附件4 吉林大学本科毕业论文（设计）开题报告

|  |  |  |  |
| --- | --- | --- | --- |
| 学 院 | 动物科学学院 | 专业 | 动物科学 |
| 学生姓名 | 朱明远 | （教）学号 | 85150226 |
| 指导教师 | 赵志辉 | 职称 | 教授 |
| 合作导师 |  | 职称 |  |
| 论文题目 | 比较基因组学揭示家鸡黑白羽色变异的遗传学基础 | | |
| 1. 课题研究的背景和意义（综述国内外相关研究现状，阐述课题的研究目的、意义）   随着人类生产方式由狩猎向定居转变，在几千年的过程中超过40 余种物种被驯化，它们在驯化过程中获得了较祖先更多的表型多样性。这其中，鸡作为鸟类中多样性最多的代表，在羽毛颜色、个体大小、视觉等方面有较大的分化。 传统遗传学多以 QTL 定位，工作繁琐且假阳性高，定位的区域往往很大，无法精确定位。但随着下一代测序技术的飞速发展，从基因组水平鉴定群体遗传多样性的群体基因组学迅速发展起来。群体基因组学是在群体遗传学的基础上，基于测序技术以及基因组图谱，应用统计学和数学原理在群体规模上对全基因组进行研究，探究基因频率和基因型频率的位点特异性效应以及迁移和遗传漂变等与遗传结构的关系的全基因组效应，探究背后的选择效应和突变作用。在重测序的基础上，鸡的许多多样性已经被解释，例如藏鸡的高原机制以及体型的大小多样性，  清楚解释了鸡驯化过程中的遗传学基础。元宝鸡是一种着名的观赏名鸡，它有着的金元宝外观，从唐代以来便进行培育。元宝鸡是优良生物模型，尤其是小体型和纯色羽毛​​等表型被广泛应用于研究之中。为了更好地理解影响家畜羽毛颜色变化的突变，我们用的基于NGS的比较群体基因组学研究了元宝鸡的基因组。我们将确定一系列可能影响羽色的基因突变以及区域，为在家鸡中的色素沉着模式提供新的生物学间接。 | | | |
| 1. 课题研究已有的工作基础，附证书、报告、文献翻译（总结归纳本人的学习、科研、实习等成果，以及已掌握的前人资料，简述自己初步的学术见解）   工作基础:本人经过在吉林大学动物科学学院的4年学习，掌握了动物生化、生理、遗传学、生物统计等生命科学知识和实验基础。假期于中科院昆明动物所、中科院广州生物医药与健康研究中心进行长时间实习，积累了大量生物信息学相关知识。  文献翻译：  **比较群体基因组学揭示了家养鸡体型的遗传基础**  Ming-Shan Wang1,2, Yong-Xia Huo1,3, Yan Li4, Newton O. Otecko1,2, Ling-Yan Su2,5, Hai-Bo Xu1,2, Shi-Fang Wu1,2, Min-Sheng Peng1,2, He-Qun Liu1,2, Lin Zeng1,2, David M. Irwin1,6,7, Yong-Gang Yao2,5, Dong-Dong Wu1,2,\*, and Ya-Ping Zhang1,2,4,\*  **介绍**  家养动物作为优秀的生物学模型，广泛用于发育生物学、表型进化和医学研究。它们被驯化为不同品种，在形态学、生理学、行为和适应方面表现出显着差异。作为一种经济性状，家养动物的体型对人类和人类文明的发展极为重要。在家养动物中可以看到数量巨大的外表变异，远远高于其野生祖先。包括育种研究者在内，进化和发育生物学家都对发现和控制家畜外形变异遗传的调控基础机制感兴趣。  家鸡在鸟类中拥有最多的表型变异。例如，矮脚鸡和浦东鸡是两种极端的成年体重分别为0.5和5千克左右的神奇的鸡种。作为全球分布最广泛且数量最多的家养动物，家鸡也被用于遗传和医学研究。已经被证实的多个特定性状的遗传变异，特别是体型，在研究和育种中具有重要意义-已经绘制了数百个数量性状基因座并报告其与鸡的生长和体重相关。尽管取得了这些成就，但由于低密度基因座的低分辨率和微卫星和SNP组标记的数量有限，大多数这些QTL被定位到大的基因组区域。因此，仅鉴定了有限数量的基因座。例如，一些基因，包括IGF1，TBC1D1，FOXO1A，KPNA3，INTS6和HNF4G，与鸡的生长和体重有关。这些研究主要基于变异非常有限的商品代鸡，并且大多数控制外形的突变个体已经丢失。全基因组关联研究有望阐明这种复杂性状的遗传基础，虽然这些方法的难度和收集表型数据的高昂费用妨碍了其广泛应用。此外，不同品种之间的巨大表型多样性及其复杂的群体统计学历史也阻碍了对鸡体型变异的遗传机制的研究。幸运的是，由比较群体基因组学补充的下一代基因组测序数据研究彻底改变了数量遗传学和进化领域，因此已被证明是解释家畜中复杂性状的遗传基础的有力工具，例如野鸽的驯化，高纬度中国猪的冷适应，以及狗适应的富含淀粉的食物的机制。  元宝鸡是中国着名的观赏鸡品种，以其体型小巧着称，成年雄性体重约800克，成年雌性体重约500克。它具有悠久的繁殖历史，可以追溯到唐朝。小巧的体型使其易于把握，外观类似于中国古代金元宝，使元宝鸡在古代被视为财富的象征。迄今为止，元宝鸡无疑是中国最受人类器重的鸡种之一。在这里，我们采用比较群体基因组学来研究元宝鸡小型体型的遗传基础。我们确定了四个可能控制家养鸡体型变异的新基因座。  **结果**  **对89个鸡基因组的分析鉴定了超过2000万个SNP**  在该研究中，获得89个个体的基因组，其中包括7只红色丛林鸡，24只元宝鸡和58只其他家养鸡，每个个体具有约12.2×序列覆盖。基因组序列之间的比较鉴定了总共21286312个SNP，其中51.8％映射到基因间区域，42.6％映射到内含子区域，并且仅有一小部分映射到基因组的外显子区域。分配给蛋白质编码区的SNP的功能注释鉴定了产生非同义氨基酸取代的101999个SNP和同义的226713个SNP，其中704个基因具有导致终止密码子获得或丧失的SNP。芯片阵列中使用的SNP和基因分型阵列，分别包含在我们的新的数据集。我们获得的SNP数据集远远大于鸡SNP数据库中的数据。这些新的SNP可能补充鸡变种的目录。  与其他鸟类相比，元宝鸡的核苷酸多样性水平较低。所有个体的系统发生树，使用加权邻接法构建。结果表明，元宝鸡形成相对均匀的祖簇。主成分分析、杂合子和基于单倍型的结构分析表明，元宝鸡和其他鸡一样属于混合血统。  **比较群体基因组学作为识别控制鸡体型变异的位点的方案**  群体变异度的比较分析是一个强大的工具，能够成功调查复杂性状下的遗传机制。由于元宝鸡的体型与鸡的平均体型相比要小得多，因此元宝和其他鸡的比较基因组分析将是识别鸡体型变异的遗传基础的有效策略。在这里，我们采用了FST和LSBL评价从其它鸡。首先，进行滑动窗口分析，具有50kb窗口大小和25kb步长，分别从经验数据中鉴定268和275个基因，其中FST和LSBL作为基于异常值方法的筛选候选基因。这些候选基因的功能富集分析未揭示与体型发展特异性相关的任何途径。通过组合FST的信号和LSBL，我们确定了四个基因组的区域，表现出极端的群体分化，可能是人工选择的结果。  chr22：0.25Mb-0.33Mb的分析显示BMP10可能控制元宝鸡的体型；基因组区域chr22：0.25 MB-0.33M是与种群分化的最高水平。有三个基因GKN1，GKN2和BMP10位于该区域。GKN1和GKN2是在胃中大量且独特表达的旁系同源物。这两个基因都证明了维持胃粘膜完整性和正常功能的功能重要性，它们的异常与胃癌有关。在元宝和其他鸡中，GKN1和GKN2在热，肾，脾，肌肉，肝和肺中均未表现或极低的表达水平。BMP10，转化生长因子β家族的成员，FST和LSBL分析和等位基因频率有较高的差异。单倍型比较分析揭示的一致分化BMP10用于从其它鸡。到目前为止，BMP10在体型中的作用尚未见报道。此外，没有与鸡体型相关的QTL定位于该基因组区域。  进一步的研究表明，BMP10在心脏中具有高表达，并且与其他鸡相比，元宝鸡的表达水平显着上调。基于桑格测序演化网络分析验证数据还支持这样的结论的启动子区域BMP10相比其他鸡。为了检测启动子活性，我们用元宝鸡的BMP10启动子序列和参考序列进行荧光素酶报告基因测定。同样地，我们观察到由BMP10驱动的报启动子活动增加元宝鸡的启动子与参考启动子相比。接下来，基于我们的系统发育网络，我们鉴定了位于BMP10上游的21个SNP，并显示了元宝鸡与其他鸡之间的高度分化。进一步检查这些SNP以通过电泳迁移率变动分析与来自鸡心的核提取物来研究DNA-蛋白质相互作用的差异。对于这些SNP五个探针表现出元宝和其它鸡在chr22：274606和chr22：274758的突变导致DNA和蛋白质之间相互作用的减少或丧失。另一方面，元宝鸡在chr22：274670，chr22：276118和chr22：276140的突变增加了DNA-蛋白质相互作用。根据这些观察结果，我们推断，由于潜在的SNP，蛋白质结合的差异可能有助于元宝鸡心脏中BMP10表达的上调。我们这个强连锁区域，其显示出与体重。SNP可以解释五种鸡系中总重量变异的22.41％，即济宁白鸡，鲁花鸡，矮脚鸡，元宝鸡和观赏鸡。  **BMP10过表达抑制血管生成并诱导斑马鱼体长短**  BMP10的过表达诱导小鼠体重减轻。为了检查它是否是BMP10的一个保守功能区域来控制脊椎动物的体型，我们在斑马鱼中进行了野生型BMP10的过表达测定。来自fli1a-EGFP转基因系的受精单细胞斑马鱼胚胎分别每个胚胎注射200pg，野生型斑马鱼BMP10 mRNA。与未注射的对照胚胎相比，斑马鱼中BMP10的过表达导致体长减小和体轴弯曲。这些结果证实了这一点BMP10在决定体型方面具有重要作用。血管生成是生长和发育过程中正常而重要的过程。为了研究BMP10过表达对斑马鱼血管生成的表型后果，我们用0.016％甲磺酸三卡因麻醉转基因入胚胎，并在受精后32小时计数完整节间血管的数量。与对照斑马鱼相比，过表达BMP10的斑马鱼显示出更多的不完全ISV并且仅偶尔出现背主动脉的芽。这些结果表明BMP10的过表达抑制斑马鱼血管生成血管生长，抑制生长，导致身体缩短。所有这些数据表明，BMP10的上调可能有助于减少元宝鸡的体型。  BMP10的过度表达诱导斑马鱼的发育缺陷。 32 h的总形态。 与未注射的野生型对照胚胎相比，具有野生型斑马鱼BMP10过表达的胚胎表现出体长减小和弯曲的体轴。  **chr1的基因：147.55Mb-147.82Mb，chr2：57.05Mb-57.22Mb，chr24：6.17Mb-6.25Mb区域可能参与体型的发育**  CHR1：147.55Mb-147.82Mb是选择性扫描SNP的一个群体集合，表现出显著更高水平的种群分化水平-更高的FST和LSBL。但是没有注释基因位于该区域。然而，该区域位于先前报道的与“体重”和“生长”相关的QTL内。在该映射位置附近发现了在控制细胞分裂和生长调节中起作用的基因GPC5。RNA-seq分析显示GPC5的表达在元宝鸡的心脏中下调。  与chr1：147.55Mb-147.82Mb中的相似，没有蛋白质编码基因位于chr2：57.05Mb-57.22Mb基因组区域，尽管它也显示出强烈的选择信号，具有高水平的群体分化，如FST和LSBL所示。QTL作图显示该区域与鸡的“体重”和“生长速率”密切相关。Chr2：57.05Mb-57.22Mb是基因spalt样转录因子3的上游，这是一种在动物发育中起基础作用的转录因子。与村鸡和Red Junglefowl 相比，我们观察到元宝鸡肾脏中SALL3的表达上调。  第四个定位区域chr24：6.17Mb-6.25Mb含有13个蛋白质编码基因。该区域没有与体重或生长速率相关的QTL。这13种基因参与各种生物过程。例如，BCO2与鸡的皮肤颜色相关，而DIXDC1和CRYAB参与神经系统。HSPB2热休克蛋白基因在心脏和骨骼肌中高度表达，这对于维持一些小鼠骨骼肌的肌细胞完整性至关重要。敲除HSPB2导致小鼠骨骼肌退化。在我们的研究中，与家鸡和红原鸡相比，元宝鸡肌肉中HSPB2的表达下调。  **讨论**  鸡在全球许多社区中具有不可或缺的饮食。它们也被用于生物研究和娱乐等其他目的。对于食物和经济收入来源，育种者已经做出巨大努力来开发体型庞大且肉类生产快速增长的鸡。我们的研究报告了四个可能控制体型的基因座，为鸡的繁殖提供了重要信息和候选遗传标记。我们的研究还提供了一种比较群体基因组学的策略，以确定考虑到鸡体大小变化的候选基因/变体。与以前的方法相比，该策略更具成本效益和节省时间。  在几种驯养动物中观察到体型的巨大变化，包括狗，猪和鸡。体尺不仅是食品生产的重要商业特性，也是进化和发育生物学研究的重要课题。控制体型变异的基因/遗传变异的研究引起了动物育种者，进化和发育生物学家甚至医学科学家的关注。  身体大小，典型的许多复杂性状，通常被认为受到涉及类似功能途径的许多基因的影响。例如，在人类研究中，确定了97个与体重指数相关的基因座，但这些基因座仅占BMI变异的~2.7％。与之形成鲜明对比的是，据报道很少有基因参与家畜体型的进化。例如，六个基因的衍生变体解释了一些狗品种中大小减少的近一半。在马中，报告了类似的模式，其中四个位点解释了83％的体型变异。在这里，我们还发现BMP10可以解释五种鸡系22.41％的体型变异，包括济宁白鸡，鲁花鸡，矮脚鸡，元宝鸡和观赏鸡。人类与家畜之间的这些对比模式可能是由自然选择与人工选择的差异所解释的。  在评估元宝鸡体型较小的遗传基础时，我们鉴定了几个与形态发生基因相关的基因组区域。在染色体1上鉴定出非基因区域。该序列位于蛋白质编码基因GPC5的上游，并显示出阳性选择的证据，其表达在元宝鸡的心脏中下调。据报道，人GPC5基因座与高度有关。基于Illumina 60 K Chicken SNP Beadchip的GWAS也发现了GPC5与家养鸡体重的潜在关联的证据。类似地，在染色体2的选定区域中没有发现蛋白质编码基因，其位于另一个参与发育的基因SRL3的上游。表达SALL3在元宝鸡肾脏上调。SALL3是否具有控制体型的功能尚不清楚。但是SALL3蛋白直接结合DNA甲基转移酶3α并减少DNMT3A介导的CpG岛甲基化。DNMT3A是控制体重和能量稳态所必需的，与人体身高有关。此外，SALL3的表达可以由BMP4诱导，骨形态发生蛋白在骨骼系统的发育中起重要作用。具有双失活Sall1 / Sall3的小鼠突变体在肢体形态发生中表现出畸形。在24号染色体的选择性扫描区域，有13种蛋白质编码基因具有不同的生物学功能。HSPB2是一种必不可少的骨骼肌发育基因，在元宝鸡中下调，可能与元宝鸡独特的生长特性有关。在染色体22的选定区域中发现了三种蛋白质编码基因GKN1，GKN2和BMP10。无论是GKN1还是GKN2都在胃中高度和独特的表达，在维持其正常功能的重要作用。BMP10在心脏中特异性表达，在调节小鼠心脏发育中起关键作用。BMP10- 缺陷小鼠通过E10.0-E10.5存活，然后由于严重受损的心脏发育和功能而死亡。BMP10在小鼠中的过度表达导致胚胎中的心肌过度生长和过度蠕动。在心肌中出生后过度表达BMP10的转基因小鼠在1个月大时显示心脏大小减少50％并且体重和体积减小。据报道，BMP10还具有诱导细胞凋亡，增殖和生长的作用。例如，BMP10在前列腺肿瘤中观察到表达降低或不存在，并且强制BMP10过表达降低了体外生长，细胞基质粘附，侵袭和前列腺癌细胞的迁移。进一步检查显示，元宝鸡心脏中BMP10表达上调，可能是由于BMP10上游的5个潜在突变可能增加启动子活性。与小鼠相似，BMP10的过度表达诱导斑马鱼体长减少，这意味着BMP10的保守功能控制脊椎动物的体型。在我们的研究中发现的四个位点，在元宝和其他鸡之间具有高的群体分化，可能影响基因表达而不是蛋白质编码序列。这可能支持一种模型，即基因表达的变化对体型的进化有显着贡献，这一观点与基因表达变化在形态学演变中特别重要的假设一致。  我们的研究仍有一些局限性。首先，我们认为5个突变可能会改变BMP10的启动子活性，导致元宝鸡BMP10的高表达。两个突变chr22：274606和chr22：274758导致DNA-蛋白质相互作用的减少或丧失，而其他三个突变chr22：274670，chr22：276118和chr22：276140增加DNA-蛋白质相互作用。我们的研究无法指出哪些蛋白质参与这些相互作用，无论这些蛋白质中的所有蛋白质是否全部共同作用以激活BMP10的启动子。此外，一些微型家养鸡品种的体型与元宝鸡的体型相似，但在我们的研究中我们只包括元宝鸡作为小体型鸡品种。此外，大围山鸡中含有BMP10基因的染色体区域与红原鸡和其他家养鸡品系处于相同的野生型状态。因此，我们不能肯定地得出结论，BMP10在其他国内鸡系中具有共同的结果，特别是由于家养鸡的复杂起源和群体统计学。更广泛的抽样包括更多体型小的鸡品种和额外的工作将有助于解决这些问题。  材料和方法  动物实验伦理  所有动物实验程序均按照昆明动物研究所伦理委员会批准的指导方针进行。  采样和基因组数据收集  本研究对多达42个基因组进行了测序，其中包括24只元宝鸡，8只大围山鸡，1只红色丛林鸡，6只广州本地鸡和3只云南当地鸡。 使用酚 - 氯仿提取法提取DNA，并通过电泳和NanoDrop分光光度计2000测量质量。根据Illumina标准基因组文库制备管道，仅使用高质量DNA构建基因组测序文库。测序在Illumina Hiseq 2000平台上进行，读取长度为101bp。来自我们之前研究的33只鸡的基因组和Fan等人的研究中的2只鸡和来自Yi等人的研究中的12只鸡，被纳入我们的研究。总体而言，获得了7只Red Junglefowls和82只家养鸡的89个基因组。  基因组序列比对，SNP调用和注释  使用cutadaptor和Btrim软件通过去去除接头和低质量碱基，过滤原始序列读数。使用具有默认设置的BWA-MEM将合格的读数与鸡参考基因组比对。然后采用一系列后处理来处理对齐BAM格式文件，包括排序，重复标记，局部重新校准和基本质量重新校准，这些都是使用Picards中的SortSam和MarkDuplicates函数进行包，以及Genome Analysis Toolkit中的RealignerTargetCreator，IndelRealigner和BaseRecalibrator工具。使用GATK中的UnifiedGenotyper和VariantFiltration命令调用和过滤SNP和indel。RMS映射质量<25且基因型质量<40的基因座被删除，其中具有零映射质量的读数占该站点所有读数的> 10％。移除具有> 2个等位基因并且在簇内的基因座。使用基于ENSEMBL鸡注释的ANNOVAR将所有SNP分配到特定基因组区域和基因。估计缺失频率<10％的SNP，并且每个染色体的单倍型由BEAGLE推导。  种群变异和种群遗传分析  使用VCF工具使用50kb滑动窗口以25kb逐步递增计算元宝鸡、红原鸡、大围山鸡和其他鸡群的全基因组遗传多样性。采用几种方法推断元宝鸡的种群结构。首先，我们使用软件PHYLIP基于从所有SNP位点的简单匹配距离导出的成对距离矩阵构建了邻接树。使用MEGA5观察树。其次，为了最大限度地减少由广泛的强连锁不平衡区域贡献的SNP的影响，我们根据使用PLINK观察到的样本相关系数来修剪SNP，并且使用GCTA进行PCA。第三基于修剪的数据，使用ADMIXTURE进行混合物分析以观察群体结构，其祖先群体大小为2至5。第四，我们使用基于单倍型的方法ChromoPainter和fineSTRUCTURE来推断种群结构。  全基因组选择性扫描分析  我们采用三项试验来研究元宝鸡阳性选择足迹的基因组区域。如其他地方所述，估计每个SNP的F ST值在元宝和其他鸡之间。基于三组之间的FST值计算每个SNP的LSBL统计量。由于大围山鸡具有与红色丛林鸟类相似的特征，因此我们将元宝鸡定义为A组，红原鸡和大围山鸡为B组。其他家养鸡系被指定为C组。使用LSBL公式计算每种变体的LSBL统计数，对于每个50kb窗口中的 FST和LSBL，以25kb逐步递增进行滑动窗口分析。此外，我们计算了元宝与其他鸡之间每个SNP的绝对等位基因频率差异，以确认阳性选择的信号。通过以上方法检测到的推导候选选择性扫描使用 http://asia.ensembl.org/info/docs/tools/index.html上提供的变体效应预测器进行注释。使用g：Profiler分析蛋白质编码基因的功能富集，包括基因本体论类别，KEGG途径和人类表型本体。  SNP验证和网络建设  在Appled Biosystems ABI 3730XL遗传分析仪上进行Sanger重测序，用于验证BMP10基因上游区域和第一外显子区域的SNP。共有54只鸡，包括元宝鸡和当地土着鸡，被用于确认。用于扩增和测序的引物列于补充表S7中。使用PHASE程序分阶段进行单倍型。使用网络构建了中间连接网络。  基因分型和关联分析  为了进一步确定BMP10的变异如何影响体型，我们对来自五个鸡系的301只鸡进行了一个SNP的基因分型。使用Sanger重测序法获得可用的体重信息。解释的重量变化比例使用PLINK与线性模型估算。  RNA提取和实时定量PCR检测  使用TRNzol-A +试剂从成年鸡的心脏，肝脏，脾脏，肺，肌肉，肾脏和脑组织中分离总RNA，并使用RNeasy Micro Kit纯化。使用电泳和NanoDrop分光光度计2000测量RNA的浓度和完整性。根据制造商的说明，使用PrimeScript RT-PCR试剂盒以25μl的终体积使用总RNA合成单链cDNA。 。使用实时定量PCR和相对标准曲线方法以及对持家基因GAPDH的归一化来测量鸡心脏中BMP10的相对mRNA表达水平。  RNA-seq分析  对于RNA-seq分析，我们在我们的一个项目中使用Hiseq平台生成了来自元宝鸡，乡村鸡和红色丛林禽的肺，心脏，肌肉，脾脏，肝脏和肾脏组织的47个转录组表S9）。首先，使用Trimmomatic过滤掉质量差的读数，参数设置为'LEADING：5 TRAILING：5 SLIDINGWINDOW：4：10 MINLEN：50'。其次，使用HISAT2将干净的读数与鸡参考基因组比对，参数设置。Cuffcompare用于测量红原鸡和元宝鸡之间以及家鸡和元宝鸡之间每种组织的基因表达差异的显着性。基于泊松离散分散模型计算P值。  荧光素酶报告分析  为了推断元宝鸡BMP10上游高度分化的SNP是否增加了BMP10启动子活性，我们进行了荧光素酶报告基因检测。分别通过PCR产生BMP10基因的两个上游片段，长2449bp片段和短949bp片段。并克隆到pGL3 Basic载体中。Kpn I和XhoI酶位点用于构建载体。使用Lipofectamine TM 2000在每个孔中用pGL3报告质粒和80ng pRL-TK Renilla萤光素酶构建体以60％-70％汇合转染24孔中的人胚肾293T细胞。萤光素酶活性使用GloMax转染后在第24个小时测定96微孔板发光。以Basic载体为参照计算萤火虫发光/海肾发光的比率。进行了三次技术重复。t检验用于衡量元宝与其他鸡之间的统计学意义。  电泳迁移率变动分析  从BMP10的起始位点起2249-bp上游区域内总共21个SNP通过Sanger测序验证的基因，使用EMSA的功能测定以揭示DNA-蛋白质相互作用的潜在差异。合成了总共20对5'标记的探针和chr22：276331突变共享一个探针。在将鸡处死并在-70℃下储存直至进一步分析后，收集来自4只鸡的心脏组织。使用NucBuster蛋白质提取试剂盒从心脏组织制备核提取物。将核提取物加入到结合反应中，然后在冰上预孵育20分钟。对于竞争反应，将20pmol未标记的双链寡聚物加入到反应中。预温育后，将20fmol生物素化的寡聚物加入到反应中并在室温下温育20分钟。通过在0.5％非变性聚丙烯酰胺凝胶上在120V下在0.5×TBE运行缓冲液中电泳分离DNA-蛋白质复合物90分钟。将分离的复合物在冷的0.5×TBE中以390mA转移至结合膜40分钟。使用Stratalinker UV交联剂交联DNA-蛋白质复合物，并使用Lighten检测生物素化的探针 将分离的复合物在冷的0.5×TBE中以390mA转移至结合膜40分钟。使用Stratalinker UV交联剂交联DNA-蛋白质复合物，并使用Lighten检测生物素化的探针 将分离的复合物在冷的0.5×TBE中以390mA转移至结合膜40分钟。使用Stratalinker UV交联剂交联DNA-蛋白质复合物，并使用Lighten检测生物素化的探针® HRP-B底物溶液A和B连接。  斑马鱼的饲养和保存  成年斑马鱼在14小时光照/ 10小时黑暗周期中维持在28.5℃。每对设置四到五对斑马鱼进行自然交配。平均而言，产生了100-200个胚胎。将胚胎在鱼水中保持在28.5℃。根据Kimmel洗涤并分级胚胎。fli1a-EGFP转基因系的建立和表征。上海生物模型生物科技发展有限公司的斑马鱼设施获得了国际实验动物管理评估与认证协会的认可。  斑马鱼显微注射  对于过表达测定，受精的单细胞胚胎每个胚胎注射200pg野生型斑马鱼BMP10 mRNA。  斑马鱼血管生成研究  为了评估斑马鱼中的血管形成，向受精的单细胞fli1a-EGFP转基因胚胎每个胚胎注射200pg野生型BMP10 mRNA。在32h时，用0.016％MS-222麻醉胚胎，并计数将DA连接到DLAV的完整ISV的数目。使用公式测定抗血管生成作用  图像采集  用Nikon SMZ 1500荧光显微镜检查胚胎和幼虫，然后用数码相机拍照。使用Adobe Photoshop 7.0软件调整图像子集的亮度，对比度，色调和饱和度，以最佳地可视化表达模式。使用基于图像的形态测量分析处理定量图像分析。每种处理定量10只动物。  统计分析  所有数据均以平均值±SEM表示。使用GraphPad Prism 5.0进行统计分析和数据的图形表示。使用学生进行统计显着性测试，ANOVA或卡方检验进行。统计显着性用\*表示。  原文：  Comparative population genomics reveals genetic basis underlying body size of domestic chickens  Ming-Shan Wang1,2, Yong-Xia Huo1,3, Yan Li4, Newton O. Otecko1,2, Ling-Yan Su2,5, Hai-Bo Xu1,2, Shi-Fang Wu1,2, Min-Sheng Peng1,2, He-Qun Liu1,2, Lin Zeng1,2, David M. Irwin1,6,7, Yong-Gang Yao2,5, Dong-Dong Wu1,2,\*, and Ya-Ping Zhang1,2,4,\*  Introduction  Domestic animals are excellent biological models widely used in developmental biology, phenotypic evolution, and medical research studies. They have been developed as different breeds exhibiting remarkable differences in morphology, physiology, behavior, and adaptations (Darwin, 1868; Roots, 2007; Sutter et al., 2007; Menheniott et al., 2013; Gou et al., 2014; Yoon et al., 2014; Wang et al., 2015a, 2016). As an economic character, body size of domestic animals is extremely important for humans and the development of human civilization. An amazing amount of body size variation is seen within domestic animals, which is much higher than that seen in their wild ancestors (Roots, 2007). In addition to breeders, both evolutionary and developmental biologists are interested in discovering and characterizing the mechanisms that underlie the genetic control of variation in body size of domestic animals (Sutter et al., 2007; Makvandi-Nejad et al., 2012; Gou et al., 2014).  Domestic chickens are the most phenotypically variable bird (Darwin, 1868). For instance, bantam and cochin are amazing chicken breeds with adult body sizes at ~0.5 and ~5 kg, respectively, on the two extremes. As the farm animal with the widest distribution globally and raised in the largest number, domestic chickens have also been used in genetic and medical studies (Lawler, 2014). Genetic variants of specific traits, especially for body size, have been characterized, as they have major implications in both research and breeding (Sutter et al., 2007; Makvandi-Nejad et al., 2012; Gou et al., 2014). Several hundreds of quantitative trait loci (QTL) have been mapped and reported to be associated with growth and body weight of chickens (http://www.animalgenome.org/cgi-bin/QTLdb/GG/index). Despite these achievements, most of these QTLs are mapped to large genomic regions due to the low resolution of low-density loci and limited number of microsatellite and SNP panel markers. Thus, only a limited number of causative loci have been identified. For example, some genes, including IGF1, TBC1D1, FOXO1A, KPNA3, INTS6, and HNF4G, have been associated with growth and body weight in chickens (Rubin et al., 2010; Gu et al., 2011; Elferink et al., 2012; Xie et al., 2012; Wang et al., 2015b). These studies were mostly based on commercial chickens with very limited variations, and most of the variants controlling body size could have likely been missed. Genome-wide association studies (GWAS) hold a promise for elucidating the quantitative genetic basis of this complex trait (Gu et al., 2011; Elferink et al., 2012; Xie et al., 2012; Wang et al., 2015b), although the difficulty of the methods and the high expense of collecting phenotypic data hamper its wide application. In addition, the great phenotypic diversity among the diverse breeds and their complicated demographic histories (Miao et al., 2013) have also impeded the study for genetic mechanisms underlying the variation of body size in chickens. Fortunately, next-generation genome sequencing data supplemented by comparative population genomics have revolutionized the fields of quantitative genetics and evolution, and thus have proved to be a powerful tool for interpreting the genetic underpinnings of complex traits in domestic animals, e.g. the head crest in the rock pigeon (Shapiro et al., 2013), cold adaptation of high latitude Chinese pigs (Ai et al., 2015), and adaptation to starch-rich foods by dogs (Axelsson et al., 2013).  Yuanbao chicken, a famous Chinese ornamental chicken breed, is known for its miniature body size, with adult male weight ~800 g and adult female ~500 g. It has a long breeding history that can be traced back to the Tang dynasty (Supplementary Figure S1). Both the small body size that makes it easily handled in the palm and the appearance similar to ‘Yuanbao’, a metallic ingot used in ancient China as money, made Yuanbao chicken be treated as a symbol of wealth at-hand in ancient times (Supplementary Figure S1). To date, Yuanbao chicken is indisputably one of the most esteemed chicken breeds in China. Here, we employed comparative population genomics to study the genetic basis underlying the small body size of Yuanbao chicken. We identified four novel loci that potentially control the variation in body size of domestic chickens.  Results  Analyses of 89 chicken genomes identify >20 million SNPs  In this study, 89 genomes were obtained representing 7 Red Junglefowls, 24 Yuanbao chickens (Supplementary Figure S1), and 58 other domestic chickens, with ~12.2× sequence coverage for each individual (Supplementary Figure S2 and Table S1). Comparisons among the genome sequences identified a total of 21286312 SNPs, with 51.8% of them mapping to intergenic regions, 42.6% to intronic regions, and only a small proportion (1.5%) mapping to exonic regions of the genome (Supplementary Table S2). Functional annotation of the SNPs assigned to protein-coding regions identified 101999 SNPs that produce non-synonymous amino acid substitutions and 226713 SNPs that were synonymous, with 704 genes having SNPs that cause gain or loss of a stop codon (Supplementary Table S3). Further comparisons indicated that 88% and 90% of the SNPs used in the 60 K Illumina BeadChip genotyping array and the 600 K Affymetrix® Axiom® HD genotyping array, respectively, were contained in our new dataset. Our dataset of SNPs is much larger than those available in the chicken SNP database: 14353694 and 8670333 of our SNPs were not reported in BUILD 138 and BUILD 145 of the chicken dbSNP databases (ftp://ftp.ncbi.nih.gov/snp/organisms/chicken\_9031), respectively. These novel SNPs potentially supplement the catalog of chicken variants.  Compared with other birds, Yuanbao chicken showed a lower level of nucleotide diversity (mean value: 4.56E-03) (Supplementary Figure S3). A phylogenetic tree of all individuals was constructed using weighted neighbour-joining method (Bruno et al., 2000), which revealed that Yuanbao chicken formed a relatively homogeneous ancestral cluster (Figure 1A). Principle component analysis (PCA) (Figure 1B), admixture (Supplementary Figure S4), and haplotype-based structure analyses (Supplementary Figure S5) indicated that several Yuanbao chickens had mixed ancestry with other chicken breeds.  Comparative population genomics as a strategy to identify loci controlling body size variation in chickens  Comparative analysis of population variants is a powerful tool that has enabled successful investigation into genetic mechanisms underlying complex traits (Axelsson et al., 2013; Kamberov et al., 2013; Shapiro et al., 2013; Ai et al., 2015; Lamichhaney et al., 2015). Since Yuanbao chicken has a remarkably smaller body size compared to the average body size of chickens, comparative genome analysis of Yuanbao and other chickens would be an effective strategy to identify the genetic basis underlying the variation in body size among chickens. Here, we employed FST and LSBL (Shriver et al., 2004) to evaluate the population differentiation of Yuanbao chicken from other chickens (Figure 1C and D). First, a sliding window analysis was performed, with 50 kb window size and 25 kb step size, identifying 268 and 275 genes from the empirical data with FST and LSBL, respectively, as candidates based on the outlier approach (99th percentile cutoff). Functional enrichment analysis of these candidate genes did not reveal any pathway specifically associated with the development of body size (Supplementary Tables S4 and S5). By combining the signals of FST and LSBL, we identified four regions of the genome (chr22:0.25Mb–0.33Mb, chr1:147.55Mb–147.82Mb, chr2:57.05Mb–57.22Mb, and chr24:6.1Mb–6.3Mb) that exhibited extreme population differentiation, likely as the result of artificial selection.  Analysis of chr22:0.25Mb–0.33Mb shows BMP10 potentially controlling the body size of Yuanbao chicken  The genomic region chr22:0.25Mb–0.33Mb stands out as the most extremely candidate selective sweep with the highest level of population differentiation (Figure 1C). There are three genes GKN1, GKN2, and BMP10 located in this region (Figure 2A). GKN1 and GKN2 are paralogues abundantly and uniquely expressed in the stomach (Menheniott et al., 2013). Both genes have documented functional importance in maintaining integrity and normal function of gastric mucosa, and their anomaly is associated with gastric cancer (Kim et al., 2014; Yoon et al., 2014). In Yuanbao and other chickens, both GKN1 and GKN2 exhibited no or extremely low expression levels in the heat, kidney, spleen, muscle, liver, and lung (Supplementary Figure S6).  BMP10, a member of the transforming growth factor β (TGFβ) family, showed consistently higher values in FST and LSBL analyses and high differences in allele frequencies (Figure 2A−C). A haplotype comparison analysis revealed a consistent differentiation of BMP10 for Yuanbao chicken from other chickens (Figure 2D). Until now, a role of BMP10 in body size has not been reported in chickens. In addition, no QTL associated with body size in chickens was mapped to this genomic region.  Further investigation showed that BMP10 had a high expression in the heart (Supplementary Figure S7), and the expression level was significantly upregulated in Yuanbao chicken compared to other chickens (Figure 3A). Phylogenetic network analysis based on the Sanger resequencing verified data also supported the conclusion that the promoter region of BMP10 has become highly differentiated in Yuanbao chicken compared to other chickens (Figure 3B). To examine promoter activity, we performed luciferase reporter gene assays with BMP10 promoter sequence from Yuanbao chicken and the reference sequence. Consistently, we observed an increased reporter activity driven by the BMP10 promoter of Yuanbao chicken compared to the reference promoter (Figure 3C and D). Next, based on our phylogenetic network, we identified 21 SNPs that were located upstream of BMP10 and showed high differentiation between Yuanbao and other chickens. These SNPs were further examined to investigate differences in DNA−protein interactions by electrophoretic mobility shift assays (EMSA) with nuclear extracts from chicken hearts. Probes for five of these SNPs showed differences in gel shift between Yuanbao and other chickens (Figure 3E and Supplementary Figure S8). Mutations in Yuanbao chicken at chr22:274606(T→C) and chr22:274758(C→deletion) led to decrease or loss of interactions between DNA and protein. On the other hand, mutations in Yuanbao chicken at chr22:274670(A→G), chr22:276118(A→G), and chr22:276140(C→T) increased the DNA−protein interaction. From these observations, we inferred that the differences in protein binding, due to the underlying SNPs, likely contribute to the upregulation of BMP10 expression in the heart of Yuanbao chicken (Figure 3A). We further genotyped one SNP within this strong linkage region, which showed a significant association with body weight (Figure 3F, P = 3.249E-18). The SNP could explain 22.41% of the overall weight variance in five chicken lines, i.e. Jiningbairi chicken, Luhua chicken, bantam, Yuanbao chicken, and ornamental chicken.  BMP10 overexpression inhibits angiogenesis and induces short body length in zebrafish  Overexpression of BMP10 induced a decrease in body weight in mice (Chen et al., 2006). To examine whether it is a conserved function of BMP10 to control body size in vertebrates, we performed an overexpression assay of wild-type BMP10 in zebrafish. Fertilized one-cell zebrafish embryos from the fli1a-EGFP transgenic line were injected with 200 pg wild-type zebrafish BMP10 mRNA per embryo respectively. Overexpression of BMP10 in zebrafish resulted in a decreased body length and a curved body axis compared to uninjected control embryos (Figure 4 and Supplementary Figure S9). These results affirm that BMP10 has an important role in determining body size. Angiogenesis is a normal and vital process in growth and development (Folkman and Shing, 1992). To study the phenotypic consequence of BMP10 overexpression on angiogenesis in zebrafish, we anesthetized transgenic embryos with 0.016% tricaine methanesulfonate (MS-222) and counted the number of complete intersegmental vessels (ISVs) at 32 h post-fertilization (hpf). Zebrafish with overexpression of BMP10 showed a larger number of incomplete ISVs and only occasional sprouts of dorsal aorta (DA) compared with the control zebrafish (Figure 5 and Supplementary Figure S10). These results indicate that overexpression of BMP10 inhibits angiogenic vessel growth in zebrafish, which would inhibit growth and result in a shorter body. All these data suggest that upregulation of BMP10 likely contributes to a smaller body size of Yuanbao chicken. Genes at chr1:147.55Mb–147.82Mb, chr2:57.05Mb–57.22Mb, and chr24:6.17Mb–6.25Mb are potentially involved in the development of body size  Chr1:147.55Mb–147.82Mb, harbouring a cluster of selective sweep SNPs, demonstrates significantly higher levels of population differentiation as revealed by FST (Figure 1C) and LSBL (Figure 1D). No annotated gene is located in this region. However, this region is located within a previously reported QTL associated with ‘Body weight’ and ‘Growth’ (Carlborg et al., 2003; Gu et al., 2011). The gene GPC5, which plays a role in the control of cell division and growth regulation (Yang et al., 2013), is found adjacent to this mapped location. RNA-seq analysis showed that expression of GPC5 was downregulated in the heart of Yuanbao chicken (Supplementary Figure S11A, P < 0.05).  Similar to that in chr1:147.55Mb–147.82Mb, no protein-coding gene is located in the chr2:57.05Mb–57.22Mb genomic region, although it also displayed strong signals of selection with high levels of population differentiation as revealed by FST (Figure 1C) and LSBL (Figure 1D). QTL mapping has shown that this region is strongly associated with ‘Body weight’ and ‘Growth rate’ in chickens (Carlborg et al., 2003; Siwek et al., 2004; Tercic et al., 2009). Chr2:57.05Mb–57.22Mb is upstream of the gene spalt-like transcription factor 3 (SALL3), a transcription factor that plays a fundamental role in animal development (de Celis and Barrio, 2008). We observed an upregulated expression of SALL3 in kidneys of Yuanbao chicken compared to village domestic chicken (P = 0.0208) and Red Junglefowl (P = 0.24795) (Supplementary Figure S11B).  The forth mapped region chr24:6.17Mb–6.25Mb contains 13 protein-coding genes (Supplementary Table S6). There is no QTL associated with body weight or growth rate in this region. These 13 genes are involved in various biological processes (Supplementary Table S6). For example, BCO2 is associated with skin colour in chickens (Eriksson et al., 2008), while DIXDC1 and CRYAB are involved in nervous system (Ousman et al., 2007; Kivimae et al., 2011). HSPB2, a heat shock protein (HSPs) gene, is highly expressed in the heart and skeletal muscle, which is essential in maintaining muscle cell integrity in some mouse skeletal muscles. Knockdown of HSPB2 results in degeneration of skeletal muscle in mice (Brady et al., 2001). In our study, the expression of HSPB2 was downregulated in the muscle of Yuanbao chicken compared to village domestic chicken (P = 0.0682) and Red Junglefowl (P = 0.06205) (Supplementary Figure S12).  Discussion  Chickens are of integral dietary importance (e.g. egg and meat) in many communities globally. They are also raised for other purposes like biological research and entertainment. For the source of food and economic income, breeders have made great effort to develop chickens with a large body size and rapid growth rate for meat production (Lawler, 2014). Our study reports four loci that potentially control body size, providing important information and candidate genetic markers for chicken breeding. Our study also provides a strategy with comparative population genomics to identify candidate genes/variants accounting for the variation in the body size of chickens. This strategy is much more cost-effective and timesaving than previous methods such as QTL mapping and GWAS analysis.  A great variation in body size has been observed in several domesticated animals, including dogs, pigs, and chickens (Roots, 2007). Body size is not only an important commercial trait for food production, but also a key topic for evolutionary and developmental biology studies (Sutter et al., 2007; Makvandi-Nejad et al., 2012; Rubin et al., 2012; Rimbault et al., 2013; Qanbari et al., 2014). The investigation in genes/genetic variants controlling variation in body size has attracted attentions from animal breeders, evolutionary and developmental biologists, and even medical scientists (Sutter et al., 2007; Makvandi-Nejad et al., 2012; Gou et al., 2014)  Body size, typical of many complex traits, is commonly believed to be influenced by many genes involved in similar functional pathways (Devlin et al., 2009). For example, in a study of humans, 97 loci associated with body mass index (BMI) were identified, yet these loci only account for ~2.7% of BMI variation (Locke et al., 2015). In stark contrast, very few genes have been reported to be involved in the evolution of body size in domestic animals. For example, derived variants at six genes explain nearly half of the size reduction seen in some dog breeds (Rimbault et al., 2013). In horses, a similar pattern was reported, where four loci explained 83% of the variation in body size (Makvandi-Nejad et al., 2012). Here, we also found that BMP10 could explain 22.41% of body size variation in five chicken lines, including Jiningbairi chicken, Luhua chicken, bantam, Yuanbao chicken, and ornamental chicken. These contrasting patterns between humans and domestic animals are likely explained by the differences in natural vs. artificial selection.  In assessing the genetic basis underlying the small body size of Yuanbao chicken, we identified several genomic regions associated with morphogenic genes. A non-genic region was identified on chromosome 1 (chr1:147.55Mb–147.82Mb). This sequence is upstream of the protein-coding gene GPC5 and showed evidence of positive selection, whose expression was downregulated in the heart of Yuanbao chicken. The human GPC5 locus has been reported to be associated with height (Lango Allen et al., 2015). A GWAS based on the Illumina 60 K Chicken SNP Beadchip also found evidence for a potential association of GPC5 with body weight in domestic chickens (Sewalem et al., 2002; Carlborg et al., 2004; Gu et al., 2011). Similarly, no protein-coding gene was found in the selected region of chromosome 2 (chr2:57.05Mb–57.22Mb), which is located upstream of another gene involved in development, SALL3 (Parrish et al., 2004; Kojima et al., 2013). The expression of SALL3 was upregulated in kidneys of Yuanbao chicken. Whether SALL3 has a function in controlling body size is unclear. But SALL3 protein directly binds to DNA methyltransferase 3 alpha (DNMT3A) and reduces DNMT3A-mediated CpG island methylation (Shikauchi et al., 2009). DNMT3A is necessary for the control of body weight and energy homoeostasis (Kohno et al., 2014), and is associated with height in humans (Gudbjartsson et al., 2008). In addition, expression of SALL3 can be induced by BMP4 (Shikauchi et al., 2009), a bone morphogenetic protein (BMP) that plays an important role in the development of the skeletal system. Mouse mutants with double-null Sall1/Sall3 exhibited malformation in limb morphogenesis (Kawakami et al., 2009). In a selective sweep region in chromosome 24 (chr24:6.17Mb–6.25Mb), there were 13 protein-coding genes with diverse biological functions. HSPB2, a gene essential for skeletal muscle development, was downregulated in Yuanbao chicken, likely showing an association with the unique growth properties of Yuanbao chicken. Three protein-coding genes GKN1, GKN2, and BMP10 were found in the selected region of chromosome 22 (chr22:0.25Mb–0.33Mb). Both GKN1 and GKN2 are highly and uniquely expressed in the stomach, with important roles in maintaining its normal function. BMP10 is specifically expressed in the heart and plays a crucial role in regulating the development of the heart in mice (Neuhaus et al., 1999). BMP10-deficient mice survived through E10.0–E10.5 and then died due to severely impaired cardiac development and function (Chen et al., 2004). Overexpression of BMP10 in mice leads to myocardial overgrowth and hypertrabeculation in embryos (Pashmforoush et al., 2004). Transgenic mice with postnatal overexpression of BMP10 in the myocardium display a 50% deduction in the heart size and a reduction in body weight and size at the age of 1 month (Chen et al., 2006). BMP10 is also reported to have a role in inducing apoptosis, proliferation, and growth of cells (Kawakami et al., 2009; Kim et al., 2014). For example, BMP10 expression was observed to be decreased or absent in prostate tumours, and forced BMP10 overexpression decreased in vitro growth, cell matrix adhesion, invasion, and migration of prostate cancer cells (Kawakami et al., 2009). Further examination showed that BMP10 expression was upregulated in the heart of Yuanbao chicken, probably as a result of five potential mutations upstream of BMP10 that likely increased the promoter activity. Similar to that in mice (Chen et al., 2006), an overexpression of BMP10 induced a decrease in body length in zebrafish, implying a conserved function of BMP10 in controlling body size in vertebrates. The four loci identified in our study, with high population differentiation between Yuanbao and other chickens, potentially influence gene expression rather than protein-coding sequence. This potentially supports a model that changes of gene expression contribute significantly to the evolution of body size, a view consistent with the hypothesis that changes in gene expression are particularly important in morphological evolution (Carroll, 2008; Young et al., 2015).  There are still some limitations in our study. First, we supposed that five mutations could have likely changed the promoter activity of BMP10 leading to higher expression of BMP10 in Yuanbao chicken. Two mutations chr22:274606(T→C) and chr22:274758(C→deletion) lead to decrease or loss of DNA−protein interactions, while the other three mutations chr22:274670(A→G), chr22:276118(A→G), and chr22:276140(C→T) increased DNA−protein interactions. Our study could not single out which proteins were involved in these interactions, neither whether all or only some of these sites work together to activate the promoter of BMP10. In addition, some miniature domestic chicken breeds have similar body size as Yuanbao chicken, but we only include Yuanbao chicken as a small body-sized chicken line in our study. Furthermore, the chromosomal region containing BMP10 gene in Daweishan chicken was in the same wild-type state as in Red Junglefowl and other domestic chicken lines. Hence, we cannot definitely conclude that BMP10 has a common consequence in other domestic chicken lines, especially due to the complex origin and demographic history of domestic chickens (Miao et al., 2013). Broader sampling to include more chicken breeds with small body size and additional work will help address these issues in the future.  Materials and methods  Animal experimental ethics  All animal experimental procedures were performed according to the guidelines approved by the Ethics Committee of Kunming Institute of Zoology.  Sampling and genomic data collection  Up to 42 genomes, including 24 Yuanbao chickens (YB), 8 Daweishan chickens (DWS, a semi-domestic and miniature breed), 1 Red Junglefowl (RJF), 6 Guangzhou local chickens, and 3 Yunnan local chickens were sequenced in this study (Supplementary Table S1). DNA was extracted using the phenol-chloroform extraction method and the quality was measured by electrophoresis and on a NanoDrop spectrophotometer 2000. Only high-quality DNA was used for the construction of genome sequencing libraries according to the Illumina standard genome library preparation pipeline. Sequencing was performed on an Illumina Hiseq 2000 platform with a read length of 101 bp. Genomes for 33 chickens from our previous study (Wang et al., 2015a), 2 chickens from the study by Fan et al. (2013), and 12 chickens from the study by Yi et al. (2014) were integrated into our study (Supplementary Table S1). Overall, 89 genomes for 7 Red Junglefowls and 82 domestic chickens were obtained.  Genomic sequence alignment, SNP calling and annotation  Raw sequence reads were filtered by removing adaptors and low-quality bases using cutadaptor and Btrim software (Kong, 2011). Qualified reads were aligned onto the chicken reference genome (Galgal4) using BWA-MEM with default settings except the ‘-t 8 -M’ options (https://github.com/lh3/bwa). A series of post-processes were then employed to process the alignment BAM format file, including sorting, duplicates marking, local realignment, and base quality recalibration, which were carried out using the SortSam and MarkDuplicates functions in the Picards (picard-tools-1.56, http://picard.sourceforge.net) package, and RealignerTargetCreator, IndelRealigner, and BaseRecalibrator tools in the Genome Analysis Toolkit (GenomeAnalysisTK-2.6-4, GATK) (McKenna et al., 2010). SNPs and indels were called and filtered using UnifiedGenotyper and VariantFiltration command in GATK. Loci with RMS mapping quality <25 and genotype quality <40, for which reads with zero mapping quality constitute >10% of all reads at this site were removed. Loci with >2 alleles and within clusters (>3 SNPs in a 10-bp window) were removed. All SNPs were assigned to specific genomic regions and genes using ANNOVAR based on the ENSEMBL chicken annotations (Wang et al., 2010). Missing SNPs with <10% frequency were imputed, and haplotypes for each chromosome were deduced by BEAGLE (BEAGLE 3.3.2.) (Browning and Browning, 2007).  Population variation and population genetic analyses  Genome-wide genetic diversity (π) was calculated for Yuanbao chicken, Red Junglefowl, Daweishan chicken, and other chicken groups using VCFtools (Danecek et al., 2011) using a 50-kb sliding window with 25-kb stepwise increments. Several methods were applied to infer the population structure of Yuanbao chicken. First, we constructed a neighbour-joining tree using the software PHYLIP (Bruno et al., 2000) based on the pairwise distance matrix derived from the simple matching distance for all SNP sites. The tree was viewed using MEGA5 (Tamura et al., 2011). Second, to minimize the effects of SNPs contributed by regions of extensive strong linkage disequilibrium (LD), we pruned the SNPs according to the observed sample correlation coefficients using PLINK (Purcell et al., 2007) with the parameter ‘--indep 100 50 0.1’, and PCA was performed using GCTA (Yang et al., 2011). Third, admixture analysis was performed to view the population structure by using ADMIXTURE (Alexander et al., 2009) with an ancestor population size ranging from 2 to 5, based on the pruned data. Fourth, we used a haplotype-based approach, ChromoPainter and fineSTRUCTURE, to infer population structure (Lawson et al., 2012).  Genome-wide selective sweep analysis  We employed three tests to investigate the genomic regions harbouring footprints of positive selection in Yuanbao chicken. FST values for each SNP were estimated between Yuanbao and other chickens as described elsewhere (Akey et al., 2002). LSBL statistics were calculated for each SNP based on the FST values between the three groups (Shriver et al., 2004). Here we defined Yuanbao chicken as group A, Red Junglefowl and Daweishan chicken as Group B since Daweishan chicken possesses features similar to Red Junglefowl (i.e. appearance, habits, and characters). Other domestic chicken lines were assigned group C. LSBL statistics for each variant was calculated using the formula: LSBL = (FST(AB) + FST(AC) − FST(BC))/2. Sliding window analysis was performed for FST and LSBL in each 50-kb window with 25-kb stepwise increments. In addition, we computed the absolute allele frequency difference (ΔAF) per SNP between Yuanbao and other chickens to confirm the signal of positive selection (Carneiro et al., 2014). Deduced candidate selective sweeps detected by above methods were annotated using the Variant Effect Predictor available at http://asia.ensembl.org/info/docs/tools/index.html. Functional enrichments of protein-coding genes including Gene Ontology (GO) categories, KEGG pathway, and Human Phenotype Ontologies (HPO) were analyzed using g:Profiler (Reimand et al., 2011).  SNP verification and network construction  Sanger resequencing on an Appled Biosystems ABI 3730XL Genetic Analyzer, was used to verify SNPs in the region upstream and in the first exon of the BMP10 gene. A total of 54 chickens, including Yuanbao chicken and local indigenous chickens, were used for confirmation. Primers used for amplification and sequencing are listed in Supplementary Table S7. Haplotypes were phased using PHASE program (Stephens and Donnelly, 2003). A median-joining network was constructed using Network (Bandelt et al., 1999).  Genotyping and association analysis  To further define how variation at BMP10 contributes to the body size, we genotyped one SNP (chr22:276457, G/C) in overall 301 chickens from five chicken lines (Jiningbairi chicken, Luhua chicken, bantam, Yuanbao chicken, and ornamental chicken) with available body weight information using Sanger resequencing method. The proportion of weight variation explained was estimated using PLINK with the linear model (Purcell et al., 2007).  RNA extraction and real-time quantitative PCR assay  Total RNA was isolated from heart, liver, spleen, lung, muscle, kidney, and brain tissues of adult chickens using TRNzol-A+ Reagent (TIANGEN) and purified using RNeasy Micro Kit (QIAGEN). The concentration and integrity of the RNA was measured using electrophoresis and NanoDrop spectrophotometer 2000. Total RNA (~2 µg) was used to synthesize single-strand cDNA using the PrimeScript RT-PCR Kit in a final volume of 25 µl according to the manufacturer's instructions. Relative mRNA expression levels of BMP10 in the chicken heart were measured using real-time quantitative PCR (qPCR) with the relative standard curve method and normalization to the housekeeping gene GAPDH. Primer pairs used for BMP10 are listed in Supplementary Table S8. qPCR was performed on the iQ2 system platform (BioRad Laboratories, Hercules) with SYBR® Premix Ex Taq™ II Kit. Student's t-test was used to measure the statistical significance.  RNA-seq analysis  For RNA-seq analysis, we included 47 transcriptomes from lung, heart, muscle, spleen, liver, and kidney tissues of Yuanbao chicken, village domestic chicken, and Red Junglefowl, which were generated using Hiseq platform in one of our projects (see Supplementary Table S9). Firstly, poor-quality reads were filtered out using Trimmomatic (Bolger et al., 2014), with parameters set to ‘LEADING:5 TRAILING:5 SLIDINGWINDOW:4:10 MINLEN:50’. Secondly, clean reads were aligned onto chicken reference genome (Galgal4) using HISAT2 (Kim et al., 2015) with parameters set to ‘--sp 1000,1000 --k 20 --no-unal --dta --dta-cufflinks --no-discordant’ StringTie (Pertea et al., 2015). Cuffcompare (Trapnell et al., 2012) were then used to assemble new transcripts and compare the assembled transcripts with the annotated reference transcripts to generate a new-merged GTF annotation file. Cuffdiff (Trapnell et al., 2012) was used to measure the significance of the gene expression difference for each tissue between Red Junglefowl and Yuanbao chicken, as well as between village domestic chicken and Yuanbao chicken. P-value was calculated based on the Poisson fragment dispersion model (default by cuffdiff program) (Trapnell et al., 2012).  Luciferase reporter analysis  To infer whether the highly differentiated SNPs in the upstream of BMP10 in Yuanbao chicken increased the BMP10 promoter activity, we performed a luciferase reporter assay. Two upstream fragments of the BMP10 gene, a long 2449-bp fragment (L-BMP10, chr22:274094–276542) and a short 949-bp fragment (S-BMP10, chr22:275594–276542), respectively, were generated by PCR and cloned into the pGL3 Basic vector (Promega). KpnI and XhoI enzyme sites were used to construct the vectors. Human embryonic kidney 293T cells (HEK 293T) plated in 24 wells were transfected at 60%–70% confluency with the pGL3 reporter plasmids and 80 ng of pRL-TK Renilla luciferase construct in each well using Lipofectamine™ 2000 (Invitrogen). Luciferase activity was measured at 24 h after transfection using the GloMax® 96 Microplate Luminometer (Promega). Ratios of Firefly luminescence/Renilla luminescence were calculated with the Basic vector as the reference. Three technical replicates were performed. Student's t-test was used to measure the statistical significance between Yuanbao and other chickens.  Electrophoretic mobility shift assays  A total of 21 SNPs within 2249-bp upstream region from start site of the BMP10 gene, which were also verified by Sanger sequencing, were selected for a functional assay using EMSA to reveal potential differences in DNA−protein interactions. A total of 20 pairs of 5′Biotin-labelled probes (mutations at chr22:276330 (T→C) and chr22:276331(G→A) shared one probe) were synthesized (Integrated DNA Technologies). Heart tissues from 4 chickens (including one Yuanbao chicken and three Chinese local domestic chickens) were collected after the chickens were sacrificed and stored at −70°C until further analysis. Nuclear extracts were prepared from the heart tissue using NucBuster Protein Extraction Kit (Viagene Biotech). The nuclear extracts (2.1 μg) were added to the binding reaction and then preincubated for 20 min on ice. For the competition reactions, 20 pmol of unlabelled double-strand oligos were added to the reactions. After preincubation, 20 fmol of the biotinylated oligos were added to the reactions and incubated for 20 min at room temperature. DNA−protein complexes were separated by electrophoresis on 6.5% non-denaturing polyacrylamide gel at 120 V for 90 min in 0.5× TBE running buffer. Separated complexes were transferred to binding-membrane (Viagene Biotech) at 390 mA for 40 min in cold 0.5× TBE. DNA−protein complexes were crosslinked using Stratalinker UV Crosslinker, and biotinylated probes were detected using Lighten® HRP-B Substrate Solution A and B (Viagene Biotech).  Zebrafish care and maintenance  Adult zebrafish were maintained at 28.5°C on a 14-h light/10-h dark cycle (Westerfield, 1993). Four to five pairs of zebrafish were set up for natural mating for every cross. On average, 100–200 embryos were generated. Embryos were maintained at 28.5°C in fish water (0.2% instant ocean salt in deionized water). Embryos were washed and staged according to Kimmel et al. (1995). The establishment and characterization of the fli1a-EGFP transgenic line has been described elsewhere (Lawson and Weinstein, 2002). The zebrafish facility at the Shanghai Biomodel Organism Science & Technology Development Co., Ltd is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.  Zebrafish microinjection  For the overexpression assay, fertilized one-cell embryos were injected with 200 pg wild-type zebrafish BMP10 mRNA per embryo.  Zebrafish angiogenesis studies  To evaluate blood vessel formation in zebrafish, fertilized one-cell fli1a-EGFP transgenic embryos were injected with 200 pg wild-type BMP10 mRNA per embryo. At 32 hpf, embryos were anesthetized with 0.016% MS-222 (Sigma-Aldrich), and the number of complete ISVs, which connect the DA to the DLAV, was counted. The anti-angiogenesis effect was determined using the following formula:  % inhibition=(1−ISVamountofexperimentgroupISVamountofwildtypecontrol)×100(a)% inhibition=(1-ISVamountofexperimentgroupISVamountofwildtypecontrol)×100(a)  Image acquisition  Embryos and larvae were examined with a Nikon SMZ 1500 Fluorescence microscope and subsequently photographed with digital cameras. A subset of images were adjusted for levels of brightness, contrast, hue, and saturation with Adobe Photoshop 7.0 software (Adobe) to optimally visualize the expression patterns. Quantitative image analyses were processed using image-based morphometric analysis (NIS-Elements D3.1). Ten animals were quantified for each treatment.  Statistical analysis  All data are presented as mean ± SEM. Statistical analysis and graphical representation of the data were performed using GraphPad Prism 5.0 (GraphPad Software). Statistical significance was performed using a Student's t-test, ANOVA, or χ2 test as appropriate. Statistical significance is indicated by \*, where P < 0.05, and \*\*\*, where P < 0.0001. | | | |

|  |
| --- |
| 1. 研究的内容及可行性分析   研究内容：  家鸡的羽色在驯化以及选择过程中获得了较大的表型变异，但背后的遗传学基础难以探究。群体遗传学以及高通量测序技术的发展带来了进展。在本次研究中，我们尝试利用NGS（下一代测序技术）使用群体基因组来识别潜在的决定家鸡羽毛颜色变化的遗传学基础。  我们采取的主要物种-元宝鸡，是一种著名的观赏鸡种，它的人工培育历史可以追溯到唐朝，其纯净的毛色以及元宝大小的小巧体型受到了人们的喜爱，因此也被广泛用作生物模型，进行进化的研究。为了更好地解析元宝鸡为代表的家鸡在驯化过程中导致的遗传突变，我们基于NGS和比较群体基因组学研究了元宝鸡的重测序数据。我们将会确定可能控制颜色变化的新基因座区域，将提供有关基础色素沉着的新的生物学见解。  可行性分析：  本实验原理上完全可行，并且当前学校实验室所提供的条件完全可以满足实验要求，所以实验可行性很高。 |
| 1. 论文拟解决的关键问题及难点   关键问题：  选择具有较高质量的元宝鸡基因组二代测序数据集  遗传育种实验室高性能服务器的环境配置与维护  根据测序数据选择合适的统计学数论方法以及生物信息学分析流程  难点  作为二代基因组测序数据集，数据量非常大，对服务器的性能要求以及使用人员的合理规划提出了考验  根据数据的实际情况进行质量控制以及选择合适分析软件  生物信息学的相关操作对实验人员的统计学以及计算机技能提出了较高的考验   1. 研究方法与技术路线(重点论述技术方案)   流程图如下：  1、基因组测序数据的获取  分析数据所有24只鸡基因组均来自文章“Comparative population genomics reveals genetic basis underlying body size of domestic chickens”中的部分数据集，该研究由8只黑色元宝鸡，8只白色元宝鸡以及8只其他家养鸡组成。通过文末提供的SRA号，SRAtoolkit工具批量下载NCBI的数据集，存储于高性能服务器。   |  |  | | --- | --- | | 鸡的类型 | 样本ID | | yuanbao\_black | 140401\_Ypt581-700\_AGTCAA\_L005 | | yuanbao\_black | 140401\_Ypt582-700\_CCGTCC\_L005 | | yuanbao\_black | 140403\_Ypt588-650\_GCCAAT\_L006 | | yuanbao\_black | 140430\_Ypt589-650\_CAGATC\_L004 | | yuanbao\_black | 140403\_Ypt595-700\_GCCAAT\_L003 | | yuanbao\_black | 140403\_Ypt596-700\_CAGATC\_L004 | | yuanbao\_black | 140403\_Ypt597-700\_ACTTGA\_L004 | | yuanbao\_black | 140403\_Ypt598-700\_GATCAG\_L004 | | yuanbao\_white | 140401\_Ypt578-700\_GCCAAT\_L004 | | yuanbao\_white | 140401\_Ypt580-700\_CTTGTA\_L005 | | yuanbao\_white | 140403\_Ypt583-650\_ATCACG\_L005 | | yuanbao\_white | 140403\_Ypt584-650\_CGATGT\_L005 | | yuanbao\_white | 140403\_Ypt591-700\_CGATGT\_L003 | | yuanbao\_white | 140403\_Ypt593-700\_TGACCA\_L003 | | yuanbao\_white | 140403\_Ypt594-700\_ACAGTG\_L003 | | yuanbao\_white | 140430\_YPt601-700\_CTTGTA\_L001 | | yuanbao\_other | 140403\_Ypt585-650\_TTAGGC\_L005 | | yuanbao\_other | 140403\_Ypt586-650\_TGACCA\_L005 | | yuanbao\_other | 140416\_Ypt590-650\_ATCACG\_L004 | | yuanbao\_other | 140401\_Ypt579-700\_CAGATC\_L004 | | yuanbao\_other | 140403\_Ypt587-650\_ACAGTG\_L005 | | yuanbao\_other | 140403\_Ypt592-700\_TTAGGC\_L003 | | yuanbao\_other | 140403\_Ypt599-700\_TAGCTT\_L004 | | yuanbao\_other | 140403\_YPt600-700\_GGCTAC\_L004 |   2、基因组比对、变异位点获取以及注释  原始的基因组测序数据去掉接头序列后用 Btrim （http://graphics.med.yale.edu/trim/）去掉低质量的碱基和序列。利用 BWA  （https://github.com/lh3/bwa）的 MEM 算法将过滤后的双端测序 reads 比对到家鸡的参考基因组上（Galgal4）， 除“-t 8 -M”之外，其余参数均为默认。接着，用Picards（http://picard.sourceforge.net）和 Genome Analysis Toolkit（GATK）软件包中相应的工具对比对后的 bam 文件进行一系列的处理，包括排序、去除其重复序列、INDEL 附近的 reads 重新比对以及矫正其比对质量等。其次，利用 GATK中的 UnifiedGenotyper 工具获得群体的 SNP，并使用 VariantFiltration 对 SNP 进行过滤。最终得到SNP用 ANNOVAR 软件对其进行注释。  3、全基因组选择性清除分析  为了全面挖掘家鸡在驯化过程中受人工选择作用的基因，用基于不同算法的  FST、Pi、LSBL三种方法比较家鸡（VC）和元宝鸡的群体基因组数据进行比较。首先，根据 FST算法衡量家鸡和红原鸡的基因组分化程度，并计算了家鸡和红原鸡中每一个 SNP 的 FST值。接着，以50Kb 的窗口、25Kb 的步长分别计算了家鸡和红原鸡的核苷酸多态性，根据（Δπ 或者 ΔPi）=πRJF –πvc 计算了二者的差值。其次，根据公式进行LSBL的计算。计算公式为：LSBL=(FST(AB)+FST(AC)-FST(BC))/2。  4、 正选择基因注释  选取 FST、Pi、LSBL分析中的前1%的值所相对应的基因组区域为候选的选择性清除的基因座位，通过在线工具 Variant Effect Predictor（http://asia.ensembl.org/info/docs/tools/index.html）进行注释。获得的蛋白质编码基因利用 g: Profiler 进行功能注释，获得富集显著的 GO、KEGG 和 HP 功能通路。  六、论文的进度安排   |  |  |  | | --- | --- | --- | | 时间 | 实验项目 | 备注 | | 2018.9 | 查阅相献，咨询导师，撰写开题报告 | 已完成 | | 2018.9-10 | 学生学习实验的基本技能，参与课题组实验 | 已完成 | | 2018.10－11 | 阅读文献，参与相关实验，掌握技能 | 已完成 | | 2018.10－12 | 进行实验 分析实验内容。 | 进行中 | | 2019.1-6 | 数据分析，查阅相关文献，撰写论文，参与答辩 |  | |
| 七、毕业设计研制报告或毕业论文撰写提纲（初步）  摘要  引 言  第一部分 文献综述  第二部分 研究内容  1 材料与方法  2 数据与分析  3 讨论  4 小结  结 论  参考文献  八、主要参考文献  [1]中国科学院中国动物志编辑委员会主编.中国动物志 鸟纲 第4卷 鸡形目[M].1978  [2]Carl-Johan Rubin;Michael C. Zody;Jonas Eriksson;Jennifer R. S. Meadows;Ellen Sherwood;Matthew T. Webster;Lin Jiang;Max Ingman;Ted Sharpe;Sojeong Ka;Finn Hallböök;Francois Besnier;Örjan Carlbord;Bertrand Bed'Hom;Michèle Tixier-Boichard;Per Jensen;Paul Siegel;Kerstin Lindblad-Toh;Leif Andersson.Whole-genome resequencing reveals loci under selection during chicken domestication[J].Nature,2010,Vol.464(25): 587-593  [3]刘益平，朱庆，曾凡同，邱祥聘.原鸡线粒体DNA部分序列多态性分析[J].畜牧兽医学报,2004,第35卷(2): 134-140  [4]陆俊贤，贾晓旭，唐修君，樊艳凤，唐梦君，高玉时，苏一军.2个云南原始鸡种遗传多样性及其与红色原鸡的亲缘关系[J].浙江大学学报(农业与生命科学版),2016,第42卷(3): 385-390  [5]包文斌，束婧婷，王存波，张红霞，Steffen Weigend，陈国宏，BAO Wen-bin，SHU Jing-ting，WANG Cun-bo，ZHANG Hong-xia，Steffen Weigend，CHEN Guo-hong.中国家鸡和红色原鸡mtDNA控制区遗传多态性及系统进化分析[J].畜牧兽医学报,2008,(11): 1449-1459  [6]胡日查，满初日嘎，赵建国，王学梅，李笑春，吴科榜.红色原鸡及其研究进展[J].中国家禽,2010,(1)  [7]霍海龙，霍金龙，李大林，苗永旺，伍革民，李月体.红色原鸡群体遗传多样性[J].动物学杂志,2007,第42卷(5): 131-135  [8]王晓峰，钱勇.活禽市场规范经营的有效举措[J].中国禽业导刊,2010,(16): 5-11  [9]Chunyan Mou;Frederique Pitel;David Gourichon;Florence Vignoles;Athanasia Tzika;Patricia Tato;Le Yu;Dave W. Burt;Bertrand Bed'hom;Michele Tixier-Boichard;Kevin J. Painter;Denis J. Headon.Cryptic Patterning of Avian Skin Confers a Developmental Facility for Loss of Neck Feathering[J].PLOS Biology,2012,  [10]Jonas Eriksson;Greger Larson;Ulrika Gunnarsson;Bertrand Bed'hom;Michele Tixier-Boichard;Lina Strömstedt;Dominic Wright;Annemieke Jungerius;Addie Vereijken;Ettore Randi;Per Jensen;Leif Andersson.Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken.[J].PLoS genetics,2008,Vol.4: e1000010  [11]Chen Siang Ng;Ping Wu;John Foley;Anne Foley;Merry-Lynn McDonald;Wen-Tau Juan;Chih-Jen Huang;Yu-Ting Lai;Wen-Sui Lo;Chih-Feng Chen;Suzanne M. Leal;Huanmin Zhang;Randall B. Widelitz;Pragna I. Patel;Wen-Hsiung Li;Cheng-Ming Chuong.The Chicken Frizzle Feather Is Due to an α-Keratin (KRT75) Mutation That Causes a Defective Rachis[J].PLoS Genetics,2012,Vol.8(7): e1002748  [12]Hubbard, Joanna K. 1 ( Joanna.Hubbard@colorado.edu);Uy, J. Albert C. 2 ;Hauber, Mark E. 3 ;Hoekstra, Hopi E. 4 ;Safran, Rebecca J. 1.Vertebrate pigmentation: from underlying genes to adaptive function[J].Trends in Genetics,2010,Vol.26(5): 231-239  [13]郭军;曲亮;王克华;贺兴龙;.鸡羽色性状基因定位的研究进展[J].中国畜牧兽医,2012,(12): 45-50  [14]王艳，舒鼎铭.家禽及哺乳动物类胡萝卜素氧化酶BCMO1及BCO2研究进展[J].中国家禽,2015,(20): 43-47  [15]张静，刘毅，刘安芳.畜禽羽色候选基因ASIP和TYRP1的研究进展[J].中国家禽,2015,(1): 55-58  [16]孟浩浩，许瑞霞，代蓉，李辉，李良远，万鹏程，石国庆.绵羊黑色素合成相关基因的研究进展[J].生物技术通报,2014,(8): 34-39  [17]徐伟，封竣淇，黄兰，蔡慧芬，罗卫星.TYR基因研究进展[J].中国畜牧杂志,2017,第53卷(4): 23-27  [18]刘小辉，周荣艳，张传生，彭永东，李祥龙.坝上长尾鸡TYR基因核心启动子鉴定与单核苷酸多态性分析[J].农业生物技术学报,2018,第26卷(6): 959-969  [19]刘薇.丝羽乌骨鸡BAC文库的构建和黑色素相关基因TYRP1和ID的研究[D].中国农业大学,2004  [20]崔丽君，张桂贤，王雪娇，王烨，刘宇，刘伟.鸡形目黑素皮质素受体1基因多态性研究[J].畜牧与兽医,2014,(7): 10-16  [21]Sakae Takeuchi;Hideyuki Suzuki;Sayoko Hirose;Masafumi Yabuuchia;Chikara Sato;Hiroaki Yamamoto;Sumio Takahashi.Molecular cloning and sequence analysis of the chick melanocortin 1-receptor gene[J].Biochimica et Biophysica Acta: Gene Structure and Expression,1996,Vol.1306: 122-126  [22]Takeuchi S;Suzuki H;Yabuuchi M;Takahashi S..A possible involvement of melanocortin 1-receptor in regulating feather color pigmentation in the chicken[J].Biochimica et Biophysica Acta,1996,Vol.1308(2): 164-168  [23]Marie A Pointer;Nicholas I Mundy.Testing whether macroevolution follows microevolution: Are colour differences among swans (Cygnus) attributable to variation at the MC1R locus?[J].BMC Evolutionary Biology,2008,Vol.8: 249  [24]Eizirik, Eduardo 1,2 ;Yuhki, Naoya 1 ;Johnson, Warren E. 1 ;Menotti-Raymond, Marilyn 1 ;Hannah, Steven S. 3 ;O'Brien, Stephen J. 1 obrien@ncifcrf.gov.Molecular genetics and evolution of melanism in the cat family.[J].Current Biology,2003,Vol.13(5): 448-453  [25]S. Kerje 1 ;J. Lind 1 ;K. Schütz 2 ;P. Jensen 2 ;L. Andersson 1,3.Melanocortin 1-receptor (MC1R) mutations are associated with plumage colour in chicken[J].Animal Genetics,2003,Vol.34(4): 241-248  [26]Maria K. Ling 1 ;Malin C. Lagerström 1 ;Robert Fredriksson 1 ;Ronald Okimoto 2 ;Nicholas I. Mundy 3 ;Sakae Takeuchi 4 ;Helgi B. Schiöth 1.Association of feather colour with constitutively active melanocortin 1 receptors in chicken.[J].European Journal of Biochemistry,2003,Vol.270(7): 1441-1449  [27]翟正晓.基于RAD简化基因组测序技术的13种中国地方优良鸡品种SNPs多态性图谱构建及群体遗传学分析[D].上海交通大学,2014  九、指导教师意见  签名： 20 年 月 日  十、开题审查小组意见  （要求具体意见，对前7项进行评价，结论：通过，不通过）    开题审查小组组长签名： 20 年 月 日 |