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Genomic diversity and evolution of the head crest in the rock pigeon

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Abstract

The geographic origins of breeds and genetic basis of variation within the widely distributed and phenotypically diverse domestic rock pigeon (*Columba livia*) remain largely unknown. We generated a rock pigeon reference genome and additional genome sequences representing domestic and feral populations. We find evidence for the origins of major breed groups in the Middle East, and contributions from a racing breed to North American feral populations. We identify *EphB2* as a strong candidate for the derived head crest phenotype shared by numerous breeds, an important trait in mate selection in many avian species. We also find evidence that this trait evolved just once and spread throughout the species, and that the crest originates early in development by the localized molecular reversal of feather bud polarity.

Since the initial domestication of the rock pigeon in Neolithic times (1), breeders have selected striking differences in behavior, vocalizations, skeletal morphology, feather ornaments, colors, and color patterns to establish over 350 breeds (2). In many cases, the number and magnitude of differences among breeds are more characteristic of macroevolutionary changes than of changes within a single species (2, 3). Indeed, Charles Darwin was so fascinated by domestic pigeons that he repeatedly called attention to this

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dramatic example of diversity within a species to communicate his ideas about natural selection (3, 4).

The genetic architecture for many derived traits in pigeons is probably relatively simple (5, 6), likely more so than interspecific trait variation among many wild species, as breeders often focus on qualitative rather than quantitative variation; this increases the chance of identifying genes responsible for differences among breeds. Additionally, several morphological traits show similar patterns of variation in different breeds, making it possible to test whether the same or different genes underlie similar phenotypes. Despite these advantages, the pigeon is underused as a model for the molecular genetic basis of avian variation due to the paucity of genetic and genomic resources for this bird.

We examined genomic diversity, genetic structure, and phylogenetic relationships among domestic breeds and feral populations (free-living birds descended from escaped domestics) of the rock pigeon. The pigeon reference genome was sequenced from a male Danish tumbler with the Illumina HiSeq2000 platform, and we also resequenced 40 additional *C. livia* genomes to 8-to 26-fold coverage (38 individuals from 36 domestic breeds and two feral pigeons) (7). Genome-wide nucleotide diversity in the rock pigeon ($\pi=3.6\times10^{-3}$) and the mutation rate estimate in the pigeon lineage (1.42×10^{-9} substitutions site^{-1} year^{-1} ± 2.60×10^{-12} SE) are comparable to other avian species (8, 9). Observed heterozygosity indicates a large effective population size for the rock pigeon of $N_e\approx521{,}000$; demographic inferences based on the allele frequency spectrum indicate that, aside from a very recent bottleneck, N_e has been remarkably stable over the past 1.5 million generations (7).

Patterns of linkage disequilibrium (LD) are indicative of haplotype sizes and genome-wide recombination rates, and inform decisions about genetic mapping strategies. Using genotype data from the 40 resequenced *C. livia* genomes, we found that mean “useful LD” (10) ($r^2>0.3$) decays in 2.2 kb (Fig. S10J). This suggests that we should expect little LD between typical pairs of genes in an analysis across breeds; thus, the pigeon is well suited for association-mapping strategies.

We leveraged our whole-genome data to determine breed relationships using 1.48 million variable loci. A neighbor-joining tree rooted on *C. rupestris*, the sister species of *C. livia* (11), yielded several well-supported groups (Figs. 1, S16). Notably, the two feral pigeons grouped with the wattle and homer breeds (Fig. 1, pink branches), supporting the idea that escaped racing homers are probably major contributors to feral populations (12). As with many domesticated species, pigeon evolution is probably not exclusively linear or hierarchical (12). We therefore examined genetic structure among breeds by analyzing 3,950 loci with ADMIXTURE (13), and found a best model fit at K=1 (a single population, where K is the number of assumed ancestral populations). However, higher values of K can also be biologically informative (Figs. S17–S20). Our analysis includes some of the oldest lineages of domestic pigeons and breeds that were not exported from the Middle East until the late nineteenth or early twentieth centuries (14), providing information about likely geographic origins of breeds and their exchange along ancient trade routes (7).

Derived traits in domesticated birds tend to evolve along a predictable temporal trajectory, with color variation appearing in the earliest stages of domestication, followed by plumage and structural (skeletal and soft tissue) variation, and finally behavioral differences (2). One of the genetically simplest derived traits of pigeons is the head crest. Head crests are common ornaments in many bird species (2) and are important display structures in mate selection (15). In pigeons, head crests consist of neck and occipital feathers with reversed growth polarity, such that the feathers grow toward the top of the head instead of down the neck. Crests can be as small and simple as a peak of feathers, or as elaborate as the hood of

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the Jacobin that envelops the head (Fig. 2A). Remarkably, classical genetics experiments suggest that the head crest segregates as a simple Mendelian recessive trait (6, 14). Moreover, previous studies suggest that the *same* locus controls the presence of a crest in numerous breeds, either with alternative alleles at this locus or additional modifier loci controlling the extent of crest development (6, 14).

We resequenced 8 individuals with head crests to directly test if the same mutation controls crest development in different breeds. We sorted genomic variants from birds with and without head crests into separate bins, and calculated allele frequency differentiation (F_{ST}) across the genome (Fig. 2B). We identified a region of high differentiation between crested and uncrested birds in the pigeon ortholog of *Ephrin receptor B2* (*EphB2*; F_{ST}=0.94, top hit genome wide; Fig. S22A) (Fig. 2D). The role of *EphB2* in feather growth is not known, but it plays important roles in tissue patterning and morphogenesis, and is a member of a receptor tyrosine kinase family that mediates development of the feather cytoskeleton (16, 17). All 8 crested birds were homozygous for a T nucleotide at scaffold 612, position 596613 (hereafter, "*cr*" allele), while uncrested birds were heterozygous (n=3) or homozygous (n=30, including the uncrested outgroup *C. rupestris*) for the putatively ancestral C nucleotide ("+" allele). These results were consistent with the known simple recessive architecture of the trait, and implicated a common polymorphism associated with head crest development in multiple breeds with different genetic histories (Fig. 1). This trend extended well beyond our resequencing panel: we genotyped an additional 61 crested birds from 22 breeds, and 69 uncrested birds from 57 breeds, and found a perfect association between *cr/cr* genotype and the crest phenotype (Fig. 2F). By treating the genomes of crested and uncrested birds as separate populations, we also found suggestive evidence for positive selection around the *cr* allele using cross-population extended haplotype homozygosity analysis (Figs. 2D, S21,S22B).

We then used the Variant Annotation, Analysis, and Search Tool (VAAST (18)) to interrogate the pigeon genomes for additional coding changes associated with the head crest phenotype. This identified one gene with genome-wide significance: *EphB2*, and specifically the *cr* SNP ($P_{genome} = 2.0\times10^{-8}$) (Fig. 2C,D). The *cr* allele has a predicted charge-changing arginine (basic) to cysteine (polar uncharged) transition in the catalytic loop of the intracellular tyrosine kinase domain of *EphB2* (Fig. 2E). This amino acid position is invariant among other vertebrates suggesting strong purifying selection for conserved protein function. Notably, the same DLAARN to DLAACN motif change we observe in EphB2 is sufficient to abrogate kinase activity in human and mouse orthologs of the protein tyrosine kinase ZAP-70, and in both mammals and pigeons the mutant phenotypes are inherited recessively (19). Hence, the pigeon *cr* mutation probably abrogates kinase activity in EphB2 and disrupts downstream signal propagation, consistent with the high VAAST score for this gene. *EphB2* is therefore a convincing candidate for the *cr* locus of classical pigeon genetics (5–7, 14).

In several wild and domesticated species, the repeated evolution of a derived trait has occurred by selection on the same gene, possibly due to the repeated selection on the same allele or haplotype (20–22). Similarly, the *cr* SNP is part of a 27.4-kb haplotype that is shared by all crested pigeons, suggesting that the mutation occurred just once and spread to multiple breeds by introgression among domestic breeds, or was selected repeatedly from a standing variant in wild rock pigeons (Figs. 2G, S23; the core haplotype containing the *cr* mutation is reduced to 11 kb with the inclusion of uncrested heterozygotes). The only gene present in the shared *cr* haplotype is *EphB2* (Fig. 2D, green bar), although at this time we cannot rule out the presence of regulatory variants that might alter the expression of another gene. Crested members of the toy, fantail, Iranian, Jacobin, and owl breed groups are not more closely related to each other than to uncrested breeds (Fig. 1). Nevertheless, members

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of these groups had head crests hundreds of years ago (14), so some of these introgression events must have occurred in the distant past. Breeds with a wide variety of crest phenotypes share the same derived allele; therefore, allelic variation at the *cr* locus alone does not control all aspects of crest development (14). Other genetic and developmental factors beyond this locus must contribute to variation in crest morphology, akin to the presumed complex genetic architecture of species-level divergence in feather ornaments (2).

In crested pigeons, feather placode polarity and bud outgrowth are inverted during embryogenesis (Fig. 3). Expression of *EphB2* is not polarized in early placodes (Fig. S26), so the effects of the *cr* mutation on feather polarity are probably exerted earlier in development. Why might the crest phenotype be limited to the head and neck? In Naked neck chicken mutants, regionalized production of retinoic acid allows uniform upregulation of *Bmp7* expression to change skin phenotypes in the neck but not the body (23). Similarly, the head crests of several chicken breeds, in which feathers are elongated but do not have a reversed growth trajectory as in pigeons, are localized to the top of the head probably due to ectopic expression of *Hox* positional cues (24). Together, these examples provide evidence for regionalization of the developing head and neck skin in the chicken. We propose that analogous mechanisms might underlie skin regionalization in the pigeon and allow *cr* to change feather polarity in the occiput and neck, but not elsewhere.

Our study of domestic rock pigeons illustrates how combining comparative genomics and population-based analyses forwards our understanding of genetic relationships and the genomic basis of traits. Many of the traits that vary among pigeon breeds also vary among wild species of birds and other animals (2, 25); thus, pigeons represent a model for identifying the genetic basis of variation in traits of general interest. Moreover, variation in many traits in domestic pigeons, including the head crest phenotype described here, is constructive rather than regressive: breeds derived from the ancestral rock pigeon possess traits that the ancestor does not have. While adaptive regressive traits are important, the genetic basis of constructive traits in vertebrates remains comparatively poorly understood. The domestic pigeon is thus a promising model to explore the genetic architecture of derived, constructive phenotypes in a bird that is amenable to genetic, genomic, and developmental investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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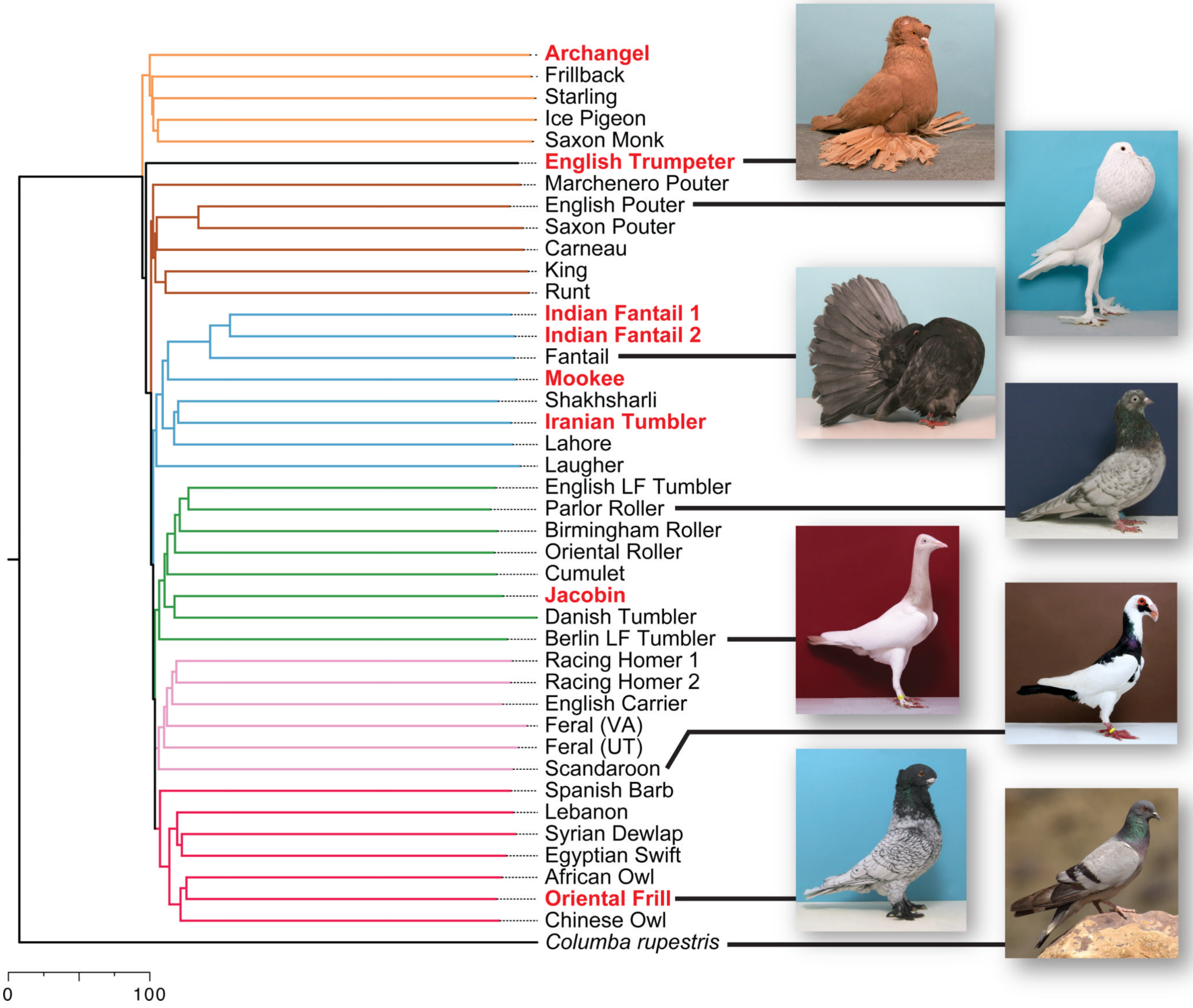

Fig. 1.

Relationships among rock pigeons and the hill pigeon *Columba rupestris*. Consensus neighbor-joining tree based on 1.48 million genomic SNPs and 1000 bootstrap replicates (see Fig. S16 for bootstrap support). Branches are colored by traditional breed group (12) and/or geographic affinities: orange, toy breeds; brown, pouters and utility breeds; light blue, Indian and Iranian breeds; green, tumblers and highflyers; pink, homers and wattle breeds; red, Mediterranean and owl breeds; black, voice characteristics (14). Bold, red lettering indicates breeds with the head crest phenotype. Scale bar, Euclidean distance. Photo credits: T. Hellmann (domestic breeds) and M.V. Shreeram (*C. rupestris*).

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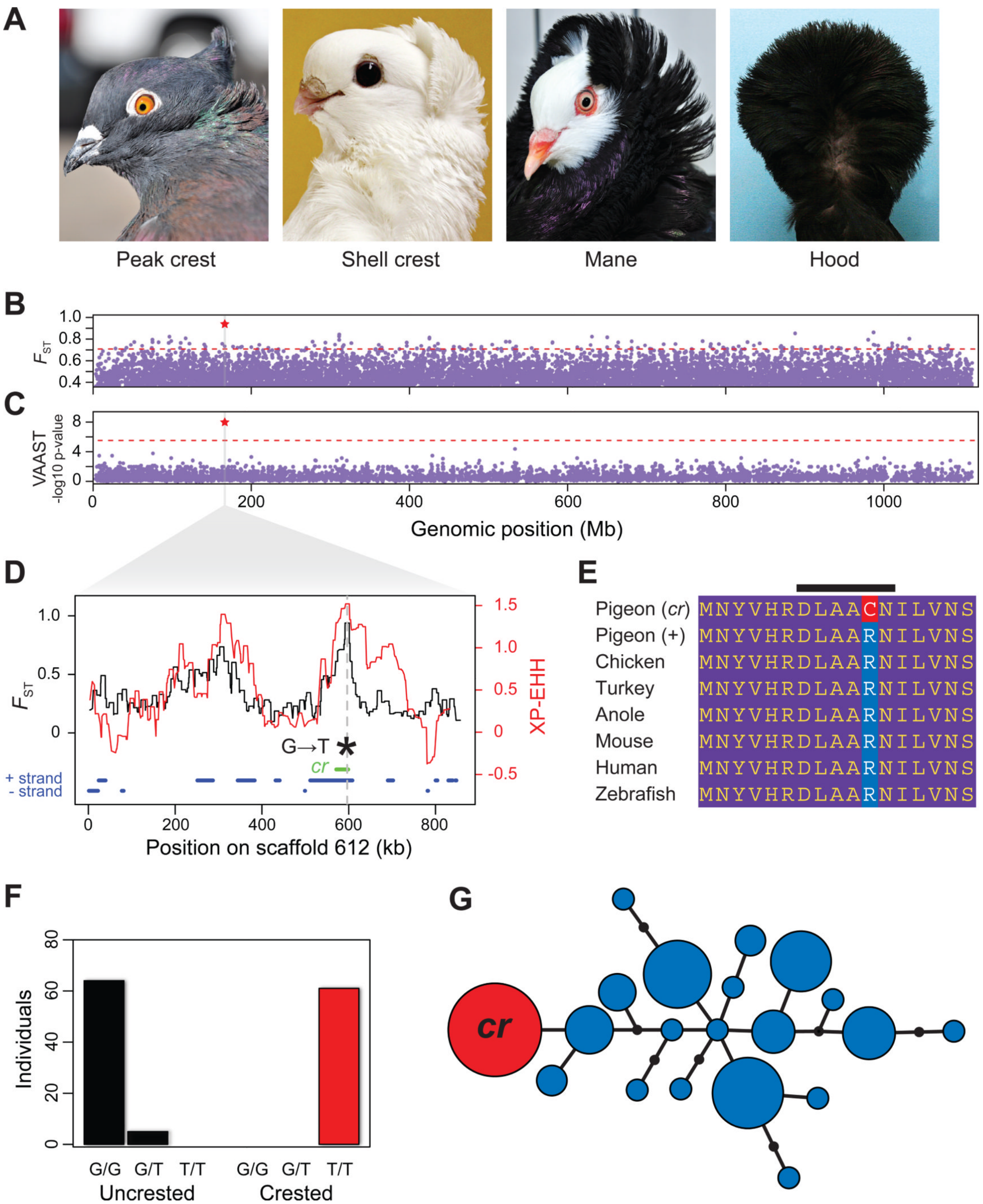

Fig. 2.

EphB2 is associated with the derived head crest phenotype. (**A**) Head crests are variable among breeds (left to right: Indian fantail, Old German owl, Old Dutch capuchin, Jacobin). (**B**) F_{ST} between crested and uncrested pigeons, maximum value for individual SNPs plotted for non-overlapping 100-kb windows across the genome. Red star, window with the highest score. Dashed red line, top 1% of scores. (**C**) Genome-wide VAAST scan. Each dot represents a single gene. Red star, gene with the highest score. Dashed red line, genome-wide significance cutoff. (**D**) Magnification of scaffold 612 in shaded region of (**B–C**). Black trace, maximum F_{ST} between crested and uncrested birds over a 300-SNP window.

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Red trace, unstandardized cross-population extended haplotype homozygosity (XP-EHH); higher values are evidence of selection (see Fig. S21, genome-wide plot). Dashed vertical line, position of the lone genome-wide significant VAAST hit. Green bar, 27.4-kb haplotype shared by all crested birds, includes only the *EphB2* gene. Blue bars, gene predictions on + and − DNA strands. (**E**) The *cr* mutation induces a charge-changing amino acid substitution; black bar, highly conserved DLAARN motif of catalytic loop. (**F**) Genotypes of 159 birds from 79 breeds at the *cr* locus are perfectly associated with the crest phenotype under a recessive model. (**G**) Network diagram of the minimal 11-kb haplotype shared by all resequenced rock pigeons with the *cr* mutation (also see Fig. S23). Many haplotypes contain the + allele (blue), but only one contains the *cr* SNP (red). Sizes of circles are proportional to the number of chromosomes containing a haplotype. Line segments represent single nucleotide differences. Jacobin photo credit: T. Hellmann.

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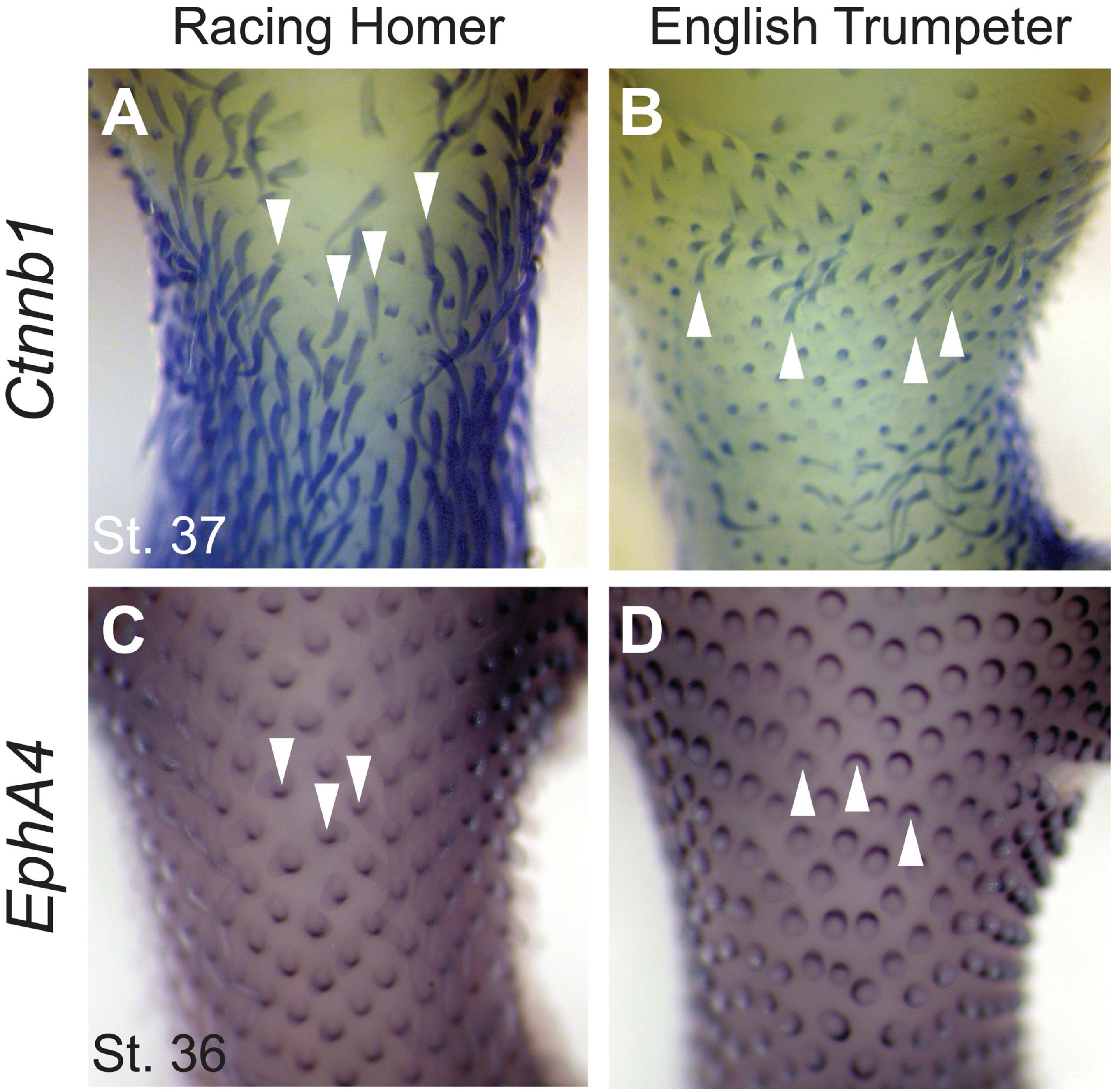

Fig. 3.

Feather bud polarity is reversed in the *cr* mutant. (**A,B**) Expression of the feather structural gene *Ctnnb1* reveals the direction of outgrowth of early feather buds. (**A**) Neck and occipital head expression of *Ctnnb1* in an embryo of the uncrested racing homer. Feather buds point downward along the contour of the head and neck (arrowheads). (**B**) Occipital feathers buds point upward in the equivalent region of the crested English trumpeter, indicating morphological reversal of feather orientation. (**C,D**) Expression of the polarity marker *EphA4* was assayed at an earlier developmental stage to test if feather placodes, the ectodermal thickenings that give rise to feather buds, are also reversed. (**C**) Polarity marker *EphA4* is expressed posteriorly (arrowheads) in feather placodes of the racing homer. (**D**)

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Polarity of placodes is reversed in the English trumpeter. Expression of *EphB2* in the skin is weak and unpolarized at this stage in both morphs (Fig. S26).