The domesticated brain:

genetics of brain mass and brain structure in an avian species

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1   **ABSTRACT**

As brain size usually increases with body size it has been assumed that the two are tightly constrained and evolutionary studies have therefore often been based on relative brain size (i.e. brain size proportional to body size) instead of absolute brain size. The process of domestication offers an excellent opportunity to disentangle the linkage between body and brain mass due to the extreme selection for increased body mass that has occurred. By breeding an intercross between domestic chicken and their wild progenitor, we address this relationship by simultaneously mapping the genes that control inter-population variation in brain mass and body mass. Loci controlling variation in brain mass and body mass have separate genetic architectures and are therefore not directly constrained. Genetic mapping of brain regions in the intercross indicates that domestication has led to a larger body mass and to a lesser extent a larger absolute brain mass in chickens, mainly due to enlargement of the cerebellum. Domestication has traditionally been linked to brain mass regression, based on measurements of relative brain mass, which confounds the large body mass augmentation due to domestication. Our results refute this concept in chicken and confirm recent studies that show that different genetic architectures underlie these traits.

KEY WORDS Brain-mass evolution, domestication, cerebellum, QTL

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3 **INTRODUCTION**

4   Brain size variation across vertebrate species continues to fascinate evolutionary biologists, due

5   to the cognitive and behavioral phenotypes it is thought to underlie. Most studies on brain size

6   differences suggest some kind of trade-off between the costs of developing and maintaining

7   energetically expensive brains and certain physiological variables (such as body size [1],

8   metabolic rate [2], development time [3]) or lifestyle variables (e.g. foraging ecology [4] and social

9   environment [5]). One physiological variable that correlates notably with brain size is body size [1].

10   As brain size usually increases with increasing body size [6] it has been assumed that the two are

11   tightly constrained during developmental growth [7]. Researchers have therefore often relied on

12   relative rather than absolute brain size in correlative studies. The relationship between body size

13   and brain size is, however, poorly understood and the use of allometry in brain size evolution

14   studies has been criticized [8-10]. Understanding the genetics of brain size evolution is extremely

15   pertinent to determine the relationship between brain size and body size. Most importantly, to

16   what degree there is overlap (and potential pleiotropy) between the genes responsible for both.

17   To date, studies on the genetic relationship between brain and body size are almost entirely

18   limited to phylogenetic comparisons and measures of selection, and have failed to identify the

19   overlap of the genetic architecture between these traits (brain size and body size), especially

20   using a within-species approach. The analysis of the rates of evolution in a between-species

21   analysis of cichlids can indicate that brain size and body size can have distinct rates of evolution

22   [11]. Similarly, selection on body mass can potentially drive reduced relative brain mass [12], whilst

23   different orders of animals may have different brain-body mass variation, which is driven

24   primarily by variability in body mass [13]. Genetic correlations between brain and body size in

25   stickleback (*Gasterosteus aculeatus*) showed a positive correlation between the two traits [14], but

26   also a large standard error to this estimate (with the correlation being between 12-96%). In the

27   principle study to actually assess the genetic architecture of quantitative variation in brain size, a

28   quantitative trait loci (QTL) study in inbred mice strains identified genomic regions associated

29   with overall brain mass [15], though was primarily focussed on identifying QTL for separate brain

30   sub-regions. In this study, however, the genetic architecture for body mass was almost entirely

31   unresolved (identifying only one suggestive locus), making it impossible to assess any potential

32   overlap between brain and body mass loci. Therefore, nothing is known regarding the combined

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33 genetic architectures for brain mass and body mass. Thus, to date, no studies exist that

34 comprehensively examine the genetic architecture of both brain mass and body mass in an intra-

35 species specific manner.

36 The genetics underlying loci affecting overall brain size and body size in avian (or indeed

37 any other) species have yet to be explored, although the genes underpinning certain brain

38 regions in birds have been investigated. Most genetic work on avian brain regions has been on

39 genes relating to variation in the brain structures governing song learning [16-18], and genetic

40 programs involved in determining the basic architecture of the telencephalon [19-21]. The genetic

41 basis of overall brain composition differences has yet to be investigated in birds. The degree to

42 which different brain regions can develop independently is highly debated. According to the

43 'mosaic evolution' hypothesis individual brain regions can develop and grow independently in

44 size [22] while the 'concerted evolution' hypothesis argues that different brain regions have been

45 limited by developmental constraints and that brain size alters predominantly as a whole [3]. In

46 the case of the latter hypothesis therefore individual regions cannot change in size

47 independently. To date, only two studies have attempted to identify gene regions that affect

48 variation in brain substructure size within species. The previously mentioned QTL study in

49 inbred mice strains [15], and a Genome Wide Association Study (GWAS) study by Hibar et al. [23]

50 that identified eight loci affecting putamen and caudate nucleus volumes in humans.

51

52 Brain size differs substantially within species [24]. Since evolution operates through

53 intraspecific variation, within species differences can help disentangle the relationship between

54 brain and body size and the degree to which different brain structures are constrained

55 developmentally. The process of domestication is especially interesting because of the huge

56 differences in both brain and particularly body size in domesticated animals as compared to

57 their wild progenitors, and the reduction in relative brain size. This generates a perfect model

58 for assessing the effects of variation in brain and body size, how body size is constrained by

59 brain size and vice-versa. A classical effect of domestication is reduced relative brain size, with

60 this believed to reflect the reduced functional needs of domesticated animal's brains. Studies

61 have reported smaller brain size in both domesticated mammalian (sheep: [25], pig: [26,27], mink: [28]),

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62   and avian species (turkeys: [29], chickens: [30], pigeons: [31], ducks: [32]), as compared to their wild

63   progenitors. However, the methods used in virtually all studies on brain size reduction in

64   domesticated animals confound the effect of brain reduction with the often very large body size

65   augmentation that has occurred during domestication. The effect of reduced relative brain size

66   with directional selection is not limited to domestication, with studies on fish showing that

67   reduced relative brain mass can be potentially driven by increased selection for body mass [12].

68   Thus results from the genetic dissection of domestication may be pertinent in a wider

69   perspective.

70   Genomic sequencing of the wild progenitor of all domesticated chickens, the Red

71   Junglefowl (RJF) *Gallus gallus*, as well as different domestic chicken breeds has enabled the

72   identification of selective sweeps – gene regions that have been strongly selected upon during

73   domestication [33]. Although the domestic chicken and RJF differ both physiologically and

74   behaviourally they still belong to the same species and can interbreed. This allows us to perform

75   a similar QTL analysis as that by Hager and colleagues in mice [15] but with the added advantage

76   that large differences in the domestic chicken and RJF brain and body mass and genome

77   provides us with an *a priori* hypotheses regarding brain and body phenotype (mass) and which

78   genes might be involved (underlying selective sweep-regions).

79   In this study we use a domestic (White Leghorn chicken, *Gallus gallus domesticus*) x wild

80   (RJF) advanced intercross to fine-map quantitative trait loci (QTL) pertaining to brain mass and

81   body mass differences between wild and domestic populations, as well as ontogenetical

82   analyses of brain and body mass development in wild and domestic birds. This intercross allows

83   us to separately map both brain mass and body mass QTL and determine the genetic

84   architecture of both traits. Brains from this intercross population were further subdivided into 4

85   brain regions and weighed. To access potential differences in cerebrotype, each brain region

86   was mapped both as a whole-weight measure and in terms of the proportion of total brain mass

87   accounted for by each region (termed 'proportional QTL'). It is important to note that the QTL

88   mapping procedure only detects loci that differ between the parental populations, i.e. those loci

89   that are distinct between wild and domestic birds. Loci that both populations have in common

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90   will not be detected. In this way, we can therefore specifically detect those loci that control

91   brain mass and body mass that have been selected upon by domestication. However, the general

92   loci that both domestic and Red Junglefowl share will be undetected, and therefore missed.

93   Ontogenetic brain and body mass development analyses were performed using populations of

94   wild and domestic birds. These allow the developmental growth trajectories of both brain mass

95   and body mass to be compared between wild and domestic birds. If brain mass is indeed

96   inextricably linked with body mass, the growth trajectories of both brain mass and body mass

97   should mirror one another, in both wild and domestic populations. This enables a separate

98   analysis of the relationship between brain and body mass in an inter-population context. To our

99   knowledge, this is the first study to actually identify multiple genomic regions underpinning the

100 mass differences in both whole brain and brain region mass, whilst simultaneously mapping

101 body mass, not only between a wild and domesticated population but also in vertebrates in

102 general. This approach separates and decouples the detected brain loci at a genetic level from

103 those loci affecting only body mass.

104 **METHODS**

105 **Chicken Study population and cross design**

106 The intercross population was an eighth generation intercross between a population of RJF,

107 derived originally from Thailand [34,35] and a line of selected White Leghorn (WL) chickens, with

108 a total of 470 adult F_8 individuals were used in this study. Females were assayed for brooding

109 prior to culling. In addition a total of 61 RJF and 65 WL chickens were reared (in conditions

110 identical to the intercross birds) to assess brain development and growth differences from age

111 one, two, four, ten and fifteen weeks as well as at adult age, between RJF and WL. Eleven

112 broiler birds were additionally dissected at two weeks of age, as a comparison for the other

113 major domesticated strain. Volumetric measurements were taken on the brains of two adult

114 male RJF and 2 adult male WL individuals. The study was approved by the local Ethical

115 Committee of the Swedish National Board for Laboratory Animals. All methods were

116 performed in accordance with the relevant guidelines and regulations.

117 **Phenotyping**

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118   **Brain measurements and dissection**

119   Immediately after culling, the brains were removed from the birds and weighed, after being

120   dissected into four pieces for 315 individuals and nine pieces for 129 individuals. Total brain

121   mass values were available for 439 of these individuals. Four piece dissections involved

122   dividing the brain into the cerebral hemispheres, optic tectum, cerebellum and a brainstem

123   region (which included thalamus, the rest of the midbrain and the hindbrain; for more

124   information on the dissected brain regions see supplementary figure 1 and the supplementary

125   methods). Nine-piece dissections were only used for QTL analysis of body mass and whole

126   brain mass, with these dissections being used for a different study ([36] – with the brainstem

127   region further subdivided into the optic chiasma, thalamus, hypothalamus, medulla

128   oblongata, pons and nucleus tractus solitarii). Each brain region was weighed to the nearest

129   0.001g. To ascertain whether a heavier brain also equates to a larger brain, brain mass was

130   compared with volume in a subset of RJF and WL birds. The proportional brain region mass

131   was calculated by dividing the mass for each specific region by the total brain mass. Details of

132   volumetric measures are given in the supplementary methods section as is the technique for

133   assessment of brooding. Brain volume was found to scale linearly with mass (see supplementary

134   figure 2A). In all four brain regions, regions with a larger mass had a greater volume, with brain

135   mass explaining 98-100% of the variation when regressed on brain volume (supplementary

136   figure 2B).

137   **Genotyping, QTL and mapping**

138   All individuals were genotyped for 652 SNP markers, with QTL analysis performed using R/Qtl

139   [37] for both standard interval mapping and epistatic analyses. See supplementary methods for full

140   details of the mapping and models used.

141   **RESULTS**

142   **Total brain mass is larger in domestic birds with a different growth trajectory relative to**

143   **body mass in comparison to wild birds**

144   Absolute brain mass is larger in domestic (WL) chickens relative to wild RJF (figure 1), with a

145   consistent difference of approximate ~0.2g up to 5 weeks of age, with this increasing to ~0.4g at

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146 sexual maturity. In the case of both domestic chickens and RJF brain growth is linear up to

147 adulthood. In contrast body mass differences diverge rapidly after 4 weeks of age between

148 domestic chickens and RJF, with domestic chickens showing a sharp exponential increase in

149 mass, and at sexual maturity they weigh almost twice as much as RJF (figure 1). Thus whilst

150 domestic chickens exhibit the classic effects of domestication (reduced relative brain mass,

151 massively increased body mass), the differences between brain growth and body growth

152 trajectories between these two populations suggests that different genetic systems are at play

153 governing these different traits. If this is the case, then the genetic architecture of brain mass and

154 body mass in the intercross population should be non-overlapping. Additionally, the brain

155 composition of wild birds and domestic birds was also found to differ, with domestic birds

156 possessing a larger cerebellum and cerebral hemispheres and smaller optic tecta and brainstem

157 with these differences being largely consistent throughout post-hatch growth (Supplementary

158 figure 3). Therefore, non-overlapping architecture for different brain sub-regions in the

159 intercross would also favour the mosaic brain theory over the concerted brain hypothesis.

160 **The detected architectures for inter-population brain mass and body mass appear**

161 **separate**

162 A total of 20 QTL relating to inter-population brain mass and proportional brain mass variation

163 were identified (table 1 and supplementary table 2). One of the first things of note is that the

164 QTL for brain mass and proportional brain region mass and absolute brain region mass are

165 entirely separate from the QTL for body mass. There is no overlap for any of the loci involved,

166 indicating that the genes selected by domestication (i.e. those leading to inter-population

167 variation) for body mass and brain region mass are at least partially separate, i.e. it is possible to

168 select for increased brain growth without increased body mass and vice-versa during

169 domestication (see figures 2 and 3). Although body mass was not used as a covariate for the

170 brain mass QTL analysis presented above (due to the possibility that including it could mask

171 QTL that overlap body mass by already factoring body mass out the model), its inclusion lead to

172 no changes in the detected QTL position, and in fact strengthened the QTL in some cases,

173 further highlighting the separate nature of the two architectures. A general caveat with QTL

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174   analysis is that the genes controlling brain mass and body mass that are shared between

175   domestic and wild populations (i.e. intra-population variation) will not be detected as QTL in

176   this analysis. However, the phenotypic correlation between brain and body mass in this cross is

177   relatively low and undetected loci causing intra-population variation that also overlapped would

178   increase this correlation. Although significant, sex and body mass together account for only

179   18% of the total variation in brain mass, with sex alone accounting for 16% of the variation.

180   Similarly, when a linear model fitting all the detected QTL as well as body mass was regressed

181   onto brain mass, body mass still only accounted for 2% of the variation in brain mass variation,

182   whilst the detected genetic loci accounted for 22% of the brain mass variation. A further caveat

183   is that genes with effects too small to be detected may yet be pleiotropic between brain and

184   body mass, though given the lack of any phenotypic correlations as noted above, this seems less

185   probable. In the case of the genetic architecture for body mass, the detected QTL account for

186   over 36% of the total variation for body mass (excluding sex differences), whilst the total GLM

187   that included all body mass QTL and other covariates explained 84% of the variation for body

188   mass. Therefore, relatively little unexplained variation is present in the model for body mass. A

189   significant sex interaction can indicate that a QTL has greater effect on one sex as compared to

190   the other. In this case, very few sex interaction effects were seen for cerebellum and total brain

191   mass traits, with the only interactions being on chromosomes 3 (total brain mass) and 5

192   (proportional cerebellum mass), and none were found for any other traits (see supplementary

193   table 3).

194   **Domestic genotypes cause larger brains and larger cerebella than wild genotypes**

195   In the case of the cerebellum and total brain mass QTL, the observed allelic effect is in

196   accordance with the phenotypic differences seen in the wild and domestic breeds (see

197   supplementary figures 3-4). Domestic alleles correlate with an increase in cerebellum mass and

198   total brain mass in 6 out of 7 of the cerebellum QTL and 3 out of 4 of the brain mass QTL

199   identified, i.e. the QTL are not transgressive (see the additive and dominance QTL effect sizes

200   in table 1), meaning that the alleles correlating with an increase in the phenotype come from the

201   parental strain possessing the larger phenotype. In combination with the observation that brain

202   mass has a separate genetic architecture to body mass in this cross, this indicates that once the

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203   genes underpinning brain mass and body mass are de-coupled, domestic chickens have larger,

204   not smaller, brains as compared to their wild counterparts. Further to this, the cerebellum is also

205   larger in mass in the domestic chickens (both totally and as a proportion of total brain mass),

206   with the genetic control of this trait separate from overall body mass. There is a strong overlap

207   between the observed genetic architectures for the cerebellum and total brain mass, with three of

208   the four brain mass QTL overlapping total cerebellar mass QTL (on chromosomes 1,3, and 7,

209   see table 1).

210   **Brain regions each have their own unique genetic architecture**

211   The different brain regions have separate genetic architectures from one another for both total

212   and proportional mass, as determined by the loci detected. Although in the case of the optic

213   tectum and brainstem regions very few QTL were identified (only two loci in total). For both

214   cerebral hemispheres and the cerebellum multiple QTL were identified (18 in total), explaining

215   a relatively large amount of the variation in the cross (26% of total variation for proportional

216   cerebral hemispheres mass, 26% of total variation for total cerebellum mass, 22% of the

217   variation for total brain mass). Even with these more complete genetic architectures, there was

218   nevertheless no overlap between different brain regions QTL with one another, indicating that,

219   at least partially, this supports the mosaic theory of brain evolution [22].

220   **Brain mass is selected for during domestication**

221   Selective sweeps have been putatively identified in the chicken genome, representing regions

222   that have reduced heterozygosity during the periods of selection that occurred during

223   domestication [33]. By using a clustering analysis based on the number of sweeps detected in the

224   genome, the confidence intervals of the brain QTL and the number of overlaps between brain

225   QTL and selective sweep loci, we found that the QTL relating to cerebellum mass (7 loci in

226   total, marked in bold in table 1) contained a significant enrichment of selective sweeps (P=0.04,

227   using the clustering test as described in the supplementary methods), as did the regions relating

228   to total brain mass (4 loci overlapped, P=0.01, clustering test). These results indicate that loci

229   affecting cerebellum and total brain mass may have been directly selected upon during

230   domestication, or that these loci are closely linked with other genes targeted by domestication

231 selection. Given this significant overlap between selective sweeps and QTL regions, genes

232 contained within these sweeps are good candidates for the genes causal to the QTL (often

233 referred to as Quantitative Trait Genes). Twelve genes are present in the selective sweeps within

234 total brain and cerebellar QTL (see table 1). Four of these (*FGF3, FGF9, BIN1, SHANK1*) have

235 previous associations with neuronal conditions or neurogenesis (see discussion).

236

237 **Brooding behaviour correlates with proportional cerebellum mass**

238 Given the strong selection on the mass of the cerebellum in domestic chickens, we correlated

239 brooding behaviour with proportional cerebellum mass. We found a strong negative relationship

240 between brooding behaviour and proportional cerebellum mass (GLM, n=123, F=-2.6,

241 P=0.009), indicating that birds with a larger cerebellum and thereby a more domesticated brain

242 cerebrotype displayed less brooding behaviour, characteristic of domestic chickens. Body mass

243 was included in the model as a covariate, so these results are not due to overall body mass

244 effects. Four QTL were detected for brooding behaviour (see supplementary table 2). Although

245 none overlapped total brain mass QTL, the confidence interval of one (on chromosome 9) was

246 within 32cM of a total brain mass QTL, whilst another (on chromosome 4) was within 38cM of

247 a QTL for proportional optic tectum mass. In the case of the latter this distance corresponds to

248 around 200kb, whilst in the case of the latter it is around 10Mb. No correlations were found

249 between other brain regions and brooding.

250

251 **RJF and WL fat/ lean/ bone body tissue proportions**

252 The body composition, and in particular the lean mass to fat mass percentage, could potentially

253 be important in explaining intra-species brain mass differences, especially if lean mass requires

254 a proportional increase in neurons. If domestic birds have a far greater percentage of fat mass,

255 then departures from the usual brain allometry may be due to large brain mass not being

256 required for the extra fat tissue. Using data from a previous study by Rubin and colleagues [38]

257 that measured overall fat, lean and bone mass in both wild and domestic chickens using Dual X-

258 ray Absorbancy (DXA) techniques it is possible to calculate the relative proportions of these

259 tissue types (see supplementary table 4). Although domestic females have a higher percentage

260 of body fat (65% lean mass in WL females to 84% lean mass in RJF females), the domestic

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261   males (that show the largest decrease in relative brain mass) actually possess a far lower

262   percentage body fat than their wild counterparts, with a correspondingly higher proportion of

263   lean muscle mass (86% lean mass in WL males to 77% lean mass in RJF males).

264

265   **DISCUSSION**

266   Domesticated chickens (WL) have a larger brain mass and body mass than their wild progenitor,

267   but whereas body mass has increased by ~85% during domestication, brain mass has only

268   increased by ~15%. This indicates that brain mass has been altered less by selection during

269   domestication than body mass and that in chickens reduced relative brain mass during

270   domestication has mainly been caused by an increase in body mass. By separating the linkage

271   between loci affecting inter-population variation in brain mass and body mass in an advanced

272   intercross we demonstrate that domestication selection has acted on apparently separate loci to

273   increase brain mass and body mass in domesticated individuals. Therefore selection on body

274   mass is not limited by brain mass and vice-versa in the chicken, with domestication leading to

275   (at least partially) separate genetic architectures for these traits. It is important to note that brain

276   and body mass architectures that are common to both wild and domestic populations will not be

277   detected with this analysis. This means we cannot infer that no loci affecting brain mass and

278   body mass are pleiotropic or overlap, only that those that are due to domestication selection do

279   not. Similarly, with any QTL study there is always the possibility that loci with effect sizes too

280   small to detect exist and do overlap and exhibit pleiotropy. However, the relatively small

281   percentage of variation of brain mass explained by body mass suggests that these within-

282   population loci are of smaller effect than the between-population loci. In the combined model of

283   QTL and body mass covariates affecting brain mass, body mass explains only 2% of brain mass

284   variation whilst the QTL explains around 22%, and sex explains 4% of the variation. Likewise,

285   if numerous undetected, small-effect loci were also pleiotropic we would still expect the brain

286   and body mass correlation to be higher. It is also possible that developmental constraints

287   between brain and body mass may not necessarily exist at a pleiotropic level, but there could

288   still be a physiological constraint. In this case, however, a stronger correlation between brain

289   mass and body mass would still be expected in the above linear model. Our study is the first to

290   provide evidence for a relatively distinct genetic architecture for body mass and brain mass in

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291   an avian species and also to demonstrate that separate loci underlie the mass of the different

292   brain regions, thereby showing that the mass of an individual brain regions can be selected upon

293   without being strictly constrained by the mass of the other brain regions, as predicted by the

294   mosaic brain evolution hypothesis. Support for mosaic brain evolution has also been reported in

295   mammals [15] and fish [14] and together with our findings in birds suggest that mosaic brain

296   evolution is possible across vertebrate species.

297

298   The nature of the body and brain mass increases during domestication is of relevance when

299   considering allometric scaling and the relationship between brain mass and body mass. It has

300   been proposed that the developmental constraints limiting brain and body mass are related to the

301   overall musculature (see the trophic theory of neural connections [39], amongst others), with

302   increased neural circuitry required for an increase in muscular anatomy. A possibility therefore

303   is that domestic birds have a lower relative brain mass due to increased fat reserves making up a

304   larger percentage of their overall body mass. The majority of the mass gain in domestics would

305   then be due to increased fat deposits and thus not require any increase in brain mass. However,

306   the calculations we performed using the data from a previous study by Rubin and colleagues [38]

307   indicates that this does not appear to be a limiting factor in this instance, with male WL birds

308   having a higher lean body mass percentage than their RJF counterparts. Similarly, we have also

309   shown that in chickens an increase in brain mass also correlates with an increase in brain

310   volume. Similar strong correlations between brain mass and brain volume have recently been

311   shown in stickleback fish [14]. This suggests that the density of brain tissue is constant, however it

312   remains an open and intriguing question whether the increased brain mass induced by

313   domestication relates to the incorporation of more neurons, or alternatively non-neuronal cells

314   such as blood cells or glia, connections and the like.

315   Although the brain and the four different brain regions grow continuously until adulthood in

316   both RJF and WL, the proportional mass of each brain region changes during posthatch

317   development and domestic and wild birds differ in the proportional mass of certain brain

318   regions. These differences in cerebrotype between RJF and WL, caused by selection during

319   domestication, must therefore occur initially during prehatch development or during the first

13

320   week posthatch. Our findings suggest that selection during domestication in chickens has been

321   stronger on the cerebral hemispheres and cerebellum than on the brainstem region and optic

322   tectum. This is further supported by the enrichment of selective sweeps (gene regions that have

323   been strongly selected upon during chicken domestication), which are present in the QTL

324   regions for both cerebellum and overall brain mass. Increased proportional cerebellum mass,

325   compared to their wild counterparts, is seen in most studies on domesticated birds, including

326   domesticated geese [40], turkeys [29] and pigeons [41] (but see also [31] for opposite results), but not in

327   ducks [32], suggesting that proportional enlargement of the cerebellum may have played an

328   important role during domestication in several birds species.

329

330   We find that brooding behaviour is inversely correlated with the proportional mass of the

331   cerebellum. These findings suggest that the cerebellum could help govern this behaviour, and

332   therefore provide a link between domestication effects on brain composition (an enlarged

333   cerebellum) and domestication effects on behaviour (reduced broodiness). Proportional

334   enlargement of the cerebellum and cerebral hemispheres could potentially have been important

335   for the chicken to adapt to several aspects of the domesticated environment. In birds increased

336   cerebral hemispheres mass has been linked with increased social complexity [42] and the

337   cerebellum has been linked with foraging strategy [43]. Wild RJF live in small group sizes of

338   around 4-10 individuals [44], whereas domestic chickens are kept in far larger groups. Although

339   these theories are tempting to extrapolate upon, care must be taken in their interpretation given

340   the purely correlative data.

341   The ultimate goal of a QTL study is often to identify the genes themselves that underlie the

342   trait in question. In standard (F_2 or similar) QTL analyses, the confidence intervals of detected

343   QTL are so large (typically in the region of 20-30 megabases) that identifying putative

344   candidate genes is virtually impossible. However, in the study presented here the combination

345   of the advanced intercross and the overlap with selective sweeps for domestication yield a

346   number of candidate genes for brain growth. In regards to the QTL for cerebellum mass and

347   total brain mass, 12 genes in total are present within sweeps in these regions. Of these, four

348   have prior associations with neural conditions and neuronal generation, reinforcing them as

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349 excellent candidate genes for increased brain and cerebellum mass. The chromosome 1 sweep at

350 182.6Mb, in the middle of the QTL region for total cerebellum mass, contains the gene *FGF9*,

351 which regulates the generation and positioning of Bergmann glia cells in the developing mouse

352 cerebella [45]. *FGF9* also has a crucial role in embryonic neurological development [46-49]. Similarly

353 the QTL for proportional cerebellum mass on chromosome 5 contains 4 sweeps, with three

354 genes present in those sweeps. Once again an *FGF* gene, in this case *FGF3*, is present. Also

355 present in a separate sweep is *SHANK1*, mutations in which are associated with dysfunction of

356 glutamatergic synapses that lead to a variety of neuropsychiatric disorders including autism and

357 schizophrenia [50]. The sweep located at 25.42Mb on chromosome 7 that overlaps with the total

358 brain mass QTL is located within 100kb of the gene *BIN1* that has effects on working memory,

359 hippocampal volume and functional connectivity [51]. The QTL for total cerebellum mass on

360 chromosome 1 at 2204cM has an interval of 1.1Mb and is therefore sufficiently highly resolved

361 to also address the genes contained within for potential functionality, containing as it does 15

362 reference sequence genes. Of these, a number of highly interesting genes are identified for

363 further investigation. *MAP6* is in the centre of the QTL confidence interval and mediates

364 neuronal connectivity for axonal growth [52]. It has been linked with synaptic plasticity anomalies

365 and has associations with schizophrenia through neuronal transport defects [53] and cognitive

366 impairment [54]. *CCKBR* is linked with posttraumatic stress disorder and synaptic plasticity [55] and

367 *PLEKHB1* is associated with Attention Deficit Hyperactivity Disorder [56].

368   Our findings reinforce the concerns from recent years regarding the use of relative brain

369 mass in evolutionary studies. Although brain mass can co-vary with body mass, and allometric

370 effects still exist, if the differing genetic architectures are confounded together meaningful

371 differences will be masked. Most notably, selection can increase body mass irrespective of brain

372 mass, and vice versa, so the use of relative measures can potentially be flawed. Our results also

373 refute the common notion that domestication leads to the regression of brain mass in

374 domesticated chicken as a result of reduced functional needs. The combination of an advanced

375 intercross and the presence of selective sweeps give a number of high confidence candidate

376 genes with putative effects on total brain and cerebella growth. This demonstrates that the RJF

377 and the domestic chicken provide an interesting animal model for studying brain mass evolution

378   during domestication, and also a general model for studying evolution in brain mass and

379   composition.

AUTHORS' CONTRIBUTIONS

381   Conceived and designed the experiments: DW PJ LA RH. Performed the experiments: DW RH.

382   Analyzed the data: RH MJ DW. Contributed reagents/materials/analysis tools: DW LA PJ.

383   Wrote the paper: RH MJ DW.

COMPETING FINANCIAL INTERESTS

385   All authors have no competing interests.

DATA ACCESSIBILITY STATEMENT

387   Full genotype and phenotype data is available on dryad with the following doi: XXXXX.

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538   **TABLE LEGENDS**

539   **Table 1**. QTL information for body mass, total brain mass and cerebellum QTL. Includes

540   locations (both the chromosome and the position in centiMorgans), % variance explained by

541   each QTL (r-squared), additive and dominance effect sizes (positive values for additive values

542   indicate a larger QTL effect size in domestic genotype birds, negative a larger value in wild

543   genotype birds). The lower and upper bounds of the 95% confidence interval (C.I) are noted.

544   The total QTL region is therefore the region bounded between these two limits. Locations of

545   selective sweeps are also provided, with AD indicating the sweep is present in both Broiler and

546   Layer birds, and LR indicating the sweep is specific to Layer birds. For sweeps present in

547   cerebellum and total brain mass QTL any genes present within sweeps are also provided after

548   the sweep location. Cerebellum QTL are marked in bold.

549   **FIGURE LEGENDS**

550   **Figure 1.** Changes in body mass (solid lines: mean±SE) and brain mass (dotted lines:
551   mean±SE) in White leghorn (black lines) and Red Junglefowls (red lines) from 1-week of age
552   until adulthood.

553   **Figure 2.** QTL supports intervals (as determined by the 1.8 LOD drop method) in the genome

554   separating out loci for proportional and total brain mass and body mass. X-axis represents the

555   chromosome containing the QTL, whilst the size of the each bar represents the total QTL region

556   size (i.e. the 95% C.I. of the QTL location).

557   **Figure 3.** Comparison of LOD graphs for total brain mass QTL and body mass QTL

558

20

ait	chr	pos	LOD	r-sq	add +/- s.e	dom +/- s.e.	lower CI	upper CI	selective sweeps pre
ɔdy mass (212 days)	1	510	43.5	21.5	235 +/- 28	-20 +/- 38	507	516	
ɔtal brain mass	1	1516	5.3	3.3	0.06 +/- 0.02	-0.06 +/- 0.04	1494	1583	119.46 LR (*SPAC17A2*
ɔtal Cerebellum mass	1	1593	13.5	9.9	0.012 +/- 0.005	-0.023 +/- 0.007	1586	1598	127.88 LR
ɔportional Cerebellum	1	1945	4.8	4.8	0.003 +/- 0.001	0.005 +/- 0.002	1931	1956	179.66 LR (*UBL3*), 18
ɔtal Cerebellum mass	1	2204	9.3	6.6	0.0004 +/- 0.004	-0.004 +/- 0.005	2196	2224	*MAP6, CCKBR, PLEKF*
ɔtal Cerebellum mass	3	448	8.5	6	0.005 +/- 0.005	0.022 +/- 0.007	442	458	62,62 LR (*KNF217*), 6
ɔtal brain mass	3	448	8.7	5.6	-0.12 +/- 0.07	0.25 +/- 0.09	444	454	62,62 LR (*KNF217*), 6
ɔdy mass (212 days)	4	265	8.6	4.7	8.1 +/- 9.8	19.8 +/- 12.3	254	274	
ɔportional Cerebellum	5	124	4.8	4.8	0.002 +/- 0.004	-0.03 +/- 0.006	106	144	18.8 LR (*FGF3*), 19.4
ɔdy mass (212 days)	6	207	5	1.9	26 +/- 8	30 +/- 11	195	214	
ɔportional Cerebellum	7	159	7.4	7.6	0.005 +/- 0.002	0.004 +/- 0.002	150	171	
ɔtal Cerebellum mass	7	174	5.9	4.1	0.019 +/- 0.005	0.003 +/- 0.005	150	176	23.04 AD (*TANK1*)
ɔtal brain mass	7	200	6.6	4.2	0.02 +/- 0.018	-0.04 +/- 0.03	190	212	23.04 AD (*TANK1*), 2:
ɔtal brain mass	9	51.1	13.6	8.9	0.018 +/- 0.017	-0.04 +/- 0.02	48	60	
ɔdy mass (212 days)	12	64	4.4	1.7	35 +/- 10	20 +/- 14	45	79	
ɔdy mass (212 days)	24	14	9.5	5	0.8 +/- 9.1	38 +/- 11	6	18	
ɔdy mass (212 days)	27	68	4.2	1.6	38 +/- 11	-1.8 +/- 9.8	56	80	

559   Table 1.

560

561

Figure 1

563

Brain and Body mass

564

Figure 2.

QTL support intervals
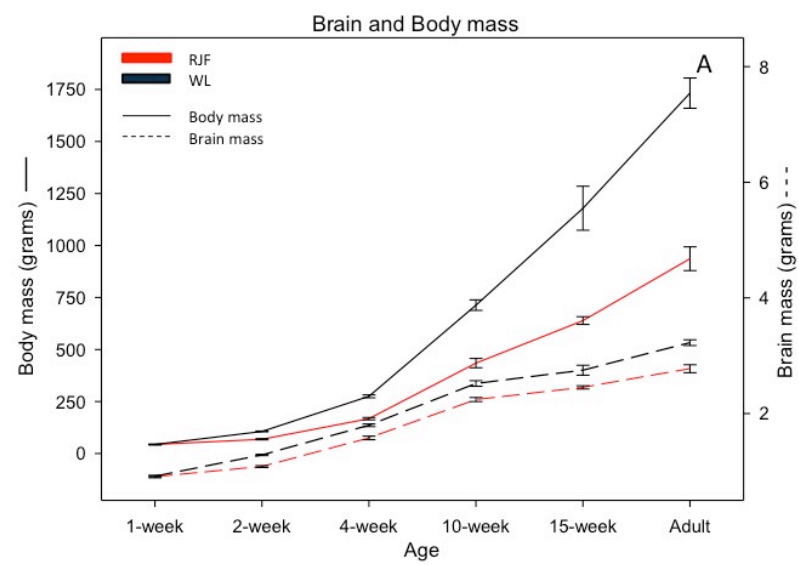
565

566

567   Figure 3.

568