Genetic variation interacts with experience to determine interindividual differences in learned song

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Learning reflects the influence of experience on genetically determined circuitry, but little is known about how experience and genetics interact to determine complex learned phenotypes. Here, we used vocal learning in songbirds to study how experience and genetics contribute to interindividual differences in learned song. Previous work has established that such differences in song within a species depend on learning, but in principle some of these differences could also depend on genetic variation. We focused on song tempo, a learned and quantifiable feature that is controlled by central neural circuitry. To identify genetic contributions to tempo we computer-tutored juvenile Bengalese finches (*Lonchura striata domestica*) from different genetic backgrounds with synthetic songs in which tempo was systematically varied. Computer-tutored birds exhibited unexpectedly strong heritability for song tempo and comparatively weak influence of experience. We then tested whether heritability was fixed and independent of experience by providing a second group of birds with enriched instruction via live social tutoring. Live tutoring resulted in not only a significant increase in the influence of experience on tempo but also a dramatic decrease in the influence of genetics, indicating that enriched instruction could overcome genetic biases evident under computer tutoring. Our results reveal strong heritable genetic contributions to interindividual variation in song tempo but that the degree of heritability depends profoundly on the quality of instruction. They suggest that for more complex learned phenotypes, where it can be difficult to identify and control relevant experiential variables, heritability may similarly be contingent on the specifics of experience.

behavior | genetics | learning | songbird | GXE

Learning reflects the influence of experience on genetically determined neural circuitry, and it has long been appreciated that the specific conditions of experience under which a particular behavior develops may modulate the impact of the relevant genetic constraints (1). However, our understanding of how experience and genetics interact to shape learned behaviors remains scant. Indeed, there are few cases where it has been possible to systematically control genetic and experiential factors and quantify behavioral outcomes to test such interactions. Here, we take advantage of the quantifiable nature of birdsong and the ability to control relevant instructive experience to investigate how genetic and environmental influences interact to shape a complex learned phenotype.

Birdsong provides a particularly attractive experimental framework for studying sources of individual variation in learning. Song is acquired in a manner similar to human speech (2) but offers the advantages of experimental control over the nature of experience that drives learning and a well-understood neural circuitry. Young birds learn to produce songs that closely resemble those of an adult "tutor," typically their father, to which they are exposed during early life (3). However, tutor song experience can be manipulated by presenting juvenile birds with recorded songs or cross-fostering them into different nests where they will be tutored by the songs of resident males other than their father (4). Moreover, both the acoustic structure of tutor songs and the resultant copies learned by juveniles are readily recorded and quantified, facilitating analysis of learning.

The presence of some genetic constraints on the capacity for song learning already has been well established at the species level. Consistent differences in song structure are present across different species of birds, and birds reared either in isolation, or in the presence of abnormal songs (synthetic or heterospecific), still exhibit some species-typical aspects of song structure (3, 5–11). For example, swamp sparrows can learn song tempo from synthetic tutor songs, but only over a range of values that deviate by a limited amount from species-typical values (7). Consistent with these observations, hybridization of phenotypically distinct breeds of canaries has demonstrated that predispositions to produce specific types of syllables can be genetically determined (12, 13). Together, these observations indicate that differences in song structure between species are in part shaped by genetic differences.

These species-level observations raise the possibility that genetic contributions to song variation may extend to an interindividual level. Indeed, differences in song structure are conspicuous across individuals within a given species. However, previous studies have found only limited evidence for heritable genetic contributions to interindividual differences in song within species (14, 15), and such individual-to-individual variation is typically construed as reflecting the influence of learning. Here, we investigate whether some of the individual differences in the learned songs of Bengalese finches (*Lonchura striata domestica*) reflect within-species genetic variation, and whether the magnitude of any genetic contributions to learned song structure is influenced by the nature of instruction.

Significance

Learning reflects the influence of experience on genetically determined circuitry, but little is known about how experience and genetics interact to determine learned phenotypes. Here, we use vocal learning in songbirds to study genetic influences on learned behavior. We first show that the tempo of learned song is strongly influenced by genetics. However, increasing richness of the learning experience from weak (tutoring by computer) to strong (tutoring by a live bird) reduces this genetic influence in favor of experiential influence. Our results demonstrate a strong, heritable contribution to individual variation in song learning but that the degree of heritability depends profoundly on the quality of instructive experience. Therefore, increasing the richness of instruction can overcome even strong genetic bias.

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Results

To probe potential genetic contributions to interindividual variation in learned song structure we first sought to define an aspect of song that both displays variability across individuals and is likely to be centrally controlled. While many studies of song learning focus on the spectral structure of individual syllables, including pitch and amplitude, these features are subject to peripheral contributions from syringeal and respiratory motor control. We therefore instead focused our examination on song tempo—a readily quantifiable feature that is shaped by learning (7) but for which the mechanistic implementation is likely to reside primarily within a recurrent circuit of the central song system (16, 17). Song tempo exhibits systematic differences across species, suggesting at least species-level genetic constraints (6, 7), and female birds exhibit preferences for songs based on their tempos, indicating that tempo is a behaviorally relevant feature of song (18) that may be subject to selective pressure. Moreover, systematic differences in the tempos of songs—here defined as the average number of syllables produced per second of song (*Materials and Methods*)—were clearly present across nests in our Bengalese finch colony; the mean song tempo for a given nest ranged from ∼6–12 syllables per s (syl/s) (Fig. 1 *A* and *B*). Such differences in song structure are typically attributed to the influence of learning but could in principle reflect, at least in part, genetic variation across nests.

To create an experimental framework for testing potential genetic and experiential influences on learned song tempo and for determining how they interact with tutor song experience we provided individual Bengalese finches from different genetic backgrounds (corresponding to different nests) with controlled learning experiences through a computer tutoring system (19) (Fig. 1*C*). To ensure a diversity of genetic backgrounds we tutored 47 juveniles from 15 different nests in our colony for which the genetic fathers produced songs with a broad range of tempos. To prevent exposure to the genetic fathers' songs these juveniles were transferred from their home nests as eggs within 36 h of laying, well before the development of any peripheral or central components of the auditory system (20, 21). Eggs were hatched, and juveniles were raised to independence, by nonsinging female fosters such that these juveniles had no exposure to songs before they were transferred to sound-isolation boxes at ∼45 d of age. Juveniles were then computer-tutored with a synthetic song that was delivered at either a slow, medium, or fast tempo, corresponding to the lower, middle, and upper range of tempos present in the colony (6.5, 8.5, and 10.5 syl/s). This design established a population of genetically diverse birds that were tutored with songs that had systematically controlled tempo. As previously demonstrated, birds that were computer-tutored exhibited a broad range in the quality of song learning (ref. 19 and Fig. S1), but in all cases song tempo could be readily measured. The learned adult songs of this population of computer-tutored birds were then analyzed to assess how genetic and experiential factors contributed to individual differences in song tempo.

To test for heritable genetic influences on learned song tempo we first examined the group of birds from different nests that were each tutored with an 8.5-syl/s song, near the median value for our colony. Although all of these birds experienced an identical tutor song tempo they developed learned songs that exhibited a broad range of tempos (6–10 syl/s) that was nearly as great as that present across the entire colony (6–12 syl/s). Moreover, the tempos of the learned songs and the tempos of the genetic fathers' songs were strongly correlated, even though these birds had never heard their fathers sing (Fig. 1 *D* and *E*, slope 0.544 ± 0.083, r^2 0.704, P < 0.001). These data indicate a markedly strong heritability for individual differences in the tempo of song (38–70% paternal contribution, 95% confidence interval; *Materials and Methods*).

Fig. 1. When experience is held constant, song tempo is strongly influenced by genetics. (*A*) Spectrograms illustrating songs from two Bengalese finch father–son pairs. The two fathers' songs (*Top*: *i* and *iii*) had distinct syllable structure and tempo that were also present in the corresponding songs of their sons (*Bottom*: *ii* and *iv*). (*B*) Regression between the father's song tempo and the mean song tempo of all of his offspring (error bars represent SE; n = 281 individuals from 18 nests, slope = 0.356, r^2 = 0.366, P < 0.001, two-tailed *t* test). The significant correspondence between father and offspring songs reflects the influence of learning as well as potential genetic contributions. (*C*) Schematic of computer tutoring. Individuals from different genetic backgrounds (red and blue nests) were raised by nonsinging females to prevent exposure to the fathers' songs and were then computer-tutored with a synthetic song in which tempo was controlled. (*D*, *Top*) Example of synthetic tutor song with 8.5-syl/s tempo. (*D*, *Bottom*) Examples of tutee songs that displayed variation in learned song tempo. For simplicity of comparison only a portion of the full tutor stimulus and learned songs are shown. (*E*) Correlation between tutee song tempo and the genetic father's song tempo for all birds tutored with the 8.5-syl/s song (n = 20 individuals from 13 nests; slope = 0.544, r^2 = 0.704, P < 0.001, two-tailed *t* test). Interindividual variation in song tempo was well explained by genetic fathers' song tempo. Examples from *D* are shown in red.

For this group of computer-tutored birds, song tempo was held constant across individuals to clearly isolate genetic contributions to learned song. However, in a more natural setting song tempo will depend on both genetic and experiential variation. We therefore next assessed how individual differences in learned song tempo depended jointly on influences of heritable genetic contributions and experience when those factors were independently varied over a naturalistic range (Fig. 2*A*). We used multiple linear regression to fit each individual's song
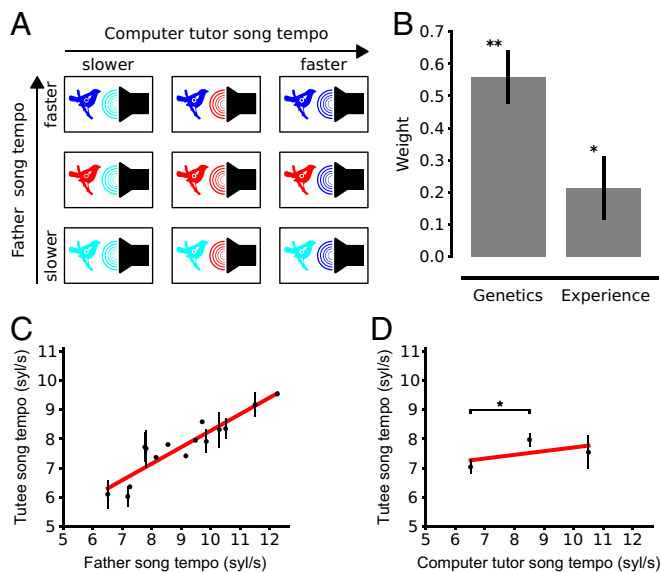
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Fig. 2. For computer-tutor birds song tempo is influenced strongly by genetics and only weakly by experience. (*A*) Birds from nests with father song tempos ranging from slow (cyan) to medium (red) to fast (blue) were computer-tutored with songs having slow (6.5 syl/s, cyan), medium (8.5 syl/s, red), or fast (10.5 syl/s, blue) tempos. (*B*) Multiple regression analysis revealed that the tutee song tempo was strongly influenced by the genetic father's song tempo but only weakly influenced by the tutor song tempo [parameter estimates by ordinary least squares (OLS)]; * $P < 0.05$, ** $P < 0.001$, two-tailed t test). (*C*) Plot illustrating the strong relationship between tutee song tempo and genetic father's song tempo when tutor song tempo is ignored ($n = 15$ father–offspring pairs; 47 birds from 15 nests tutored with three song tempos; $r^2 = 0.82$, slope = 0.57, $P < 0.001$). (*D*) Plot illustrating the weak relationship between tutee song tempo and tutor song tempo when genetic background is ignored ($n = 3$ tutor song–offspring pairs; 47 birds from 15 nests tutored with one of three song tempos; slope = 0.13, $P = 0.64$). Consistent with a weak influence of experience, the only significant pairwise difference across groups was for birds tutored at 8.5 syl/s vs. 6.5 syl/s (* $P < 0.01$, two-tailed t test). Error bars indicate SEs.

tempo as a weighted function of genetic and experiential contributions (*Materials and Methods*; individual's learned song tempo = W_genetics*G + W_experience*E + noise). For each individual, we used the tempo of the genetic father's song as the value for genetic contribution (G) and the tempo of the synthetic tutor song as the value for the experiential contribution (E). Across the entire computer-tutored population, the best-fit weight for the genetic contribution to individual variation in tempo was 55% (W_genetics = 0.55 ± 0.098, $P < 0.001$), indicating strong heritability from the father. In contrast, the best-fit weight for tutor song tempo was only 21% (W_experience = 0.21 ± 0.104, $P < 0.05$), indicating a weaker but still significant influence of experience (Fig. 2*B*). Consistent with a significantly stronger influence of genetics than experience, the learned song tempo was strongly correlated with the genetic father's song tempo when experiential differences were ignored (Fig. 2*C*), while there was only a weak relationship between learned song tempo and tutor song tempo when genetic differences were ignored (Fig. 2*D*). These results demonstrate a surprisingly strong influence of genetics and weak influence of experience in shaping individual differences in the tempo of song, a complex phenotype that is studied specifically because it is learned.

We next wondered whether the relative contributions of genetics and experience to the tempo of learned songs could be altered under conditions of more enriched instruction. In particular, numerous previous studies have shown that live, social tutoring drives learning more effectively than does tutoring via computer or recorded playback (4, 5, 22–24). Hence, we were interested in the possibility that

live tutoring might similarly increase the influence of experience on tutee song tempo. Such an increase in the influence of experience could occur with or without altering the genetic contributions to song tempo; while genetic and experiential contributions to complex human phenotypes are often treated as additive and independent (25, 26), prior work has suggested that for learned behaviors more supportive environments may increase (27–29) or decrease (30) heritable contributions to individual differences in performance. We therefore were also interested in assessing whether any increased weight on experience would be additive to fixed genetic contributions or would interact nonlinearly to alter genetic contributions to song tempo.

To test the hypothesis that enriched instruction would alter the balance of genetic and experiential influences on song tempo we carried out a second experiment in which 58 juveniles from a variety of genetic backgrounds were socially tutored by unrelated adult males (*Materials and Methods*). As before, eggs were transferred to foster nests 36 h postlaying to ensure that there was no exposure to the genetic fathers' songs. Hatchlings were then tutored by resident adult males that, across the population, displayed almost the entire range of song tempos (Fig. 3*A*; range of live-tutor song tempos 5–11 syl/s). Live tutoring in this fashion provides an enriched experience, both because of greater diversity in acoustic experience of the tutor song and because tutoring occurs in a more natural setting, where there is opportunity for aspects of visual and social stimulation to modulate learning. Consistent with previous studies indicating that live tutoring drives better learning (4, 5, 22–24), there was qualitatively better copying of syllable spectral features in the live-tutored birds than in the computer-tutored birds (e.g., Fig. 3*B* vs. Fig. 1*D* and Fig. S1).

For live-tutored birds we again estimated the relative weights of genetic and experiential contributions to song tempo by multiple regression, but with the value of experiential contributions now reflecting the tempos of the live tutors' songs. As hypothesized, the weight on experience was significantly increased, from 21% for computer-tutored birds to 53% for live-tutored birds, consistent with the notion that live tutoring provides more effective instruction (Fig. 3*C*; W_experience = 0.529 ± 0.147, $P < 0.001$; increase in weight relative to computer-tutored: $P < 0.002$). Strikingly, the weight on genetics for this population was also dramatically reduced, from 55% for computer-tutored birds to 16% for live-tutored birds (W_genetics = 0.157 ± 0.082, $P < 0.05$; decrease in weight relative to computer-tutored: $P < 0.05$). Consistent with a shift in the balance of genetic and experiential contributions to song tempo, univariate regressions also indicated that the heritability of tempo for live-tutored birds was decreased compared with heritability for computer-tutored birds (Fig. 3*D* vs. Fig. 2*C*), while the influence of experience was increased (Fig. 3*E* vs. Fig. 2*D*). This reversal of the relative influences of genetics and experience with live tutoring indicates that enriched instruction provided by live tutoring cannot only increase the influence of experience but also overcome strong genetic contributions to individual differences in song that are expressed under conditions of computer tutoring.

The linear regression analysis that we used to analyze sources of variation in song tempo implicitly assumes independent and additive contributions of genetics and experience in shaping an individual's phenotype. However, the markedly different estimates of heritability obtained with live and computer tutoring indicate a nonlinear interaction between genetics and the type of tutoring experience. Hence, we wondered whether there might also be nonlinear interactions between genetics and other aspects of experience within each of the two experimental groups considered individually. In contrast to the common assumption of independence, we found that strong nonlinear interactions between genetics and experience were embedded within these datasets. In particular, for the computer-tutored birds, estimates of genetic influence on the tempo of learned song were contingent on the tempo of the tutor song, with significantly greater
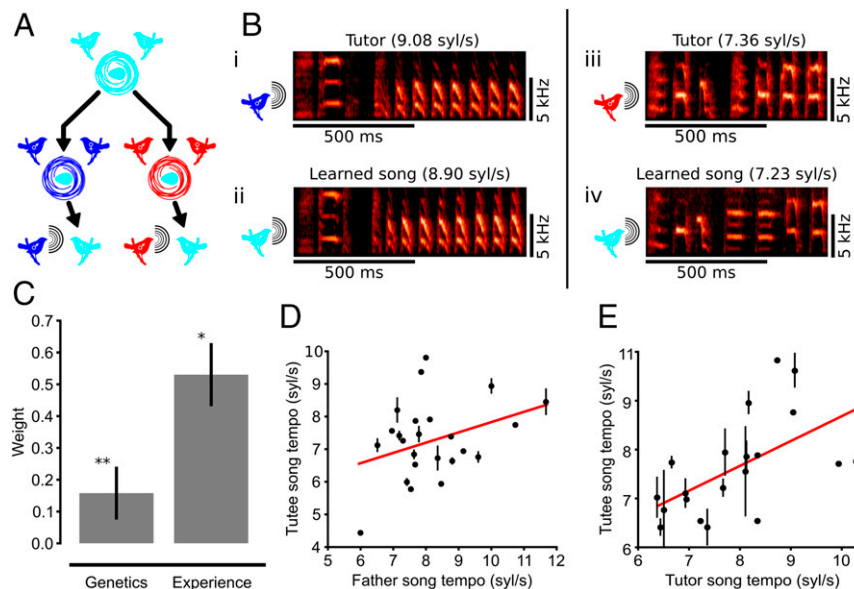
Fig. 3. For live-tutored birds, the influence of experience is increased and the influence of genetics is decreased. (*A*) Schematic representation of cross-fostering experiments in which birds from different genetic backgrounds are transferred to foster nests where they are reared and learn song from a live tutor. (*B*) Exemplar songs from two foster father–tutee pairs (*i–iv*) demonstrate close correspondence between the tutee's learned songs and the songs of their foster fathers. In this example, both tutees are from the same genetic background (nest). (*C*) Multiple regression revealed that when birds were live-tutored, tutee song tempos were strongly influenced by experience and weakly influenced by genetics (parameter estimates by OLS; *$P < 0.05$, **$P < 0.001$, two-tailed t test). (*D*) Plot illustrating the relationship between tutee song tempo and genetic father's song tempo when tutor song tempo is ignored ($n = 24$ father–offspring pairs; 58 birds from 24 nests fostered into 20 tutor nests; $r^2 = 0.12$, slope $= 0.32$, $P < 0.05$, one-tailed t test). (*E*) Plot illustrating the relationship between tutee song tempo and the tutor song tempo of the foster father when genetic background is ignored ($n = 20$ tutor–offspring pairs; birds from 24 nests fostered into 20 tutor nests; $r^2 = 0.32$, slope $= 0.50$, $P < 0.005$, one-tailed t test). Error bars indicate SEs.

heritability manifest for birds tutored at 10.5 and 8.5 syl/s than for birds tutored at 6.5 syl/s ($P < 0.02$, two-tailed t test; Fig. 4). Indeed, if we included a nonlinear interaction term in the regression analysis (genetics × experience, GXE) there was a significant weight on this term for both computer-tutored birds (W_GXE $= 0.152 \pm 0.065$, $P < 0.02$) and live-tutored birds (W_GXE $= 0.168 \pm 0.095$, $P < 0.05$). However, the inclusion of this GXE interaction term did not alter the conclusion that for computer-tutored birds heritable contributions greatly outweighed experiential contributions to individual variation in song tempo (W_genetics $= 0.523 \pm 0.093$, $P < 0.001$; W_experience $= 0.277 \pm 0.102$, $P < 0.01$; W_GXE $= 0.152 \pm 0.065$, $P < 0.02$), and that for live-tutored birds this pattern was reversed (W_genetics $= 0.116 \pm 0.14$, $P = 0.112$; W_experience $= 0.445 \pm 0.154$, $P < 0.005$; W_GXE $= 0.168 \pm 0.095$, $P < 0.05$). Hence, these data confirm strong nonlinear interactions between genetics and tutoring modality (computer vs. live) and provide a further independent indication that genetics and experiential factors (tutor song tempo) interact nonlinearly to shape learned song.

Discussion

Our results establish the songbird as a rich model system for investigating how interactions between genetics and experience contribute to interindividual variation in learned phenotypes. Individual differences for many complex human phenotypes such as cognitive abilities and personality traits depend on both genetics and experience (26–28, 30). However, because of the difficulty in quantifying such phenotypes as well as in identifying and controlling the relevant genetic and experiential factors it has been challenging to probe how these factors interact to shape behavioral outcomes. Here, we take advantage of the robust nature of song learning in Bengalese finches, and of the ability to vary in a controlled fashion the nature and the richness of specific instruction experienced by juveniles during learning, to demonstrate that individual differences in a key aspect of song

structure—the tempo—are subject to a complex interplay between genetic and experiential influences. Whereas previous studies have shown that song tempo can be shaped by experience (6, 7), we demonstrate that interindividual differences in tempo are also highly heritable. Critically, we found that the strength of
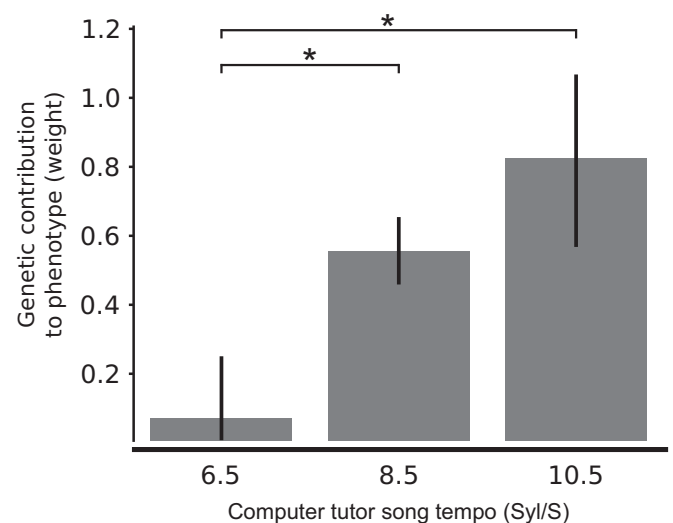

Fig. 4. Tutor song tempo modulates genetic influence on learned tempo. Birds tutored with synthetic songs at a tempo of 6.5 syl/s displayed no significant influence of genetics on learned tempo ($n = 11$, slope $= 0.105$, $P = 0.589$, OLS) while birds tutored at 8.5 syl/s ($n = 20$, slope $= 0.528$, $P < 0.001$, OLS) and 10.5 syl/s ($n = 13$, slope $= 0.818$, $P < 0.01$, OLS) both displayed significant genetic influence on song tempo. For cohorts tutored on 8.5 syl/s and 10.5 syl/s, the degree of genetic influence was significantly greater than that found in birds tutored on songs with a 6.5-syl-per-s tempo. (*$P < 0.05$, two-tailed t test).

heritable genetic influences on learned song were not fixed but instead were highly contingent on the quality of instruction. For birds that experienced a computerized tutor stimulus interindividual differences in tempo were strongly influenced by genetics and only weakly influenced by experience. Conversely, for birds that experienced a more enriched live-tutor stimulus tempos were only weakly heritable but strongly influenced by experience. The prominent, yet variable, contribution of genetic differences to song structure, coupled with the well-elucidated and experimentally accessible neural substrates for song production, provides an ideal opportunity in this system for future mechanistic studies of how genetics and experience interact to shape learned behavior.

Our observation that song tempos for computer-tutored juveniles correlated strongly with the song tempos of their absent genetic fathers indicates an unexpectedly high degree of heritability (~50% or more) for a behavioral phenotype that is studied specifically because it is shaped by experience. Indeed, our analysis—based solely on paternal contributions as reflected in the tempos of the fathers' songs—provides a lower bound on heritability, since there may be additional maternal contribution to individual variation in song tempo. This finding is particularly striking in light of previously unsuccessful attempts to expose strong genetic contributions to individual differences in song structure (14, 15). These previous studies used statistical analyses across large breeding populations of zebra finches to test for heritability of multiple aspects of song structure. In contrast, our experimental paradigm, using controlled tutoring with synthetic songs in which features other than tempo were held constant, likely enhanced the detection of genetic contributions to tempo. Moreover, as demonstrated by our findings, the use of computer tutoring enabled a much stronger, and more readily detected, expression of genetic contributions to tempo than are apparent for live tutoring (as was used in previous studies of heritability). For humans, some aspects of vocal behavior (speech tonality) may also be influenced by genetics, but the degree of heritability has not been quantified (31). However, it is noteworthy that our estimate of heritability for interindividual differences in learned song tempo is on par with what has been reported for other complex human phenotypes, including academic achievement and reading proficiency (29, 32), where there similarly is an expectation of an important role for experience.

In human studies of learned phenotypes there have been differing perspectives on whether and how the degree of heritability might vary with experience. Although it is widely accepted that better instruction will leave a stronger imprint on performance of learned behaviors, it has variously been suggested that such increased influence of experience could be accompanied by an increase, decrease, or no change in the genetic contributions to the behavior (27–30). In birds, live tutoring has previously been shown at the species level to enhance—relative to tutoring with song playback—the influence of experience on song structure, including enabling birds to learn species-atypical features of song (6, 22). Consistent with this, we found that live tutoring significantly increased the influence of experience on learned song tempo. Importantly, our results additionally indicate that live tutoring did not merely change the phenotype toward instructed values (as it could, if it exerted an independent contribution) but also diminished the weight placed on genetics.

A variety of factors could account for the increased influence of live tutoring vs. computer tutoring on learned song tempo. Live tutoring provides earlier and quantitatively more exposure to song than does our computer-tutoring paradigm. In particular, live tutoring was provided throughout development and the number of tutor-song exposures was unconstrained, while computer tutoring was initiated at 35 d posthatch and was limited to 30 song presentations per day (see also *Materials and Methods*). However, previous work has shown that for similar computer tutoring in zebra finches increasing the number of song presentations can actually decrease the quality of tutor-song copying

(19). Further, early song exposure has been shown to have little impact on learned song; restricting exposure to a live male tutor to the first 35 d posthatch results in poorly structured song with little resemblance to the tutor song (33). Hence, factors other than the amount and timing of song exposure are likely instrumental in the increased efficacy of live tutoring. This might include other aspects of acoustic structure that are important for learning, such as the presence of species-appropriate structure and variation in spectral and temporal aspects of song (8, 11, 34–36). Additionally, social aspects of live tutoring are likely to be crucial in engaging attentional and motivational processes that enhance learning (24, 37–39). Indeed, it is striking that visual isolation from a live tutor, despite otherwise normal exposure to the tutor's repertoire, can be sufficient to grossly impair learning (23, 24). Regardless of the specific factors responsible for the increased influence of live tutoring, our findings indicate that potential genetic constraints imposed on learning should not be viewed as fixed. Rather, they demonstrate that sufficiently enriched instruction can overcome otherwise strong genetic contributions to individual variation in learning outcomes.

More broadly, our results provide a striking demonstration that estimates of heritability for complex learned behaviors can be highly contingent on experiential conditions. We found that heritability of song tempo depended not only on the richness of instruction but also on the specific tempo of the tutor-song stimulus, further revealing nonlinear interactions between genetics and experience. Such nonlinear interactions between genetic and experiential factors have long been postulated for complex phenotypes that depend on both genetics and instruction (1). However, there is only limited and inconsistent evidence for such interactions (25, 40–43). While it is difficult to exclude the possibility that the degree of gene–environment interactions differs greatly for different learned phenotypes, the lack of consistent evidence for such interactions in human studies may simply reflect a greater challenge of quantifying phenotypes and controlling experience in human studies of complex traits. Our ability to uncover prominent gene–environment interactions in the context of the comparatively simple phenotype of learned song supports the latter interpretation and argues that many complex human phenotypes are likely shaped by even more pronounced interactions between genetic and experiential factors.

Materials and Methods

Subjects. Subjects were male Bengalese finches (*L. striata domestica*); 47 birds from 15 nests were raised with computer tutoring and an additional 58 birds from 24 nests were raised with foster tutoring, as described in the text. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of California, San Francisco.

Audio Recording and Initial Processing. All recordings used for analysis were acquired during early adulthood (90–120 d posthatch). Further details are provided in *SI Materials and Methods*.

Calculation of Song Tempo. Song tempo was quantified as the average number of syllables produced per second of song. Further details are provided in *SI Materials and Methods*.

Computer Tutoring. To create populations of birds that had controlled tutoring experiences, eggs were taken from parents 36 h after laying and were then raised by nonsinging foster mothers housed in sound-isolation chambers. These foster mothers raised the juveniles until they were able to feed themselves. At independence (usually 35–40 d posthatch) birds were moved to an acoustic-isolation chamber with an audio recording system and a computer tutoring apparatus, based on an approach that previously has been demonstrated to drive song learning (19). At 45 d post hatch, the tutoring apparatus was activated, allowing birds to access a tutor song. The apparatus consisted of a perch-activated switch that caused playback of a tutor stimulus (discussed below). Each perch hop elicited a single playback of the tutor stimulus. Birds were allowed to play back 10 songs, three times a day (morning, noon, and evening). Playback of a tutor song was limited to 30 songs per day based on previous work indicating that this was near an

optimal value to maximize the quality of song learning in this paradigm (19). This computer tutoring apparatus was implemented with custom LabView (National Instruments) software. Birds remained in the tutoring apparatus until an age of 120 d post hatch. For experiments involving different tutor-song tempos, the tempo for an individual was randomly selected from three possible tempos (discussed below).

Computer Tutor Stimulus. To create a naturalistic but controlled learning stimulus a synthetic song used for computer tutoring was derived from songs sampled from our Bengalese finch colony. The synthetic song (Fig. S1) was composed of nine categorically distinct syllables that were chosen to reflect a range of different syllable types found in Bengalese finch song (i.e., short "introductory" syllables, noisy syllables, syllables with harmonic structure and constant or modulated frequency, etc.). The tutor stimulus consisted of a series of introductory syllables followed by three repetitions of a stereo-typed sequence of syllables, or "motif." The gaps between syllables were chosen to reflect naturalistic means and SDs based on the distribution of gap durations found in normal Bengalese finch song. Correspondingly, the tutor song stimulus had a relatively natural prosody compared with a stimulus in which there was a fixed time between syllable onsets. The 8.5-syl/s tutor song stimulus arose naturally out of this process, as 8.5 syl/s is close to the median song tempo present in our colony. The 6.5- and 10.5-syl/s learning stimuli were created by proportionally increasing or decreasing only the intersyllable gap durations, resulting in songs with identical spectral content presented at different tempos.

Cross-Fostering. To create populations of birds that were tutored by a live bird but never heard the song of their genetic fathers, eggs were taken from parents 36 h postlaying and transferred to foster nests. For each individual, the specific foster nest was randomly selected from a set of 20 possible nests within our breeding colony, excepting the nest of the genetic father. In addition to the presence of a live tutor, the cross-fostered birds experienced a variety of other differences from computer-tutored birds that potentially contributed to enhanced efficacy of live tutoring. These included a richer social environment that included the presence of both the tutor and his mate, as well as up to two additional juvenile birds. Additionally, for cross-fostered birds exposure to tutor song began as soon as birds were transferred to foster cages, whereas for computer-tutored birds song exposure did not begin until activation of the playback apparatus at 45 d of age. Hence, an earlier and more extended period of tutor song exposure could have contributed to the enhanced influence of experience on song tempo for the live-tutored population. To test this possibility we analyzed data from an additional set of nine birds from five genetic backgrounds that were treated identically to the computer-tutored birds until 45 d of age, at which point they were transferred randomly into one of six live foster nests. Consistent with the cross-fostered dataset presented in *Results*, multiple regression for this additional set of birds revealed a strong influence of experience on tempo ($W\_experience = 0.51 \pm 0.18$, $P < 0.02$ one tailed t test) and no influence of genetics ($P > 0.1$ one tailed t test). This suggests that the earlier exposure to tutor song in cross-fostered birds was not a major contribution to the enhanced influence of experience for this population. Aspects of the rearing environment outside those specifically detailed above were kept similar between computer tutoring and cross-fostering conditions.

Heritability Calculations. Heritability is reported as the fraction of the total phenotypic variance that can be attributed to additive genetic factors (narrow sense heritability) contributed from the father (44). Further details are provided in *SI Materials and Methods*.

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