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Mapping genes for phosphorus utilization and correlated traits using a 4k SNP linkage map in Japanese quail (*Coturnix japonica*)

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Summary

A large F2 cross with 920 Japanese quail was used to map QTL for phosphorus utilization, calcium utilization, feed per gain and body weight gain. In addition, four bone ash traits were included, because it is known that they are genetically correlated with the focal trait of phosphorus utilization. Trait recording was done at the juvenile stage of the birds. The individuals were genotyped genome-wide for about 4k SNPs and a linkage map constructed, which agreed well with the reference genome. QTL linkage mapping was performed using multimarker regression analysis in a line cross model. Single marker association mapping was done within the mapped QTL regions. The results revealed several genome-wide significant QTL. For the focal trait phosphorus utilization, a QTL on chromosome CJA3 could be detected by linkage mapping, which was substantiated by the results of the SNP association mapping. Four candidate genes were identified for this QTL, which should be investigated in future functional studies. Some overlap of QTL regions for different traits was detected, which is in agreement with the corresponding genetic correlations. It seems that all traits investigated are polygenic in nature with some significant QTL and probably many other small-effect QTL that were not detectable in this study.

Keywords feed utilization, Japanese quail, linkage map, quantitative trait loci

Introduction

Phosphorus is an essential mineral for all living organisms. It is important for energy metabolism, nucleic acid synthesis, enzyme activity and bone mineralization. Most of the phosphorus in plant seeds and feedstuffs produced thereof is present as phytic acid and its salts, called phytates (Eeckhout & Paepe 1994). Owing to low endogenous phytase activity in the digestive tract of poultry, phytate-P sources can only partially be utilized. Therefore, poultry diets are usually supplemented with mineral phosphorus, often in combination with exogenous phytase, which results in additional costs. Additionally, global mineral phosphorus resources are limited, and the phosphorus in excreta has an environmental impact. Therefore, it is desirable to minimize mineral phosphorus supplementation without compromising animal health and performance. Thus, high phosphorus utilization (PU) by animals is desirable.

Japanese quail (*Coturnix japonica*) has long been an important model species in poultry studies because of its short generation intervals, small body size, which results in a smaller space requirement (Kayang *et al.* 2004; Cheng *et al.* 2010), and similarity to other poultry species (Stock & Bunch 1982; Shibusawa *et al.* 2001). A recent study implemented an F2 experimental design with approximately 1000 Japanese quail and phenotyped the F2 individuals for PU and related traits (Beck *et al.* 2016a). The coefficient of variation for PU was 0.11, which indicated substantial variation, with a heritability of 0.14 (±0.06). By applying structural equation models some complex relationships of PU were detected with body weight gain and feed per gain ratio (Beck *et al.* 2016a). A subsequent study of the ileum microbiota composition of those birds estimated a significant microbiability for PU (Borda-Molina *et al.* 2020; Vollmar *et al.* 2020). In addition, ileal transcriptome profiles, miRNA–mRNA and gut microbiome interactions of subsets of quails with divergent PU have been studied (Oster *et al.* 2020; Ponsuksili *et al.* 2020).

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Because calculation of PU involves quantitative measurement of feed intake and excretion over several days, PU is a very-hard-to-measure trait in a routine breeding enterprise. Therefore, proxy traits and genetically correlated traits are desirable and convenient to measure. Bone ash traits are features that have been used to determine the bioavailability of phosphorus in quail (Vali & Jalali 2011) and chicken (Li *et al*. 2017). Several bone ash traits were analyzed using samples from the experiment of Beck *et al*. (2016a) and the genetic correlations with PU were estimated, which were between 0.5 and 0.6 (Künzel *et al*. 2019). Thus, it might be possible to consider bone ash traits as proxy traits to breed for the improvement of PU.

Until now, it has been largely unknown whether the genetic variance of PU is caused by many QTL with small effects or if there are some large QTL that might be of special interest for breeding purposes. Therefore, the aim of this study was to map the QTL associated with the focal trait PU as well as other performance traits and bone ash traits in Japanese quail using an F2 cross. The individuals were genotyped genome-wide with 4k SNPs, and we used these data to establish a linkage map and subsequently to conduct QTL linkage and association mapping.

Materials and methods

Experimental design

The experiment was conducted in accordance with the German Animal Welfare Legislation approved by the Animal Welfare Commissioner of the University Hohenheim (approval number S371/13TE). An F2 cross of Japanese quail (*C. japonica*) was established. The details of the F2 design can be found in Beck *et al*. (2016a), and only the essential steps are described in the following. The founder lines were divergently selected for social reinstatement behavior in an earlier experiment conducted at the INRA, France (Mills & Faure 1991). The selection of these founder individuals is thus not related to the focal trait PU. Twelve males from founder line A (B) were mated to 12 females from founder line B (A) to produce the F1 generation. From this generation, 17 males and 34 females were selected, and one male was mated with two females, resulting in 920 F2 individuals. These individuals belonged to 34 full-sib families and 17 paternal half-sib families, with approximatively the same family size. A low-P-content diet was provided to allow the quails to exhibit their full PU potential. The diet did not contain mineral P supplement or phytase.

Trait records

Body weight gain (BWG) was calculated as the difference in body weight at days 10 and 15. Feed per gain ratio (F:G) was calculated as feed intake (FI) within this 5-day period divided by BWG. PU and calcium utilization (CaU) were calculated for this period based on quantitative intake and excretion of the elements as described in Beck *et al*. (2016a). The quails were slaughtered on day 15, and the right tibia and the right foot were preserved. The total amount of ash in the tibia and foot (TA and FA) as well as ash concentrations in the dry matter of the bones (TA% and FA%) were recorded as described in detail in Künzel *et al*. (2019). Descriptive statistical parameters, heritabilities and trait abbreviations are provided in Table 1. The heritabilities of the traits were estimated by Beck *et al*. (2016a) and Künzel *et al*. (2019) using mixed linear animal models.

DNA collection and SNP genotyping

One milliliter of blood was collected from each animal using EDTA-K tubes and stored at −20°C until DNA extraction was performed using the Maxwell 16 Blood DNA Purification Kit (Promega). The DNA concentration was adjusted to 50 ng/μl to ensure consistent measurements. Using a customer's Illumina iSelect chip, we genotyped 5388 SNPs. The SNP markers were mapped through the chicken genome using the method described in Recoquillay *et al*. (2015), as no quail genome was available at the time of

Table 1 Descriptive statistics and heritabilities of the traits.

Trait[1,2]	Abbreviation	Unit	Minimum	Maximum	Mean	h^2 (SE)
Phosphorus utilization†	PU	%	21.49	87.43	71.41	0.14 (0.06)
Calcium utilization*	CaU	%	19.42	84.31	60.56	0.17 (≤0.10)
Feed per gain†	F:G	g/g	1.21	3.92	1.78	0.12 (0.06)
Feed intake*	FI	g	16.11	62.35	42.65	0.11 (≤0.10)
Body weight gain†	BWG	g	5.80	37.85	24.50	0.09 (0.14)
Tibia ash (mg)*	TA	mg	19.20	83.50	45.82	0.23 (≤0.10)
Tibia ash (%)*	TA%	%	35.53	55.71	45.26	0.23 (≤0.10)
Foot ash (mg)*	FA	mg	19.60	83.60	44.76	0.34 (≤0.10)
Foot ash (%)*	FA%	%	12.10	21.91	17.30	0.31 (≤0.10)

[1]From days 10–15 of life.
[2]Measurements and heritabilities from Beck *et al*. (2016a)† and Künzel *et al*. (2019)* and SEs are in parentheses.

genotyping. The following criteria were applied to filter the genotypes: one or more conflicting genotypes between parent and offspring, a MAF ≤0.03, an SNP call frequency ≤0.9 and cluster separation ≤0.4. This led to the exclusion of 842 SNPs. Furthermore, we rejected SNPs on the sex chromosomes Z or W and in the linkage group (LG) LGE22C19W28_E50C23 or E64 (information obtained from the *C. japonica* reference genome assembly (NCBI GCA_001577835.1)). This filtering resulted in a total of 3986 SNP markers for further analysis.

Linkage map construction

The linkage mapping software LEP-MAP2 (Rastas *et al.* 2015) was used to build a sex-averaged Japanese quail map. The software uses pedigree and marker information to assign SNP markers to LGs and computes the likelihood of the marker order within each LG using standard hidden Markov models (Rastas *et al.* 2013; Rastas *et al.* 2015). In the first step, the module *SeparateChromosomes* was used to assign markers to the LG. We used the option LOD = 1–20 to test lodLimits with a sizeLimit = 5 so that LGs with fewer than five markers were removed. A lodLimit of 5 resulted in 27 LGs with 3975 markers assigned to them. The remaining markers were assigned to LGs by using the module *JoinSingles* with lodLimit = 1–15 and lodDifference = 2. A lodLimit = 1 was selected because there was no difference compared with other lodLimits in terms of results, and additional nine SNPs could be assigned. The module *OrderMarkers* orders the markers within each LG. This step was replicated five times to select the best order with the highest likelihood. The module was run with the options polishWindow = 30, filterWindow = 10 (both parameters are used for speeding up the computations), numThreads = 10 (maximum number of threads to use), useKosambi = 1 (using Kosambi mapping function), minError = 0.15 (because genotyping errors can lead to large map distances) and sexAveraged = 1 (to compute the sex-averaged map distances).

To compare the calculated genetic map with the reference genome *C. japonica* (NCBI GCA_001577835.1), the flanking sequences for each SNP were aligned by performing BLAST searches of the reference genome. This led to the assignment of our LGs to the chromosomes. These assignments were used throughout the rest of the study.

QTL linkage and association analysis

A line cross model was applied in this study. For this purpose, we used the package RQTL2 (Broman *et al.* 2019). This program was developed for inbred line crosses. We estimated the F_{ST} value for each SNP in the two founder populations using eq (8) in Weir & Cockerham (1984). Subsequently, we selected only those SNPs with an $F_{ST} > 0.23$, which comprised approximately half of the SNPs, and the selected SNPs were used for QTL linkage mapping. This filtering ensured that the assumptions regarding the inbred founder lines made by the software were approximatively fulfilled. In addition, we selected only those chromosomes with >40 SNPs because we applied multimarker linkage mapping. These two filter steps resulted in 1968 SNPs that were used for QTL linkage mapping on 19 chromosomes. Subsequently, we applied the RQTL2 software package and estimated QTL genotype probabilities for each F2 individual and each marker position. These probabilities were used in a regression analysis to map the QTL. We included the hatches as fixed effects in the regression model. The LOD score was used as a test statistic, and correction for multiple testing was done using the permutation test (10 000 permutations). We considered two significance criteria for each trait, i.e. 1 and 5% genome-wide significance (LOD scores 4.9–5.9 and 4.2–4.7 respectively). The QTL support intervals (SI) were approximated using the LOD drop off method with a drop of 1.5 LOD (Manichaikul *et al.* 2006). The upper and lower bounds of the SI were extended by 5 cM to be conservative. Because the

Table 2 Numbers of markers (*n* SNPs) on each chromosome (*Coturnix japonica,* CJA), length in cM and in Mb, average number of markers per cM and per Mb, and correlation between the cM and the bp positions

		Length		Markers		
CJA	*n* SNPs	cM	Mb[1]	per cM	per Mb	Correlation
1	769	253.09	177	0.33	0.23	0.96
2	650	189.57	136	0.29	0.20	0.98
3	457	153.11	101	0.34	0.22	0.94
4	436	116.80	83	0.27	0.19	0.98
5	278	97.64	54	0.35	0.19	0.89
6	145	64.45	32	0.44	0.19	0.97
7	152	68.45	34	0.45	0.20	0.93
8	138	54.70	27	0.40	0.15	0.92
9	121	53.67	21	0.44	0.15	0.99
10	88	44.22	19	0.50	0.17	0.96
11	91	43.40	18	0.48	0.18	0.95
12	90	47.72	17	0.53	0.15	0.99
13	63	38.74	16	0.61	0.19	0.96
14	75	47.11	13	0.63	0.16	0.92
15	55	46.04	12	0.84	0.19	0.98
16	–	–	0.3	–	–	–
17	52	45.19	9	0.87	0.14	0.98
18	40	38.77	10	0.97	0.13	0.95
19	56	45.78	9	0.82	0.12	0.97
20	62	51.92	13	0.84	0.20	0.96
21	26	31.08	6	1.20	0.15	0.96
22	18	39.11	4	2.17	0.13	0.96
23	27	39.93	5	1.48	0.13	0.96
24	36	46.08	6	1.32	0.15	0.98
25	5	3.33	3	0.67	0.10	0.88
26	19	37.30	5	2.33	0.14	0.95
27	17	35.41	5	2.08	0.19	0.89
28	9	2.77	4	0.31	0.13	0.95
Total	3975	1735.36	839.30	—	—	—
Average	147	64.27	29.98	0.81	0.17	0.95

[1]Size in Mb based on the reference genome *Coturnix japonica* 2.0 (NCBI GCA_001577835.1).

assumptions of the linkage QTL mapping approach regarding the inbred founder lines were only approximated fulfilled (i.e. not every SNP with $F_{ST} > 0.23$ was divergently fixated in the two founder lines), we conducted an SNP association analyses. For this purpose, we tested all markers within the SI (i.e. also those with an $F_{ST} < 0.23$) for trait associations to support the presence of a QTL. We repeated this process for each SNP within the intervals separately by applying a mixed linear model using the software GCTA (Yang *et al.* 2011). The hatches were considered as fixed effects, and correction for putative population stratification effects was performed by including a random animal effect based on a genomic relationship matrix that was calculated using all markers except those on the chromosome under consideration (i.e. the leave-one-chromosome-out option in GCTA). As only those markers in the SI were tested for associations, no correction for multiple testing was performed.

To identify positional candidate genes in the 0.5 Mbp regions up- and downstream of significant SNPs, we used Genome Data viewer from NCBI and the reference genome assembly (NCBI GCA_001577835.1).

Results

Construction of the linkage map

The summary of the linkage map is shown in Table 2, and a list of SNPs with their chromosomal position was made public available (see Data availability statement). The linkage map is plotted in Fig. S1. A total number of 3975 SNPs were assigned to 27 LGs. The map covers 1735 cM with individual LG lengths that range from approximately 3 cM [*C. japonica* chromosome (CJA) 28] to 253 cM (CJA1) (Table 2). The number of markers per chromosome varied from 5 (CJA25) to 769 SNPs (CJA1), and the average density was 0.81 markers per cM across all chromosomes. We estimated a high correlation between the genetic (cM) position of the calculated linkage map and the physical (bp) position of the reference genome assembly (NCBI GCA_001577835.1), ranging from 0.88 to 0.99 (Table 2). Overall, the order of the markers of the genetic map agreed well with the order of the physical positions of the reference genome. No LGs could be assigned to chromosome 16, because this chromosome is poorly characterized so far and no SNP could be assigned to it. Figure 1 shows the comparison between the physical (bp) and genetic map (cM) for chromosome 2, and some outliers are visible. These outlier markers were either identified at other positions within an LG compared with the reference genome or had positions that were not yet known. The comparisons of the remaining chromosomes are shown in Fig. S1.

Identification of QTL

The test statistic plots of the analyzed chromosomes are shown in Figs 2 & 3 for the performance and the bone ash traits respectively. A total of 21 QTL (eight QTL for 1%, 13

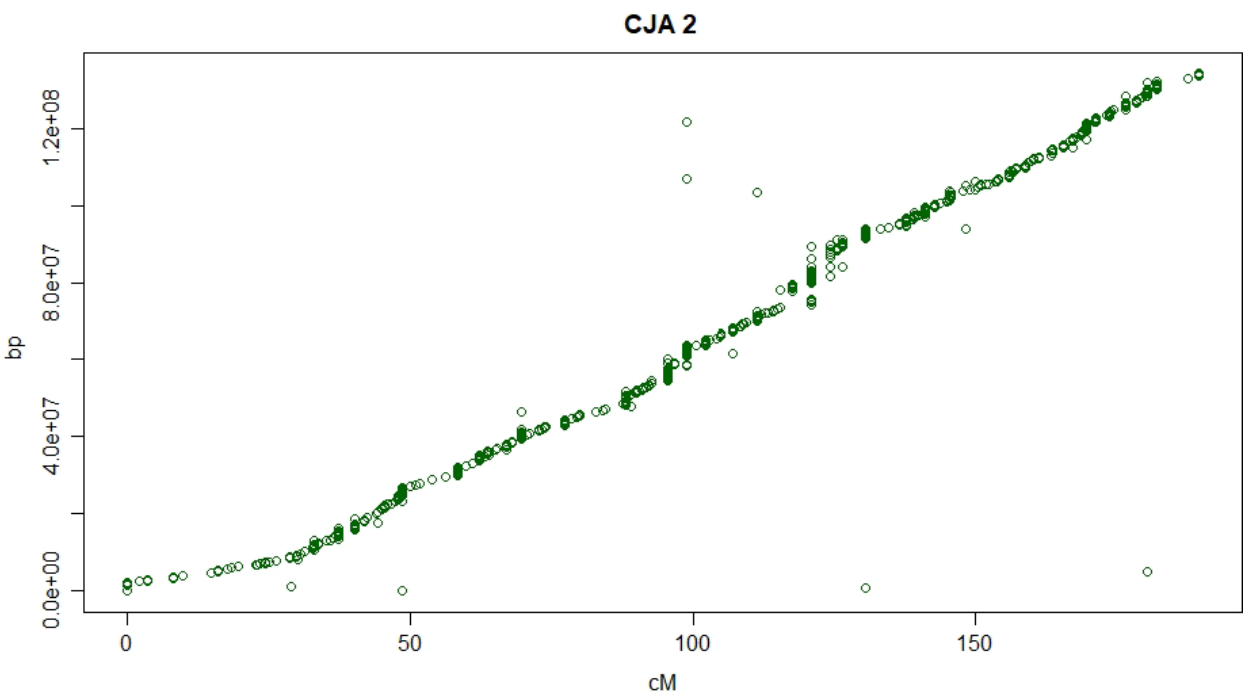

Figure 1 Plot of SNPs that were assigned to chromosome 2. The *y*-axis shows the physical position (bp), which is based on the reference genome assembly (NCBI GCA_001577835.1), and the *x*-axis shows the genetic position (cM). Note that the SNP positions at 0 bp refer to an as yet unknown position

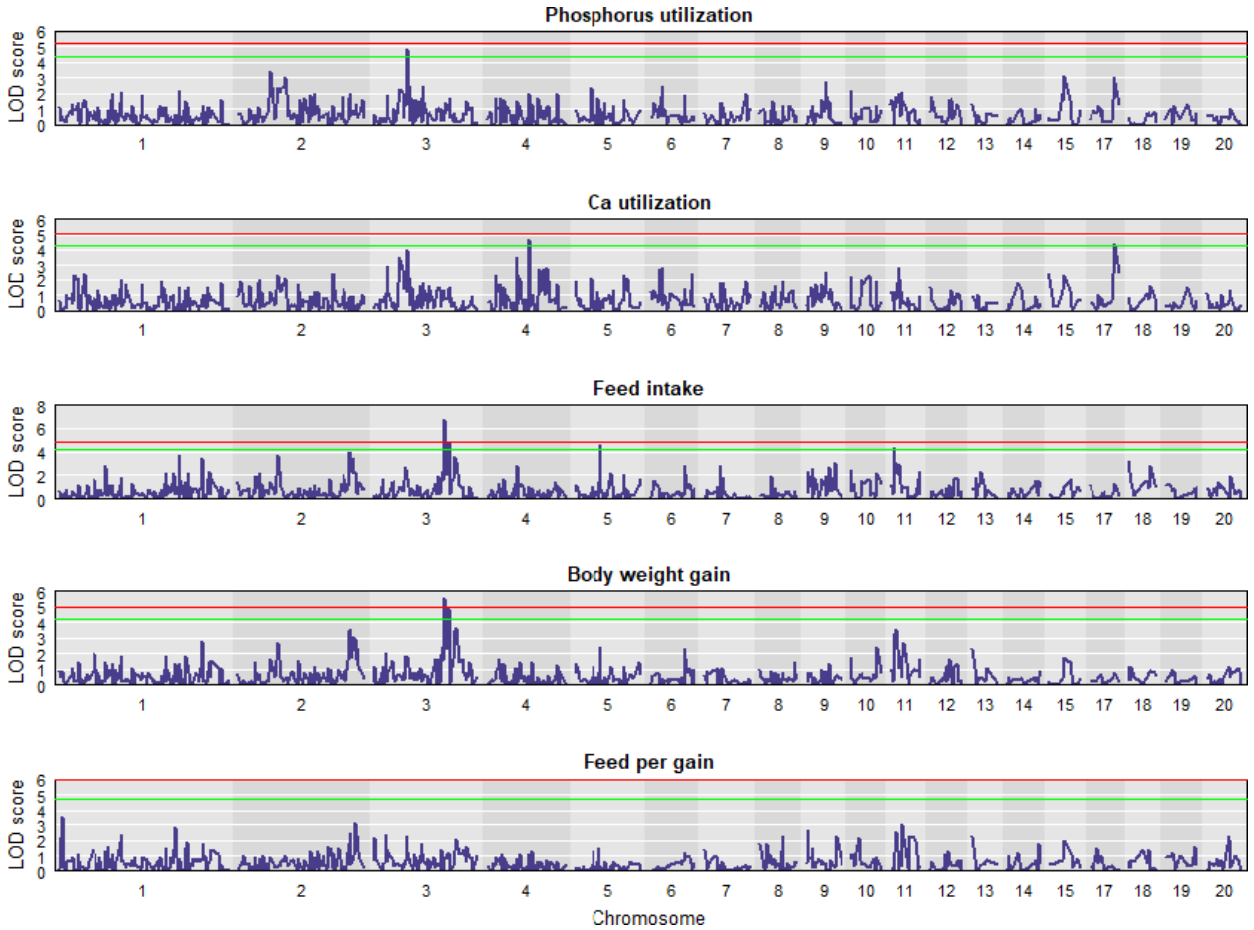

Figure 2 Plot of the QTL linkage mapping scan of growth and efficiency traits with LOD score test statistics. The green and red lines correspond to genome-wide significance levels of 5 and 1% respectively

QTL for 5%) were mapped for all traits at a 1% (5%) genome-wide significance level. For all traits, QTL could be mapped, except for F:G. A detailed description of the QTL is given in Table 3. For PU, we identified one QTL on CJA3, and for BWG, we found one QTL on CJA3, whereas all other traits were associated with two or more QTL (Table 3, Figs. 2 & 3). Some SI overlapped for several traits. For example, the SI on CJA3 for PU and FA% and the SI on CJA4 for CaU, TA and FA overlapped (Table 3).

The results of the SNP association analyses are shown as the numbers of significant SNPs in the QTL regions, and the significant SNPs are listed in Table S1. A total of 127 SNPs were shown to be significant in QTL regions for all traits. Significant SNPs were found in all QTL regions for the traits PU, CaU, FI, BWG and TA (Table 3, Table S1). Although the SI on CJA3 overlapped for PU and FA%, no significant identical SNPs could be found in this region (Table S1). PU was associated with five significant SNPs, and FA% was associated with three SNPs on CJA3. Several SNPs were significantly associated with several traits. The QTL on CJA3 for FI shared five significant SNPs with the QTL for BWG (id12506 at 91 cM, id10670 at 95 cM, id10683 at 97 cM, id06748 at 101 cM and id14876 at 102 cM). Nine SNPs on CJA11 (id06872 and id32446 at 7 cM, id15452 at 11 cM, id32451, id07827, id09706, id05659 and id08551 at 13 cM, and id05029 at 16 cM) were significant within the QTL region for FI and FA (Table S1). One SNP (id08651 at 39 cM) on CJA18 was significant within the QTL region for TA and TA%. No other common significant SNP similarities could be found despite the presence of overlapping SI.

Candidate genes associated with PU, performance and bone ash traits

We identified numerous genes in a 0.5 Mbp region up- and downstream of the significant SNPs in all QTL regions. For the PU QTL on CJA3 we identified 73 positional genes (see Table S2). Of these genes, 51 have known functions. No functional annotation analyses were conducted. No SNP within exon regions could be identified. Therefore, we looked for SNPs that were either intronic or obvious and were related to metabolic processes in which phosphorus might play a role. This filtering led to four genes (from the initial 73 ; Table 4), which were discussed in detail.

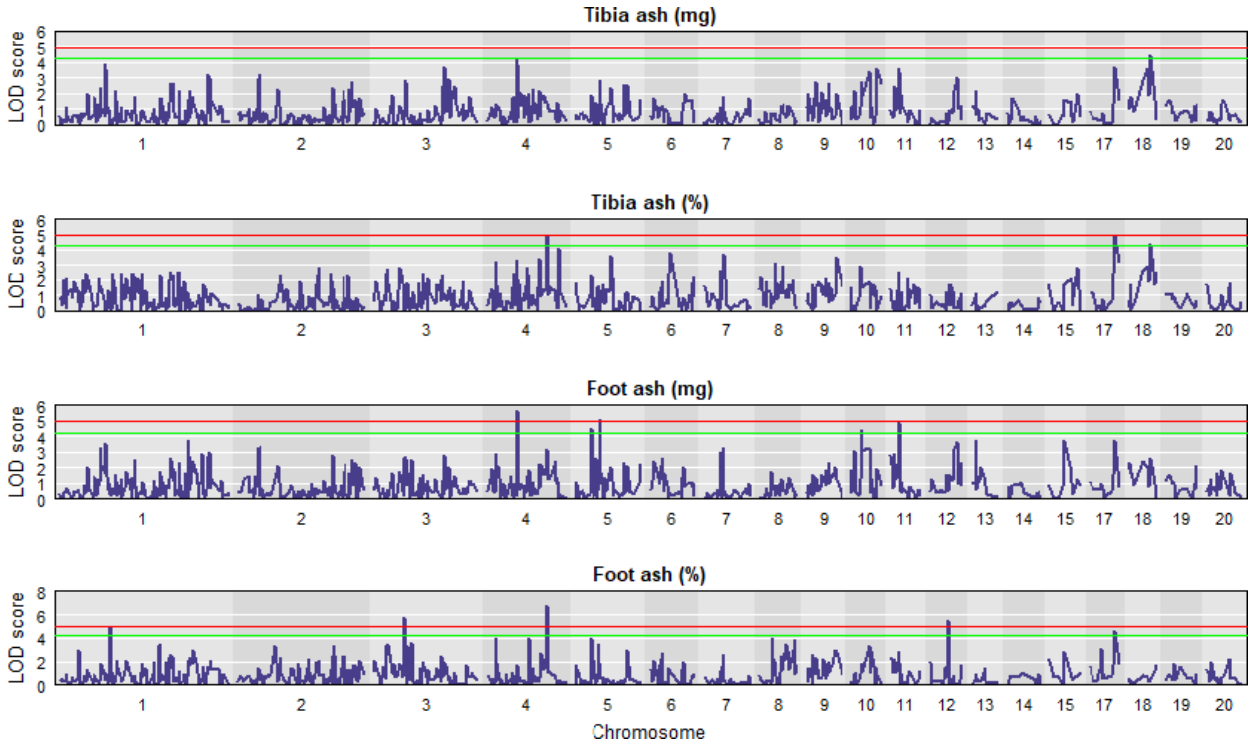

Figure 3 Plot of the QTL linkage mapping scan of bone ash traits with LOD score test statistics. The green and red lines correspond to genome-wide significance levels of 5 and 1% respectively

Table 3 Trait specific positions of significant QTL (Pos) on the chromosomes (CJA), LOD score test statistics (LOD) at the 1% (**) and 5% (*) genome-wide significance level, and the corresponding support intervals (SI). SI_low and SI_high = beginning and end of the support interval respectively, with the number (*n*) of significant SNPs identified by the association analysis

Trait[1]	CJA	Pos	LOD	SI_low	SI_high	*n* of SNPs
PU	3	48.9	4.82*	35.89	65.71	5
CaU	4	62.6	4.64*	31.71	75.64	14
	17	36.8	4.35*	20.21	57.69	6
FI	3	104.6	6.73**	90.27	119.49	7
	5	38.6	4.63*	25.99	51.10	4
	11	5.5	4.34*	0.00	27.44	10
BWG	3	104.6	5.59**	90.27	124.58	12
TA	4	44.7	4.23*	31.71	57.82	28
	18	30.6	4.43*	6.11	49.06	1
TA%	4	88.6	4.98*	72.78	120.10	0
	17	36.8	4.95*	20.21	57.69	1
	18	30.6	4.38*	6.11	49.06	1
FA	4	45.2	5.63**	32.68	57.82	0
	5	38.6	5.05**	11.00	51.10	0
	10	15.5	4.45*	0.00	42.25	6
	11	13.2	4.92*	0.00	27.44	10
FA%	1	77.1	4.99**	63.95	90.56	4
	3	44.2	5.76**	31.22	58.10	3
	4	88.6	6.74**	72.78	103.79	12
	12	27.5	5.45**	13.05	41.16	0
	17	36.8	4.62*	4.58	57.69	3

[1]For trait abbreviations, see Table 1.

Discussion

In previous studies, we analyzed the impact of the quail genome (Beck *et al.* 2016a; Künzel *et al.* 2019), ileum microbiota composition (Borda-Molina *et al.* 2020; Vollmar *et al.* 2020) and transcriptomic profiles (Oster *et al.* 2020) as well as miRNA–mRNA and gut microbiota (Ponsuksili *et al.* 2020) on the focal trait PU and other related traits. Preliminary QTL mapping was done on few chromosomes and markers, without reporting any clear signals (Beck *et al.* 2016b). Hence, a thorough QTL mapping has not been done previously. This study filled this gap by conducting QTL linkage and association mapping for these traits in the same experimental design. The results clearly showed that all of the the investigated traits are polygenic in nature and are associated with several significant QTL as well as many other small-effect QTL that were not detectable.

Linkage map

Until the publication of the reference genome in 2016, only a few low-density genetic maps were available. The map calculations were based on ALFP markers (Roussot *et al.* 2003) or microsatellites (Kayang *et al.* 2004), or both types of markers (Kikuchi *et al.* 2005). Recoquillay *et al.* (2015) were the first to calculate a genome-wide linkage map based

Table 4 Genes and their functions[1] and positions in the reference genome within the PU QTL region of CJA3

Official gene symbol	Gene name	Function[1]
BMP2	*Bone morphogenetic protein 2*	Ligand of TGF-beta superfamily, induces cartilage and bone formation
PLCB1	*Phospholipase Cβ1*	Hydrolyzes phospholipids into fatty acids and other lipophilic molecules, catalyzes the formation of inositol-1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol-4,5-bisphosphate, uses calcium as a cofactor, involved in intracellular transduction of many extracellular signals
PLCB4	*Phospholipase Cβ4*	Hydrolyzes phospholipids into fatty acids and other lipophilic molecules, catalyzes the formation of inositol-1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol-4,5-bisphosphate, uses calcium as a cofactor, involved in intracellular transduction of many extracellular signals
TGFB2	*Transforming Growth Factor β2*	Involved in TGF-β-2 chains, involved in many processes, e.g. cell differentiation, growth and morphogenesis processes

[1]According to GeneCards and UniProt.

on SNP markers. Our genetic map agreed well with the map from Recoquillay *et al.* (2015). Next, based on the reference genome *C. japonica* 2.0 (NCBI GCA_001577835.1), genome assemblies for other quail species (Wu *et al.* 2018) and Japanese quail (Morris *et al.* 2020) were developed. As our experiment with several full- and half-sib families and approximately 1000 animals across three generations can be seen as a powerful linkage mapping design, we developed a further linkage map.

The coverage and density of SNP markers were low for some LGs (Table 2, Fig. S1). This is especially noticeable for the smaller LGs (assigned to CJA25 and 28) with fewer than 10 markers. This is a result of the chosen sizeLimit = 5 in the module SeparateChromosome of the software LEPMAP2, as a larger sizeLimit resulted in a larger number of markers that could not be assigned to any LG. In addition, this sizeLimit was chosen to obtain the best fit based on the karyotype of Japanese quail. The genome of Japanese quail is closely related to that of the domestic chicken (*Gallus gallus domesticus*) (Wu *et al.* 2018) and shows a typical avian species karyotype that includes 10 pairs of macrochromosomes and numerous small microchromosomes (Schmid *et al.* 1989; Zlotina *et al.* 2019).

After comparison with the reference genome (e.g. Fig. 1), only a few markers could not be assigned to physical positions. However, most marker positions in the LGs were consistent with the chromosomes of the reference genome. The good fit of the map is also demonstrated by the high correlation of the linkage and physical marker positions (Table 2). Overall, the present linkage map seems to be of good quality and consistent with the reference genome. This justified the use of this map for the QTL linkage analysis.

QTL results and candidate genes

Our study adds new information for QTL in Japanese quail and provides novel QTL affecting PU, i.e. the PU QTL on CJA3 (Table 3). Owing to the use of different methods, experimental designs and trait definitions and recordings, a sophisticated comparison of QTL linkage mapping results across studies is difficult and thus was not performed in this study. QTL associated with other traits in Japanese quail have been reported by Minvielle *et al.* (2005), Esmailizadeh *et al.* (2012), Ori *et al.* (2014), Sohrabi *et al.* (2012), Recoquillay *et al.* (2015) and Knaga *et al.* (2018).

Some trait interrelationships could be identified by studying the genetic and phenotypic correlations (Beck *et al.* 2016a; Künzel *et al.* 2019) as well as the overlapping of the QTL SI (Table 3). For example, on CJA3, we detected QTL associated with PU and FA% in the same chromosomal region (Table 3). These traits are genetically correlated (0.46) (Künzel *et al.* 2019). On CJA4, we mapped QTL associated with CaU, TA and FA (Table 3), and these traits also showed substantial genetic correlations. The strong genetic correlation between BWG and FI (approximately 0.87, Künzel *et al.* 2019) can be partly explained by the QTL on CJA3, which mapped to both traits (Table 3).

Two of the four most interesting candidate genes in the PU QTL on CJA3 (Table 4) are *transforming growth factor-β 2* (*TGFB2*) and *bone morphogenetic protein 2* (*BMP2*). Both genes are members of the TGFB superfamily (Iqbal *et al.* 2018; Loozen *et al.* 2019), which is known to encode multifunctional growth factors involved in cell differentiation, growth and morphogenesis processes (Li *et al.* 2003; Darzi Niarami *et al.* 2014). *TGFB2* is also involved in the mitogen-activated protein kinase (MAPK) signaling pathway (Kyoto Encyclopedia of Genes and Genomes, KEGG). This pathway is associated with many tissue-building and -rebuilding processes in organisms. The other two candidate genes are *phospholipase Cβ1* and *4* (*PLCB1* and *PLCB4*) (Table 4). According to KEGG analysis, they are involved in a broad spectrum of biological processes, including inositol phosphate metabolism, the calcium signaling pathway, the phosphatidylinositol signaling system, the GnRH signaling pathway and the Wnt signaling pathway. Involvement in inositol phosphate-related pathways is of specific interest,

because phytate provided the main source of P in the diet. Variation in PU likewise was caused by differences in digestive phytate breakdown, thus providing a different amounts of inositol and inositol phosphates for the quail's metabolism. Also far-reaching and as an example, the Wnt signaling pathway is known to be involved in bone metabolism, which supports the connection of PU and bone ash traits (Robling 2013; Maeda *et al.* 2019; Ponsuksili *et al.* 2020). This partially explains the genetic correlation of the traits.

Conclusions

The experimental design used in this study proved to be powerful for the calculation of an SNP linkage map. Several genome-wide significant QTL could be mapped by linkage and subsequent association analyses. It seems that the focal trait PU and the other performance and bone ash traits are polygenic in nature and are associated with some significant QTL and probably many other small-effect QTL that were not detectable in this study. Some overlap of QTL regions for different traits was detected, which is in agreement with the corresponding genetic correlations. For PU, a QTL on CJA3 could be detected by linkage mapping, which was substantiated by the results of the SNP association mapping. Four candidate genes were identified for this QTL, which should be investigated in further functional studies.

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Data availability statement

Genotype and phenotype data, pedigree information and the genetic map are available through OSF and can be accessed at https://osf.io/57nty/.

References

Beck P., Piepho H.-P., Rodehutscord M. & Bennewitz J. (2016a) Inferring relationships between phosphorus utilization, feed per gain, and bodyweight gain in an F2 cross of Japanese quail using recursive models. *Poultry Science* **95**, 764–73.

Beck P., Stratz P., Preuß S., Pitel F., Recoquillay J., Duval E., Rodehutscord M. & Bennewitz J. (2016b) Linkage mapping of quantitative trait loci for phosphorus utilization and growth related traits in an F2-cross of Japanese quail (*Coturnix japonica*). *European Poultry Science* **80**. https://www.european-poultry-science.com/Linkage-mapping-of-quantitative-trait-loci-for-phosphorus-utilization-and-growth-related-traits-in-an-Fsub2sub-cross-of-Japanese-quail-span-classws-name-Coturnix-japonica,QUlEPTUwNTg4NjkmTUlEPTE2MTAxNA.html. https://doi.org/10.1399/eps.2016.133

Borda-Molina D., Roth C., Hérnandez-Arriaga A., Rissi D., Vollmar S., Rodehutscord M., Bennewitz J. & Camarinha-Silva A. (2020) Effects on the ileal microbiota of phosphorus and calcium utilization, bird performance, and gender in Japanese Quail. *Animals* **10**, 885.

Broman K.W., Gatti D.M., Simecek P., Furlotte N.A., Prins P., Sen Ś., Yandell B.S. & Churchill G.A. (2019) R/qtl2: software for mapping quantitative trait loci with high-dimensional data and multiparent populations. *Genetics* **211**, 495–502.

Cheng K.M., Bennett D.C. & Mills A.D. (2010) The Japanese Quail. In: *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*, 8th edn (Ed. by J.K. Kirkwood & R. Hubrecht), pp. 655–673. Wiley-Blackwell, Chichester, West Sussex, Ames, IA.

Darzi Niarami M., Masoudi A.A. & Torshizi R.V. (2014) Association of single nucleotide polymorphism of GHSR and TGFB2 genes with growth and body composition traits in sire and dam lines of a broiler chicken. *Animal Biotechnology* **25**, 13–22.

Eeckhout W. & de Paepe M. (1994) Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Animal Feed Science and Technology* **47**, 19–29.

Esmailizadeh A.K., Baghizadeh A. & Ahmadizadeh M. (2012) Genetic mapping of quantitative trait loci affecting bodyweight on chromosome 1 in a commercial strain of Japanese quail. *Animal Production Science* **52**, 64–8.

Iqbal M., Zhang H., Mehmood K. *et al.* (2018) Icariin: a potential compound for the recovery of tibial dyschondroplasia affected chicken via up-regulating BMP-2 expression. *Biological Procedures Online* **20**, 15.

Kayang B.B., Vignal A., Inoue-Murayama M., Miwa M., Monvoisin J.L., Ito S. & Minvielle F. (2004) A first-generation microsatellite linkage map of the Japanese quail. *Animal Genetics* **35**, 195–200.

Kikuchi S., Fujima D., Sasazaki S., Tsuji S., Mizutani M., Fujiwara A. & Mannen H. (2005) Construction of a genetic linkage map of Japanese quail (*Coturnix japonica*) based on AFLP and microsatellite markers. *Animal Genetics* **36**, 227–31.

Knaga S., Siwek M., Tavaniello S., Maiorano G., Witkowski A., Jezewska-Witkowska G., Bednarczyk M. & Zieba G. (2018) Identification of quantitative trait loci affecting production and biochemical traits in a unique Japanese quail resource population. *Poultry Science* **97**, 2267–77.

Künzel S., Bennewitz J. & Rodehutscord M. (2019) Genetic parameters for bone ash and phosphorus utilization in an F2 cross of Japanese quail. *Poultry Science* **98**, 4369–72.

Li H., Deeb N., Zhou H., Mitchell A.D., Ashwell C.M. & Lamont S.J. (2003) Chicken quantitative trait loci for growth and body composition associated with transforming growth factor-beta genes. *Poultry Science* **82**, 347–56.

Li X., Zhang D. & Bryden W.L. (2017) Calcium and phosphorus metabolism and nutrition of poultry: are current diets formulated in excess? *Animal Production Science* **57**, 2304–10.

Loozen L.D., Kruyt M.C., Kragten A.H.M., Schoenfeldt T., Croes M., Oner C.F., Dhert W.J.A. & Alblas J. (2019) BMP-2 gene delivery

in cell-loaded and cell-free constructs for bone regeneration. *PLOS ONE* **14**, e0220028.

Maeda K., Kobayashi Y., Koide M., Uehara S., Okamoto M., Ishihara A., Kayama T., Saito M. & Marumo K. (2019) The regulation of bone metabolism and disorders by Wnt signaling. *International Journal of Molecular Sciences* **20**, 5525.

Manichaikul A., Dupuis J., Sen S. & Broman K.W. (2006) Poor performance of bootstrap confidence intervals for the location of a quantitative trait locus. *Genetics* **174**, 481–9.

Mills A.D. & Faure J.-M. (1991) Divergent selection for duration of tonic immobility and social reinstatement behavior in Japanese quail (*Coturnix coturnix japonica*) chicks. *Journal of Comparative Psychology* **105**, 25–38.

Minvielle F., Kayang B.B., Inoue-Murayama M., Miwa M., Vignal A., Gourichon D., Neau A., Monvoisin J.-L. & Ito S. (2005) Microsatellite mapping of QTL affecting growth, feed consumption, egg production, tonic immobility and body temperature of Japanese quail. *BMC Genomics* **6**, 87.

Morris K.M., Hindle M.M., Boitard S. *et al*. (2020) The quail genome: insights into social behaviour, seasonal biology and infectious disease response. *BMC Biology* **18**, 14.

Ori R.J., Esmailizadeh A.K., Charati H., Mohammadabadi M.R. & Sohrabi S.S. (2014) Identification of QTL for live weight and growth rate using DNA markers on chromosome 3 in an F2 population of Japanese quail. *Molecular Biology Reports* **41**, 1049–57.

Oster M., Reyer H., Trakooljul N., Weber F.M., Xi L., Muráni E., Ponsuksili S., Rodehutscord M., Bennewitz J. & Wimmers K (2020) Ileal transcriptome profiles of Japanese quail divergent in phosphorus utilization. *International Journal of Molecular Sciences* **21**, 2762.

Ponsuksili S., Reyer H., Hadlich F. *et al*. (2020) Identification of the key molecular drivers of phosphorus utilization based on host miRNA-mRNA and gut microbiome interactions. *International Journal of Molecular Sciences* **21**, 2818.

Rastas P., Calboli F.C.F., Guo B., Shikano T. & Merilä J. (2015) Construction of ultra-dense linkage maps with Lep-MAP2: stickleback F2 recombinant crosses as an example // Data from: Construction of ultra-dense linkage maps with Lep-MAP2: stickleback F2 recombinant crosses as an example. *Genome Biology and Evolution* **8**, 78–93.

Rastas P., Paulin L., Hanski I., Lehtonen R. & Auvinen P. (2013) Lep-MAP: fast and accurate linkage map construction for large SNP datasets. *Bioinformatics (Oxford, England)* **29**, 3128–34.

Recoquillay J., Pitel F., Arnould C. *et al*. (2015) A medium density genetic map and QTL for behavioral and production traits in Japanese quail. *BMC Genomics* **16**, 10.

Robling A.G. (2013) The expanding role of Wnt signaling in bone metabolism. *Bone* **55**, 256–7.

Roussot O., Feve K., Plisson-Petit F., Pitel F., Faure J.-M., Beaumont C. & Vignal A. (2003) AFLP linkage map of the Japanese quail *Coturnix japonica*. *Genetics Selection Evolution* **35**, 559–72.

Schmid M., Enderle E., Schindler D. & Schempp W. (1989) Chromosome banding and DNA replication patterns in bird karyotypes. *Cytogenetics and Cell Genetics* **52**, 139–46.

Shibusawa M., Minai S., Nishida-Umehara C., Suzuki T., Mano T., Yamada K., Namikawa T. & Matsuda Y. (2001) A comparative cytogenetic study of chromosome homology between chicken and Japanese quail. *Cytogenetics and Cell Genetics* **95**, 103–9.

Sohrabi S.S., Esmailizadeh A.K., Baghizadeh A., Moradian H., Mohammadabadi M.R., Askari N. & Nasirifar E. (2012) Quantitative trait loci underlying hatching weight and growth traits in an F2 intercross between two strains of Japanese quail. *Animal Production Science* **52**, 1012.

Stock A.D. & Bunch T.D. (1982) The evolutionary implications of chromosome banding pattern homologies in the bird order Galliformes. *Cytogenetics and Cell Genetics* **34**, 136–48.

Vali N. & Jalali M.A. (2011) Influence of different levels of phytase enzyme on japanese quail (*Coturnix japonica*) performance. *Agriculturae Conspectus Scientificus* **76**, 387–390.

Vollmar S., Wellmann R., Borda-Molina D., Rodehutscord M., Camarinha-Silva A. & Bennewitz J. (2020) The gut microbial architecture of efficiency traits in the domestic poultry model species Japanese quail (*Coturnix japonica*) assessed by mixed linear models. *Genes Genomes Genetics* **10**, 2553–62.

Weir B.S. & Cockerham C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358.

Wu Y., Zhang Y., Hou Z. *et al*. (2018) Population genomic data reveal genes related to important traits of quail. *GigaScience* **7**, 1–16.

Yang J., Lee S.H., Goddard M.E. & Visscher P.M. (2011) GCTA: a tool for genome-wide complex trait analysis. *American Journal of Human Genetics* **88**, 76–82.

Zlotina A., Maslova A., Kosyakova N., Al-Rikabi A.B.H., Liehr T. & Krasikova A. (2019) Heterochromatic regions in Japanese quail chromosomes: comprehensive molecular-cytogenetic characterization and 3D mapping in interphase nucleus. *Chromosome Research* **27**, 253–70.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Comparison of genetic and physical maps

Table S1. Summary of trait-associated markers ($P \leq 0.05$) within the significant QTL regions

Table S2. Summary of 73 identified genes for PU in a 0.5 Mbp region up- and downstream of significant SNPs