

## Population differences in complexity of a learned skill are correlated with the brain space involved

(birdsong/evolution of learning/brain size/marsh wren)

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**ABSTRACT** The song of marsh wrens (*Cistothorus palustris*) is a learned trait passed on from generation to generation. Male marsh wrens from California and New York learn about 150 and 50 different songs apiece, respectively. The volumes of the hyperstriatum ventralis, pars caudalis and the robust nucleus of the archistriatum, two telencephalic nuclei involved in song control, are larger by an average of 40% and 30%, respectively, in the population with a larger song repertoire.

Oscine songbirds (order Passeriformes) learn their song by modifying their vocal output until it matches an auditory model (1, 2). Two forebrain song control nuclei, the hyperstriatum ventralis, pars caudalis (HVC) and the robust nucleus of the archistriatum (RA), are large in canaries with a large song repertoire; canaries with small HVC and RA tend to have small song repertoires (3). These anatomical observations suggested that there may be a causal link between brain space for a learned skill and how much of that skill is learned. If so, then two populations of a same species diverging for one of those traits would also diverge for the other one. We set to test this prediction in free-living populations of marsh wrens, a songbird inhabiting cattail and bullrush marshes across North America. Marsh wrens learn their song repertoire by imitating the song of older marsh wrens (4).

### MATERIALS AND METHODS

Two marsh wren populations known to differ greatly in average song repertoire size were chosen for comparison of brain anatomical traits that relate to song control. Between 300 and 800 songs from each individual wren were recorded on tape during the 1- to 2-hr period beginning just before dawn, when singing is most intense. For this we used Nagra IS and Uher 4200IC recorders at 7½ ips (19 cm/sec) with a Sennheiser MKH-816 directional microphone. These recordings were converted to sonograms through the use of a Princeton Applied Research model 4512 FFT real-time spectrum analyzer with modifications by Unigon and the Rockefeller University electronics and instrument shops to allow continuous photographic recording of sound spectrograms. This improved upon a system for real-time sound analysis described earlier (5). The number of different song patterns occurring were determined from these samples.

The basic structure of marsh wren song is stereotyped, having an introductory note, a long trill segment, and usually a concluding segment (Fig. 1 Upper). Songs with different trills can share introductory notes or concluding segments. The song types of an individual were classified by the structure and spacing of the trill notes.

Song sequences in male marsh wrens are nonrandom. A given song type may recur soon after an initial occurrence,

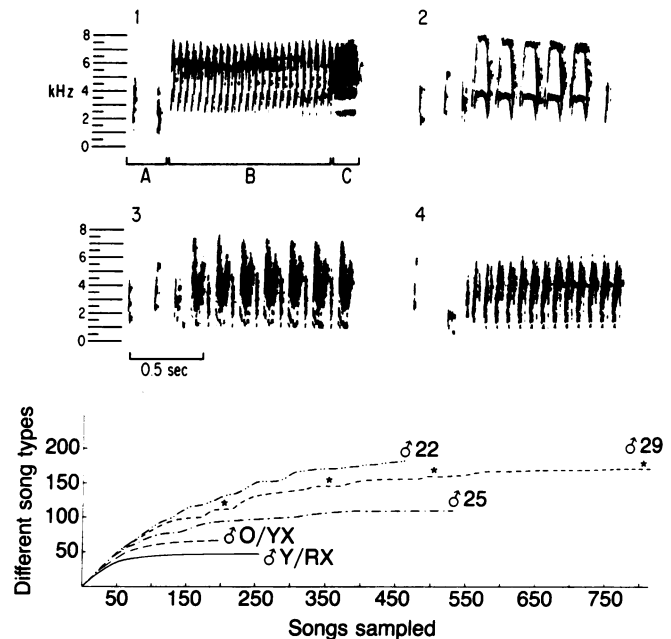


FIG. 1. Song repertoire estimation. (Upper) Examples of marsh wren song: trace 1 shows introduction (A), long trill (B), and conclusion (C). Traces 2, 3, and 4 show three other examples of marsh wren song. (Lower) Cumulative plots of novel song-type occurrences for five of the marsh wrens used in this study. To check the estimation method used, repertoire estimates were calculated at four sample sizes (\*) for male 29: at 200, 350, 500, and 810 songs,  $\theta$  (see text) was 0.745, 0.857, 0.914, and 0.977, corresponding to repertoire estimates of 150.3, 169.2, 175, and 175, respectively. Birds 22, 25, and 29 were from the western population; Y/RX and O/YX were from the eastern population.

after which the bird tends to run through one-third to one-half of its complete repertoire before returning to that song type. Only "independent" song occurrences were considered for purposes of counting the number of songs recorded from each bird and then were used to estimate the size of its song repertoire. We call the occurrence of a song type "independent" if nine other song types intervened since its last occurrence (a 10-song "window"). In both populations being examined, the frequency of recurrence of a given song type fell abruptly at the fifth song in sequence following the song being examined. Varying the window from 5 to 15 songs in four wrens that represented extremes of sampling and repertoire size had a negligible effect on repertoire estimates. The use of a "window" and "independent" song occurrences decreased the size of song samples used for individuals, so that they ranged from 124 to 248 independent occurrences of

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Abbreviations: HVC, hyperstriatum ventralis, pars caudalis; RA, robust nucleus of the archistriatum; Rot, rotundus; SpM, spiriformis medialis.

song types for the "eastern" wrens and from 463 to 787 independent occurrences for the "western" wrens.

The sequencing peculiarities described, along with the possibility that individuals may favor certain song themes over others, makes repertoire estimation a difficult problem. We compared several methods of estimating repertoire size and found that all gave essentially the same results. These methods were: (i) exponential curve-fitting, using  $n = R(1 - e^{-N/R})$ , where "n" is the number of distinct song types in the sample, "R" is the number of distinct song types in the repertoire, and "N" is the number of independent song occurrences in the sample (6); (ii) correction for sample coverage by using  $R = n/\theta$  (see below); and (iii) best fit and estimation of the "zero class" by assuming a Poisson log-normal distribution of song-type occurrence frequencies [as described by M. G. Bulmer (7)]. The estimate  $R = n/\theta$  was used in this report because it seemed to give more consistent, sample-independent results (Fig. 1 Lower) (8, 9). The "sample coverage" was estimated by using the formula  $\theta = 1 - S/N$ , where "S" is the number of song types that occurred only once in our samples (i.e., one independent occurrence). The sample coverage as used here is an estimate of the probability that the next sampled song will be of a type already encountered (10). Sample coverage for our data ranged from 0.857 to 0.996 (median, 0.971).

Males in each population were in full song when studied and at very similar stages in the breeding cycle. Males of both populations were actively building nests. There were indications at each study site of recent fledging of early broods. Despite these similarities, testes weight differed between the two populations; the difference was in the same direction as that seen in body weight (Table 1). Fifteen males were recorded and captured from a marsh located on the Hudson River in Dutchess County, New York ("eastern" sample). Another 15 males were captured, but only 7 were recorded, from a marsh located in the Grizzly Island Wildlife Area, Fairfield, California ("western" sample). One of the eastern song repertoires was not used because of poor recording quality. The samples from an additional four eastern wrens were not used in the intrapopulation correlations because of some uncertainty that the recordings were, in fact, of the individual captured. One to two hours after they were recorded, the wrens were weighed, given an overdose of anesthesia, and perfused intracardially with 0.9% saline, followed by 10% formalin in 0.9% saline. Brains were then excised, weighed, and processed for histology (3). (Brain weight was not obtained for two of the eastern wrens; body weight was not obtained for one western wren.) Brain sections 50  $\mu$ m thick were stained with cresyl violet.

Two telencephalic nuclei, HVC and RA, are known to be involved in song control (12). The boundaries of these nuclei

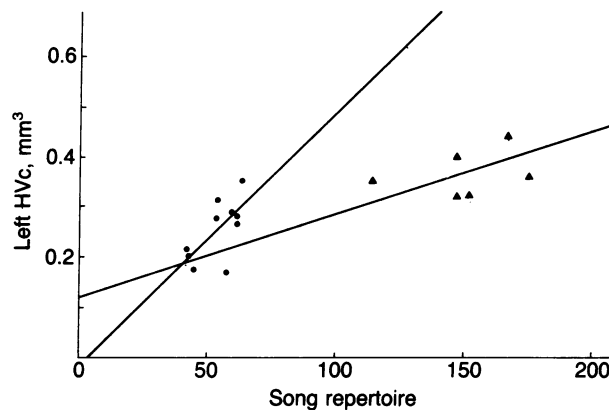


FIG. 2. Scatter plot and regression lines relating size of song repertoire and size of left HVC in two populations of marsh wrens. Correlation coefficient "r" values for the relation between song repertoire size and left HVC volume are 0.6 ( $n = 10$ ) and 0.718 ( $n = 7$ ) (9, 13) for the eastern and western samples, respectively, corresponding in both cases to a probability of  $<0.05$ . Because the two samples are small, we do not wish to attach significance to the population differences in "r" slope. ●, Eastern wrens; ▲, western wrens.

and two others, rotundus (Rot) and spiriformis medialis (SpM), were traced from microprojector images. We chose Rot and SpM for comparison because they have discrete boundaries and are not part of the vocal control pathway. Methods used in this laboratory for estimating volume of brain nuclei have been described (3). Volume reconstruction was done for nuclei in both the right and left hemispheres in the case of HVC. As in a previous study with canaries, no systematic right-left differences were observed (3). Only the left-side volume was reconstructed for nuclei RA, Rot, and SpM.

## RESULTS

There was considerable intrapopulation variability in the size of the song repertoire and in the volume of HVC (Fig. 2). As predicted, there was a positive and significant correlation between the size of HVC and the size of the song repertoire in both eastern and western populations (Fig. 2). None of the other traits measured, listed in Table 1, led to statistically significant correlations with the size of the song repertoire for both wren populations.

In addition to the intrapopulation differences, there was a striking difference between the eastern and western populations in song repertoire size (Table 1 and Fig. 2). The average estimated repertoire size in the western sample was three times that in the eastern sample, with a range of 42–208 in the combined sample.

Table 1. Population differences in behavioral and anatomical measurements

Variable	West $\bar{X}$ (SEM)	East $\bar{X}$ (SEM)	Ratio W/E	P
Song repertoire	158.5 (11.0)	53.6 (2.2)	2.96	<0.001
Volume measurements				
Left HVC, mm <sup>3</sup>	0.353 (0.022)	0.259 (0.017)	1.36	<0.01
Right HVC, mm <sup>3</sup>	0.362 (0.023)	0.246 (0.016)	1.47	<0.001
Left RA, mm <sup>3</sup>	0.129 (0.005)	0.104 (0.004)	1.24	<0.01
Left Rot, mm <sup>3</sup>	0.623 (0.023)	0.716 (0.022)	0.87	<0.01
Left SpM, mm <sup>3</sup>	0.075 (0.003)	0.081 (0.005)	0.93	<0.5
Brain, g	0.504 (0.015)	0.529 (0.013)	0.95	<0.1
Body, g	10.89 (0.14)	11.48 (0.14)	0.95	<0.01
Testes, g	0.134 (0.005)	0.192 (0.012)	0.70	<0.001

Sample means ( $\bar{X}$ ) compared by using the Student two-sided *t* test to test the significance (11). Sample sizes were:  $n = 15$  for HVC, RA, Rot, and SpM volume measurements of western and eastern samples;  $n = 7$  and 14 for western and eastern song repertoires, respectively;  $n = 14$  for body weight of the western sample;  $n = 13$  for the brain weight of the eastern sample. Figures in brackets correspond to one SEM. Ratio W/E, ratio of  $\bar{X}$  West to  $\bar{X}$  East values.

The eastern marsh wrens had slightly larger bodies and testes. Whole-brain weight and the volume of the two control nuclei followed the same trend, though only the difference in Rot was significant, with the eastern sample averaging 15% more in volume than the western sample (Table 1).

Contrary to the direction of East–West differences in body size and brain weight, HVC and RA averaged 40% and 30% larger, respectively, in the western sample than in the eastern sample, and this difference was significant (Table 1). That is, wrens from the population with larger, more varied song repertoires also had larger absolute volumes of brain tissue devoted to vocal control.

## DISCUSSION

The observation of a correlation between the size of learned song repertoire and the size of HVC and RA in canaries led to the inference that learned song occupied space in these parts of the brain (3). From this we predicted that when two populations of a same species differ in the size of their learned repertoire, the size of HVC and RA would differ accordingly. This prediction was met. The principle of brain space for a learned skill first enunciated for a close-bred, domesticated stock of canaries seems to apply to other species and to wild populations.

The results presented are thought-provoking in other ways. The size distribution of HVC showed partial overlap between the eastern and western populations, and this was also true for RA. Some of the western marsh wrens, which had repertoires 2 or 3 times larger than those of their eastern counterparts, had HVC volumes which were no bigger than those at the upper end of the eastern distribution. We can infer that factors other than HVC or RA volume also influence the size of the song repertoire. In addition, it is possible that the functions of HVC and RA are broader than just song control.

Auditory information reaches HVC (13–16), and some HVC neurons respond selectively to conspecific song and to playbacks of the bird's own song (15, 16). Even the hypoglossal motoneurons that innervate the syrinx respond to sound, this response being mediated by HVC, through RA (17). Thus, the role of HVC and RA may be a dual one, song production and perception—encoding and decoding (18). A similar situation occurs in the human brain, where Broca's area partakes in the encoding and decoding of speech (19). The decoding function of HVC and RA may be more comparable between our eastern and western populations than the observed differences in song production. Circuitry used in production may be a subset of that used in perception. With all this in mind, the most parsimonious interpretation may be that the size of HVC and RA relate to an individual's ability for mastering and remembering a diversity of motor *and/or* perceptual song patterns.

The observations reported here are preliminary in that they focus on a gross measure of "brain space." The volume of HVC and RA could vary between populations as a result of any of a number of anatomical differences: in neuronal numbers, in neuronal size, in neuropil, in number of glia, or even in the amount of vascularization. We have not shown, yet, that a larger HVC or RA has more "network space" than a smaller one, though this seems likely.

In a separate experiment, marsh wren nestlings from the same eastern and western populations described here were hand-reared under laboratory conditions and tutored with a common set of song patterns. The song repertoire developed by the birds from the western population was 2.5 times larger than that of the eastern birds. The western birds also had a

larger HVC (by 26%) and a larger RA (by 29%). Only the latter difference was statistically significant. Though these samples of hand-reared individuals were smaller ( $n = 6$  and  $5$  for western and eastern birds, respectively) than our samples of wild-caught adults, the similarity in behavioral and anatomical trends is encouraging. It suggests that the behavioral and anatomical differences observed reflect genetic differences between these two geographically separated populations (unpublished data).

Song is a behavior modified by learning. The models learned are passed down from one generation to the next, just as our language and dialects are. Both birdsong and language are examples of a behavior modified by culture. The size of a bird's song repertoire is not learned (unpublished data) but is a vehicle for culture, and the two are related: the larger the repertoire, the more complex the cultural trait. It has been suggested that brain processes favoring the acquisition of adaptive cultural traits are favored in turn by natural selection, leading to a coevolution of culture and brain (20, 21). The observations we present here do not test this hypothesis. For example, we have not yet proven that when other conditions are equal, the size of HVC or RA set limits to the size of the learned song repertoire. What our observations do point to is an animal example that, once understood, might shed light on how nervous systems evolve in response to pressures for the acquisition of an increasingly complex culture.

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