MOLECULAR ECOLOGY

Molecular Ecology (2013) **22**, 3949–3962 doi: 10.1111/mec.12376

Genomic dissection of variation in clutch size and egg mass in a wild great tit (*Parus major*) population

ANNA W. SANTURE,*[1] ISABELLE DE CAUWER,*†[1] MATTHEW R. ROBINSON,* JOCELYN POISSANT,* BEN C. SHELDON‡ and JON SLATE*

Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK, †Laboratoire de Génétique et Evolution des Populations Végétales, UMR CNRS 8198, Bâtiment SN2, Université des Sciences et Technologies de Lille - Lille 1, F-59655, Villeneuve d'Ascq Cedex, France, ‡Department of Zoology, Edward Grey Institute, University of Oxford, Oxford, OX1 3PS, UK

Abstract

Clutch size and egg mass are life history traits that have been extensively studied in wild bird populations, as life history theory predicts a negative trade-off between them, either at the phenotypic or at the genetic level. Here, we analyse the genomic architecture of these heritable traits in a wild great tit (*Parus major*) population, using three marker-based approaches – chromosome partitioning, quantitative trait locus (QTL) mapping and a genome-wide association study (GWAS). The variance explained by each great tit chromosome scales with predicted chromosome size, no location in the genome contains genome-wide significant QTL, and no individual SNPs are associated with a large proportion of phenotypic variation, all of which may suggest that variation in both traits is due to many loci of small effect, located across the genome. There is no evidence that any regions of the genome contribute significantly to both traits, which combined with a small, nonsignificant negative genetic covariance between the traits, suggests the absence of genetic constraints on the independent evolution of these traits. Our findings support the hypothesis that variation in life history traits in natural populations is likely to be determined by many loci of small effect spread throughout the genome, which are subject to continued input of variation by mutation and migration, although we cannot exclude the possibility of an additional input of major effect genes influencing either trait.

Keywords: association study, genomics, life history evolution, QTL mapping, quantitative genetics

Received 6 December 2012; revision received 10 February 2013; accepted 20 February 2013

Introduction

Clutch size and egg mass are reproductive traits with strong links to fitness in natural bird populations (Bennett & Owens 2002; Krist 2011). Life history theory predicts that when the amount of resources dedicated to a reproductive event is limited, individuals can invest in a either few large eggs or many small eggs (Smith & Fretwell 1974; Bernardo 1996), and across species, there appears to be such a trade-off (Blackburn 1991). However, the relationship within species is less clear, with evidence for both negative and positive relationships (Christians 2002). Positive relationships could result from variation in individual quality, such that high-condition individuals produce large clutches of large eggs, while poor-condition individuals produce small clutches of small eggs (van Noordwijk & de Jong 1986; Blackburn 1991). Of particular interest is whether an observed correlation, whether positive or negative, has a genetic basis, which could influence how the two traits (and the relationship between them) are able to respond to selection. There is some evidence of a genetic trade-off between offspring number and size in fish (Snyder 1991), reptiles (Sinervo & Doughty 1996) and

Correspondence: Anna W. Santure, Fax: +44 114 222 0002; E-mail: asanture@gmail.com

[1]These authors contributed equally to this work.

mammals (Roff 1992; Mappes & Koskela 2004; Wilson *et al.* 2005) (although no evidence of genetic trade-offs was found in a number of other studies, see Brown & Shine 2007; Gall & Neira 2004; Schroderus *et al.* 2012). In birds, a genetic covariance between clutch size and egg mass has previously only been estimated for great tits (Garant *et al.* 2008).

Great tits, *Parus major*, are described as an 'ecological model organism' owing to a wealth of studies of their behaviour, ecology and evolutionary biology in numerous (>12) populations across their range (Visser *et al.* 2003). Their extensive Eurasian distribution, abundance and amenity to using nest boxes have facilitated their choice as a field study organism. One long-term study of the species is located in Wytham Woods, near Oxford, which has been intensively monitored since the 1960s, with life history data and social pedigree relationships recorded for most individuals [see, for example, McCleery *et al.* (2004) and references therein]. Clutch size and egg mass in the Wytham great tits have been the focus of a number of pedigree-based quantitative genetic studies. It is known that both traits have moderate heritabilities (0.26–0.34 for clutch size and 0.40–0.51 for egg mass: Garant *et al.* 2008; McCleery *et al.* 2004; Quinn *et al.* 2006) and that there is a significant negative phenotypic correlation [± standard error (SE)] of −0.070 (0.013) between the two, which is at least partly driven by a significant genetic component [genetic correlation −0.210 (0.100): Garant *et al.* 2008].

Recently, genomic resources have become available for the great tit, including a 'SNP chip' with 9193 SNPs (van Bers *et al.* 2012), a genetic linkage map (K. van Oers, A. W. Santure, I. De Cauwer, N. E. M. van Bers, R. P. M. A. Crooijmans, B. C. Sheldon, M. E. Visser, J. Slate & M. A. M. Groenen, in preparation), a description of the pattern of linkage disequilibrium (I. De Cauwer, A. W. Santure, K. van Oers, N. E. M. van Bers, R. P. M. A. Crooijmans, B. C. Sheldon, M. E. Visser, J. Slate & M. A. M. Groenen, in preparation) and ongoing high-coverage whole-genome sequencing (M. A. M. Groenen, personal communication). The availability of markers distributed throughout the great tit genome gives the opportunity to conduct marker-based quantitative genetic approaches that complement 'classical' quantitative genetic studies. This offers the possibility of dissection of the genetic architecture of traits (Slate *et al.* 2010), that is, to investigate where additive genetic variation is located in the genome, and whether a trait is influenced by many genes of small effect distributed throughout the genome (polygenicity) or by a few genes of major effect (oligogenicity). Such questions have, in the past, been addressed by quantitative trait locus (QTL) scans and genome-wide association studies (Mackay 2001; Stinchcombe & Hoekstra 2008; Flint & Mackay 2009; Slate *et al.* 2010). However, one disadvantage of such approaches is that in the absence of significant results, one cannot discriminate between (i) a lack of power to detect loci of large effect in an oligogenic trait and (ii) a polygenic trait with loci of undetectably small effect distributed throughout the genome. A solution that provides an explicit test of polygenicity is to partition additive genetic variance for complex traits across genomic regions, for example across individual chromosomes. In particular, if the additive genetic contribution of a region scales with its size (and/or gene content), this provides strong support for a polygenic basis to the trait. This approach has been widely applied in human genetics (Visscher *et al.* 2007; Yang *et al.* 2011b) and in animal breeding (Hayes *et al.* 2010). A recent study has adapted this approach to complex pedigree structures encountered in studies of wild populations and applied it to the dissection of the genetic architecture of a morphological trait in the Wytham great tits (Robinson *et al.* 2013).

In this study, a large pool of SNP markers was used to dissect the genetic architecture of two female life history traits, clutch size and egg mass, and the genetic basis of the relationship between them, using three different approaches: (i) the variance explained by each great tit chromosome was estimated from the covariance between phenotypic similarity and sharing of SNP alleles and compared with chromosome size to determine whether each chromosome's contribution scales with size; (ii) QTL scans were performed to search for regions of the genome contributing to trait variation between individuals; and (iii) SNPs across the genome were tested for association with phenotypic variation.

Methods

Study population

Great tits have been studied at Wytham Woods, near Oxford, United Kingdom (UK) (51°46′N, 1°20′W), since the 1940s, with nest boxes first erected in 1947 (Lack 1964; Savill *et al.* 2010). Since the early 1960s, a wide range of phenotypic traits have been recorded, including morphological traits, life history information and social pedigree relationships [see, for example, McCleery *et al.* (2004) and references therein]. Blood has been collected for a limited subsample of birds between 1985 until 2005 and for most birds since 2005.

Genotyping

A total of 2644 individuals were successfully genotyped on an Illumina iSelect BeadChip ('SNP chip'); 7203 of

the 9193 SNPs included on the SNP chip were polymorphic in the study population (see van Bers *et al.* 2012 for a full description of methods and outcomes).

Pedigree and identity checking

Because pedigree errors can bias estimates of heritability (Charmantier & Reale 2005), the pedigree links among the 2644 genotyped individuals were checked using a set of molecular-based approaches (see K. van Oers, A. W. Santure, I. De Cauwer, N. E. M. van Bers, R. P. M. A. Crooijmans, B. C. Sheldon, M. E. Visser, J. Slate & M. A. M. Groenen, in preparation). A total of 2497 individuals of confirmed identity were included in further analyses.

Genetic maps

A genetic linkage map based on 1656 individuals from Wytham Woods was constructed for 32 of the 39 great tit chromosomes (1–15, 17–24, 26–28, 1A, 4A, 25A, 25B, LGE422 and Z; a number of very small microchromosomes, including chromosome 16, could not be mapped as no SNPs were genotyped on these chromosomes) (described in K. van Oers, A. W. Santure, I. De Cauwer, N. E. M. van Bers, R. P. M. A. Crooijmans, B. C. Sheldon, M. E. Visser, J. Slate & M. A. M. Groenen, in preparation). The different downstream analyses required linkage maps with different marker densities. Therefore, the final maps were as follows: (i) for QTL mapping, a 'framework' linkage map of 1674 markers, covering 1893 cM, where markers were placed in map positions where the best order was 1000 times [logarithm of odds (LOD) = 3] more likely than any other order, and (ii) for GWAS and chromosome partitioning, a set of 5591 'chromosome-assigned' markers, which included 4878 markers placed in a 'parsimonious' linkage map [1916 cM, where the best marker order was 1.002 times (LOD = 0.001) more likely than any other order], plus an additional 713 markers that were linked to markers in the parsimonious map and could be assigned a putative mapping position (in cM) based on comparative genomics with their predicted zebra finch genome location (see Appendix S1).

Clutch size and egg mass phenotypes

Data were available for 15 219 breeding events in Wytham Woods from 1958 to 2011. After filtering the data to remove nests that had been experimentally manipulated, second clutches and clutches for which the female's (*i.e.* mother of the nest) age was unknown, 10 532 records remained. This data set included the number of eggs, total mass of the eggs and the lay date of the nest; the three phenotypes were defined as follows:

1. *number of eggs*: the total number of eggs laid by a single female in a single reproductive attempt. Rare cases with more than one female inferred to be laying in the same nest (occurrence of more than one egg in a 24-h period) were excluded, as were (more frequently) clutches that could not be confirmed to be complete (defined as a clutch size recorded in a nest where incubation had begun).
2. *total mass of the eggs*: the fresh mass of a sample of between 1 and 13 eggs weighed before incubation had begun. The mean number of eggs measured per nest was 4.11 (standard deviation = 1.42, median = 4).
3. *lay date of the nest*: the date on which the first egg of a clutch was laid, inferred, if necessary, by back-calculation based on the assumption of one egg laid per day.

To account for year-to-year variation, the number of eggs, mean egg mass and lay date of the nest were all standardized by the mean and standard deviation each year and will hereafter be referred to as 'clutch size', 'egg mass' and 'lay date', respectively.

If available, clutch size, egg mass and lay date were extracted for all genotyped females for each year (*i.e.* each reproductive event) and merged with a number of environmental and individual variables as indicated in Appendix S2. The effects and significance of these variables on clutch size and egg mass were tested in two ways. First, a linear model was constructed using the 'lm' function in R (R Development Core Team 2012) and the significance of fixed terms tested using the 'dropterm' function to identify variables with a significant effect on clutch size or egg mass. Second, the significance of the random effects was tested by constructing a linear mixed model with and without each of the random effects, using the 'lmer' function in the lme4 package (Bates *et al.* 2011) in R. The log likelihood of the models was compared with a chi-squared statistic with one degree of freedom to assess the significance of each random effect.

All significant terms were included in subsequent models, with the nest box the eggs were laid in and the mother's identity (*i.e.* permanent environment effect) fitted as random effects and female age, the section within Wytham Woods, the area surrounding the nest box, the altitude of the nest box and the lay date of the nest fitted as fixed effects (see Appendix S2).

A total of 1610 clutch size measurements for 969 genotyped individuals and 1424 egg mass measurements for 902 genotyped individuals were available. All egg mass records also had clutch size measured.

Partitioning genetic variation across chromosomes

Chromosome partitioning was performed as described in Robinson *et al.* (2013). Under the null hypothesis of a polygenic additive genetic model, a large number of locations in the genome each contribute a small amount to the overall variance. The total genetic variance can therefore be partitioned as the sum of the effects of many markers across the genome in a mixed model framework (VanRaden 2008). To estimate the contribution of all SNP effects to the overall variance, the genome-wide sharing of SNP alleles (genome-wide relatedness, GWR) between two individuals was included as a random effect where the phenotype (y) is partitioned as

$$y = \mathbf{X}\beta + \mathbf{Z}u + \varepsilon$$

where X and Z are incidence matrices relating trait records to vectors of fixed effects β and random effects u, respectively, and ε is a vector of residual effects, where marker information is included in the random effects.

The genome-wide relationship matrix (GWRM) approach described in Robinson *et al.* (2013) is similar to that employed in human genetics to partition additive genetic variance for complex traits across genomic regions (reviewed in Powell *et al.* 2010; Yang *et al.* 2011a), but is applicable to data sets with complex pedigrees and close relatives. Here, Method 3 from Robinson *et al.* (2013) was used to estimate $\mathbf{G_3}$, a matrix of marker relatedness between individuals, using the 5591 'chromosome-assigned' markers; note that this method is the same, to a factor of two, as methods commonly used to calculate identity-by-state (IBS) allele sharing [for example, PLINK (Purcell *et al.* 2007) and GenABEL (Aulchenko *et al.* 2007b)]. The matrix $\mathbf{G_3}$ was then weighted by the expected relatedness from the pedigree following the study by Goddard *et al.* (2011) to give the genome-wide relatedness matrix **G** (Robinson *et al.* 2013).

Variance components were estimated in a restricted maximum-likelihood (REML) framework using ASReml version 3 (Gilmour *et al.* 2009). Under the null hypothesis of a polygenic model, it was predicted that the total additive genetic variance is distributed across the chromosomes in the genome according to the gene content of each chromosome. To test the alternative hypothesis that a small number of genes account for most of the genetic variance (*i.e.* an oligogenic model), it was first necessary to examine the contributions of each chromosome. For every chromosome, four models sets (Robinson *et al.* 2013) were constructed as follows:

1 mixed model with **G** constructed excluding markers on that chromosome.
2 mixed model with **G** constructed excluding markers on that chromosome, plus **G** constructed with only markers on that chromosome.
3 mixed model with **G** constructed with all markers
4 mixed model with **G** constructed with all markers, plus **G** constructed with only markers on that chromosome.

Subsequently, two likelihood ratio tests were performed.

1 contrast 1: for each autosome, model (ii) was compared with model (i), to test whether the chromosome explains any variation in the trait. Across all chromosomes, the expectation is that there is a positive linear relationship between the number of genes and the amount of variance explained per chromosome if the trait is polygenic. Given that each variance component is a point estimate (*i.e.* it may be over- or under-estimated, Robinson *et al.* 2013), the significance of the relationship is tested by fitting a linear regression between the number of genes and the variance explained by each chromosome.
2 contrast 2: for each autosomal chromosome and the Z chromosome, model (iv) was compared with model (iii) to test whether there is evidence that the variance explained by the chromosome is greater than the amount expected given its size (*i.e.* gene content).

The contribution of each chromosome to the overall phenotypic variance was tested by comparing the log likelihood of the genome-wide model (model i or iii; L_0) with the log likelihood of the genome-wide plus chromosome model (model ii or iv; L_1), with a likelihood ratio test (LRT);

$$\mathrm{LRT} = -2(L_0 - L_1)$$

Under the null hypothesis, the *LRT* follows a 50:50 distribution of a chi-square test with zero degrees of freedom (which is a point mass at 0; equivalent to the Dirac delta function) and a chi-square test with one degree of freedom. Given the small number of markers (<60) on some chromosomes, a total of 23 chromosomes or chromosome sets were constructed; chromosomes 1–15, 17–20, 1A, 4A and Z were each fitted as single chromosomes, while a chromosome set was obtained by combining all markers from chromosomes 21–28 and linkage group LGE422. These regions contained a total of 15 448 genes predicted from homology with the zebra finch genome (see K. van Oers, A. W. Santure, I. De Cauwer, N. E. M. van Bers, R. P. M. A. Crooijmans, B. C. Sheldon, M. E. Visser, J. Slate & M. A. M. Groenen, in preparation) (Table 1).

Table 1 Contributions of individual chromosomes (chr) to heritability (h^2) of clutch size (1610 records, 969 individuals) and egg mass (1424 records, 902 individuals). The likelihood ratio test, LRT1, tests whether the chromosome explains significant variation and can be used to test whether there is a linear relationship between chromosome size and contribution to overall additive genetic variance (see Fig. 1). LRT2 tests whether the chromosome contributes more to overall heritability than expected

					Clutch size			Egg mass		
Chr	N markers	Length (cM)	N Genes†	Size (Mbp)†	h^2	LRT1	LRT2	h^2	LRT1	LRT2
1	579	139.9	1254	119.6	0.141 (0.073)	4.512*	1.946	0.118 (0.080)	2.34	0.824
1A	415	93.6	972	73.7	0	0	0	0.075 (0.063)	1.830	0.506
2	700	139.7	1450	156.4	0.078 (0.073)	1.302	0.098	0.194 (0.085)	6.394**	3.054*
3	596	114.9	1290	112.6	0	0	0	0.025 (0.068)	0.148	0
4	356	97.6	811	69.8	0	0	0	0.007 (0.052)	0.022	0
4A	103	59.4	39	20.7	0.019 (0.030)	0.538	0.156	0.022 (0.035)	0.468	0.172
5	346	98.6	998	62.4	0.107 (0.062)	3.140*	1.67	0	0	0
6	177	78.0	596	36.3	0	0	0	0.027 (0.041)	0.542	0.104
7	176	72.6	562	39.8	0.058 (0.043)	2.510	1.35	0.010 (0.038)	0.078	0
8	134	53.8	575	28.0	0.030 (0.039)	0.670	0.262	0.057 (0.044)	2.198	1.380
9	130	54.2	497	27.2	0.024 (0.036)	0.518	0.152	0	0	0
10	148	50.5	444	20.8	0	0	0	0.006 (0.038)	0.032	0
11	135	58.2	397	21.4	0	0	0	0	0	0
12	152	51.9	369	21.6	0.109 (0.048)	8.270**	6.200**	0	0	0
13	117	40.9	379	17.0	0.028 (0.034)	0.930	0.392	0	0	0
14	126	49.2	426	16.4	0	0	0	0.019 (0.037)	0.304	0.060
15	173	49.1	381	14.4	0	0	0	0.015 (0.042)	0.138	0
17	96	45.4	336	11.6	0	0	0	0	0	0
18	93	49.9	334	11.2	0.004 (0.030)	0.018	0	0	0	0
19	97	49.4	348	11.6	0.011 (0.033)	0.104	0.006	0	0	0
20	155	49.4	356	15.7	0.015 (0.039)	0.146	0.002	0	0	0
Micros	308	411.0	1808	40.2	0.096 (0.061)	2.798*	1.490	0.051 (0.058)	0.906	0.226
Z	279	51.2	826	72.8	–	–	0	–	–	0

Terms significant at $P = 0.05$ and $P = 0.01$ are marked * and **, respectively. Numbers in parentheses are standard errors.
†Predicted from homology with the zebra finch genome.

Bivariate analysis

By fitting clutch size and egg mass in a mixed model jointly, it is possible to estimate both the variance parameters for each trait and the covariance between the traits for each random effect. In this way, the total phenotypic covariance and the polygenic additive genetic covariance can be partitioned. Components were estimated in ASReml version 3 using both the genome-wide relatedness matrix calculated from all SNPs and using the pedigree.

QTL analysis

A two-step variance components analysis (George *et al.* 2000) was performed to map the genome locations of loci contributing to variance in clutch size and egg mass. Because this approach requires pedigree information, only a subsample of genotyped individuals was used. By including all first- to fourth-degree family links, 1733 individuals could be linked into a 'QTL pedigree'. Of the 1733 genotyped individuals in the QTL pedigree, a total of 1202 clutch size measurements were available for 682 females, while for egg mass, 1058 measurements were available for 635 individuals.

Variance components analysis was performed as described in the study by George *et al.* (2000) and Slate (2005), where a mixed linear model was fitted to partition variance into fixed and random effects. Briefly, this is a standard animal model (Henderson 1975)

$$y = \mathbf{X}\beta + \mathbf{Z}u + \varepsilon$$

where **X** and **Z** are incidence matrices relating trait records to vectors of fixed effects β and random effects u, respectively, and e is a vector of residual effects. Variance components were estimated in a restricted maximum-likelihood (REML) framework using ASReml version 3. The heritability (h^2) is defined as the ratio of additive genetic (V_A) to total phenotypic variance (V_P, the sum of all variance components); $h^2 = V_A/V_P$.

To test the contribution of a putative QTL effect to the overall variance in the trait, a second linear mixed model was fitted, where variance is partitioned as

$$y = \mathbf{X}\beta + \mathbf{Z}u + \mathbf{Z}q + \varepsilon$$

where q is a vector of additive QTL effects. The effect of the QTL was tested by comparing the log likelihood of the polygenic model (L_0) with the log likelihood of the polygenic plus QTL model (L_1), with a likelihood ratio test (LRT);

$$\text{LRT} = -2(L_0 - L_1)$$

Under the null hypothesis of no QTL, the LRT follows a 50:50 distribution of a chi-square test with zero degrees of freedom and a chi-square test with one degree of freedom. To account for multiple tests genome-wide, the approach of Lander & Kruglyak (1995) was used to adjust the significance thresholds based on the genome size and number of mapped chromosomes. For this data set, a logarithm of odds (LOD) score [where LOD = LRT/2ln(10)] of 1.620, corresponding to a nominal P-value (P) of 0.003, is expected to occur once by chance in every genome scan and is termed 'genome-wide suggestive linkage', while a LOD score of 3.062 ($P = 9 \times 10^{-5}$) is expected with probability 0.050 times every time a genome scan is performed and is termed 'genome-wide significant linkage' (Lander & Kruglyak 1995; Nyholt 2000). Nominal significance ($P < 0.050$) requires a LOD score of ≥0.588.

To construct the polygenic plus QTL model, it is first necessary to use markers to estimate IBD (identity by descent) sharing between all individuals in the pedigree at each genomic location. The markers were first screened to remove one marker from each pair of markers with moderate to high linkage disequilibrium between them. To do so, the genotypes of all genotyped individuals were phased into haplotypes for each chromosome using BEAGLE version 3.3 (Browning & Browning 2007, 2009). For each chromosome, linkage disequilibrium between all pairs of markers was estimated with the r^2 statistic in GOLD (Abecasis & Cookson 2000), for further details, see I. De Cauwer, A. W. Santure, K. van Oers, N. E. M. van Bers, R. P. M. A. Crooijmans, B. C. Sheldon, M. E. Visser, J. Slate and M. A. M. Groenen (in preparation). Eighteen marker pairs were identified with $r^2 > 0.1$, and the marker with the lowest minor allele frequency from each pair was excluded.

Using the remaining 1656 markers in the framework map, the IBD coefficients between all pairs of individuals were derived at 1-cM and 5-cM intervals across the genome using the software LOKI v2.4.5 (Heath 1997; Heath *et al.* 1997), with 100 000 iterations for each position. For the Z chromosome, the IBD coefficients at 1-cM and 5-cM intervals were calculated using LOKI after amending the pedigree to remove all mother–daughter links (because in a ZW sex determination system, a daughter cannot inherit the Z chromosomes from her mother). The amended pedigree was also used in the estimation of variance components and likelihoods for the polygenic and QTL models; by including both the full pedigree and the amended pedigree in **Z**, variance was partitioned into polygenic additive genetic effects on the autosomes plus polygenic additive genetic effects on the Z chromosome; that is, the models are as follows:

$$y = \mathbf{X}\beta + \mathbf{Z}u + \mathbf{Z}'u + \varepsilon$$

and

$$y = \mathbf{X}\beta + \mathbf{Z}u + \mathbf{Z}'u + \mathbf{Z}'q + \varepsilon$$

where **Z′** includes the amended pedigree.

Quantitative trait locus scans were performed for clutch size and for egg mass every 5 cM across the genome, with neighbourhoods (±5 cM) of nominally significant QTL peaks scanned at 1-cM intervals for each trait. Approximate 95% confidence intervals for QTL peaks were defined by a drop of one in LOD score from the peak (Lander & Botstein 1989) as well as a more conservative 1.5-LOD drop. The correspondence between the genome-wide test statistics obtained from the clutch size and egg mass QTL scans were tested using the permutation approach of Keightley & Knott (1999) (see Appendix S3).

QTL mapping power analysis

Conclusions from the QTL mapping analysis rely on the power to detect regions of large effect (and hence the power to reject the hypothesis that traits are influenced by QTL of large effect that fail to reach genome-wide suggestive or significant linkage). Therefore, a simulation approach was used to determine the power to detect QTL explaining 5%, 12.5%, 20% and 35% of the overall phenotypic variance (Appendix S4).

Genome-wide association study (GWAS)

Genome-wide association studies (GWAS) aim to identify markers that are in strong linkage disequilibrium with causal variants affecting the trait value. A mixed model framework can also be used to test the effect of each SNP marker across the genome

$$y = \mathbf{X}\beta + \mathbf{Z}u + \varepsilon$$

where the SNP is fitted as a fixed effect β rather than fitting a local identity-by-descent (IBD) matrix as a random effect in the QTL mixed model. Thus, rather than partitioning variance due to variation in relatedness between individuals at a position in the genome,

association analysis fits the marker itself as a fixed effect and tests whether different marker alleles cause differences in the trait mean across individuals (Stinchcombe & Hoekstra 2008; Slate *et al.* 2010).

To test the effects of each SNP, a full mixed model can be run for each marker across the genome. A quicker alternative is to run a simple mixed model with SNP information and use the predicted breeding values (*i.e.* the predicted additive genetic value) of individuals as a proxy for the phenotype; this removes all variance due to fixed and random effects and substantially reduces the computational time to subsequently analyse each SNP (Aulchenko *et al.* 2007a). The simplified mixed model then fits SNPs as a fixed effect and partitions variance in breeding value into genome-wide relatedness as a random effect (analogous to the polygenic effect in QTL mapping). Fitting the marker-based genome-wide relatedness between individuals controls for similarity in phenotypes of related individuals due to overall genome sharing caused by population substructure (Amin *et al.* 2007).

ASReml version 3 was run to fit the initial mixed model in order to predict breeding values for the 969 and 902 genotyped females with clutch size and egg mass measurements. A number of these females had no known pedigree links with other genotyped individuals; therefore, rather than fitting the matrix of pedigree relatedness in the mixed model to account for the polygenic additive effect, the matrix of genome-wide relatedness was fitted, calculated using the approach outlined in the study by Robinson *et al.* (2013). SNPs were tested for allelic association with egg mass and clutch size breeding values using the 'polygenic' and 'mmscore' functions in GenABEL (Aulchenko *et al.* 2007b), adjusting for population stratification by fitting the internally calculated genome-wide kinship matrix (this kinship matrix is identical to $\mathbf{G}_3$, except for a factor of two). The significance of the fixed SNP effects was tested by extracting the *P*-values from the test for allelic association between SNP and trait with one degree of freedom in GenABEL, where the statistic is corrected for population stratification. The significance threshold was further adjusted for multiple testing by calculating the effective number of genome-wide tests, taking into account linkage disequilibrium between markers in the package Keffective (Moskvina & Schmidt 2008); for the panel of 5591 chromosome-assigned SNPs, the effective number of tests is 5573. For a significance threshold of 0.050, this gives a genome-wide significant value of $P = 9.0 \times 10^{-6}$.

It is known that breeding values should be used with caution, particularly in situations where the response to selection is being predicted, or when the relationship between breeding values and fitness is estimated (Postma 2006; Hadfield *et al.* 2010). To test whether using breeding values influenced the results of the GWAS, full mixed models were also fitted for each SNP. GenABEL is not able to incorporate repeated measures; therefore, two sets of models were run for each trait: (i) the nesting event of a female in her first year and (ii) a randomly selected nesting event of female from her second and subsequent years. Further details are provided in Appendix S5.

Concordance between chromosome partitioning and GWAS results, and QTL mapping

The three approaches described above exploit slightly different marker and phenotype information. QTL mapping relies on recombination events within families to define the 'boundaries' around a causal locus. Both chromosome partitioning and GWAS mapping exploit ancestral recombination events in the population, which have broken down associations between markers and phenotype for all loci except those in close physical linkage to causal loci. Because the power to detect QTL is dependent on their magnitude (Lynch & Walsh 1998; Sham *et al.* 2000) and the power of GWAS and chromosome partitioning is additionally dependent on the amount of LD between causal variants and the markers (Pritchard & Przeworski 2001), a genome scan may not detect true QTL of small to moderate effect because they fail to reach suggestive or genome-wide significance. However, concordance of nominally significant regions of the genome across QTL mapping and the GWAS scan and/or chromosome partitioning approach may provide some independent support for a putative QTL at that location. In particular, it is expected that the overall variance explained by a chromosome should scale with the sum of GWAS SNP effects across that chromosome.

The concordance between the analyses was tested in a number of ways for each trait. First, the concordance of effect size estimates and chromosomes contributing to overall variance in the chromosome partitioning approach was verified by calculating the correlation between the summed GWAS effect size estimates and the amount of variance explained by each chromosome. The significance of the correlation was tested by permuting SNPs across the genome 2000 times to give a null distribution of summed effects for each chromosome.

The concordance between results from QTL mapping with the GWAS and chromosome partitioning results was tested in three ways. First, the LOD score at the mapping position (in cM) of each SNP marker was predicted from a linear regression of the LOD scores of neighbouring QTL positions (for example, LOD scores

at 4 cM and 5 cM were used to predict the LOD score of a SNP mapped to 4.6 cM), and the *P*-value for each inferred LOD score was calculated and compared with the *P*-value from the GWAS. To determine whether the number of autosomal positions nominally significant in both analyses was greater than expected, the observed and expected counts were compared with a chi-square test. Second, a chi-square test was used to test whether autosomes with nominally significant QTL peaks were more likely to contain SNPs that reached nominal significance in the GWAS. Finally, those chromosomes with nominally significant ($P < 0.05$) QTL peaks were compared with the nominally significant chromosomes from the partitioning approach with a Fisher's exact test (*i.e.* with counts of nominal significance in both, one or neither approach).

Results

Partitioning genetic variation across chromosomes

The variance component partitioning of clutch size and egg mass using the genome-wide relatedness matrix calculated from all SNPs gave a heritability of 0.42 [standard error (SE) = 0.08] for clutch size and 0.42 (0.04) for egg mass, in broad agreement with previous work using 'classical' quantitative genetic methods using a social pedigree (McCleery *et al.* 2004; Quinn *et al.* 2006; Garant *et al.* 2008). The nest box and the permanent environment component contributed some variation for clutch size, but very little for egg mass (Table 2).

For both traits, there was a significantly positive relationship between the variance explained and the predicted size (in bp) of each chromosome (for clutch size: slope = 4.705×10^{-10}, $R^2 = 0.182$, $P = 0.048$ (Spearman rank correlation = 0.316, $P = 0.076$); for egg mass: slope = 9.402×10^{-10}, $R^2 = 0.637$, $P = 0.000$ (Spearman rank correlation = 0.577, $P = 0.002$, Fig. 1) (contrast 1, see Methods). Chromosome length (cM) and number of genes have a correlation of 0.810, and the number of markers, number of genes and chromosome size (bp) are also all highly correlated ($r = 0.774$–0.975, see Table 1). For clutch size, the number of genes (slope = 5.168×10^{-5}, $R^2 = 0.270$, $P = 0.013$) and chromosome length (slope = 2.691×10^{-4}, $R^2 = 0.227$, $P = 0.013$) were the best predictors of variance explained. For egg mass, chromosome size and the number of markers (slope = 1.653×10^{-4}, $R^2 = 0.651$, $P = 0.000$) were the best predictors of variance explained.

Chromosomes 12 and 2 contributed significantly more than expected from their size to clutch size and egg mass additive genetic variation, respectively (Table 1, LRT2, see Methods). A number of chromosomes did not contribute significantly greater than zero genetic variance (Table 1). Markers on the Z chromosome did not explain any additional variance in clutch size or egg mass (variance contributed from Z chromosome markers was 0 for both traits), giving the same likelihood and the same variance component partitioning as a model fitting all autosomal markers (LRT2, see Methods).

Table 2 Proportion of variance explained of random effects in models of clutch size (1610 records, 969 individuals) and egg mass (1424 records, 902 individuals) using the full marker set of 5591 SNPs (see 'partitioning genetic variation across chromosomes')

Term	Clutch size	Egg mass
Nest box	0.034 (0.027)	0
Additive genetic	0.423(0.079)**	0.424 (0.036)**
Permanent environment	0.016 (0.076)	0
Residual	0.527 (0.039)	0.576 (0.036)

**Terms significant at $P < 0.01$. Numbers in parentheses are standard errors.

QTL analysis

For clutch size, no regions of the genome were genome-wide significant. One region of the genome reached genome-wide suggestive linkage (LOD score = 2.01, $P = 0.001$) on chromosome 20 at 6 cM. The one-LOD drop interval was between 2.77 and 11.39 cM, while a more conservative 1.5-LOD drop gives an interval of 0–22.08 cM. Seven additional regions were nominally significant ($P = 0.050$): chromosome 1 at 21 cM, chromosome 3 at 0 cM, chromosome 12 at 45 cM, chromosome 19 at 8 cM, chromosome 19 at 35 cM, chromosome 20 at 19 cM and chromosome 20 at 25 cM (Fig. 2).

No regions of the genome reached genome-wide suggestive or significant linkage for egg mass. There were seven nominally significant peaks on chromosome 1A at 0 cM, chromosome 1A at 12 cM, chromosome 4 at 14 cM, chromosome 8 at 10 cM, chromosome 22 at 2 cM, chromosome 25B at 0 cM and chromosome 26 at 37 cM (Fig. 2).

There was no overlap of suggestive or nominally significant QTL peaks for clutch size and egg mass or evidence of any correspondence between the test statistics obtained from the two QTL scans [correlation = −0.072, tested using the approach of Keightley & Knott (1999), see Appendix S3].

QTL mapping power analysis

The results of the simulations indicate very low power to detect QTL of even moderate effect, suggesting that the data sets do not allow reliable identification of QTL for clutch size and egg mass (Appendix S4).

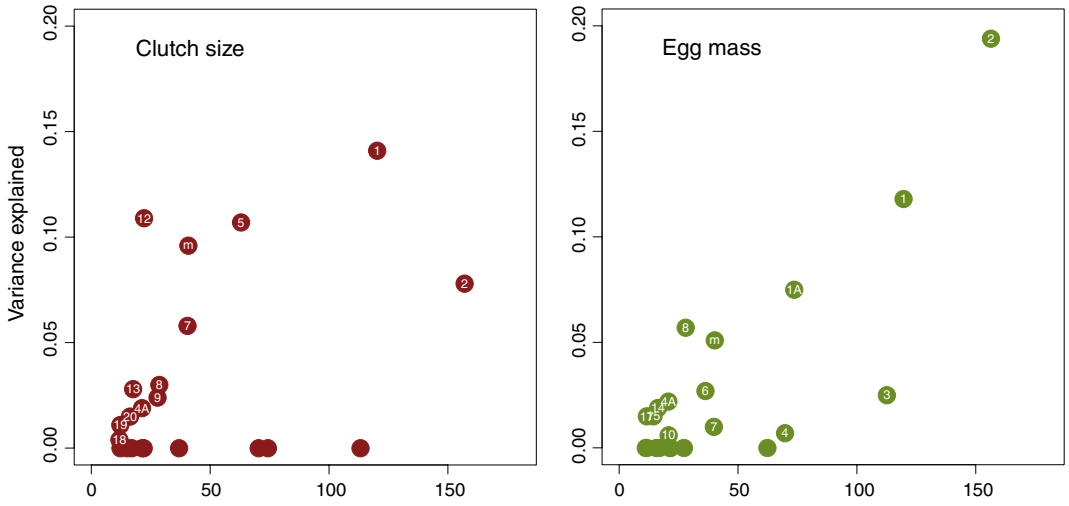

Fig. 1 Relationship between chromosome size (Mbp) and variance explained for clutch size and egg mass; for clarity, chromosomes that explain variance of <0.02 are not labelled.

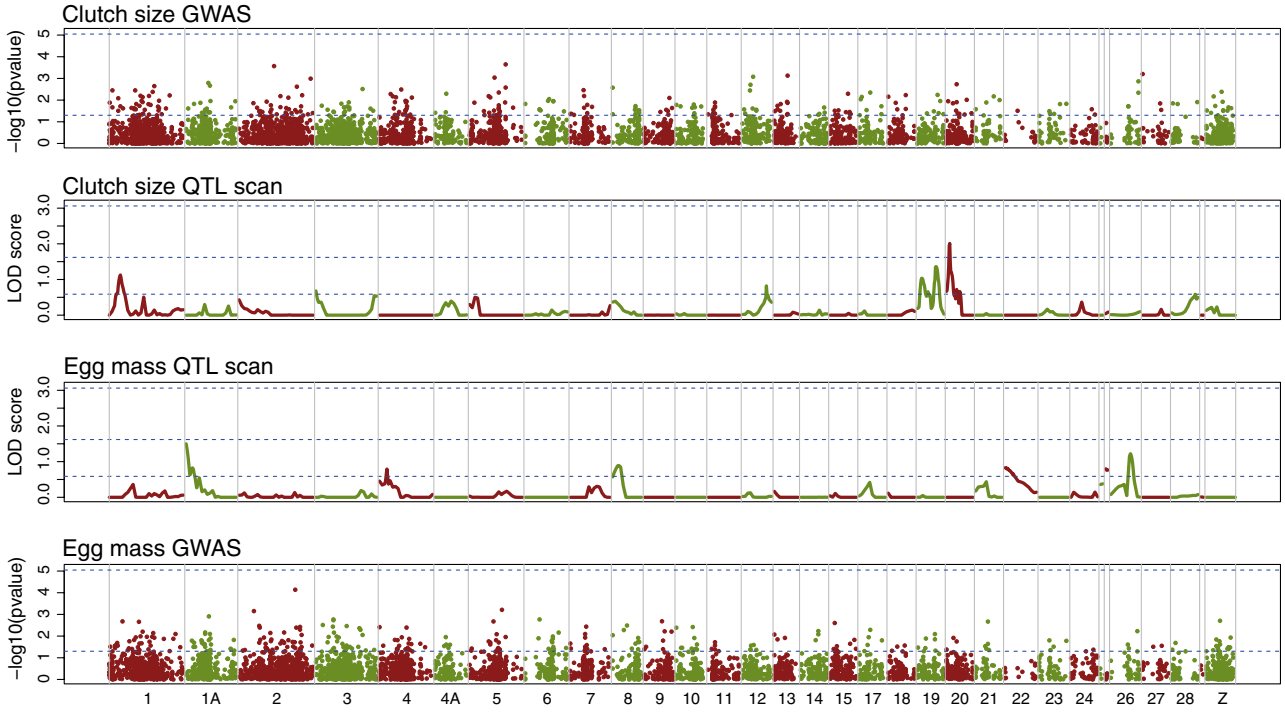

Fig. 2 Genome QTL and GWAS scans for regions of the genome affecting variation in clutch size and egg mass. For the QTL maps, the blue lines show nominal (LOD = 0.588), suggestive (LOD = 1.620) and significant (LOD = 3.062) scores. For the GWAS maps, the blue lines show thresholds for nominal [*P*-value = 0.050, $-\log_{10}$(*P*-value) = 1.301] and significant [*P*-value = 9×10^{-6}, $-\log_{10}$(*P*-value) = 5.046] *P*-values. Chromosome labels are shown beneath the plots; chromosomes 25A and 25B (plotted after chromosome 24) and LGE422 (after 28) are not labelled.

Bivariate analysis

The total phenotypic correlation between the traits was −0.064 (0.035) (Table 3). The additive genetic correlation between the traits was −0.120 (0.089) and did not differ significantly from zero. Similar results were apparent when using the genome-wide relatedness matrix to partition additive genetic variance and covariance [phenotypic correlation −0.049 (0.030), additive genetic correlation −0.131 (0.074), Appendix S6].

Given (i) the small and insignificant additive genetic correlation across the genome, (ii) the absence of

Table 3 Proportion of variance explained and covariance partitioning for a bivariate polygenic model (see 'bivariate analysis') including both clutch size (1058 records, 635 individuals) and egg mass (1058 records, 635 individuals), using the pedigree to partition additive genetic variance and covariance. Numbers in parentheses are standard errors

Term	Clutch size	Cross-trait covariance	Cross-trait correlation	Egg mass
Nest box	0.154 (0.042)	NA	NA	0
Additive genetic	0.404 (0.042)	−0.041 (0.031)	−0.120 (0.089)	0.396 (0.042)
Permanent environment	0	NA	NA	0
Residual	0.442 (0.046)	−0.011 (0.023)	−0.024 (0.047)	0.604 (0.042)

evidence that any region of the genome contributed to both traits in the chromosome partitioning analysis and (iii) no overlap of suggestive or nominally significant QTL peaks for clutch size and egg mass, a bivariate QTL scan of the genome was not undertaken. Running such a model would not have been justified, because at all positions in the genome, the variance for at least one trait lacked a QTL, and so the model would have been attempting to estimate the covariance between two variance components, at least one of which was zero.

Genome-wide association study

None of the 5591 SNPs tested for association with clutch size or egg mass were genome-wide significant after adjustment to account for population structure (by fitting genome-wide relatedness between individuals) and multiple testing (Fig. 2). Accounting for structure, 281 SNPs (5.025%) and 285 SNPs (5.097%) had *P*-values <0.050 for clutch size and egg mass, respectively. There was good agreement between the estimated effect sizes of nominally significant SNPs whether analysed using breeding values (see above and Fig. 2) or fitting a mixed model including standardized trait values and including all fixed effects while controlling for population structure (Appendix S5).

The distribution of the 281 and 285 nominally significant SNPs for clutch size and egg mass was random across the genome; a SNP was no more likely to be nominally significant if its neighbour was significant than if its neighbour was not (chi-square test on the observed and expected counts of neighbouring SNPs, $P = 0.964$ for clutch size and $P = 0.983$ for egg mass), and there was no evidence for some chromosomes having more nominally significant SNPs than others (chi-square test on the observed and expected counts of nominally significant SNP per chromosome, $P = 0.528$ for clutch size and $P = 0.490$ for egg mass). Sixteen SNPs were nominally significant for both clutch size and egg mass; this did not differ significantly from the number expected by chance (chi-square test on the observed and expected counts of SNPs with zero, one and two nominally significant peaks for the two traits; $P = 0.897$).

Concordance between chromosome partitioning and GWAS results, and QTL mapping

As expected (given that both approaches exploit LD using the same markers and individuals), there was a strong correlation between the GWAS summed effect sizes and the variance explained in the chromosome partitioning approach for both traits, and the correlations were stronger than expected from a random genome distribution of observed effect sizes (clutch size: $r = 0.490$, $P = 0.000$, egg mass: $r = 0.789$, $P = 0.031$).

There was no evidence that nominally significant GWAS SNPs colocalized with nominally significant QTL peaks (chi-square test on the observed and expected counts of SNPs being nominally significant in both, one or neither the GWAS and QTL analysis, $P = 0.257$ for clutch size and $P = 0.930$ for egg mass). Further, it was found that chromosomes with nominally significant QTL peaks were no more likely to harbour nominally significant GWAS SNPs (chi-square test on the observed and expected counts of nominally significant GWAS SNPs on chromosomes with and without nominally significant QTL peaks, $P = 0.508$ for clutch size and $P = 0.384$ for egg mass). The Z chromosome was not included in these analyses, as the QTL mapping followed a slightly different model (see Methods). However, given there were no nominally significant peaks on the Z chromosome for either clutch size or egg mass, including the Z chromosome would not affect the conclusion that there was no evidence for the co-occurrence of nominally significant GWAS SNPs and QTL peaks.

There was no evidence that chromosomes that contributed significantly to overall variance (measured as the significance of the LRT in contrast 1, see Table 1) harboured more nominally significant QTL peaks detected from the QTL mapping approach for either trait (two-tailed Fisher's exact test, $P = 0.210$ for clutch size, $P = 1.000$ for egg mass).

Discussion

Three complementary approaches were followed to study the genetic architecture of clutch size and egg mass in a wild population of great tits: all three approaches suggest that these traits have a polygenic basis, with many loci of small effect contributing to trait variation. In particular, evidence for a polygenic basis for clutch size and egg mass is provided by the observations that (i) for both traits, there was a significant positive relationship between variance explained and the size of each chromosome, (ii) there are no genome-wide significant QTL for clutch size and egg mass, and no excess of genome-wide suggestive peaks relative to the null expectation from a genome scan and (iii) none of the 5591 SNPs tested for association with clutch size or egg mass were genome-wide significant after adjustment to account for population structure and multiple testing.

While the above results appear to lend support to a polygenic basis for both traits, conclusions from these analyses rely on the power to detect regions of large effect. The QTL mapping power analysis indicated very low power to detect QTL of even moderate effect (Appendix S4), which is a sobering conclusion given that the number of animals (~650) was quite large, particularly in comparison with many other QTL studies of wild animal populations (Slate 2013). However, it is likely that because clutch size and egg mass are measured as female traits only, many of the most informative relationships between close relatives of different sexes (*e.g*. brother–sister) are missing, resulting in a substantial reduction in power.

There is very low linkage disequilibrium between SNPs in our data set (I. De Cauwer, A. W. Santure, K. van Oers, N. E. M. van Bers, R. P. M. A. Crooijmans, B. C. Sheldon, M. E. Visser, J. Slate & M. A. M. Groenen, in preparation); therefore, the power to detect significant association between markers and the traits in a GWAS is also very low. Robinson *et al*. (2013) conducted a power analysis for the chromosome partitioning approach and concluded from simulations that a polygenic architecture [simulated as one QTL of very small effect (mean variance explained = 0.002) every 10 cM] was distinguishable from multigenic [simulated as one QTL of small effect (mean variance explained = 0.018) on each of 22 chromosomes] and oligogenic architectures [simulated as five QTL each explaining 0.08 of phenotypic variance). However, it should be noted that these simulations were based on a large sample of 2000 birds, and that the power of the clutch size and egg mass data sets are likely to be lower. Given the low power of our study, caution should be taken not to overinterpret the results. In particular, there is some support of genes of large effect for clutch size on chromosome 12 and for egg mass on chromosome 2 (both chromosomes contributed significantly more to overall heritability than expected from their size, see LRT2, Table 1, and there was a nominally significant QTL for clutch size on chromosome 12). The power simulations and the nature of weak LD in the data set both indicated that (i) we have very low power to have detected QTL of major effect and (ii) some of the QTL detected in the genome scan that do not reach genome-wide suggestive or significant linkage may nonetheless be real, although it is likely that their effect sizes are not accurately estimated (see Slate 2013). Therefore, while these traits are likely to be largely polygenic, we cannot exclude the possibility that clutch size and egg mass are also influenced by some genes of major effect. However, the relationship between chromosome size and proportion of variance explained, and the failure to find QTL of very large effect would argue against these traits having an oligogenic architecture, where just a small number of genes cause *all* of the additive genetic variance.

The results indicate that moderate additive genetic variance is present for both clutch size and egg mass. Clutch size, in particular, is under directional selection in the population (Garant *et al*. 2007), and it might be expected that such selection will erode genetic variation over time. However, there are a number of reasons to expect that variation be maintained for traits under selection. First, and most importantly, the strength and direction of selection is highly variable both through time and space (Wilkin *et al*. 2006; Garant *et al*. 2007). For example, in years or environments with limited food availability, selection might act against alleles that increase clutch size, while in contrasting years and environments, such alleles may be favoured – that is, although selection may remain directional, its strength and sign can change over time and space. Second, as the traits are probably influenced by many loci, the total mutational input across these loci is high and is likely to contribute to the overall variance (Merilä & Sheldon 1999). Finally, there is a large amount of immigration into Wytham woods each year from neighbouring sites, with only around half of breeding adults born in Wytham [see, for example, Verhulst *et al*. (1997)]. Such a large input of (potentially maladapted) alleles into Wytham each year is likely to overwhelm selection at a local scale (Postma & van Noordwijk 2005; Star *et al*. 2008).

The lack of overlap between nominally significant positions across the genome for clutch size and egg mass, along with the small and insignificant genetic covariance between clutch size and egg mass, suggests that within this population, the evolution of clutch size is unlikely to be constrained by the genetic architecture of egg mass

and vice versa. Thus, the weakly negative genetic covariance between clutch size and egg mass seen in larger data sets (see Garant *et al.* 2007, which includes individuals genotyped in this analysis), is perhaps transient and, given no evidence of loci of large effect affecting either trait, could be contributed by many small local effects that are distributed genome-wide. Further dissection of the relationship between these traits will require many more genotyped individuals and markers to accurately understand the nature of the genetic covariance.

Although there is a clear negative relationship between clutch size and egg mass (standardized for body size) across bird species, such a relationship within species is less clear and is not necessarily expected given individual variation in quality and resource allocation to reproduction (van Noordwijk & de Jong 1986; Martin *et al.* 2006). This study demonstrates that, if present at all in the Wytham great tits, the genetic relationship between clutch size and egg mass is only weakly negative, and although there is moderate additive genetic variance for both traits, 'individual' choices of investment in each reproductive event are probably driven by external environmental factors including food availability and individual condition.

In summary, this is the first time a genomic and phenotypic data set of this scale has been analysed for life history traits in a wild avian population. In contrast to studies to date of wild pedigreed populations, which in many cases have located QTL of very large effect (see Slate 2013; for a discussion of the potential causes and robustness of these large effects), a combined approach of chromosome partitioning, QTL mapping and genome-wide association has failed to locate any QTLs contributing significantly to the moderate heritability for these reproductive traits. Although this finding agrees with recent conclusions from the analysis of genomic data sets of human, livestock and model organisms that many quantitative traits are influenced by a large number of loci of small effect (Hill *et al.* 2008; Allen *et al.* 2010; Hayes *et al.* 2010; Yang *et al.* 2011b), the low power in our data set suggests that we cannot rule out genes of major effect contributing to some of the genetic variation in clutch size or egg mass in the Wytham population. This analysis demonstrates the value of the long-term study of pedigreed wild populations such as the Wytham great tits in understanding the genetic basis of life history trait evolution, but also serves as a warning that even some of the longest running studies of pedigreed wild populations are not enormously powerful for gene mapping.

Acknowledgements

We thank the many people who have collected data from the Wytham Woods great tit population over the past decades and the many funding sources, too numerous to list here, that supported the collection of the data. We are very grateful for the insightful and helpful comments from three anonymous reviewers and Subject Editor Professor Staffan Bensch. This work was funded by the European Research Council (ERC), project AvianEGG, number 202487, awarded to JS, a Natural Environment Research Council (NERC) UK fellowship to MRR, and Natural Sciences and Engineering Council of Canada (NSERC) and Marie Curie fellowships to JP.

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AWS and IDC analysed the genotyping data and performed the chromosome partitioning, QTL mapping and GWAS analysis. AWS, IDC and JS drafted the manuscript. JS conceived of the study and participated in the design of the analysis. AWS, IDC, JP and MRR prepared the phenotypic data sets for analysis. JP performed the QTL power analysis. BCS provided the phenotypic data and the great tit blood samples. JP, MRR and BCS all provided comments on the manuscript. All authors read and approved the final manuscript.

Data accessibility

SNP genotypes, pedigree information, phenotype files including fixed and random effects and chromosome locations for the SNP markers: DRYAD entry doi:10.5061/dryad.ck1rq.

Supporting information

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

Appendix S1 Physical mapping of markers that could not be placed on the linkage map.

Appendix S2 Individual and environmental variables and the model coefficients and significance of their contribution to variation in clutch size and egg mass when fitted in a linear model.

Appendix S3 Correspondence between the test statistics obtained from the clutch size and egg mass QTL scans.

Appendix S4 QTL mapping power analysis.

Appendix S5 Genome wide association analysis of clutch size and egg mass, using phenotype values instead of breeding values.

Appendix S6 Variance and covariance partitioning for a bivariate polygenic model (see 'bivariate analysis') including both clutch size (1424 records, 902 individuals) and egg mass (1424 records, 902 individuals), using the full marker set of 5591 SNPs to partition additive genetic variance and covariance. Numbers in parentheses are standard errors.