9	57.6	55.3	56.2*	2.19
22 months (662 days)											
Scapula	114.7a	119.5a	135.0b	127.0ab	135.9b	99.2	115.7	119.2	113.2	122.3	6.56
Humerus	168.9a	178.5a	204.7b	191.4ab	208.7b	147.0x	162.6xy	165.7xy**	165.9xy	176.2y***	9.44
Radius/ulna	136.7a	140.1a	160.7b	152.8ab	161.7b	118.2*	131.8	127.7**	128.5*	132.7**	7.06
Metacarpal	72.3ab	65.4a	78.3b	73.9ab	80.8b	59.5*	59.8	58.7***	60.6**	63.4***	3.98
Femur	217.6a	222.5a	254.5b	234.3ab	258.0b	191.4*	211.1xy	207.1xy**	199.7xy*	222.0y**	10.3
Tibia	186.1ab	182.7a	206.0b	200.9bc	211.1c	162.9	179.5	173.5**	173.6*	186.0**	8.04
Metatarsal	77.0ab	69.1a	82.7b	78.7ab	82.5b	63.3*	62.7*	62.8***	65.4**	129.3***	3.92

Table 2. Allometric regression coefficients (where log y = log a + b log x) for limb bone length relative to total forelimb or hindlimb length, including effect of gender where significant (correction for wethers relative to ewes) and genotype

Total limb bone length (mm) = scapula + humerus + radius + metacarpal + proximal phalanx (forelimb) or femur + tibia + metatarsal + proximal phalanx (hind); constants followed by the same letter do not differ between genotypes ($P < 0.05$); s.e., standard error of mean; RMSR, root mean square of residual; d.f., numerator and denominator degrees of freedom. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant

Bone		Source *F*-value	Wether effect	*F*-value			Genotype			Av. s.e.	*F*-value	RMSR	R^2
					M × M	PD_m × M	PD_g × M	BL × M	PD_g × BLM				
d.f.		1, 580		1, 580							4, 580		
Scapula	log a	10008***	–0.01	106.9***	–1.42x	–1.17yz	–1.28xy	–1.40x	–0.99z	0.07	6.0***	0.011	0.947
	b		–	n.s.	1.28x	1.20yz	1.23xy	1.27x	1.13z	0.03	5.8***		
Humerus	log a	16497***	0.002	11.1**	–0.24z	–0.32w	–0.26y	–0.28x	–0.34w	0.04	35.8***	0.000	0.968
	b		–	n.s.	0.88	0.88	0.88	0.88	0.88	0.01	n.s.		
Radius	log a	17591***	–0.001	5.6**	–1.02xy	–1.16xy	–1.03y	–1.19x	–1.19x	0.05	2.4*	0.008	0.971
	b		–	n.s.	1.15x	1.20y	1.15x	1.21y	1.21y	0.08	2.3*		
Metacarpal	log a	4576***	0.06	36.3***	–0.19y	–0.20xy	–0.30x	–0.01y	–0.31x	0.07	2.5**	0.012	0.906
	b		–	n.s.	0.83x	0.83xy	0.86y	0.76x	0.87y	0.02	2.3*		
Femur	log a	11894***	–0.004	24.0***	–0.63x	–0.62z	–0.62z	–0.63y	–0.62z	0.03	34.7***	0.009	0.954
	b		–	n.s.	1.05	1.05	1.05	1.05	1.05	0.01	n.s.		
Tibia	log a	21091***	–0.002	8.4**	–0.74x	–0.74y	–0.74x	–0.74x	–0.74x	0.02	5.2**	0.007	0.974
	b		–	n.s.	1.11	1.11	1.11	1.11	1.11	0.01	n.s.		
Metatarsal	log a	4702***	–0.003	9.1**	–0.34y	–0.36x	–0.35y	–0.34y	–0.35y	0.04	41.0***	0.012	0.901
	b		–	n.s.	0.90	0.90	0.90	0.90	0.90	0.01	n.s.		

Table 3. Allometric regression coefficients (where log y = log a + b log x) for limb bone weight relative to hot carcass weight, including effect of gender where significant (correction for wethers relative to ewes) and genotype

Constants followed by the same letter do not differ between genotypes ($P < 0.05$); s.e., standard error of mean; RMSR, root mean square of residual; d.f., numerator and denominator degrees of freedom. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant

Bone		Source *F*-value	Wether effect	*F*-value	Genotype M × M	$PD_m \times M$	$PD_g \times M$	BL × M	$PD_g \times BLM$	Av. s.e.	*F*-value	RMSR	r^2
d.f.		1, 506		1, 506							4, 506		
Scapula	log a	4886***	0.016	21.2***	0.826x	0.814x	0.840xy	0.921y	0.908y	0.033	2.3	1.91	0.914
	b		–	n.s.	0.787y	0.769y	0.755xy	0.703x	0.702x	0.023	2.7*		
Humerus	log a	2971***	0.040	132.3***	1.335z	1.313x	1.322xy	1.327yz	1.317xy	0.015	4.6**	2.13	0.871
	b		–	n.s.	0.560	0.560	0.560	0.560	0.560	0.010	n.s.		
Radius/Ulna	log a	2892***	0.041	136.2***	1.235z	1.195x	1.205xy	1.218y	1.195z	0.015	15.8***	2.02	0.865
	b		–	n.s.	0.565	0.565	0.565	0.565	0.565	0.011	n.s.		
Metacarpal	log a	1644***	–0.016	0.3	1.161z	1.100w	1.127xy	1.136y	1.118x	0.023	26.8***	1.74	0.807
	b		0.047	5.08*	0.422	0.422	0.422	0.422	0.422	0.016	n.s.		
Femur	log a	2966***	0.035	113.7***	1.485z	1.460x	1.474y	1.469xy	1.466xy	0.014	5.9***	2.24	0.870
	b		–	n.s.	0.535	0.535	0.535	0.535	0.535	0.010	n.s.		
Tibia	log a	3662***	0.029	83.9***	1.371z	1.323x	1.337y	1.338y	1.324x	0.014	25.4***	2.15	0.886
	b		–	n.s.	0.574	0.574	0.574	0.574	0.574	0.009	n.s.		
Metatarsal	log a	1562***	–0.022	0.5	1.035x	1.127xy	1.104xy	1.188y	1.163y	0.039	2.4*	1.76	0.792
	b		0.046	4.1*	0.534y	0.420x	0.458x	0.404x	0.406x	0.028	3.6**		
Total fore[A]	log a	3689***	0.037	129.6***	1.763z	1.726x	1.738y	1.746y	1.730x	0.014	14.0***	2.58	0.891
	b		–	n.s.	0.583	0.583	0.583	0.583	0.583	0.010	n.s.		
Total hind[A]	log a	3333***	0.034	116.7***	1.826z	1.787x	1.804y	1.803y	1.792x	0.013	16.0***	2.57	0.880
	b		–		0.539	0.539	0.539	0.539	0.539	0.009	n.s.		

[A]Total limb bone weight (g) = scapula + humerus + radius + metacarpal + proximal phalanx (forelimb) or femur + tibia + metatarsal + proximal phalanx (hind).

Table 4. Mean limb length maturity proportion (*M*) and bone mineral maturity indices, by genotype and sex

Values followed by the same letter do not differ between genotypes ($P < 0.05$) within wethers (*a*,*b*,*c*) or ewes (*x*, *y*, *z*); s.e., standard error

Age (days)	Limb	Wethers					Ewes					Av. s.e.
		M × M	PD_m × M	PD_g × M	BL × M	PD_g × BLM	M × M	PD_m × M	PD_g × M	BL × M	PD_g × BLM	
						Limb bone length maturity proportion (M)						
110	Fore	0.73a	0.77b	0.76ab	0.76b	0.75a	0.81xy	0.80x	0.81xy	0.80xy	0.83y	0.01
	Hind	0.75a	0.79b	0.78ab	0.78ab	0.77ab	0.83x	0.82x	0.82x	0.83xy	0.86y	0.01
236	Fore	0.81a	0.86b	0.82ab	0.85b	0.83ab	0.90	0.88	0.91	0.90	0.89	0.01
	Hind	0.85a	0.89b	0.86ab	0.88ab	0.86ab	0.91	0.91	0.93	0.92	0.93	0.01
412	Fore	0.87a	0.91bc	0.89abc	0.92c	0.88bc	0.98y	0.95xy	0.97xy	0.93x	0.95x	0.01
	Hind	0.90ab	0.95c	0.93bc	0.95c	0.91a	0.99	0.97	0.98	0.96	0.99	0.01
662	Fore	0.94	0.96	0.96	0.97	0.95	0.98	0.99	0.99	0.99	0.99	0.01
	Hind	0.96	0.98	0.98	0.98	0.97	0.98	0.99	1.00	0.99	1.01	0.02
						Bone ash Mg^{2+}/Ca^{2+} ratio ($10^{-3} \times N$)[A]						
110		18.3a	19.0b	18.3a	18.7ab	18.5ab	18.4	18.9	18.4	18.7	18.8	0.02
236		17.9ab	17.9b	17.7ab	17.9ab	17.5a	17.9	18.0	17.5	17.4	17.4	0.02

[A]Bone ash data are for combined hindlimbs and forelimbs.