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Variation in the volume of zebra finch song control nuclei is heritable: developmental and evolutionary implications

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In many songbird species, females prefer males that sing a larger repertoire of syllables. Males with more elaborate songs have a larger high vocal centre (HVC) nucleus, the highest structure in the song production pathway. HVC size is thus a potential target of sexual selection. Here we provide evidence that the size of the HVC and other song production nuclei are heritable across individual males within a species. In contrast, we find that heritabilities of other nuclei in a song-learning pathway are lower, suggesting that variation in the sizes of these structures is more closely tied to developmental and environmental differences between individuals. We find that evolvability, a statistical measure that predicts response to selection, is higher for the HVC and its target for song production, the robustus archistriatalis (RA), than for all other brain volumes measured. This suggests that selection based on the functions of these two structures would result in rapid major shifts in their anatomy. We also show that the size of each song control nucleus is significantly correlated with the song related nuclei to which it is monosynaptically connected. Finally, we find that the volume of the telencephalon is larger in males than in females. These findings begin to join theoretical analyses of the role of female choice in the evolution of bird song to neurobiological mechanisms by which the evolutionary changes in behaviour are expressed.

Keywords: zebra finch; song; high vocal centre; heritability; sexual selection; evolutionary neurobiology

1. INTRODUCTION

Evolutionary change in the brains of males in response to sexual selection by females requires several behavioural and neural attributes. First, differences in a male behaviour must form the basis of a female preference. Second, differences in the male behaviour must derive from differences in brain anatomy. Third, the brain differences must be heritable. In songbirds, various field and laboratory studies indicate that females use male song traits such as rate or complexity in choosing mates (Catchpole & Slater 1995; Searcy & Yasukawa 1996; MacDougall-Shackleton 1997). In the brains of male songbirds, the size of the forebrain song control nucleus high vocal centre (HVC) correlates positively with differences in the complexity of song (Nottebohm *et al.* 1981; Canady *et al.* 1984; Kroodsma & Canady, 1985; DeVoogd *et al.* 1993; Székely *et al.* 1996; DeVoogd & Székely 1998; Ward *et al.* 1998; Airey *et al.* 2000; Airey & DeVoogd 2000). We studied whether the size of the HVC in males is heritable, in order to determine whether sexual selection may have contributed to individual and species differences in this trait.

We chose to investigate HVC size heritability in zebra finches (*Taeniopygia guttata castanotis*), because adult females of this species show preferences for male song (Clayton & Pröve 1989; Houtman 1992), and male HVC size correlates to both song structure and learning (Airey & DeVoogd 2000; Ward *et al.* 1998). In zebra finches, and perhaps songbirds in general, the size of the HVC is impervious to differences in the early acoustic environment when song develops. Deafened zebra finches do not differ in HVC size when compared with normal hearing controls (Burek *et al.* 1991). Artificial tutoring of another songbird species, marsh wrens, with natural song also does not affect HVC size (Kroodsma & Canady 1985; Brenowitz *et al.* 1995). Thus, correlations between song and HVC size must reflect limitations in learning capacity rather than learning-derived brain growth. For determining heritability of HVC size in natural breeding settings, this is advantageous in that estimates can be assumed not to be confounded with the learning environment.

Just as beak shape changes in the face of selection pressures (Grant & Grant 1995), brain circuits will also change, and the pattern of evolutionary response will depend on both the magnitude of additive genetic variance for each brain nucleus and the extent of additive genetic covariance between brain nuclei (Arnold 1994). The song control nucleus HVC is one of several interconnected brain nuclei involved in song production (see figure 1), and it is possible that projections between nuclei might affect size variation in any one of them. Thus, in addition to assessing heritability of the major song system nuclei, we also derived genetic correlations between the HVC and its two main efferent targets, the robustus archistriatalis (RA) and Area X.

2. MATERIAL AND METHODS

Two serial breeding rounds conducted between April 1997 and April 1998 produced a total of 38 zebra finch families, each with at least one son. The sample of 190 birds included 38

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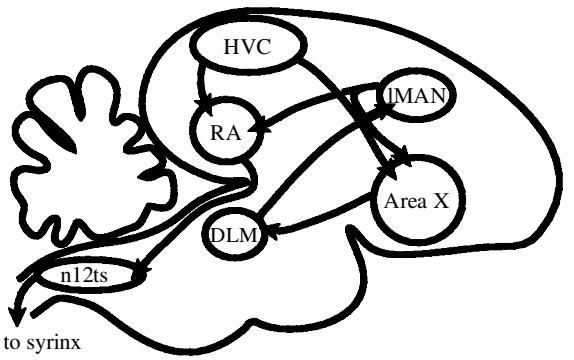

Figure 1: Schematic of the main song control pathways in the brains of songbirds. The posterior song pathway (HVC > RA > n12ts > syrinx) includes brain areas specialized for learning and producing song. Lesion of these areas removes the ability to sing. The anterior song pathway HVC > (Area X > DLM > lMAN) > RA, includes brain areas specialized for learning song. Lesion of these areas in juveniles is more disruptive to song development than lesion of these areas in adults (Bottjer *et al.* 1984; Scharff & Nottebohm 1991). HVC, high vocal centre; RA, robustus archistriatalis; n12ts, tracheosyringeal portion of the twelfth motor nucleus, syrinx–vocal organ; Area X, proper name; DLM, medial portion of the dorsolateral thalamic nucleus; lMAN, lateral portion of the magnocellular nucleus of the anterior neostriatum.

fathers, 36 mothers (two died before data collection), 68 sons, and 48 daughters. Because there were no differences (main effects) in any of the dependent measures by breeding round, the data were collapsed across breeding round to achieve a larger number of observations. To ensure paternal identity, adult breeding pairs were housed in separate apartment-style cages for at least three weeks before the pairs were supplied with nesting materials. All birds were supplied with fresh finch seed and water on a daily basis supplemented with hard-boiled chicken egg, greens, or fruit at least twice a week. Lights were set to a 14 L:10 D photoperiod and the humidity was maintained at a minimum of 50%.

Finch progeny were kept in the home cage with their parents until at least 50 days of age post-hatch. They were then housed colonially in a flight cage containing four non-breeding adult zebra finch pairs and other first-round progeny. Progeny were killed after 100 days of age. In zebra finches, song nuclei and the telencephalon attain adult volumes by 60 days (Kirn & DeVoogd 1989; Nixdorf-Bergweiler 1996; Gahr & Metzdorf 1999). Cross-fostering was not used in this experimental design, because HVC size has been shown to be impervious to experimental manipulations of song exposure (Burek *et al.* 1991; Brenowitz *et al.* 1995).

All parents and progeny were anaesthetized, weighed, and transcardially perfused with a 0.9% saline solution and then a 10% formalin in 0.9% saline solution. Brains were weighed, postfixed, embedded in gelatin, sectioned coronally at 40 μm on a freezing sliding microtome, and stained with cresyl violet.

Volumes of the four largest telencephalic song nuclei (HVC, RA, Area X, and lMAN (lateral portion of the magnocellular nucleus) and the tracheosyringeal portion of the hypoglossal nucleus innervating the syrinx (nl2ts) from male finches were estimated by summing Nissl-defined areas from every other brain section and multiplying by the sampling interval distance (80 μm). All brain nuclei were measured bilaterally. Areas of song nuclei were drawn with a camera lucida arm attached to a light microscope. Drawings were acquired with a video camera and measured with NIH-Image v. 1.61 software. Area of the telencephalon was sampled at every sixth section throughout the full extent of the brain. This sampling interval (240 μm) provided an estimate of the telencephalon volume with an error of 3–5% of that estimated by measuring every section ($n = 3$). For estimating the telencephalon area, images of the slide sections were acquired directly on a light box with a video camera.

Nissl stains, such as cresyl violet, allow division of the brain into areas based on the size and density of cells, or cytoarchitecture. Consensus evidence suggests that cytoarchitectural, cytochemical, and connectional delineations of the song control nuclei, such as the HVC, in general parcel the brain comparably (Gahr 1997; Ball & Balthazart 1997; Bottjer & Johnson 1997; Brenowitz & Smith 1997). Volumes defined by Nissl staining are therefore a useful and practical measure of individual differences in the size of song control nuclei in large samples.

Heritabilities and other genetic parameters were estimated with the UNIX multiple trait derivative-free restricted maximum-likelihood program (MTDFREML) (Boldman *et al.* 1995; Van Vleck 1998). MTDFREML estimates the additive effects of an animal's genes (breeding value) through a restricted maximum-likelihood procedure using an animal model in which the random genetic effect in the model describing the phenotypic observation is the additive genetic merit of the individual scored for the record (Henderson & Quaas 1976; Lynch & Walsh 1998). This method has the advantage that information from all relatives is used in the estimates, rather than separate father–son or sib–sib estimates typical of least-squares methods. In general, all heritability estimates should be regarded as working hypotheses to be confirmed by additional breeding designs and by selection experiments.

We used likelihood ratios to test whether the estimates of heritability and genetic correlation were significantly different from zero (Sokal & Rohlf 1995; Shaw 1987; Lynch & Walsh 1998). For single-trait animal models, a test statistic for the additive genetic variance was obtained by computing twice the difference in log-likelihoods between a complete model where the additive genetic variance is freely estimated and a reduced model where it is constrained to be zero: likelihood ratio statistic $(\mathrm{LRS}) = 2[\log(L_c)\log(L_r)]$. This LRS is compared with a χ^2-distribution with one degree of freedom using a one-tailed region of rejection. For multiple-trait animal models, a test statistic for the genetic covariance was obtained by computing twice the difference in log-likelihoods between a complete model where genetic and environmental correlations are freely estimated and a set of reduced models where (i) both genetic and environmental covariances are set to zero, and (ii) the genetic covariance is set to zero and the environmental covariance is set to the estimate from the complete model. Step (i) tests for the presence of covariance, while step (ii) tests for the significance of the genetic covariance. Since testing genetic covariance is always conditional on environmental covariance values, this test statistic is compared with a χ^2-distribution with two degrees of freedom.

Other statistical tests were performed with Data Desk v. 6.1 (Data Description, Inc., Ithaca, NY, USA). In cases where two sample tests were performed and variances were found to be different (body weight, brain weight, and telencephalon volume by sex), a two-sample separate variances *t*-test was used.

Table 1. *Anatomical summary statistics*[a]

trait	birds	mean	median	s.d.	s.e.m.	Cv[b]
body	178[c]	14.0558	13.8450	1.6386	0.1228	11.6581
brain	189	0.4539	0.4560	0.0375	0.0027	8.2632
telencephalon	177	201.92	201.10	25.0765	1.8849	12.4190
HVC	100	0.5498	0.5145	0.1424	0.0142	25.9029
RA	100	0.4397	0.4228	0.0791	0.0079	17.9904
n12ts	97	0.1126	0.1103	0.0151	0.0015	13.4397
Area X	100	2.2875	2.2676	0.4269	0.0427	18.6633
lMAN	99	0.2078	0.2066	0.0392	0.0039	18.8508

[a] Body and brain were measured in grams, other traits were measured in mm^3.
[b] Cv is coefficient of variation or $100 \times$ (s.d./mean). The statistic assists comparison of distributions.
[c] Differences in the number of birds measured and total number of subjects (190) reflect missing data (not collected or missing due to histological artefact).

Table 2. *Sex differences in body* (g), *brain* (g), *and telencephalon* (*tel.*) (mm^3)

	birds	mean	median	s.d.	s.e.m.	Cv
female						
body	79	14.448	14.400	1.786	0.201	12.363
brain	84	0.439	0.440	0.032	0.004	7.339
tel.	76	192.238	193.450	21.027	2.412	10.938
male						
body	99	13.743	13.520	1.445	0.145	10.513
brain	105	0.466	0.466	0.037	0.004	8.031
tel.	101	209.205	208.400	25.501	2.537	12.189

Table 3. *Phenotypic correlations between song nuclei* (*r-value below the diagonal, p-value above*)

	HVC	RA	n12ts	lMAN	Area X
HVC	1.000	$p < 0.00011$	n.s.	$p < 0.01$	$p < 0.0001$
RA	0.401	1.000	$p < 0.01$	$p < 0.01$	n.s.
n12ts	0.200	0.323	1.000	n.s.	n.s.
lMAN	0.282	0.360	0.094	1.000	$p < 0.0001$
Area X	0.502	0.173	0.152	0.488	1.000

3. RESULTS

All of the neuroanatomical phenotypes measured exhibited continuous variation typical of complex traits that are determined by multiple gene and environmental sources (table 1). Substantial phenotypic variance was found in all traits, but HVC volume showed the highest coefficient of variation, 25.9%.

Body weight, brain weight and telencephalon volume were found to be sexually dimorphic (table 2). Although males had 5% lower body weights ($n = 79$ females, 99 males, $t_{148} = 2.843$, $p < 0.01$), they had 6% greater brain weights ($n = 84$ females, 105 males, $t_{185} = 5.230$, $p < 0.0001$) and 8% greater telencephalon volumes compared with females ($n = 76$ females, 101 males, $t_{173} = 4.846$, $p < 0.0001$). The sex difference in telencephalon volume, *ca.* 17 mm^3, remained significant after the volumes of the four song control nuclei were subtracted from each male—*ca.* 3.5 mm^3 per male ($n = 76$ females, 99 males, $t_{171} = 3.898$, $p < 0.0001$).

The volume of each song control nucleus was found to correlate positively with brain weight and with telencephalon volume. In order of decreasing proportion of the telencephalon, each song nucleus correlated significantly with the volume of the telencephalon: Area X ($r = 0.514$, $p < 0.0001$); HVC ($r = 0.486$, $p < 0.0001$); RA ($r = 0.351$, $p < 0.001$); and lMAN ($r = 0.318$, $p < 0.01$). Brain weight and song nuclei correlations behaved somewhat differently, even though telencephalon and brain weight were moderately correlated ($r = 0.626$, $p < 0.0001$). In order of decreasing significance, brain weight correlated with RA ($r = 0.588$, $p < 0.0001$), HVC ($r = 0.395$, $p < 0.0001$), lMAN ($r = 0.359$, $p < 0.001$), Area X ($r = 0.351$, $p < 0.001$) and n12ts ($r = 0.258$, $p < 0.05$). Body weight correlated with brain weight ($r = 0.221$, $p < 0.05$) in males though not in females, and in neither sex did body weight correlate with telencephalon volume.

The volume of each song control nucleus was found to correlate positively with the volume of each monosynaptically afferent or efferent nucleus (table 3). These correlations remained significantly positive after removing variance associated with telencephalon (Tel) volume ($r_{\mathrm{HVC,RA.Tel}} = 0.281$, $p < 0.01$; $r_{\mathrm{HVC,AreaX.Tel}} = 0.336$, $p < 0.001$; $r_{\mathrm{RA,lMAN.Tel}} = 0.280$, $p < 0.01$; $r_{\mathrm{lMAN,AreaX.Tel}} = 0.399$, $p < 0.0001$).

Heritability estimates of the brain nuclei that belong to the posterior forebrain pathway (HVC > RA > n12ts) were found to be higher than heritability estimates of nuclei that belong to the anterior forebrain pathway (HVC > Area X > > lMAN > RA; table 4). The heritability estimates for the song nucleus RA ($h^2 = 0.72$, LRS = 8.48, $p < 0.01$) and n12ts ($h^2 = 0.47$, LRS = 3.29, $p < 0.05$) were significantly different from zero as indicated by likelihood ratio tests. Estimates for both Area X and lMAN were low at $h^2 = 0.23$ (LRS = 0.98) and $h^2 = 0.18$ (LRS = 0.49), respectively, and not different from zero. The heritability estimate for HVC, which contains distinct populations of RA and Area X projection neurons, was moderate at $h^2 = 0.38$ (LRS = 2.44, $p = 0.0590$).

Heritability estimates of body weight and brain weight were both significant, as was telencephalon volume (table 4). Body weight heritability was estimated at 0.32 across both sexes (LRS = 6.59, $p < 0.01$). Brain weight heritability was estimated at 0.49 across both sexes

Table 4. *REML estimates of variance components, heritability, and evolvability*

(Body and brain are measured in grams, telencephalon and song system nuclei are measured in mm^3.)

trait	variance components				
	(*G*)	(*E*)	(*P*)	heritability	evolvability
HVC	0.007 86	0.012 73	0.020 59	0.38	16.13
RA	0.004 25	0.001 69	0.005 94	0.72	14.83
n12ts	0.000 11	0.000 12	0.000 23	0.47	9.31
Area X	0.043 61	0.143 81	0.187 42	0.23	9.13
lMAN	0.000 29	0.001 34	0.001 63	0.18	8.20
telencephalon [a]	406.669	237.621	644.288	0.63	9.64
brain	0.000 66	0.000 68	0.00134	0.49	5.66
body	0.8164	1.772 59	2.588 98	0.32	6.43

[a] Male.

Table 5. *Results of a multiple trait REML model including HVC, RA, and Area X*

(Heritabilities are on the diagonal, genetic correlations are above the diagonal, and environmental correlations are below the diagonal.)

	HVC	RA	Area X
HVC	0.43	0.92	0.56
RA	− 0.07	0.72	0.19
Area X	0.47	0.27	0.23

(LRS = 14.89, $p < 0.0001$). Telencephalon volume heritability was estimated at 0.63 for males (LRS = 5.66, $p < 0.01$).

The evolvability was found to be higher for HVC and RA than for all other traits measured (table 4). Evolvability is a comparative measure of the proportional response to selection for traits measured on the same scale (Houle 1992; Roff 1997). It is defined as the coefficient of additive genetic variation or $CV_A = 100\sqrt{V/\overline{X}}$. HVC has the highest evolvability at 16.13%, followed by RA at 14.83%. Other song nuclei and the telencephalon have lower evolvabilities (n12ts = 9.31%, Area X = 9.13%, lMAN = 8.20%, male telencephalon = 9.64%, female telencephalon = 3.30%). Brain weight and body weight have similar evolvabilities at 5.66% and 6.43%, respectively.

Finally, the volume of the HVC was found to be significantly genetically correlated with the volume of nucleus RA in a multiple trait restricted maximum-likelihood (REML) model including HVC, Area X, and RA volumes (table 5). In this model, heritability estimates were comparable with the single trait REML models, although the estimate for HVC was slightly higher at $h^2 = 0.43$. The genetic correlation between HVC volume and RA volume was high at $r_G = 0.92$ and is significantly different from zero (LRS = 6.90, $p < 0.01$). The genetic correlation between HVC and Area X was lower at $r_G = 0.56$, and did not test significantly different from zero (LRS = 0.60).

4. DISCUSSION

The genetic estimates we determined for normal variation in zebra finch brain traits are the first measures of all the major components of a dedicated neural system in any avian species. Our quantitative-genetic analysis of brain nuclei controlling bird song produced several original discoveries. First, and most importantly, our evidence suggests a moderate heritability for HVC size. In addition, we find that HVC size is genetically correlated with the RA size. We find that heritability estimates differ across nuclei, and vary with the apparent functions of the anterior and posterior divisions of the circuit; for nuclei in the posterior song production pathway, heritability estimates are as high or higher than those for such traits as brain or body size. We also find that evolvability is higher for nuclei that organize song production and lower for those involved in song acquisition. We find that variation in the size of the different song nuclei is phenotypically correlated, but only for nuclei that are monosynaptically connected. We also find that variation in the size of each song control nucleus is correlated with overall telencephalon size. Finally, we find that the brains of males are larger than the brains of females, even discounting differences associated with dimorphic song control nuclei.

Heritability for volumes of song control nuclei in the posterior pathway are estimated at *ca.* 0.4 (HVC), *ca.* 0.7 (RA) and *ca.* 0.5 (n12ts). These are substantially higher than the estimates we find for lMAN (*ca.* 0.2) and Area X (*ca.* 0.2) in the anterior pathway. These results suggest that resemblance between relatives for volumes of nuclei in the anterior pathway is relatively more determined by something *other* than additive genetic variance than are nuclei in the posterior pathway, and conversely, the development of the posterior pathway is relatively less environmentally determined than the anterior pathway. Perhaps any heritable attributes of size of the anterior nuclei are expressed most clearly early in development, when the integrity of these structures is critical for song acquisition. In adulthood, when the present measures were made, variance in the size of these structures may be more closely related to differences in experience rather than to contributions of the parental genome.

The volumes of the song control nuclei, whether in the anterior or posterior pathway, are correlated with the volumes of their afferent and efferent target nuclei. This finding suggests a degree of developmental coordination between circuit nuclei. Our results suggest that this functional coordination may develop in part by shared additive genetic variance. Perhaps experience-independent specification of levels of neurotrophins, hormones and

their receptors, or a shared proliferative population, forms the developmental basis of the observed genetic correlation between HVC and RA (Bottjer 1997; Gahr & Metzdorf 1999). Because genetic correlations may result from either shared (pleiotropic) or linked genes, it is also possible that the close linkage of separate genes (physically linked or otherwise in linkage disequilibrium) is responsible for the genetic correlation between HVC and RA size.

The volumes of the song control nuclei also correlate with telencephalon volume and brain weight. Although causation cannot be determined from correlation, these results could mean that it is not possible to have a larger song system without increasing the overall size of the brain. Across species, relative HVC size is a good predictor of species diversity in song complexity (DeVoogd *et al.* 1993; Székely *et al.* 1996; DeVoogd & Székely 1998). However, larger telencephalons also covary with more complex song (Airey *et al.* 1996). Thus, elaborate song requires an elaborate neural song system, but this in turn may be linked to augmented anatomy and function in other neural systems (Airey *et al.* 1996). Within a species, it is not known whether formation and maintenance of a song system that is able to produce especially complex songs are necessarily tied to special capabilities for other brain components. The evidence for larger male telencephalons presented here suggests that integration of male song behaviour into the brain as a whole requires general neural augmentation. This in turn suggests that there may be greater developmental costs to complex song than previously suspected, or conversely that elaborate male song is an honest indicator of some aspects of overall brain capacity.

The heritability estimates of the HVC and the RA, and their high indices of evolvability, suggest the size of these structures could be affected by selection pressures like female choice for elaborate song. The pattern of evolutionary response to selection on a neural circuit is determined by both the magnitude of additive genetic variance for each brain nucleus and the extent of additive genetic covariance between brain nuclei. Depending on selection context, genetic correlations may constrain or facilitate the evolutionary response of a coordinated system (Arnold 1992; Schlichting & Pigliucci 1998). If, for example, selection is characterized by an increase in the HVC relative to the RA, the positive genetic correlation between the HVC and the RA may constitute a constraint. If selection is characterized by an increase in both the HVC and the RA, the same genetic correlation may facilitate conjoint evolution. Definitive statements about the effects that genetic correlations between song control nuclei have for song system evolution will require additional genetic parameter estimates of the song characters and measurement of the selection gradients for each trait (Arnold 1994). Such estimates remain undetermined and an important research problem.

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REFERENCES

Airey, D. C. & DeVoogd, T. J. 2000 Greater song complexity is associated with augmented song system anatomy in zebra finches. *NeuroReport* **11**, 2339–2344.

Airey, D. C., Niederer, J. K., Nelson, A. K., DeVoogd, T. J. & Finlay, B. L. 1996 High vocal center and encephalization: specialization and developmental constraints. *Soc. Neurosci. Abstr.* **22**, 1402.

Airey, D. C., Buchanan, K. L., Székely, T., Catchpole, C. K. & DeVoogd, T. J. 2000 Song, sexual selection, and a song control nucleus (HVC) in the brains of European sedge warblers. *J. Neurobiol.* **44**, 1–6.

Arnold, S. J. 1992 Constraints on phenotypic evolution. *Am. Nat.* **140**, S85–S107.

Arnold, S. J. 1994 Multivariate inheritance and evolution: a review of concepts. In *Quantitative genetic studies of behavioral evolution* (ed. C. R. Boake), pp. 17–48. University of Chicago Press.

Ball, G. F. & Balthazart, J. 1997 Letter to the editor. *Trends Neurosci.* **20**, 344.

Boldman, K. G., Kriese, L. A., Van Vleck, L. D., Van Tassell, C. P. & Kachman, S. D. 1995 *A manual for use of MTDFREML. A set of programs to obtain estimates of variances and covariances*. Revised United States Department of Agriculture–Agricultural Research Station, by R. L. Hruska. Clay Center, NB: US Meat Animal Research Center.

Bottjer, S. W. 1997 Building a bird brain: sculpting neural circuits for a learned behavior. *BioEssays* **19**, 1109–1116.

Bottjer, S. W. & Johnson, F. 1997 Letter to the editor. *Trends Neurosci.* **20**, 344–345.

Bottjer, S. W., Miesner, E. A. & Arnold, A. P. 1984 Forebrain lesions disrupt development but not maintenance of song in passerine birds. *Science* **224**, 901–903.

Brenowitz, E. A. & Smith, T. 1997 Letter to the editor. *Trends Neurosci.* **20**, 345.

Brenowitz, E. A., Lent, K. & Kroodsma, D. E. 1995 Brain space for learned song in birds develops independently of song learning. *J. Neurosci.***15**, 6281–6286.

Burek, M. J., Nordeen, K. W. & Nordeen, E. J. 1991 Neuron loss and addition in developing zebra finch song nuclei are independent of auditory experience during song learning. *J. Neurobiol.* **22**, 215–223.

Canady, R. A., Kroodsma, D. E. & Nottebohm, F. 1984 Population differences in complexity of a learned skill are correlated with the brain space involved. *Proc. Natl Acad. Sci. USA* **81**, 6232–6234.

Catchpole, C. K. & Slater, P. J. B. 1995 *Bird song: biological themes and variations*. Cambridge University Press.

Clayton, N. & Pröve, E. 1989 Song discrimination in female zebra finches and Bengalese finches. *Anim. Behav.* **38**, 352–354.

DeVoogd, T. J. & Székely, T. 1998 Causes of avian song: using neurobiology to integrate proximal and ultimate levels of analysis. In *Animal cognition in nature* (ed. I. Pepperberg, A. Kamil & R. Balda), pp. 337–380. San Diego, CA: Academic Press.

DeVoogd, T. J., Krebs, J. R., Healy, S. D. & Purvis, A. 1993 Relations between song repertoire size and the volume of brain nuclei related to song: comparative evolutionary analyses amongst oscine birds. *Proc. R. Soc. Lond.* B **254**, 75–82.

Gahr, M. 1997 How should brain nuclei be delineated? Consequences for developmental mechanisms and for correlations of area size, neuron numbers and functions of brain nuclei. *Trends Neurosci.* **20**, 58–62.

Gahr, M. & Metzdorf, R. 1999 The sexually dimorphic expression of androgen receptors in the song nucleus hyperstriatalis ventrale pars caudale of the zebra finch develops independently of gonadal steroids. *J. Neurosci.* **19**, 2628–2636.

Grant, P. R. & Grant, B. R. 1995 Predicting microevolutionary responses to directional selection on heritable variation. *Evolution* **49**, 241–251.

Henderson, C. R. & Quaas, R. L. 1976 Multiple trait evaluation using relatives' records. *J. Anim. Sci.* **43**, 1188–1197.

Houle, D. 1992 Comparing evolvability and variability of quantitative traits. *Genetics* **130**, 195–204.

Houtman, A. M. 1992 Female zebra finches choose extra-pair copulations with genetically attractive males. *Proc. R. Soc. Lond.* B **249**, 3–6.

Kirn, J. R. & DeVoogd, T. J. 1989 Genesis and death of vocal control neurons during sexual differentiation in the zebra finch. *J. Neurosci.* **9**, 3176–3187.

Kroodsma, D. E. & Canady, R. R. 1985 Differences in repertoire size, singing behavior, and associated neuroanatomy among marsh wren populations have a genetic basis. *Auk* **102**, 439–446.

Lynch, M. & Walsh, B. 1998 *Genetics and analysis of quantitative traits*. Sunderland, MA: Sinauer Associates.

MacDougall-Shackleton, S. A. 1997 Sexual selection and the evolution of song repertoires. *Curr. Ornithol.* **14**, 81–124.

Nixdorf-Bergweiler, B. E. 1996 Divergent and parallel development in volume sizes of telencephalic song nuclei in male and female zebra finches. *J. Comp. Neurol.* **375**, 445–456.

Nottebohm, F., Kasparian, S. & Patton, C. 1981 Brain space for a learned task. *Brain Res.* **213**, 99–109.

Roff, D. A. 1997 *Evolutionary quantitative genetics*. New York: Chapman & Hall.

Scharff, C. & Nottebohm, F. 1991 A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. *J. Neurosci.* **11**, 2896–2913.

Schlichting, C. D. & Pigliucci, M. 1998 *Phenotypic evolution: a reaction norm perspective*. Sunderland, MA: Sinauer Associates.

Searcy, W. A. & Yasukawa, K. 1996 Song and female choice. In *Ecology and evolution of acoustic communication in birds* (ed. D. E. Kroodsma & E. H. Miller), pp. 454–472. Ithaca, NY: Cornell University Press.

Shaw, R. G. 1987 Maximum-likelihood approaches applied to quantitative genetics of natural populations. *Evolution* **41**, 812–826.

Sokal, R. R. & Rohlf, F. J. 1995 *Biometry*, 3rd edn. New York: W. H. Freeman & Co.

Székely, T., Catchpole, C. K., DeVoogd, A., Marchl, Z. & DeVoogd, T. J. 1996 Evolutionary changes in a song control area of the brain (HVC) are associated with evolutionary changes in song repertoire among European warblers (Sylvidae). *Proc. R. Soc. Lond.* B **263**, 607–610.

Van Vleck, L. D. 1998 Development of a flexible, portable, efficient, free software program for estimation of (co)variance components for multiple models (MTDFREML). Proceedings of the Conference in honour of Shayle R. Searle, Cornell University, 9–10 August 1996, pp. 13–36. Biometrics Units.

Ward, B. C., Nordeen, E. J. & Nordeen, K. J. 1998 Individual variation in neuron number predicts differences in the propensity for avian vocal imitation. *Proc. Natl Acad. Sci. USA* **95**, 1277–1282.