

EVOLUTION ON A LOCAL SCALE: DEVELOPMENTAL, FUNCTIONAL, AND GENETIC BASES OF DIVERGENCE IN BILL FORM AND ASSOCIATED CHANGES IN SONG STRUCTURE BETWEEN ADJACENT HABITATS

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Divergent selection on traits involved in both local adaptation and the production of mating signals can strongly facilitate population differentiation. Because of its links to foraging morphologies and cultural inheritance song of birds can contribute particularly strongly to maintenance of local adaptations. In two adjacent habitats—native Sonoran desert and urban areas—house finches (*Carpodacus mexicanus*) forage on seeds that are highly distinct in size and shell hardness and require different bite forces and bill morphologies. Here, we first document strong and habitat-specific natural selection on bill traits linked to bite force and find adaptive modifications of bite force and bill morphology and associated divergence in courtship song between the two habitats. Second, we investigate the developmental basis of this divergence and find that early ontogenetic tissue transformation in bill, but not skeletal traits, is accelerated in the urban population and that the mandibular primordia of the large-beaked urban finches express bone morphogenetic proteins (BMP) earlier and at higher level than those of the desert finches. Further, we show that despite being geographically adjacent, urban and desert populations are nevertheless genetically distinct corroborating findings of early developmental divergence between them. Taken together, these results suggest that divergent selection on function and development of traits involved in production of mating signals, in combination with localized learning of such signals, can be very effective at maintaining local adaptations, even at small spatial scales and in highly mobile animals.

KEY WORDS: Bite force, BMP, bone formation, local adaptation, mating signal, natural selection, ontogeny, performance, song.

Natural selection on structures involved in both local adaptation and the production of mating signals can strongly reinforce maintenance of adaptation and facilitate population divergence (Mayr 1939, 1942; Schluter 2000). This is particularly the case in sexual displays whose production involves a significant environmental component, such as local diet-derived carotenoid pigments in integuments (Endler 1983; Ryan et al. 1994; Witmer 1996), or shared functions, such as foraging and acoustic communications in some species (Podos 2001; Patek and Oakley 2003). Although

significant environmental dependence and cultural inheritance of many sexual displays and preferences can facilitate their divergence among populations (Grant and Grant 1996; Lachlan and Servedio 2004), it can also limit their evolutionary change and thus their contribution to population divergence (e.g., Badyaev and Snell-Rood 2006). At the same time, when production of sexual displays involves well-integrated organismal structures subject to divergent natural selection between environments, the interplay between natural selection and cultural inheritance can

maintain locally adaptive ontogenies of selected traits and population divergence in these traits (e.g., Grant and Grant 1997b; Ellers and Slabbekoorn 2003; Patten et al. 2004; Irwin et al. 2005).

The diversity of bill morphologies across bird species have long been an illustration of the power of natural selection on bill function (Darwin 1859; Newton 1967; Boag and Grant 1978; Schluter 1986; Smith 1987; Grant and Grant 2002) and versatility and modularity of developmental processes producing bill morphologies (Merilä and Bjorklund 1999; Abzhanov et al. 2004; Wu et al. 2004; Grant et al. 2006; Wu et al. 2006). In seed-eating birds in particular, divergence in bill form reflects competing demands of seed manipulation and crushing (Bowman 1961; Willson 1971; Abbott et al. 1977; Boag and Grant 1981; Schluter 1988; Grenier and Greenberg 2005; Van der Meij and Bout 2006) that can have a major effect on vocal tract configurations and acoustic mating signals (Bowman 1961; Slabbekoorn and Smith 2002; Podos et al. 2004). For example, birds with stronger and larger bills might be less able to perform rapid sound modulations, such as trills, whereas bill length and gape alter the effective length of the vocal tract and corresponding frequencies of produced song (Nowicki 1987; Westneat et al. 1993; Nelson et al. 2005). Further, habitat-specific transmission of song exerts strong selection on both song structure (e.g., Wiley 1991) and the vocal apparatus (Podos et al. 2004). Such multiple selection pressures can either reinforce selection for local adaptation in bill form or, capitalizing on the versatility and complexity of the foraging structures, favor decoupling the morphologies associated with foraging and song production.

Proximally, evolution of bill morphology is enabled by ontogenetic changes in mandibular tissue proliferation, migration, and transformation—evolutionarily conserved processes governed by only a few regulatory mechanisms (Schneider and Helms 2003; Wu et al. 2006). Foremost among these mechanisms is variation in bone morphogenetic proteins (BMP) that are involved in both epigenetic growth remodeling in bone and cartilage and the evolution of major adaptive diversifications (reviewed in Young and Badyaev 2007). In birds in particular, timing and the level of expression of BMP during early development are strongly implicated in ontogenetic transformations and evolutionary diversification of beak shapes (Abzhanov et al. 2004; Wu et al. 2004; Helms and Brugmann 2007).

Here we investigate functional, morphological, acoustic, developmental, and genetic divergence between populations of house finches (*Carpodacus mexicanus*) that occupy two adjacent habitats—an urban area and the undisturbed Sonoran desert of a national park—where finches are similar in body size, but differ strongly in the foods they eat. In the desert population, the seed diet of house finches consists primarily of small seeds of cacti and grasses, whereas the main component of the seed diet of the urban population are large sunflower seeds provided at

feeders in the area (Hensley 1954; Mills et al. 1989; Hill 1993; Shochat et al. 2004). As in many other seed-eating finches, the bill form of house finches is under strong natural selection and shows extensive population divergence and rapid evolution of pre- and posthatching ontogeny across populations (Badyaev and Hill 2000; Badyaev et al. 2001a,b). Further, house finches have highly localized patterns of song learning, often resulting in delineated local neighborhoods of song structure (Mundinger 1975; Bitterbaum and Baptista 1979; Pytte 1997; Tracy and Baker 1999). Thus, if divergence in bill morphology under distinct functional demands of foraging in urban and desert habitats is associated with modifications of song structure, then local song learning might further reinforce phenotypic and genetic divergence between the two habitats despite their close geographical proximity. We first demonstrate that foraging in the two habitats requires different bite force and that habitat divergence in bill, but not body, morphology is closely linked to these functional demands. Second, we show that the population divergence in bill morphology is associated with early developmental transformations of mandibular structure. Third, we document habitat-specific patterns of natural selection on bill characteristics associated with bite force. Fourth, we examine divergence in song characteristics between the populations and the extent to which such divergence is linked to the differences in bill form and functional performance. Finally, we use neutral genetic markers to quantify population genetic parameters and the patterns of gene flow between the two populations.

Materials and Methods

STUDY POPULATIONS AND GENERAL METHODS

House finches were studied in two resident populations, 6–10 km apart, in southwestern Arizona: on the campus of the University of Arizona in central Tucson, in 2002–2007 (hereafter the urban population) and in the Mount Wesson section of Saguaro West National Park, in 2004–2007 (hereafter the desert population). Both study sites are clearly delineated by distinct vegetation and geographical features; the urban population occupies area of 2.1 km² and the desert population occupies 5.2 km². Birds were trapped year round and marked with a unique combination of four leg color bands. Upon capture, we measured (with a resolution of 0.01 mm): bill length from the anterior end of the nostril to the tip of the upper mandible, bill width at the anterior end of the nostril, bill depth in a vertical plane at the anterior end of nostrils over both mandibles, length of left and right tarsus to the base of the foot (with a resolution of 0.01 mm), and body mass (to 0.05 g). To minimize seasonal variation and for selection estimates, we used data for 1245 males ($n = 1024$ and 221 for the urban and the desert populations correspondingly) that were captured in January and February 2005–2007, during prebreeding season. Bill parameters were highly intercorrelated and thus

bill size was calculated as a linear principal component = 0.66 bill length + 0.66 bill depth + 0.40 bill width (eigenvalue $\lambda = 2.01$, explaining 76.6% variance). Small seeds of grasses and cacti that constitute the bulk of seed diet of house finches in the desert (Hensley 1954; Mills et al. 1989; Shochat et al. 2004) have average diameter in the direction of cracking = 1.2 ± 0.4 mm ($n = 377$ seeds) and average hardness = 2.7 ± 0.08 Newtons (N), whereas sunflower seeds that are the main part of seed diet in the urban population have average diameter in the direction of cracking = 6.8 ± 2.1 mm ($n = 120$) and average hardness = 8.11 ± 0.21 N.

House finches settle in their breeding population shortly after postjuvenile dispersal and typically reside in that population for the rest of their lives. Such settlement patterns and strong fidelity of adult house finches to the location of previous breeding enables estimation of survival selection among individually marked resident males. In both urban and desert populations, a male was considered to have “survived” when it was captured at least twice, or was a local breeder, during January–February of the previous year, and was observed or captured again during January–February of the following year. A resident adult that did not appear in the study site the following year was assigned “did not survive” status. The reliability of this method was confirmed in long-term studies of completely marked study populations (references in Badyaev and Duckworth 2003; Badyaev 2005). Nearly complete marking, observations, and recaptures of individually marked resident birds of the urban population under this study assures that survival selection estimates reliably reflect mortality in that population (Oh and Badyaev 2008), whereas less complete sampling and recaptures in the desert population can confound mortality estimates with some local dispersal, although this is unlikely to produce the trends reported below.

The standardized selection differentials, in units of standard deviation, were calculated for each trait as the difference in untransformed trait means “before” and “after” each selection episode, divided by a square root of the before-selection variance. No transformations were used because plots of raw values showed normal distributions. Stabilizing selection was estimated by comparing variances in each trait between the groups. Probability of survival was fitted with a spline curve by using the method of cross-validation to select the smoothing parameter with the maximum predictive power (Schluter 1989). Because of the large differences in the sample sizes between the desert and urban populations, we used both nonparametric two-tailed Wilcoxon two-sample test and general linear models to test for differences in bill morphology, bite force, and song parameters between the two populations. The results were identical and thus only Wilcoxon two-sample tests are shown hereafter. General linear models were used to assess population differences in selection patterns and in morphological determinants of bite force and song characteris-

tics. We statistically controlled for the effects of body size on bill traits and bite force estimates.

BITE FORCE AND SONG MEASURES

Upon capture, we measured the maximum bite force of males ($n = 525$ in urban population and $n = 37$ in desert population) using a Kistler type 9203 force transducer connected to charge amplifier (Herrel et al. 2001). The measurement was recorded as a bird bit the force transducer plates inserted at the point of seed manipulation and crushing (Van der Meij and Bout 2004). Two highly repeatable ($r = 0.82$, $P < 0.01$) measurements were taken from each individual and the greater of the two values was used in subsequent analyses. Courtship song recordings were made with a Sennheiser MKH 60 P48 (Old Lyme, CT) directional microphone with a Marantz PMD 660 solid state digital recorder and measured using Syrinx-PC sound analysis software (J. Burt, Seattle WA). All songs analyzed in this study included a buzz syllable, typical of courtship displays, and were recorded under fixed gain settings on the digital recorder in February–March, during displays with females present in the vicinity of a singing individual. Courtship songs performed during flights were excluded from the analyses. See Mennill et al. (2006) for further details of house finch song analyses and note differentiation. For this study, we recorded and analyzed 263 songs from 74 individual males from the urban population and 94 songs from 39 individual males from the desert population. Several (3–10, mean = 4.4) song recordings were obtained from each individual and values were averaged for each individual. For a subset of males ($n = 52$ from the urban population in winter 2005–2006, and 18 from the desert population in winter 2006–2007), morphological measurements and bite force measures were obtained within the same prebreeding season as the song recording, and these were used in subsequent analyses of morphological correlates of song production. Following previous studies of contemporary mate choice of components of house finch song (Bitterbaum and Baptista 1979; Nolan and Hill 2004; Mennill et al. 2006), we measured highest and lowest frequencies, number of distinct notes, and trill rate—number of syllables sang per second.

GENETIC VARIATION

In 2005, we collected blood samples (40–60 μ l) from a subset of adult resident males and females in both populations ($n = 167$ and 58 for the urban and desert population correspondingly). Genomic DNA was extracted using a commercial kit (Gentra Systems, Minneapolis, MN) and all adults were genotyped at 12 highly polymorphic species-specific microsatellite loci (*Hofi53*, *HofiACAG07*, *HofiACAG25*, *Hofi16*, *Hofi29*, *Hofi10*, *Hofi70*, *Hofi39*, *Hofi19*, *Hofi35*, *HofiACAG15*, and *Hofi07*; Oh and Badyaev 2006). PCR was carried out using fluorescent-labeled primers (Applied Biosystems, Foster City, CA) and product was analyzed

by capillary electrophoresis in an ABI Prism 3730 DNA analyzer. Discrete microsatellite allele sizes were determined using Genotyper software (Applied Biosystems). To test for population differentiation, we used the multilocus version of the exact genotypic G -test (Goudet et al. 1996; Petit et al. 2001) as implemented in the software FSTAT (Goudet 2001). The method performs better than F_{ST} estimators for unbalanced sample sizes between the populations (Goudet et al. 1996; Balloux and Lugon-Moulin 2002). We compared the log-likelihood ratio statistics (G) between observed and randomized datasets generated by shuffling of genotypes among the populations (10,000 iterations). For comparisons with other studies we also calculated Weir and Cockerham's F_{ST} (Weir and Cockerham 1984) with the associated 95% confidence interval generated by bootstrapping over all loci.

DEVELOPMENTAL SEQUENCES

Histology

In these study populations, oogenesis, egg-laying, and incubation were closely monitored as a part of the larger study, precise onset of incubation was recorded with incubation probes installed at the stage of nest building (Badyaev et al. 2003), and eggs of known developmental stages were removed sequentially after 5–13 days (hatching) of incubation. Embryos were fixed in 4% paraformaldehyde in phosphate buffered saline overnight at 4°C. Fixed embryonic material was embedded in paraffin and sectioned medially at 5 μ m. Sequential sections were stained in hematoxylin/eosin (H&E) to examine development of tarsus and bill and used for immunohistochemistry assessing BMP activity (see below). In addition to known developmental ages (in days), embryos were assigned to a general developmental stage (25–37) following Hamburger and Hamilton (1951) and Bellairs and Osmond (2005). For each of the 21 embryos ($n = 9$ and 12 for desert and urban populations, correspondingly), we compared developmental sequences in the quadrato-articular and Meckel's cartilages of the bill and tarsus cartilage using the steps of conserved sequence of cartilaginous bone origination, growth, and maturation: (1) a condensation and histogenesis or differentiation of mesenchyme cells to cartilaginous precursors, (2) growth due to proliferation of original cell population and production of matrix and/or swelling of cells, (3) maturation of cells, and (4) calcification and ossification.

According to these general steps, the sequential tissue transformation was partitioned into (Fig. 4): Cartilage precursor zone formed by precartilage cells, zone I (resting zone) consisting of chondroblasts, zone II (zone of proliferation) where flattened and elongated chondrocytes divide and stack up, zone III (zone of maturation and hypertrophy) where chondrocytes stop dividing and swell, and zone IV (zone of calcification) where chondrocytes are large and swollen and where matrix begins to calcify immediately prior to ossification. In the bill, cartilage precursors were distin-

guished from other mesenchyme cells by the absence of a star-like morphology and the presence of blue-staining extracellular matrix, Zone I cells had small round nuclei, cells often formed small clusters and were surrounded by large amounts of blue staining extracellular matrix, Zone II cells were slightly enlarged and elongated, these cells had large flattened nuclei, and form linear clusters associated with successive mitotic division (stages of development after Young et al. [2006]). Staging of cells was done using H&E stained sections of the bill.

Developmental sequence of the tarsus was recorded as a proportion of each of the tissue zones in the overall bone structure for each developmental stage (Fig. 4). Because of the complexity of growth and maturation of skeletal tissues in the cranium resulting from a mosaic of endochondral and intramembranous ossification and persistent cartilage (Eames and Helm 2004; Hall 2005), transitions in proportion of cells with distinct morphologies may not accurately assess developmental sequence of the bill. Thus, developmental sequence of the bill was recorded qualitatively as developmental stage at first appearance of each cell stage (asterisks in Fig. 4A, B).

Comparative analysis of both bill development (Fig. 4) and BMP activity (Fig. 3) were done using subsequent sections of quadrato-articular cartilage and Meckel's cartilage, two tissues that undergo endochondral ossification (Bellairs and Osmond 2005; Hall 2005). Meckel's cartilage cells behave differently along the proximal-distal axis. Proximally, Meckel's cartilage functions as replacement cartilage by transitioning to bone by endochondral ossification (Hall 2005). Distally, Meckel's cartilage is a persistent cartilage that can be maintained throughout an organism's lifetime covered by dermal bone (Eames and Helm 2004; Hall 2005). As a result, in older individuals, only the proximal end of the elongated Meckel's cartilage was analyzed. In younger individuals the entire Meckel's cartilage was included. Because cell stages in younger individuals have not advanced past cartilaginous precursors, use of the entire Meckel's should not bias our assessment of development or BMP activity.

Phospho-Smad immunohistochemistry

To examine activity of BMP, immunohistochemical analysis was performed on 5 μ m paraffin sections of embryos using an anti-Phosphorylated Smad1/Smad5/Smad8 (Cell Signaling Technology, Beverly, MA; Ahn et al. 2001; Sears et al. 2006). Binding of Bmps to Ser/Thr kinase receptors on the cell membrane activates receptors and subsequently leads to phosphorylation of Smad proteins (Hogan 1996; Young and Badyaev 2007). Sections were processed by immunoperoxidase labeling using the Vectastain ABC elite peroxidase kit (Vector Laboratories, Burlingame, CA). For antigen unmasking, sections on slides were incubated in a 10 mM sodium citrate solution at 92°C for 10 min. The sections were treated to quench endogenous peroxidase activity

with 3% H₂O₂ and blocked using normal goat serum for 1 h and incubated overnight at 4°C in Phospho-Smad1/Smad5/Smad8 antibody (Cell Signaling Technology) diluted 1:100 in normal goat serum. All further incubation was done in accordance with the manufacturer's instructions.

Smad stained samples for each individual were photographed and hue expression was measured at 10 randomly selected nuclei within each sample with SigmaScan Pro 5.0 (Systat Software, Inc., San Jose, CA). Specifically, nuclei were chosen by overlaying a standard-sized grid across the image of the tissue and sampling nuclei at grid intersections corresponding sequentially to 10 randomly generated numbers. Mean hue of 10 values were used for further analyses. Because BMP activity varies across the each tissue, this method allowed us to assess overall BMP in the tissue. Higher (redder) hue values indicate higher Smad staining and thus greater BMP activity.

Results

POPULATION DIVERGENCE IN MORPHOLOGY, BITE FORCE, AND COURTSHIP SONG

Desert finches had smaller bills than urban finches in all measures (Fig. 1A; bill length: Wilcoxon $\chi^2_1 = 8.68$, $P = 0.0003$; depth: $\chi^2 = 11.28$, $P = 0.0008$; width: $\chi^2 = 14.99$, $P < 0.001$) and overall bill size (Fig. 1B; $\chi^2 = 17.32$, $P < 0.001$), and had weaker bite force (Fig. 1C; $\chi^2 = 5.11$, $P = 0.02$), but similar body

mass (Fig. 1B; $\chi^2 = 0.07$, $P = 0.69$) and tarsus length (desert: 20.46 ± 0.04 SE, urban: 20.42 ± 0.02 , $\chi^2 = 0.48$, $P = 0.48$). Songs of finches in both habitats had similar high (Fig. 1D; $\chi^2 = 1.71$, $P = 0.09$) and low ($\chi^2 = 0.00$, $P = 0.99$) frequencies, but desert finches had more notes in their songs (Fig. 1E; $\chi^2 = 8.02$, $P = 0.004$) and faster trill rates ($\chi^2 = 20.39$, $P < 0.001$) than did urban finches.

MAXIMUM BITE FORCE AND BILL DIMENSIONS

In both populations, birds with deeper and wider bills had greater bite force (Fig. 2B, C; bill depth, desert: $F_{1,37} = 3.85$, $b_{ST} = 0.29$, $P = 0.05$; urban: $F_{1,523} = 15.73$, $b_{ST} = 0.19$, $P < 0.01$; bill width, desert: $F = 19.06$, $b_{ST} = 0.61$, $P < 0.01$, urban: $F = 21.37$, $b_{ST} = 0.20$, $P < 0.01$). Desert finches with longer bills had weaker bite force (Fig. 2A; $F = 22.28$, $b_{ST} = -0.63$, $P < 0.01$), whereas bill length did not correlate with bite force in urban finches ($F = 0.03$, $b_{ST} = 0.08$, $P = 0.85$). The relationship between bite force and bill length ($F_{1,560} = 13.01$, $P = 0.0003$) and bill width ($F = 4.52$, $P = 0.03$), but not bill depth ($F = 0.13$, $P = 0.72$), differed between the populations occupying different habitats.

EARLY DEVELOPMENTAL DIVERGENCE IN MORPHOLOGY

The mandibular primordia of the large-beaked urban finches expressed BMP earlier and at a higher level than those of the desert finches (Figs. 3 and 4; overall difference in BMP activity between

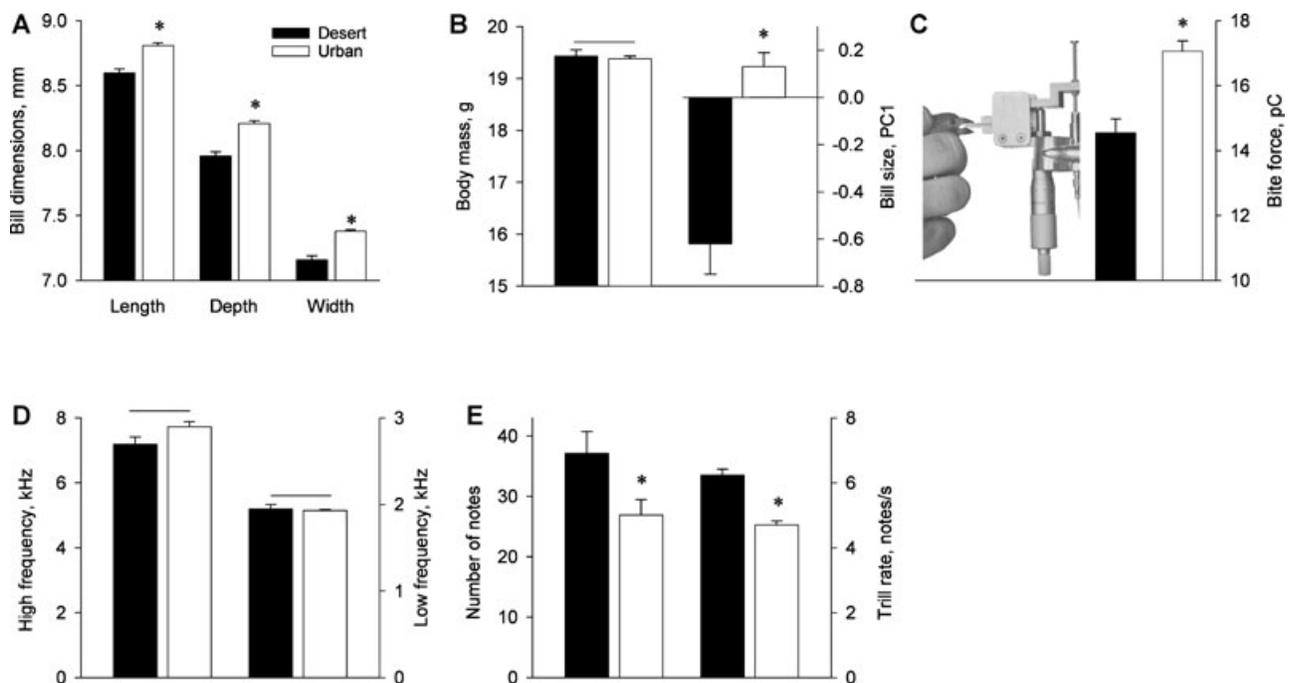


Figure 1. Divergence (mean \pm SE) of the desert and urban populations of house finches in (A) bill dimensions, (B) body mass and bill size, (C) maximum bite force, (D) high and low frequency of courtship songs, and (E) number of distinct notes and trill rate. Lines connect means that are not significantly different between the populations. Asterisks show significant difference between the populations with Wilcoxon two-sample tests at $P \leq 0.05$ level.

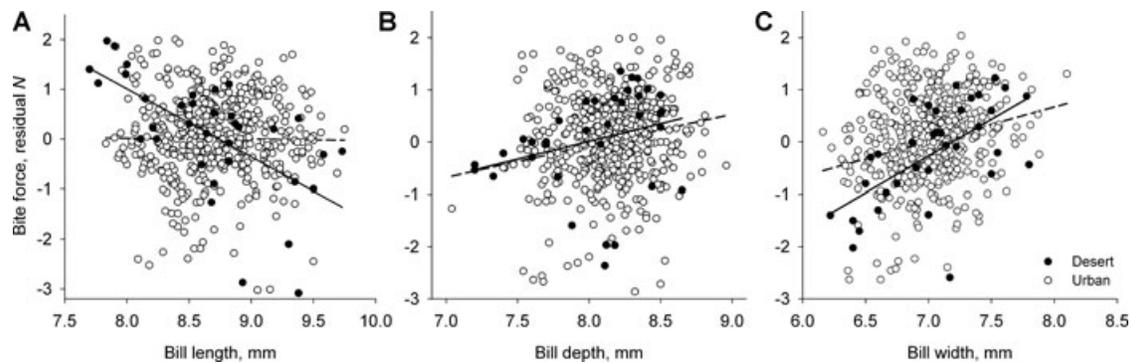


Figure 2. Relationship between bill dimensions and maximum bite force in the desert (filled circles, solid lines) and the urban (open circles, dashed lines) populations. Shown are partial regression plots for (A) bill length, (B) bill depth, and (C) bill width.

populations: $\chi^2 = 3.98$, $P = 0.04$); populations were distinct in BMP activity by embryonic day 5 (Stage 26, Figs. 3C and 4E). BMP activity increased throughout bill ontogeny, reaching the maximum level in the proliferation zones (Fig. 3E) at stage 30 (embryonic day 8, Fig. 4E). BMP activity in tarsus tissues tended to be greater in the urban population at stage 30, but overall was not significantly different between the populations either among tissue zones (Fig. 3) or across embryonic stages (Fig. 4E; overall difference in BMP activity between populations: $\chi^2 = 2.83$, $P = 0.09$). Embryos in the urban population had accelerated ontogenetic transformation of mesenchymal tissue of bill mandible (zone I; Fig. 4A, B) compared to the desert population. There were no differences in tarsus tissue transformation between desert and urban habitats ($\chi^2 > 1.51$, $P > 0.7$; Fig. 4B, C).

NATURAL SELECTION ON BILL MORPHOLOGY

Natural selection was distinct between the habitats on bill length (Fig. 5A, D; directional positive in the urban population and weakly negative in the desert population; populations were different in the patterns of directional selection: $F_{1,1244} = 32.58$, $P < 0.001$, but not stabilizing selection: $F = 1.89$, $P = 0.16$). The urban population experienced no detectable selection on bill depth, whereas the desert population was under stabilizing selection on this trait (Fig. 5B, E; populations were similar in directional selection: $F = 1.54$, $P < 0.21$, but differed in stabilizing selection: $F = 7.27$, $P = 0.007$). The urban population experienced strong positive directional selection on bill width, whereas the desert population was under stabilizing selection on this trait (Fig. 5C, F; populations were different in patterns of directional selection: $F = 5.61$, $P = 0.02$, and tended to differ in strength of stabilizing selection: $F = 3.27$, $P = 0.07$).

SONG PRODUCTION IN RELATION TO BILL MORPHOLOGY

The contribution of bill morphology to variation in song characteristics was similar between the populations (population difference,

frequency range: bill length, $F_{1,70} = 1.69$, $P = 0.19$, bill depth: $F = 0.33$, $P = 0.57$, bill width: $F = 2.37$, $P = 0.12$; number of notes: bill length, $F = 0.85$, $P = 0.35$, bill depth: $F = 1.90$, $P = 0.18$, bill width: $F = 0.14$, $P = 0.71$; trill rate: bill length, $F = 1.36$, $P = 0.25$, bill depth: $F = 0.18$, $P = 0.67$, bill width: $F = 1.59$, $P = 0.21$). However, population differences in bill morphology (Fig. 1A) strongly covaried with the strength of relationship between bill dimensions and song characteristics within each population (Fig. 6). In the urban population, where finches have longer and deeper bills compared to desert finches (Fig. 1A), the birds with longer and deeper bills had slower trill rates (Fig. 5G, H) and wider frequency range (Fig. 6A). In addition, urban birds with deeper bills sang fewer note types (Fig. 6E). In both populations, birds with wider bills had narrower frequency ranges and slower trill rates (Fig. 6C, I).

GENETIC POPULATION DIVERGENCE

The multilocus G -test revealed highly significant ($P = 0.0001$) genetic differentiation between the urban and desert populations. Similarly, F_{ST} indicated a small, but highly significant genetic differentiation between the two populations ($F_{ST} = 0.003$, 95% confidence interval = 0.001–0.006).

Discussion

Divergent natural selection on organismal structures involved in production of mating signals can strongly reinforce local adaptation (Mayr 1942; Grant and Grant 1996; Coyne and Orr 2004; Lachlan and Servedio 2004). A close link between foraging and the vocal apparatus, in combination with cultural inheritance of local vocal signals, can maintain population structure (MacDougall-Shackleton and MacDougall-Shackleton 2001; Gammon and Baker 2004; Ruegg et al. 2006), enable foraging specialization in sympatry (Groth 1993; Benkman 2003; Parchman et al. 2006), and facilitate convergence in morphology and vocalizations in areas of habitat overlap (Secondi et al. 2003). For example, a recent introduction of bird feeders might have led to modifications of bill

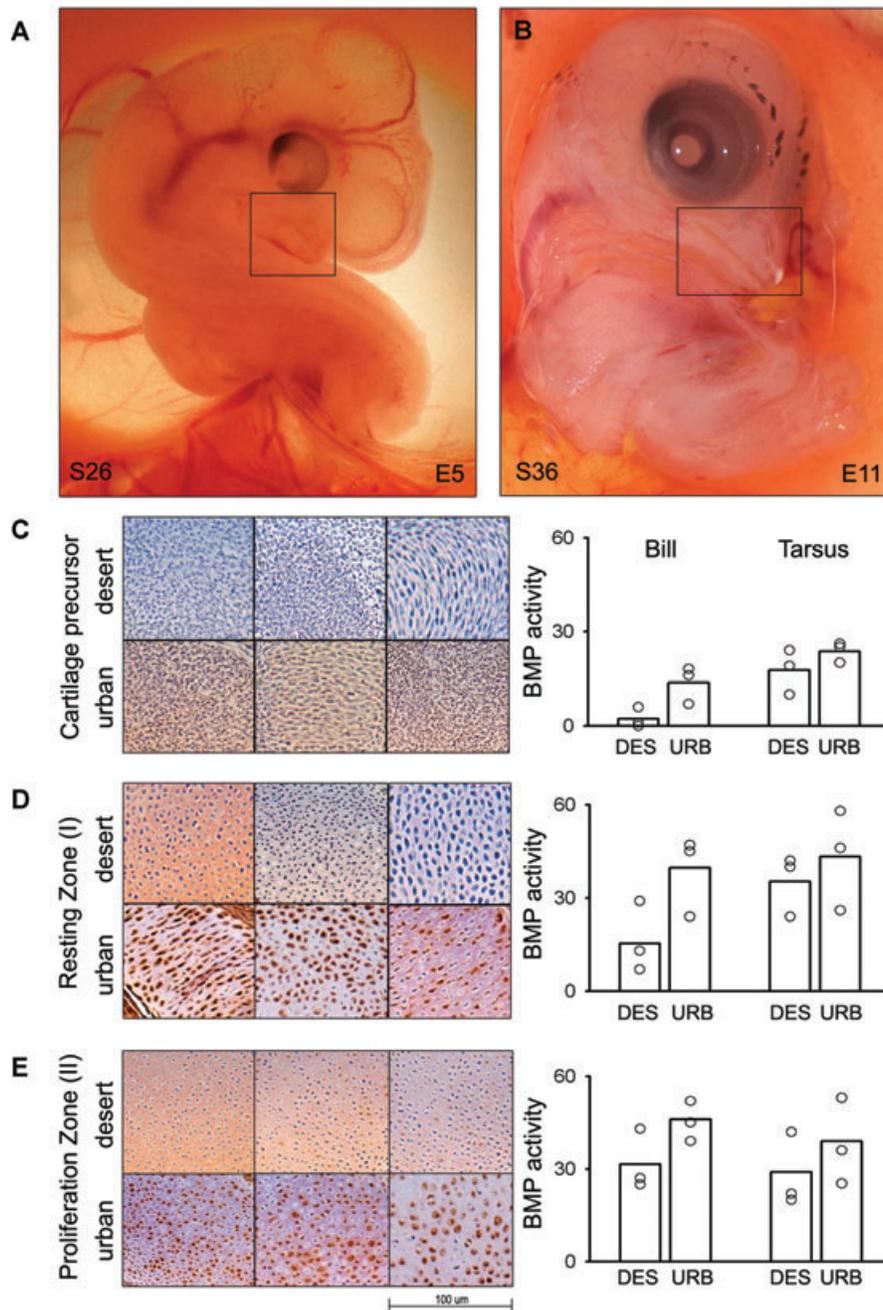


Figure 3. Early developmental divergence in BMP timing and level of activity between the urban and desert house finch populations. Embryos with bills highlighted at (A) Stage 26, embryonic day 5 and (B) Stage 36, embryonic day 11. Shown is activity of BMP in bill lower mandible's nuclei of (C) mesenchymal bill cartilage precursor, (D) resting zone, and (E) proliferation zone. Upper rows of samples show birds from the desert population and lower rows show birds from the urban population. Samples from left to right are Stage 26, Stage 30, and Stage 36. Redder and darker hue indicates higher Smad staining and thus greater BMP activity. Right column shows BMP activity (mean of actual measurements, one for each stage) in bill mandibles (left) and tarsus bones (right) for each tissue stage and population (DES—desert, URB—urban). Tarsus BMP activity images are not shown. See Methods for details of tissue identification.

morphology (Hendry et al. 2006) and bite force (e.g., Herrel et al. 2005) in a population of Darwin's finches; an effect that could be maintained subsequently by a link between bill morphology and song production in these birds (Bowman 1961; Grant and Grant 1996, 1997a; Podos 2001).

House finches in urban areas, especially in recently established populations, depend closely on feeders that supply sunflower seeds, seeds that are much larger and harder than those constituting the bulk of finch diet in natural and ancestral populations (Hensley 1954; Mills et al. 1989; Hill 1993; Shochat et al.

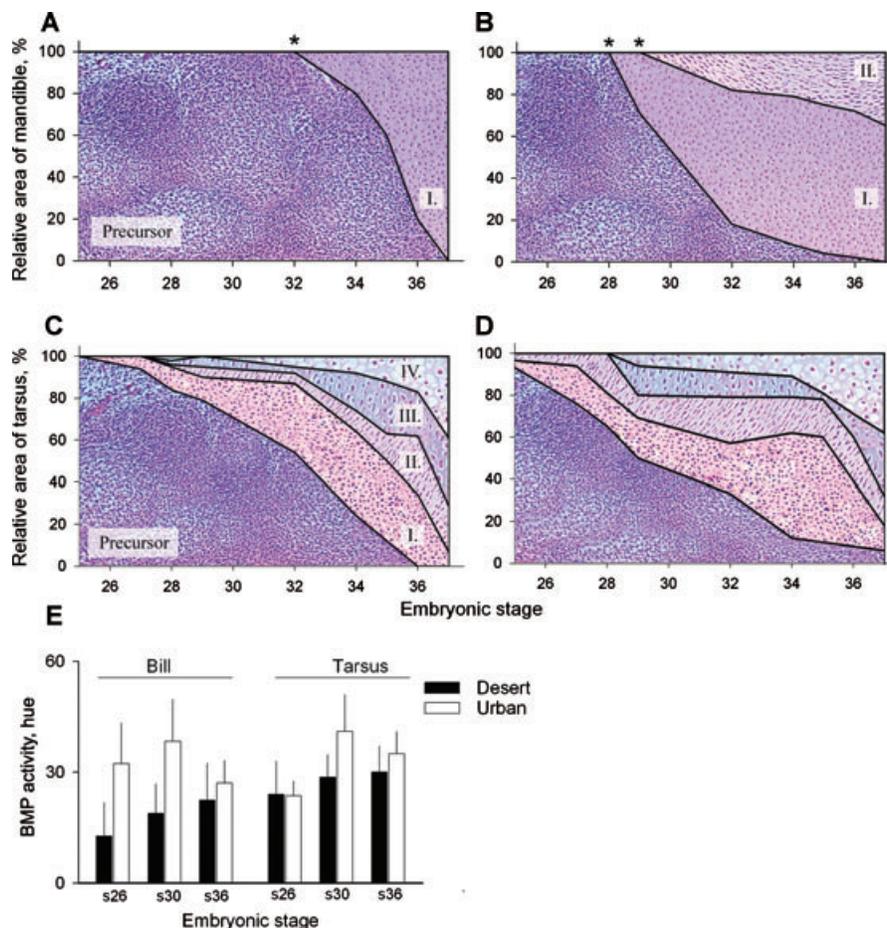


Figure 4. Ontogenetic tissue transformation in embryonic bill lower mandible in (A) the desert and (B) urban populations, and in tarsus bones in (C) the desert and (D) urban populations. Shown are relative areas of lower mandible and tarsus occupied by tissues at sequential stages of bone transformation (see Methods for details) in relation to H&H embryonic stages. Asterisks mark the earliest ages of appearance of zone I tissue in the desert finches (stage 32), and zone I (stage 28) and zone II (stage 29) appearances in the urban finches. Tissue types as fills are shown for illustration only, see Methods for details of histology. (E) Activity of BMP in early ontogeny of bill and tarsus in the desert (black) and urban (white) populations (Fig. 3). Shown are means + 1SE.

2004). Such introduction of novel food exerts strong selection on the ontogeny of bill form, and bill morphology is one of the most rapidly evolving traits in recently established populations of this species (Badyaev et al. 2001a; Badyaev 2005). In this study, we showed that adaptations for consumption of large seeds in urban areas were associated with developmental and genetic divergence between the populations and with changes in courtship song structure.

Greater bite force (contributed by bill width, Fig. 2, see also Herrel et al. 2005) and larger bill gape (longer bill, Fig. 1) were strongly favored in the urban population (Fig. 5A and 5C) where house finch's seed diet consists almost exclusively of sunflower seeds. Such modifications in bill morphology might have resulted in a greater trade-off between bill size and song characteristics in urban habitats compared to desert habitats (Fig. 5). As predicted by biomechanics of vocal communications in birds (Nowicki et al. 1992; Westneat et al. 1993), urban finches with longer and

more massive bills might be constrained to produce songs with only slower trill rates (Fig. 1; see also Podos 2001; Boncoraglio and Saino 2007). Such limitations might be particularly evident in the urban population, where high background noise should favor vocalizations at higher frequencies (Slabbekoorn and Smith 2002; Patricelli and Blickley 2006), but where longer bills might constrain temporal modulation at these frequencies in particular (Nowicki et al. 1992). Interestingly, the song characteristics most affected by divergence in bill morphology—trill rate and number of notes—are also the targets of mate choice of song in house finches (Bitterbaum and Baptista 1979; Nolan and Hill 2004; Mennill et al. 2006), which should have significant implications for reinforcement of local adaptation in this species.

Bill morphology of seed-eating birds evolves under competing demands of seed manipulation and seed crushing—manipulation of small seeds requires shorter and smaller bills, whereas crushing of hard seeds requires more massive bills (Grant

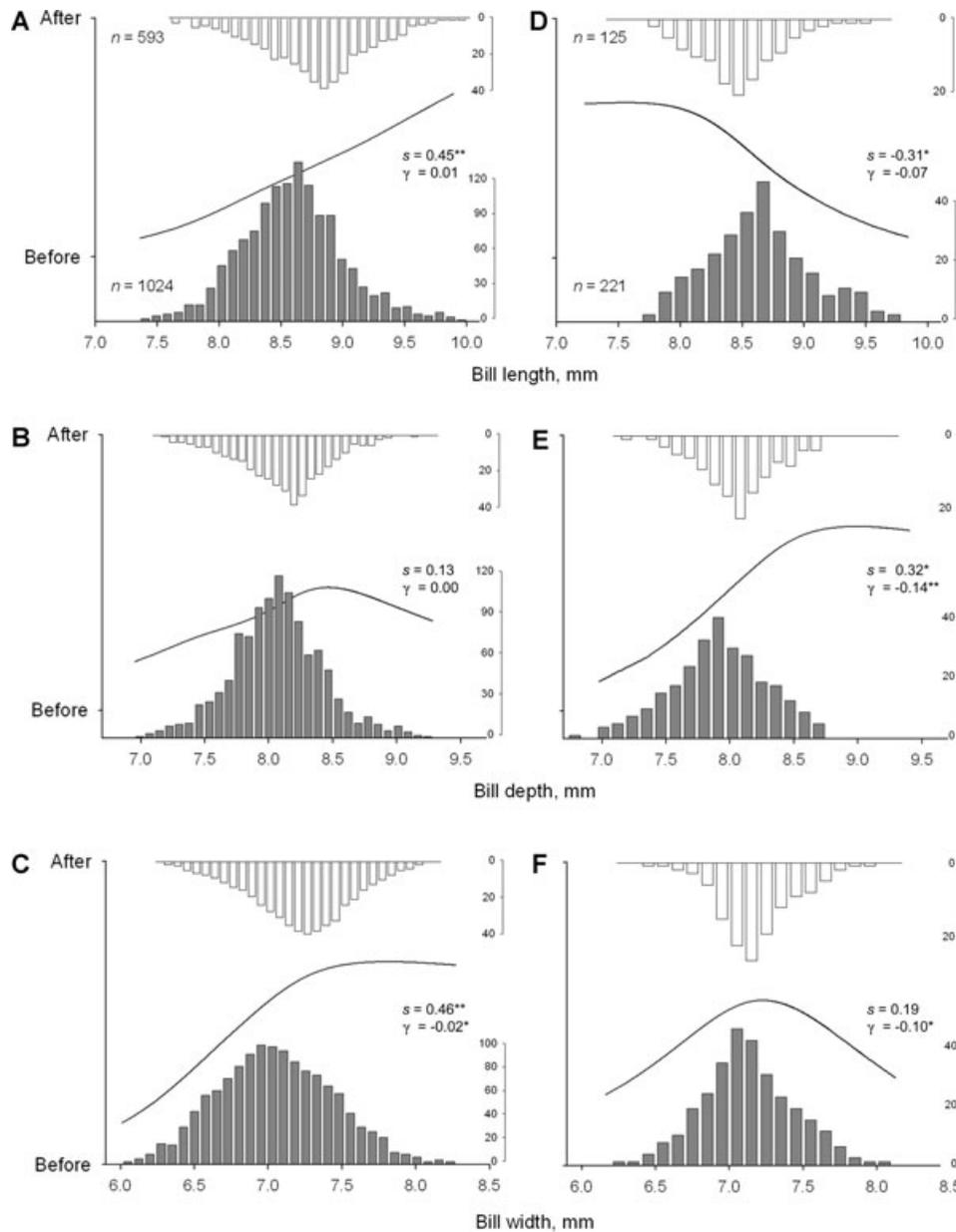


Figure 5. Survival selection on bill dimensions in the desert and the urban house finches. Shown are before-selection (filled bars) and after selection (open bars) distributions of bill dimensions and associated best-fit cubic splines of survival probability for bill dimensions of adults in (A–C) the urban population, and (D–F) the desert population. Asterisks indicate significance of directional (s) and stabilizing (γ) selection differentials: *— $P < 0.05$, **— $P < 0.01$.

1986; Smith 1990; Van der Meij and Bout 2004, 2006). In both study populations, house finches with wider and deeper bills had a stronger bite force; however the contribution of bill length to bite force and survival selection on bill length differed between populations (Fig. 5). In the urban population, longer bills might be favored because larger gape is required for manipulation of sunflower seeds (Van der Meij and Bout 2004), but bill length per se was not related to bite force in this population (Fig. 2A, see also Herrel et al. 2005 for similar results in Darwin's finches). On the contrary, in the desert population, where finches subsist

on a small seed diet, birds with longer bills had weaker bite force (Fig. 2A) and lower survival (Fig. 5D, Table 1).

Observed genetic divergence between the two ecologically distinct, but geographically adjacent habitats, might be further facilitated by highly localized patterns of song learning and ubiquitous countersinging in male house finches (e.g., Bitterbaum and Baptista 1979). Juvenile male house finches learn songs during the first 3–4 months of life, and because males typically disperse only short distances from their natal populations (Oh and Badyaev 2006, 2008) populations of this species often form localized song

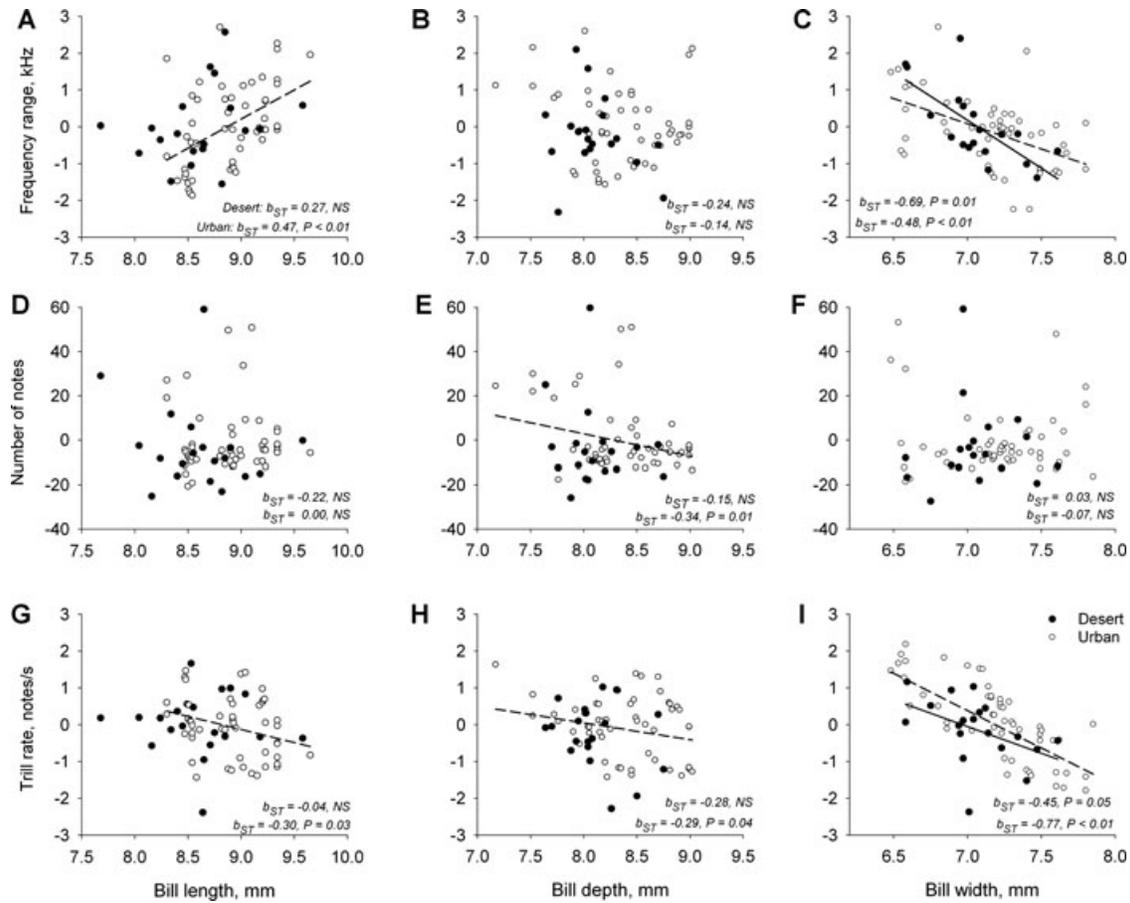


Figure 6. Relationships between bill dimensions and song characteristics in the desert (filled circles, solid line) and urban (open circles, dashed line) populations. Shown are partial regression plots, controlling for body size, for (A–C) the range of song frequencies, (D–F) number of song notes, and (G–I) trill rate. Lines are shown only for statistically significant regressions. b_{ST} is a standardized regression coefficient.

neighborhoods. In several populations, individual males were observed to share more than 90% of syllable types with other males born within 2–3 km of their natal population, but shared less than 10% of syllables with males residing 5–10 km away, and there

was no song structure sharing with males born farther than 15–20 km from the natal population (Bitterbaum and Baptista 1979; Tracy and Baker 1999). Although there is anecdotal evidence that flocks of juvenile finches are preferentially attracted to broadcasts

Table 1. Concordance between adaptive changes and observed population divergence in bill form, function, development, and song characteristics. Bill traits are larger in the urban population than in the desert population.

Bill trait	Relation to bite force ¹		Pattern of current selection ²		Relation to song parameters (#) ³		Concordance between adaptive changes in bill morphology and observed population divergence in:		
	Urban	Desert	Urban	Desert	Urban	Desert	Morphology	Song	Early ontogeny ⁴
Length	–	↓	↑	↓	++	0	yes	yes	yes
Depth	↑	↑	–	↑	++	0	no	yes	no
Width	↑	↑	↑	↓	++	++	yes	yes	yes

¹ ↑ Indicates positive relationship, ↓ indicates negative relationship, and “–” indicates no relationship.

² ↑ Indicates positive directional selection, ↓ indicates negative directional selection, ‡ indicates stabilizing selection.

³ Number of pluses indicates the number of affected song parameters.

⁴ Whole lower mandible.

of songs typical of local versus distant populations (A. Rapone and A.V. Badyaev, unpubl. ms.), no study to date have examined assortative mating in relation to habitat- and population-specific song structure.

The relative importance of local song learning versus actual inability to produce some song features due to adaptive bill modifications in urban populations requires further study, and aviary experiments with tutoring juveniles from the desert and urban populations with the song of the opposite types and examining subsequent song performance and preference could identify causal mechanisms behind the observed divergence (e.g., Podos 1996). At the same time, there is some evidence that localized patterns of song learning might have contributed to song divergence across recently established populations of house finches (Elliott and Arbib 1953; Aldrich and Weske 1978) that diverged in bill morphology (Badyaev and Hill 2000). First, studies documented reduction in song note diversity in house finch populations that were established by only a few individuals, as a result of either limited releases of captive birds (e.g., the eastern United States, Elliott and Arbib 1953; Aldrich and Weske 1978; Tracy and Baker 1999), or bottlenecks experienced by some populations during natural expansion (e.g., western North America, Wang et al. 2003). Second, populations that were established by repeated introductions of individuals from multiple sources (such as multiple releases in New England of finches caught in different locations in southern California, Munding 1975; Bitterbaum and Baptista 1979) or populations formed by admixture of distinct invasions in western North America (A.V. Badyaev, unpubl. ms) show a significant increase in diversity of song syllables (e.g., Pytte 1997). A contribution of the link between song production and local adaptation in bill morphology (Table 1) to the evolution of adaptive morphological divergence among newly established populations of house finches across diverse ecological areas of North America requires future studies.

A related important question is the developmental basis of morphological divergence between the populations in different habitats. Although population-specific allometric relationships between bill and body traits appear prior to hatching (Badyaev et al. 2001b), bill bones and jaw muscles continue to grow and gain strength during first 2–3 months of life (Badyaev and Martin 2000), so that exposure to seeds of different size and hardness, and associated bone remodelling and muscle growth, might be expected to be the main causes of population-specific changes in mandibular ossification, growth, and configuration. However, we found that the population divergence in ontogenetic transformations of bill is evident as early as day 5 of embryonic development (Fig. 3), that is, a week before hatching and a full month before the first exposure to population-specific seeds. The specific effect of earlier onset of BMP expression is dose-dependent, such that at lower levels, BMP activity results in higher rates of

cell proliferation, and at higher levels, BMP activity induces cell differentiation (Hogan 1996; Urist 1997). Our results suggest that earlier onset of BMP activity in the urban population of finches (Fig. 3) resulted in transformation of mandible tissues earlier in ontogeny (Fig. 4). Further, the pronounced population difference in the timing and levels of BMP expression and in mandibular tissue proliferation in bill ontogeny were qualitatively concordant with changes maintained by selection on functional performance (Table 1).

Whereas BMP is a ubiquitous regulator of environmentally induced bone remodelling across many vertebrates (Young and Badyaev 2007), its involvement in such early developmental divergence between the likely very recently diverged populations is puzzling. Elsewhere we reviewed a common role of BMP in both epigenetic bone remodelling and the evolution of major adaptive diversifications, and suggested that heterochronic shifts in BMP-regulated development, and not the allelic variation or altered transcription of *bmp* gene, are the main mechanism behind the rapid appearance of BMP-mediated innovations in skeletal traits (Young and Badyaev 2007). This study of birds occupying geographically adjacent habitats seems to corroborate this suggestion—greater and earlier BMP activity in the large-beaked urban birds might be due to modification of tissue susceptibility to BMP signaling or to overall level of BMP synthesis that are subsequently amplified and maintained by coordinated development of bill tissues.

A surprising finding of our study was low ($F_{ST} = 0.003$), but statistically significant genetic differentiation between geographically adjacent urban and desert populations, reporting F_{ST} values similar to genetic differentiation among house finch populations separated by much greater geographic distances (about 700–800 km) (Hawley et al. 2006). Interpreting the relative magnitude of such differentiation is difficult (Hedrick 1999; Ballou and Lugon-Moulin 2002). On the one hand, similar F_{ST} values have been shown to be associated with robust differentiation among subpopulations in some systems (Wirth and Bernatchez 2001). On the other hand, a previous study of genetic differentiation among western North American house finch populations on a continental scale using AFLP markers (Wang et al. 2003) reported F_{ST} values that were more than a magnitude greater ($F_{ST} = 0.01–0.1$) compared to our results. And although this discrepancy is not surprising due to the proximity of the populations in our study, it may also be attributed to our use of hypervariable microsatellite loci, which is generally expected to downward bias estimates of F_{ST} (Hedrick 1999; Woodhead et al. 2005).

Overall, we document adaptive modifications of bill form, function, and development, and associated changes in courtship song structure between the desert and urban populations. It is likely that such association in combination with localized patterns of song learning in this species might have contributed

to observed genetic differentiation between these geographically adjacent populations. These results show that selection on traits involved in both foraging and in acoustic communications might be exceptionally effective in enabling population's accumulation and maintenance of locally favored developmental processes leading to distinct adult morphologies even at small geographical scales and in highly mobile animals.

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