www.sciencemag.org/content/352/6284/470/suppl/DC1

# Supplementary Materials for

## A beak size locus in Darwin's finches facilitated character displacement during a drought

Sangeet Lamichhaney, Fan Han, Jonas Berglund, Chao Wang, Markus Sällman Almén, Matthew T. Webster, B. Rosemary Grant, Peter R. Grant, Leif Andersson*

*Corresponding author. E-mail: leif.andersson@imbim.uu.se

Published 22 April 2016, *Science* **352**, 470 (2016)
DOI: 10.1126/science.aad8786

**This PDF file includes:**

**Supplementary Methods**

**Samples.** Darwin's finches include triplets of co-existing species both in the ground and tree finch lineage (large, medium and small ground/tree finches). As their names imply these triplets show a continuous gradation in overall body and beak size (Fig. 1A,B); these morphological data are from Lack (*26*). Blood samples from 10 birds of each of these six taxa (altogether 60) were collected on FTA papers and stored at -80$^{o}$C. Details about these samples are in Table 1.

We also used blood samples of 71 birds from a population of medium ground finches from Daphne Major collected in 2004-05 previously included in a study on the evolution of character displacement in Darwin's finches (*11*). Roughly half of these birds had survived the severe drought on Daphne Major in 2004-05. We also included blood samples from 62 additional samples of medium ground finches collected from Daphne Major in 1989-1998.

**DNA extraction and whole genome sequencing.** DNA extraction from FTA papers was done as described (*15*). The DNA samples of 60 birds were sequenced using a Illumina HiSeq2500 platform to generate 2 x 125 bp paired-end reads. The targeted amount of sequence per sample was ~10x coverage.

**Quality filtering, sequences alignment, and variant calling.** The quality of the short sequence reads was analyzed using FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The reads were quality trimmed using FASTX (http://hannonlab.cshl.edu/fastx_toolkit/) and aligned to the medium ground finch reference genome assembly (*27*) using BWA (version 0.6.2) (*28*) and the multiple parameters of read mapping quality were assessed using PICARD (http://picard.sourceforge.net/). The sequences of the 60 individual birds together with the data on 120 birds from our previous study (*15*) were used for SNP discovery and genotyping using GATK (*29*). We used a similar in-house filtering pipeline as in our previous study (*15*) for quality filtering of raw SNP calls. Furthermore, we used BEAGLE (v.3.3.2) (*30*) to infer missing and low quality genotypes in the SNP dataset. This stringent SNP quality-filtering pipeline resulted in 44,767,199 variable sites within or between populations.

**A screen for loci affecting body and/or beak size.** We performed pairwise $F_{ST}$ scans across the whole genome in non-overlapping 15 kb windows in three separate contrasts: (i) large ground/tree finches vs. medium ground/tree finches, (ii) large ground/tree finches vs. small ground/tree finches, and (iii) medium ground/tree finches vs. small ground/tree finches. The $F_{ST}$ estimates were Z-transformed ($ZF_{ST}$) to facilitate the comparison of the genetic divergence among multiple contrasts. The windows showing high genetic divergence ($ZF_{ST} > 5$) in each of the three contrasts were selected for downstream analysis. Similar genome-wide pairwise $F_{ST}$ scans comparing large, medium, and small birds were carried out separately within ground and tree finches.

**Phylogenetic analysis.** We used the nucleotide alignment of variable positions within all 180 birds to generate maximum-likelihood phylogeny using FastTree (*31*) (http://meta.microbesonline.org/fasttree/) and default parameter settings. The local support values for each branch in the tree were computed with a Shimodaira-Hasegawa test using the in-built function in FastTree. The sequence conservation for orthologous sequences among Darwin's finches, human, and mouse were assessed using PhastCons and PhyloP scores (*32*) downloaded from the UCSC public database (https://genome.ucsc.edu/).

Regression analysis. Before running the regression analysis, standard regression diagnostics (for instance, homoscedasticity and normality of residuals) were evaluated. Leven's tests and "residuals vs. fitted plot" indicated no issues of heteroscedasticity and quantile-quantile plots showed no significant deviations from normality.

**Genotyping additional individuals for candidate loci.** Additional samples were genotyped for candidate loci using TaqMan custom genotyping (Life Technologies). We performed a standard TaqMan allele discrimination assay on an Applied Biosystems 7900 HT real-time PCR instrument. We evaluated the association of individual genotypes to various morphological measurements (body size, beak size, and beak shape) using a standard linear regression model in R and with the effect of sex in the model. Similarly, the association of the *HMGA2* locus with the survival/death of medium ground finch samples on Daphne Major during the severe drought in 2004-05 was estimated using standard Fisher's exact test. Standard errors for the percentage of surviving birds in different genotype classes were calculated according to Stuart (*33*).

The selection coefficient (s) was estimated according to standard methodology (*34*). The standard error of the selection coefficient was estimated as the square root of the variance for the ratio of the proportion of survivors with the genotype *LL* and the proportion of survivors with the *SS* genotype. The allele substitution effect at the *HMGA2* locus was estimated using linear regression analysis. The analysis indicated that the shift in frequency of the *S*-allele from 0.49 prior to the drought to 0.61 after selection is expected to reduce PC (beak size) by 0.271±0.038, which corresponds to about 30% of the total phenotypic shift (PC=0.90) due to selection.

**Supplementary Text**

**The target of selection on medium ground finches on Daphne Major Island.** As previously reported (*10, 11*), a drought that began in 2003 and ended in March 2005 resulted in the death of more than 80% of medium ground finches. Mortality in 2003 was random with respect to morphology, but from January 2004 to March 2005 small birds survived better than large birds. Selection analysis was performed on a sample of 71 birds measured prior to selection, of which 37 survived and 34 died (*11*). The first component of a principal components analysis (PCA) of weight, wing, and tarsus

measurements was used as an index of body size (table S3). First and second PCs from a separate analysis of beak length, depth, and width were used as indices of beak size and shape respectively. For the male sample ($n$=47) selection differentials ($s$) were significant for body size (-0.67, $P$<0.05) and beak size (-1.02, $P$<0.0001), whereas for females ($n$=24) selection differentials were significant only for beak size (-0.92, $P$<0.05) and not for body size (-0.52, $P$>0.05). Beak shape differentials were not significant ($P$>0.1). Selection gradient analysis that takes into account character correlations by partial regression was performed on the ensemble of original trait measurements but not on these PC indices of size and shape. Therefore it is not known which one of the three synthetic traits was most strongly and independently associated with fitness.

Here we provide the missing partial regression analysis, first with the sexes treated separately and then with them combined. As in standard selection analysis (*35*), individuals that died were given a relative fitness value of 0, and those that survived were given a value of one over the number of survivors (1/37 = 0.027). Beak size and body size are strongly correlated in males (adj $r^2$ = 0.71) and females (adj $r^2$ = 0.62). Results in table S4 are largely consistent with the previously reported selection differential analysis (*11*). Fitness is most strongly associated with beak size in both sexes. This justifies a combined analysis.

We performed an analysis of the total sample (combined sexes) with and without rescaling of female measurements to male measurements to correct for unequal sample sizes of the two sexes because females are slightly but significantly smaller than males in both body size ($F_{1,69}$ = 5.22, $P$<0.0001) and beak size ($F_{1,69}$ = 3.85, $P$=0.0003). The results with and without rescaling were almost identical, therefore we report only results without rescaling. Beak size and body size are strongly correlated in the combined sample (adj $r^2$ = 0.69), whereas beak shape (PC2 beak) varies independently of body size (adj $r^2$ = 0.02).

The analysis confirmed beak size as the main target of selection. Entries in the selection gradients were $\beta$=-0.0069±0.0014 ($t$=-4.89, $P$<0.0001) for beak size, $\beta$=0.0045±0.0016 ($t$=2.73, $P$=0.008) for body size, and $\beta$=-0.0037±0.0030 ($t$=-1.22, $P$=0.23) for beak width. Note the positive sign of the body size coefficient in this analysis and in the sex-specific analyses in table S4. The net reduction in body size means that the direct effect of selection to increase body size must have been overwhelmed by the much stronger indirect effect of selection in the opposite direction on the correlated trait beak size. Beak size variation statistically accounted for four times more of the variation in fitness than did body size. Addition of beak size to a regression model of relative fitness on body size results in a substantial increase in adjusted $r^2$ from 0.08 to 0.30, whereas addition of body size to a model with beak size has a much smaller effect, an increase from 0.24 to 0.30. Inclusion of beak shape has almost no effect. The adjusted $r^2$ of the full model is 0.30 ($F_{3,67}$ = 11.1, $P$<0.0001). Thus relative fitness is most strongly predicted by variation in beak size. The higher survival of females (0.65) than males (0.46) is consistent with the selective advantage of birds with small beaks.

Higher survival of females made a small additional contribution to the overall reduction in mean size of the two traits.

**The *HMGA2* locus and beak size**. The results of this study provide convincing evidence that the 525 kb region including the *HMGA2*, *MSRB3*, *LEMD3*, and *WIF1* genes constitutes a locus with major effect on beak size among the Darwin's finches, but to reveal the underlying causal mechanisms will require further research. In fact, at present we cannot exclude the possibility that this region contains long-range regulatory elements affecting the expression of developmental genes located outside the region, similar to the ectopic expression of *EOMES* in chicken with duplex comb despite the fact that the causal mutation is located within an intron of a closely linked gene (*36*).

The two *HMGA2* haplotypes diverged before the split between warbler finches and other Darwin finches about one million years ago (Fig. 1C, D). Thus, this locus must have played a prominent role in beak size diversification throughout the adaptive radiation of Darwin's finches. The two haplotypes most likely involve the accumulation of multiple causal mutations within the 525 kb region, as previously suggested for the 240 kb *ALX1* region affecting beak shape (*15, 37*). It is also plausible that there are multiple *HMGA2* alleles among the Darwin's finches carrying different sets of causal mutations. The very strong linkage disequilibrium in the region is most likely maintained by suppressed recombination and/or selection against recombinant haplotypes. We used our paired reads and the Manta method (*38*) to search for the presence of an inversion that could explain the large haplotype blocks but found no indication of a structural rearrangement.

*HMGA2* is our prime candidate gene at this locus because of the previously reported associations with the pygmy phenotype in *Hmga2* null mice (*17*), height in humans (*18*), dwarfism in chicken (*39*), body size in dogs (*40*), and height in Shetland ponies (*41*). If *HMGA2* is the only causal gene underlying the observed association, a possible explanation for the large haplotype blocks maintained for about a million years of evolution is that this 525 kb region contains long-range regulatory elements affecting *HMGA2* expression. An alternative scenario is that mutations affecting the function of one or more of the other genes (*MSRB3*, *LEMD3*, and *WIF1*) in the interval contribute to functional differences between haplotypes. Loss-of-function mutations in *LEMD3* (LEM domain-containing 3) cause osteopoikilosis in humans, and experimental studies indicate that LEMD3 affects BMP (bone morphogenetic protein) signaling (*42*), which is of critical importance for beak development (*14*). *WIF1* (*Wnt inhibitory factor 1*) encodes an inhibitor of Wnt signaling which is critical for bone mass; WIF1 is highly expressed in developing and mature mouse skeleton and affects osteoblast differentiation (*43*). Thus, this region contains a cluster of genes *HMGA2-LEMD3-WIF1* affecting growth and skeletal development. The fourth gene in the region *MSRB3* (*Methionine-R-sulfoxide reductases*) has no established link to growth or skeletal development; *Msrb3*-null mice show hearing loss but no effects on morphology or growth were reported (*44*).

Our results suggest that it will be very difficult to distinguish these two alternative scenarios based on genetics due to the strong linkage disequilibrium in the region. A better alternative would probably be to study the expression of these genes during beak development as previously done for calmodulin and BMP signaling (*14, 45, 46*).

**A test of pleiotropic effects of *HMGA2*.** Variation in *HMGA2* genotype is strongly associated with variation in beak size (Fig. 2E) and with fitness (Fig. 2F). The question this raises is whether variation in genotype has pleiotropic effects on fitness in addition to its direct effect upon beak size. To answer this question we used partial regression analysis to estimate the independent effects of beak size and *HMGA2* genotype on fitness during the selection event of 2004-05. Following the procedure of Rennison *et al.* (*47*), and assuming additivity, we gave a value of 1.0 to the *SS* genotypes, 0 to the *LS* genotypes and -1.0 to the *LL* genotypes. We did not transform to standard deviates because the goal was to assess the statistical significance of each independent variable and not to quantify the respective selection coefficients. Results in table S5 show that *HMGA2* has no independent effect on fitness; its effect is brought about solely through its association with beak size. Therefore there is no evidence of pleiotropic effects of *HMGA2* on unmeasured traits that might affect fitness, such as components of physiology.

**Beak shape and survival.** Survivors and non-survivors did not differ in beak shape (table S4). Therefore an association with *ALX1*, a gene known to be associated with beak shape variation in medium ground finches (*15*), is not expected. The expectation was tested and confirmed: survivors and non-survivors did not differ in allele frequencies at this locus ($P = 0.72$, Fisher's exact test, two-sided).

**Table S1 Mean body weights in grams of Darwin's finches**

| Common name | Species | Island | Weight (g) |
|---|---|---|---|
| Large ground finch | *Geospiza magnirostris* | Rábida (R) | 34 |
| | | Daphne Major (DM) | 33 |
| | | Marchena (M) | 33 |
| | | Santiago (S) | 39 |
| | | Pinta (P) | 37 |
| | | Genovesa (G) | 36 |
| Medium ground finch | *Geospiza fortis* | Pinta (P) | 18 |
| | | Daphne Major (DM) | 17 |
| | | Marchena (M) | 17 |
| | | Fernandina (F) | 20 |
| | | Santiago (S) | 21 |
| | | Isabela (I) | 19 |
| | | Santa Cruz (Z) | 23 |
| Small ground finch | *Geospiza fuliginosa* | Marchena (M) | 10 |
| | | Pinta (P) | 11 |
| | | Fernandina (F) | 13 |
| | | Santiago (S) | 15 |
| | | Santa Cruz (Z) | 14 |
| | | Isabela (I) | 15 |
| | | Rábida (R) | 14 |
| | | Santa Fe (Sf) | 13 |
| | | Española (E) | 15 |
| Large cactus finch | *Geospiza conirostris* | Española (E) | 32 |
| Medium cactus finch[4] | *Geospiza propinqua* | Genevosa (G) | 25 |
| Cactus finch | *Geospiza scandens* | Rábida (R) | 22 |
| | | Santa Fe (Sf) | 20 |
| | | Santa Cruz (Z) | 23 |
| | | Pinta (P) | 23 |
| | | Marchena (M) | 24 |
| | | Daphne Major (DM) | 21 |
| Eastern Sharp-beaked ground finch[3] | *Geospiza acutirostris* | Genovesa (G) | 12 |
| Central Sharp-beaked ground finch[2] | *Geospiza difficilis* | Pinta (P) | 19 |
| | | Santiago (S) | 27 |
| | | Fernandina (F) | 20 |
| Northern Sharp-beaked ground finch[1] | *Geospiza septentrionalis* | Wolf (W) | 21 |
| | | Darwin (D) | 26 |
| Large tree finch | *Camarhynchus psittacula* | Pinta (P) | 19 |
| | | Santa Cruz (Z) | 18 |
| Medium tree finch | *Camarhynchus pauper* | Floreana (Fl) | 16 |
| Small tree finch | *Camarhynchus parvulus* | Santa Cruz (Z) | 13 |
| | | Floreana (Fl) | 13 |
| Woodpecker finch | *Camarhynchus pallidus* | Santa Cruz (Z) | 20 |
| | | Isabela (I) | 21 |
| Mangrove finch | *Camarhynchus heliobates* | Isabela (I) | 18 |

| | | | |
|---|---|---|---|
| Vegetarian finch | *Platyspiza crassirostris* | Santa Cruz (Z) | 34 |
| | | Pinta (P) | 30 |
| | | Isabela (I) | 32 |
| | | Santiago (S) | 34 |
| Cocos finch | *Pinaroloxias inornata* | Cocos (C) | 13 |
| Green warbler finch | *Certhidea olivacea* | Santiago (S) | 9 |
| | | Santa Cruz (Z) | 9 |
| | | Isabela (I) | 9 |
| | | Rábida (R) | 9 |
| Grey warbler finch | *Certhidea fusca* | Pinta (P) | 9 |
| | | Española (E) | 8 |
| | | Genovesa (G) | 9 |
| | | San Cristóbal (L) | 8 |

For sharp-beaked ground finches and medium cactus finch from Genovesa, the revised taxonomy as proposed in (15) is used; [1]northern sharp-beaked ground finch from Wolf and Darwin *(Geospiza septentrionalis),* [2]central sharp-beaked ground finch from Pinta, Santiago and Fernandina *(Geospiza difficilis),* [3]eastern sharp-beaked ground finch from Genovesa *(Geospiza acutirostris),* [4]medium cactus finch from Genovesa *(Geospiza propinqua)*

**Table S2 List of genomic regions with high genetic divergence ($ZF_{ST}$ > 5) among large, medium and small birds**

| Scaffold | Start | End | Candidate genes* | Gene start | Gene end |
|---|---|---|---|---|---|
| JH739888 | 12,915,001 | 13,110,000 | *IGFBP2* | 12,970,635 | 12,975,694 |
| | | | *IGFBP5* | 12,978,375 | 13,001,682 |
| JH739895 | 10,770,001 | 10,785,000 | *PLAG1* | 10,736,496 | 10,742,497 |
| | | | *SDR16C5* | 10,807,776 | 10,815,173 |
| JH739900 | 6,945,001 | 7,470,000 | *HMGA2* | 7,006,417 | 7,115,390 |
| | | | *MSRB3* | 7,255,089 | 7,314,199 |
| | | | *LEMD3* | 7,337,367 | 7,376,654 |
| | | | *WIF1* | 7,414,267 | 7,434,283 |
| JH739911 | 2,520,001 | 2,535,000 | *EIF3H* | 2,500,329 | 2,566,732 |
| JH739967 | 390,001 | 405,000 | *RUNX2* | 190,214 | 341,656 |
| | | | *SUPT3H* | 428,487 | 558,156 |
| JH739975 | 1,290,001 | 1,305,000 | *MUSK* | 1,301,980 | 1,353,248 |
| JH739987 | 2,385,001 | 2,415,000 | *FOXF2* | 2,277,448 | 2,283,090 |
| | | | *EXOC2* | 2,569,086 | 2,680,796 |

* Candidate genes in regions. If the region do not contain any genes, the one closest to the region was used instead

**Table S3 Results of two principal components analyses based on the correlation matrix of 71 *G. fortis* that survived or died in the drought of 2004-05. The first analysis was restricted to weight (g), wing and tarsus length measurements in mm. The second analysis was performed with three beak measurements in mm. The first principal component of each analysis is interpreted as a measure of overall size since all loadings are uniformly positive and high. The second principal component is interpreted as a measure of beak shape because the factor loadings are of opposite sign.**

| | Factor loadings | | |
|---|---|---|---|
| **Trait** | **PC1 body** | **PC1 beak** | **PC2 beak** |
| Weight | 0.89 | - | - |
| Wing length | 0.89 | - | - |
| Tarsus length | 0.87 | - | - |
| Beak length | - | 0.95 | 0.31 |
| Beak depth | - | 0.98 | -0.04 |
| Beak width | - | 0.96 | -0.26 |
| % variance | 78.40 | 92.30 | 5.39 |

**Table S4 Partial regression coefficients (*ß*) and standard errors (*SE*) for sex-specific selection gradient analysis: males $F_{3,44} = 4.1$, $P=0.01$, $r^2$ adj = 0.17; females $F_{3,19} = 13.2$, $P<0.0001$, $r^2$ adj = 0.62**

| Trait | *ß* | *SE* | *t* | *P* |
|---|---|---|---|---|
| Males | | | | |
| Body size | 0.0050 | 0.0023 | 2.14 | 0.04 |
| Beak size | -0.0060 | 0.0020 | 3.03 | 0.004 |
| Beak shape | -0.0051 | 0.0038 | 1.34 | 0.19 |
| Females | | | | |
| Body size | 0.0037 | 0.0023 | 1.62 | 0.12 |
| Beak size | -0.0088 | 0.0018 | 4.85 | 0.0001 |
| Beak shape | -0.0062 | 0.0049 | 1.27 | 0.22 |

**Table S5 Partial regression coefficients (*ß*) and standard errors (*SE*) for selection gradient analysis of beak size and *HMGA2* genotype: $F_{2,68} = 12.2$, $P = 0.0001$, $r^2$ adj = 0.24**

| Trait | *ß* | *SE* | *t* | *P* |
|---|---|---|---|---|
| Beak size | -0.0032 | 0.0009 | 3.30 | 0.0015 |
| Genotype | -0.0022 | 0.0022 | 0.97 | 0.34 |

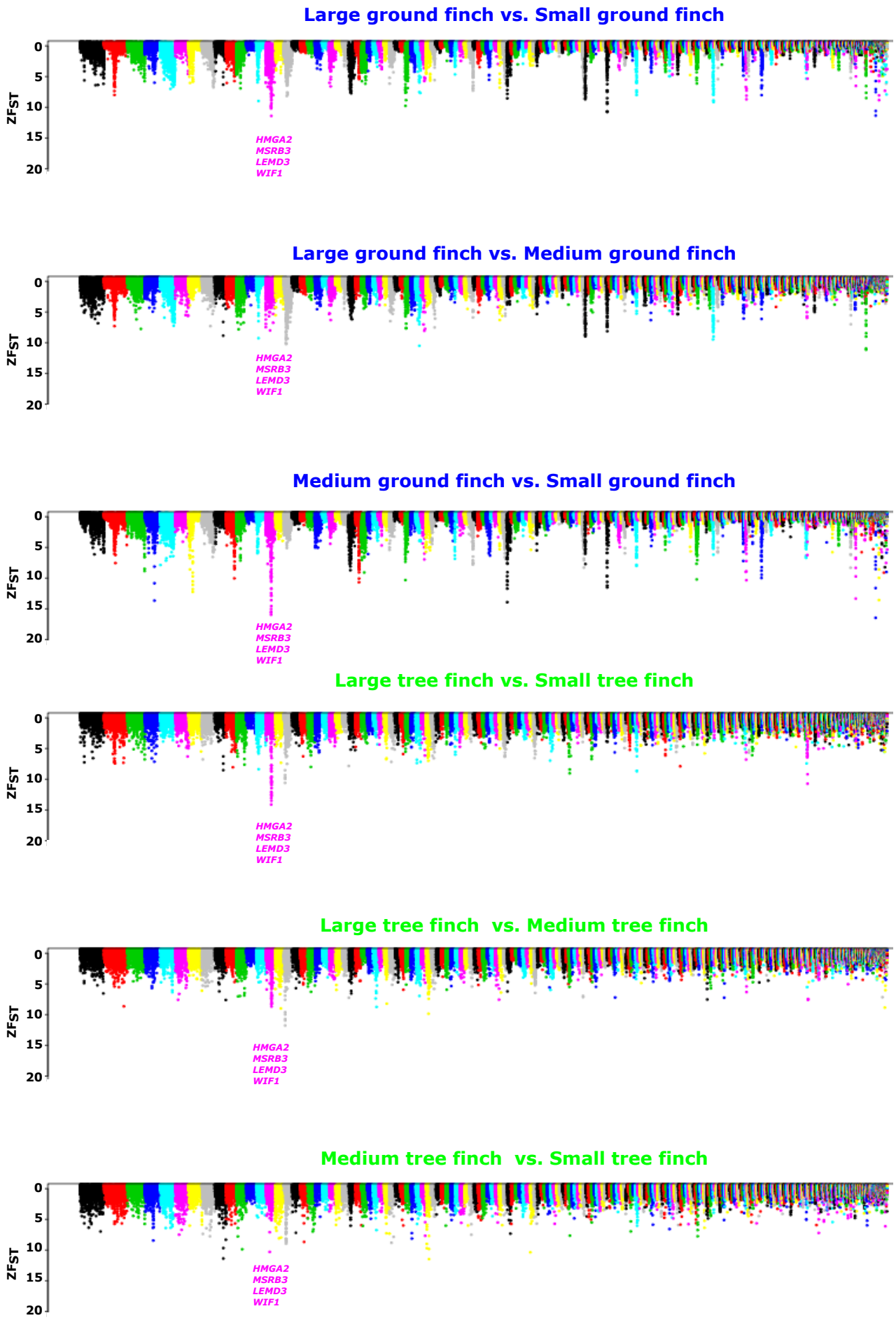


**Fig. S1. Screening for signature of selection for body/beak size in pairwise contrasts within ground and tree finches, respectively.**

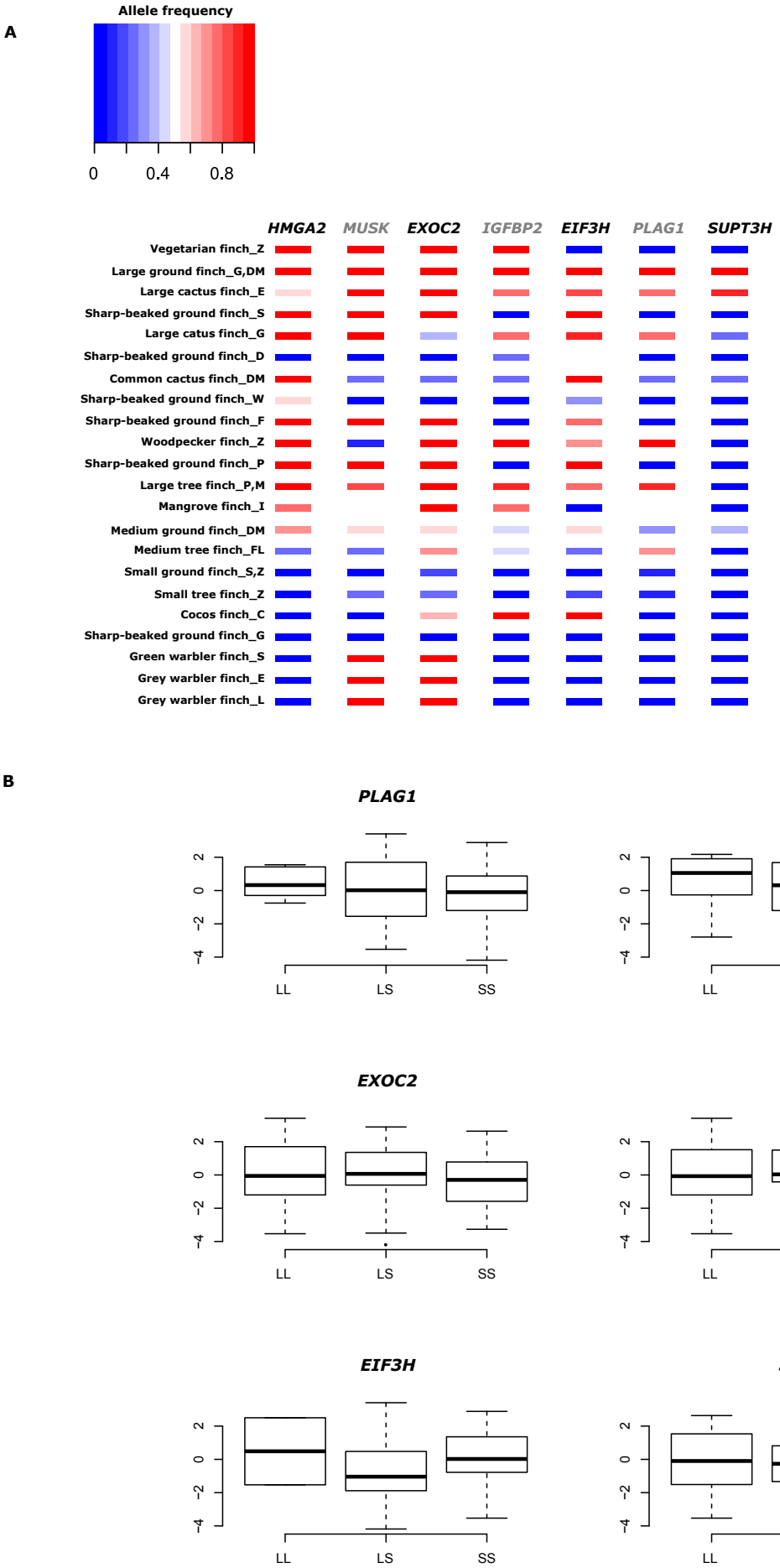


**Fig. S2. Evaluation of candidate regions associated with body size/beak size (highlighted in Fig. 2A). (A)** Allele frequency of the most strongly associated SNP in each of the seven candidate regions; body weight and island for each population are given in table S1. Medium ground finches are segregating for all seven loci. **(B)** Linear regression analysis of beak size among 133 Medium ground finches classified according to genotypes of the most strongly associated SNPs (fig. S2A) (L = allele associated with large size/large beak, S = alternative allele). The distribution of beak size scores in each genotype class is shown as a boxplot, none of the six loci showed significant association to beak size ($P > 0.05$).

**References**


1. P. R. Grant, B. R. Grant, *How and Why Species Multiply: The Radiation of Darwin's Finches* (Princeton Univ. Press, 2008).

2. J. B. Losos, *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles* (Univ. of California Press, 2009).

3. W. L. Brown Jr., E. O. Wilson, Character displacement. *Syst. Zool.* **5**, 49–64 (1956). doi:10.2307/2411924

4. P. R. Grant, Convergent and divergent character displacement. *Biol. J. Linn. Soc. London* **4**, 39–68 (1972). doi:10.1111/j.1095-8312.1972.tb00690.x

5. D. Schluter, *The Ecology of Adaptive Radiation* (Oxford Univ. Press, 2000).

6. D. W. Pfennig, K. S. Pfennig, *Evolution's Edge: Competition and the Origins of Diversity* (Univ. of California Press, 2012).

7. M. E. Arnegard, M. D. McGee, B. Matthews, K. B. Marchinko, G. L. Conte, S. Kabir, N. Bedford, S. Bergek, Y. F. Chan, F. C. Jones, D. M. Kingsley, C. L. Peichel, D. Schluter, Genetics of ecological divergence during speciation. *Nature* **511**, 307–311 (2014). Medline doi:10.1038/nature13301

8. Y. E. Stuart, J. B. Losos, Ecological character displacement: Glass half full or half empty? *Trends Ecol. Evol.* **28**, 402–408 (2013). Medline doi:10.1016/j.tree.2013.02.014

9. J. A. Tobias, C. K. Cornwallis, E. P. Derryberry, S. Claramunt, R. T. Brumfield, N. Seddon, Species coexistence and the dynamics of phenotypic evolution in adaptive radiation. *Nature* **506**, 359–363 (2014). Medline doi:10.1038/nature12874

10. P. R. Grant, B. R. Grant, *40 Years of Evolution: Darwin's Finches on Daphne Major Island* (Princeton Univ. Press, 2014).

11. P. R. Grant, B. R. Grant, Evolution of character displacement in Darwin's finches. *Science* **313**, 224–226 (2006). Medline doi:10.1126/science.1128374

12. See supplementary materials on *Science* Online.

13. P. R. Grant, B. R. Grant, Phenotypic and genetic characteristics of Darwin's finches. *Evolution* **48**, 297–316 (1994). doi:10.2307/2410094

14. A. Abzhanov, M. Protas, B. R. Grant, P. R. Grant, C. J. Tabin, Bmp4 and morphological variation of beaks in Darwin's finches. *Science* **305**, 1462–1465 (2004). Medline doi:10.1126/science.1098095

15. S. Lamichhaney, J. Berglund, M. S. Almén, K. Maqbool, M. Grabherr, A. Martinez-Barrio, M. Promerová, C. J. Rubin, C. Wang, N. Zamani, B. R. Grant, P. R. Grant, M. T.

Webster, L. Andersson, Evolution of Darwin's finches and their beaks revealed by genome sequencing. *Nature* **518**, 371–375 (2015). Medline doi:10.1038/nature14181

16. K. Pfannkuche, H. Summer, O. Li, J. Hescheler, P. Dröge, The high mobility group protein HMGA2: A co-regulator of chromatin structure and pluripotency in stem cells? *Stem Cell Rev. Rep.* **5**, 224–230 (2009). Medline doi:10.1007/s12015-009-9078-9

17. X. Zhou, K. F. Benson, H. R. Ashar, K. Chada, Mutation responsible for the mouse pygmy phenotype in the developmentally regulated factor HMGI-C. *Nature* **376**, 771–774 (1995). Medline doi:10.1038/376771a0

18. M. N. Weedon, H. Lango, C. M. Lindgren, C. Wallace, D. M. Evans, M. Mangino, R. M. Freathy, J. R. Perry, S. Stevens, A. S. Hall, N. J. Samani, B. Shields, I. Prokopenko, M. Farrall, A. Dominiczak, T. Johnson, S. Bergmann, J. S. Beckmann, P. Vollenweider, D. M. Waterworth, V. Mooser, C. N. Palmer, A. D. Morris, W. H. Ouwehand, J. H. Zhao, S. Li, R. J. Loos, I. Barroso, P. Deloukas, M. S. Sandhu, E. Wheeler, N. Soranzo, M. Inouye, N. J. Wareham, M. Caulfield, P. B. Munroe, A. T. Hattersley, M. I. McCarthy, T. M. Frayling, Genome-wide association analysis identifies 20 loci that influence adult height. *Nat. Genet.* **40**, 575–583 (2008). Medline

19. G. Fatemifar, C. J. Hoggart, L. Paternoster, J. P. Kemp, I. Prokopenko, M. Horikoshi, V. J. Wright, J. H. Tobias, S. Richmond, A. I. Zhurov, A. M. Toma, A. Pouta, A. Taanila, K. Sipila, R. Lähdesmäki, D. Pillas, F. Geller, B. Feenstra, M. Melbye, E. A. Nohr, S. M. Ring, B. St Pourcain, N. J. Timpson, G. Davey Smith, M. R. Jarvelin, D. M. Evans, Genome-wide association study of primary tooth eruption identifies pleiotropic loci associated with height and craniofacial distances. *Hum. Mol. Genet.* **22**, 3807–3817 (2013). Medline doi:10.1093/hmg/ddt231

20. R. C. Lewontin, L. C. Birch, Hybridization as a source of variation for adaptation to new environments. *Evolution* **20**, 315–336 (1966). doi:10.2307/2406633

21. P. W. Hedrick, Adaptive introgression in animals: Examples and comparison to new mutation and standing variation as sources of adaptive variation. *Mol. Ecol.* **22**, 4606–4618 (2013). Medline doi:10.1111/mec.12415

22. K. J. Liu, E. Steinberg, A. Yozzo, Y. Song, M. H. Kohn, L. Nakhleh, Interspecific introgressive origin of genomic diversity in the house mouse. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 196–201 (2015). Medline doi:10.1073/pnas.1406298111

23. P. R. Grant, B. R. Grant, Introgressive hybridization and natural selection in Darwin's finches. *Biol. J. Linn. Soc. London* **117**, 812–822 (2016). doi:10.1111/bij.12702

24. G. Zhang, C. Li, Q. Li, B. Li, D. M. Larkin, C. Lee, J. F. Storz, A. Antunes, M. J. Greenwold, R. W. Meredith, A. Ödeen, J. Cui, Q. Zhou, L. Xu, H. Pan, Z. Wang, L. Jin, P. Zhang, H. Hu, W. Yang, J. Hu, J. Xiao, Z. Yang, Y. Liu, Q. Xie, H. Yu, J. Lian, P. Wen, F. Zhang, H. Li, Y. Zeng, Z. Xiong, S. Liu, L. Zhou, Z. Huang, N. An, J. Wang, Q.

Zheng, Y. Xiong, G. Wang, B. Wang, J. Wang, Y. Fan, R. R. da Fonseca, A. Alfaro-Núñez, M. Schubert, L. Orlando, T. Mourier, J. T. Howard, G. Ganapathy, A. Pfenning, O. Whitney, M. V. Rivas, E. Hara, J. Smith, M. Farré, J. Narayan, G. Slavov, M. N. Romanov, R. Borges, J. P. Machado, I. Khan, M. S. Springer, J. Gatesy, F. G. Hoffmann, J. C. Opazo, O. Håstad, R. H. Sawyer, H. Kim, K. W. Kim, H. J. Kim, S. Cho, N. Li, Y. Huang, M. W. Bruford, X. Zhan, A. Dixon, M. F. Bertelsen, E. Derryberry, W. Warren, R. K. Wilson, S. Li, D. A. Ray, R. E. Green, S. J. O'Brien, D. Griffin, W. E. Johnson, D. Haussler, O. A. Ryder, E. Willerslev, G. R. Graves, P. Alström, J. Fjeldså, D. P. Mindell, S. V. Edwards, E. L. Braun, C. Rahbek, D. W. Burt, P. Houde, Y. Zhang, H. Yang, J. Wang, E. D. Jarvis, M. T. Gilbert, J. Wang, Comparative genomics reveals insights into avian genome evolution and adaptation. *Science* **346**, 1311–1320 (2014). Medline

25. C. R. Linnen, Y. P. Poh, B. K. Peterson, R. D. Barrett, J. G. Larson, J. D. Jensen, H. E. Hoekstra, Adaptive evolution of multiple traits through multiple mutations at a single gene. *Science* **339**, 1312–1316 (2013). Medline

26. D. Lack, *Darwin's Finches* (Cambridge Univ. Press, 1947).

27. B. Li, H. Li, P. Parker, J. Wang, The genome of Darwin's finch (*Geospiza fortis*). *GigaScience* 10.5524/100040 (2012). doi:10.5524/100040

28. H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009). Medline doi:10.1093/bioinformatics/btp324

29. A. McKenna, M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, M. Daly, M. A. DePristo, The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303 (2010). Medline doi:10.1101/gr.107524.110

30. S. R. Browning, B. L. Browning, Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. *Am. J. Hum. Genet.* **81**, 1084–1097 (2007). Medline doi:10.1086/521987

31. M. N. Price, P. S. Dehal, A. P. Arkin, FastTree 2—approximately maximum-likelihood trees for large alignments. *PLOS ONE* **5**, e9490 (2010). Medline doi:10.1371/journal.pone.0009490

32. A. Siepel, G. Bejerano, J. S. Pedersen, A. S. Hinrichs, M. Hou, K. Rosenbloom, H. Clawson, J. Spieth, L. W. Hillier, S. Richards, G. M. Weinstock, R. K. Wilson, R. A. Gibbs, W. J. Kent, W. Miller, D. Haussler, Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res.* **15**, 1034–1050 (2005). Medline doi:10.1101/gr.3715005

33. A. Stuart, Standard errors for percentages. *J. R. Stat. Soc. Ser. C* **12**, 87–101 (1963).

34. P. W. Hedrick, *Genetics of Populations* (Jones and Bartlett, Sudbury, MA, 2011).

35. R. Lande, S. J. Arnold, The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226 (1983). doi:10.2307/2408842

36. B. Dorshorst, M. Harun-Or-Rashid, A. J. Bagherpoor, C. J. Rubin, C. Ashwell, D. Gourichon, M. Tixier-Boichard, F. Hallböök, L. Andersson, A genomic duplication is associated with ectopic eomesodermin expression in the embryonic chicken comb and two duplex-comb phenotypes. *PLOS Genet.* **11**, e1004947 (2015). Medline

37. M. S. Almén, S. Lamichhaney, J. Berglund, B. R. Grant, P. R. Grant, M. T. Webster, L. Andersson, Adaptive radiation of Darwin's finches revisited using whole genome sequencing. *BioEssays* **38**, 14–20 (2016). Medline doi:10.1002/bies.201500079

38. X. Chen, O. Schulz-Trieglaff, R. Shaw, B. Barnes, F. Schlesinger, A. J. Cox, S. Kruglyak, C. T. Saunders, Manta: Rapid detection of structural variants and indels for clinical sequencing applications. http://biorxiv.org/content/early/2015/08/10/024232 (2015).

39. C. P. Ruyter-Spira, A. J. de Groof, J. J. van der Poel, J. Herbergs, J. Masabanda, R. Fries, M. A. Groenen, The HMGI-C gene is a likely candidate for the autosomal dwarf locus in the chicken. *J. Hered.* **89**, 295–300 (1998). Medline doi:10.1093/jhered/89.4.295

40. M. T. Webster, N. Kamgari, M. Perloski, M. P. Hoeppner, E. Axelsson, Å. Hedhammar, G. Pielberg, K. Lindblad-Toh, Linked genetic variants on chromosome 10 control ear morphology and body mass among dog breeds. *BMC Genomics* **16**, 474 (2015). Medline doi:10.1186/s12864-015-1702-2

41. M. Frischknecht, V. Jagannathan, P. Plattet, M. Neuditschko, H. Signer-Hasler, I. Bachmann, A. Pacholewska, C. Drögemüller, E. Dietschi, C. Flury, S. Rieder, T. Leeb, A non-synonymous *HMGA2* variant decreases height in Shetland ponies and other small horses. *PLOS ONE* **10**, e0140749 (2015). Medline doi:10.1371/journal.pone.0140749

42. J. Hellemans, O. Preobrazhenska, A. Willaert, P. Debeer, P. C. Verdonk, T. Costa, K. Janssens, B. Menten, N. Van Roy, S. J. Vermeulen, R. Savarirayan, W. Van Hul, F. Vanhoenacker, D. Huylebroeck, A. De Paepe, J.-M. Naeyaert, J. Vandesompele, F. Speleman, K. Verschueren, P. J. Coucke, G. R. Mortier, Loss-of-function mutations in *LEMD3* result in osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis. *Nat. Genet.* **36**, 1213–1218 (2004). Medline doi:10.1038/ng1453

43. M. Kansara, M. Tsang, L. Kodjabachian, N. A. Sims, M. K. Trivett, M. Ehrich, A. Dobrovic, J. Slavin, P. F. Choong, P. J. Simmons, I. B. Dawid, D. M. Thomas, Wnt inhibitory factor 1 is epigenetically silenced in human osteosarcoma, and targeted disruption accelerates osteosarcomagenesis in mice. *J. Clin. Invest.* **119**, 837–851 (2009). Medline doi:10.1172/JCI37175

44. T.-J. Kwon, H. J. Cho, U. K. Kim, E. Lee, S. K. Oh, J. Bok, Y. C. Bae, J. K. Yi, J. W. Lee, Z. Y. Ryoo, S. H. Lee, K. Y. Lee, H. Y. Kim, Methionine sulfoxide reductase B3 deficiency

causes hearing loss due to stereocilia degeneration and apoptotic cell death in cochlear hair cells. *Hum. Mol. Genet.* **23**, 1591–1601 (2014). Medline doi:10.1093/hmg/ddt549

45. A. Abzhanov, W. P. Kuo, C. Hartmann, B. R. Grant, P. R. Grant, C. J. Tabin, The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. *Nature* **442**, 563–567 (2006). Medline doi:10.1038/nature04843

46. R. Mallarino, P. R. Grant, B. R. Grant, A. Herrel, W. P. Kuo, A. Abzhanov, Two developmental modules establish 3D beak-shape variation in Darwin's finches. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 4057–4062 (2011). Medline doi:10.1073/pnas.1011480108

47. D. J. Rennison, K. Heilbron, R. D. H. Barrett, D. Schluter, Discriminating selection on lateral plate phenotype and its underlying gene, Ectodysplasin, in threespine stickleback. *Am. Nat.* **185**, 150–156 (2015). Medline doi:10.1086/679280