



COMMENTARY

Do bird nestmates learn the same songs?

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In recent years, ethologists have become acutely sensitive to the problems posed by pseudoreplication and the pooling of nonindependent replicates in experimental design (Kroodsma 1989). Most of the discussion concerning pseudoreplication has focused on playbacks in the field to adults in which the animal's immediate responses to stimuli are monitored. Song-learning and imprinting experiments in birds are other forms of playback experiment in which tape or live 'tutors' are presented, but the typical responses, imitation of tutors or the expression of sexual or affiliative responses, may not be evident for months. Such experiments using males reared from a young age either by hand or by parent birds have been widely used in many laboratories over the past five decades to explore issues of fundamental ethological importance (ten Cate et al. 1993). Because of the extended nature of such experiments, unless care is taken in the experimental design, many opportunities exist for subjects to interact with one another and produce correlated outcomes among individuals housed together (Nelson 1997).

A potential subtle form of pseudoreplication in song-learning experiments, equally applicable to other forms of imprinting experiments in other taxa and other stimulus modalities, was identified by West & King (1996). They pointed out that researchers often collect entire broods, either from the field or from captive breeding pairs, and that therefore the individuals within a brood share a common early environment, and to a variable extent, a common set of genes. Treating nestmates as independent observations may not be justified if their shared genes and early experience predispose nestmates to a similar developmental outcome. Here, we attempt to examine

this concern by reanalysing data we collected on four subspecies of the white-crowned sparrow, *Zonotrichia leucophrys*, over six experiments.

METHODS

We collected entire nests containing all chicks from four subspecies of white-crowned sparrow and hand-reared the birds in the laboratory as described in previous papers (listed in Table 1). We collected birds that were between 3 and 7 days of age. Fledging occurs on average at 9 days. Sample sizes and other details of housing and tutoring pertaining to each cohort are summarized in Table 1. We housed birds in groups of two sizes until we detected subsong production by males. In one method, three to four birds of both sexes, usually from the same brood, were housed together in a cage, with all cages held in one room while the birds were tutored. In the second housing method, one to two birds, chosen without regard to sex or nest origin, were housed together in a cage held within a sound isolation chamber. We sexed birds by laparotomy or by examination of the cloacal protuberance in their first spring. When males began subsong, usually at around one month of age, they were immediately housed singly in sound isolation chambers (Industrial Acoustics, Bronx, New York, U.S.A. or Acoustic Systems, Austin, Texas, U.S.A.) until they developed their crystallized songs the next spring. We tutored all birds with tape-recorded songs beginning a few days after fledging for at least 50 days and in some cohorts throughout the first year of life. We used two basic tutoring regimes (Table 1). In the 'Rich' regime, males heard 16 different song dialects for 40 consecutive days before switching to 16 novel dialects for another 40 days. The 'Lean' regime involved presenting different pairs of dialects for 10-day-long blocks. Eight to 28 blocks were presented depending on the experimental design. In the first two experiments listed in Table 1, we used one stimulus tape for all subjects. In the remaining experiments we used different tapes for each subject.

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Table 1. Details of sample sizes, housing, tutoring and subspecies of white-crowned sparrow, *Zonotrichia leucophrys*, used in the six studies reanalysed here

Subspecies	<i>N</i> broods	<i>N</i> males	<i>N</i> sib pairs	Housing prior to subsong	Tutor regime	Source
<i>nutalli</i>	7	12	4	3–4*/cage all in one room	Lean	Nelson et al. 1995
<i>oriantha</i>	8	14	4			
<i>nutalli</i>	5	9	4	3–4/cage all in one room	Rich	Nelson et al. 1996
<i>oriantha</i>	10	12	2†			
<i>pugetensis</i>	6	8	3			
<i>oriantha</i>	6	8	3	1–2/cage separately in IC	Lean	Nelson et al. 1997
<i>gambelii</i>	7	14	5	1–2/cage separately in IC	Rich	Nelson 1999
<i>oriantha</i>	11	16	6	1–2/cage separately in IC	Lean/Rich‡	Nelson 2000
<i>pugetensis</i>	9	12	4	1–2/cage separately in IC	Lean or Lean/Rich‡	Nelson et al. 2004

IC = isolation chamber.

*Males and females, usually from same brood.

†This *N* is small relative to the total *N* of males because nestmates were randomly assigned to different photoperiods and tutor treatments. We only used pairs of nestmates and non-nestmates that were treated identically.

‡Lean/rich regime involved one 10-day-long block of two dialects followed by one 40-day-long block of four or eight dialects.

Measurement Variables

The original studies documented how subspecies differences and differences in the tutoring environment (the numbers and timing of tutor presentation) influence the course of song development. As described in those papers, we counted for each male the number of tutors imitated in plastic song, the practise stage of singing preceding crystallized song, and the total number of song phrases (whistle, buzzes, syllables and note complexes) reproduced; and calculated the mean age at which all tutors were imitated. Each of these three variables was found to differ significantly due to treatment effects (subspecies and/or tutor regime) in one or more of the papers cited. In addition, for the current analysis we computed a fourth variable, the proportion of the imitated tutors that were shared between two males (see below), following McGregor & Krebs (1982):

$$2N_s/R_1 + R_2$$

where N_s is the number of tutors imitated by both birds, and R_1 and R_2 equal the plastic song repertoire sizes of the two males.

Statistical Analysis

Upon completion of all experiments, we searched our data to form pairs of males from the same nest, and pairs of males where each male came from a different nest. Each male wore a leg band numbered between 01 and 99 within each cohort. In nests that held two males we grouped the males together to form a pair of nestmates. In the seven nests that held three or more males, we searched a random numbers table for band numbers to form at most two pairs of nestmate males from one nest. We also used the random numbers table to form pairs of males taken from different nests in the same cohort (non-nestmates). We

formed 35 pairs of non-nestmates to correspond with the 35 pairs of nestmates matched for experimental treatment (subspecies, housing and tutoring). In some cases, an individual male that was included in a nestmate pair was also assigned at random as one member of a non-nestmate pair.

In order to increase the sample size and analyse all pairs together, we needed to statistically control for differences in how birds were housed and tutored among experiments (Table 1). We first calculated the absolute difference of a variable within each pair of males. As a result, we had 35 differences for each of the four variables calculated between nestmates and 35 differences for each variable between non-nestmates. To produce normal distributions, the differences in counts of tutors and phrases imitated were log + 1 transformed. To test for any subspecies or housing-dependent effects, we first performed a factorial repeated measures ANOVA using the difference variables measured on nestmates and non-nestmates as repeated measures, and subspecies and housing as between-subjects factors. With the exception of one significant two-way interaction between housing and mean age of imitation, none of the main repeated measures effects or their interactions with between-subjects factors approached significance ($P > 0.30$). To increase statistical power and to simplify presentation, we then compared the absolute difference variables between nestmates and non-nestmates treated alike in the same experiment using a paired *t* test. The full ANOVA results are available from D.A.N. upon request. All tests were two tailed with $P < 0.05$. If the genes and early environment shared by nestmates produce a measurable effect on song development, we would expect to reject the null hypothesis in favour of the alternative hypothesis predicting that the differences between nestmates should be smaller than the differences between non-nestmates. To assist with interpreting negative results, we calculated 95% confidence intervals for the observed effect sizes (Colegrave & Ruxton 2003). We took

this step rather than specify effect sizes a priori because we lacked an objective basis to specify what the expected effect sizes would be.

RESULTS

Across all cohorts, the median number of males per nest was 1 ($N = 71$ nests, first quartile = 1, third = 2). We found no evidence to suggest that the four parameters describing song development were more similar between pairs of nestmates than they were between pairs of males randomly chosen from different nests that were treated alike in the same experimental conditions (Table 2). The 95% confidence intervals for difference between the paired within-subjects variables were fairly narrow in all cases, indicating that the differences in numbers of tutors and phrases imitated are unlikely ($P < 0.025$) to be greater than about 0.5 tutors or phrases, and the difference in learning age is unlikely to be greater than 9 days, which is about the temporal resolution provided by the 'Lean' tutoring regime.

DISCUSSION

Our results do not support the concern expressed by West & King (1996) that males taken from the same nest for use in studies of song development should be considered nonindependent replicates for statistical purposes. This result is perhaps not surprising given that these birds are altricial and were collected while they were still in the nest with rudimentary sensory capabilities (Khayutin 1985). Learning song in the nest could be a concern, but as yet, there is little evidence that birds do so (Catchpole & Slater 1995). In our experimental designs, nestmates shared common experience after fledging to a variable degree up until about 30–40 days of age. Our result suggests that even this shared postfledging experience combined with any shared genetic influences did not produce a learning outcome correlated between nestmates.

In our experiments, the nestmate males shared their social parents and nesting environment, and a variable proportion of their genes. The common occurrence of

extrapair fertilizations in birds could mean that some nestmates are genetically unrelated, while some non-nestmates are half-brothers. Egg dumping by females in multiple nests could also produce non-nestmates that are half-brothers. The net effect would be to decrease the difference between nestmate and non-nestmate groups due to genetic influences on song development shared among siblings. In the mountain white-crowned sparrow, *Z. l. oriantha*, 30–56% of nestlings are the product of extrapair fertilizations exclusively with no egg dumping (Sherman & Morton 1988; MacDougall-Shackleton et al. 2002). In other species, it appears that neighbouring males are responsible for the bulk of the extrapair fertilizations that occur within a focal nest (Gibbs et al. 1990; Hasselquist et al. 1996; Forstmeier et al. 2002; Byers et al. 2004), and so half-brothers would be likely to occur among our non-nestmates if we collected many nests on neighbouring territories, which we rarely did. Of the 42 nests where we knew the locations of all surrounding territories, only five pairs of nests came from neighbouring territories. Therefore, it is unlikely that our non-nestmate category included many half-brothers, whereas the nestmate category may have included some unrelated males who nevertheless shared the same social parents and early nestling environment. The practical result of our analysis is that nestmates do develop song independently of one another under the conditions we used, although we cannot identify the possible role of genes inherited from the father that influence vocal development.

It is important that our result not be generalized too far, as it probably pertains only to the experimental conditions we have used in which nestlings and young fledglings are housed in groups of various sizes up until about one month of age. After young males begin subsong at that time, they are housed individually in sound isolation chambers until they complete song development the next spring and so cannot influence one another while in isolation. Although the practise of housing birds in sound isolation chambers has attracted criticism, it does have the advantage of ensuring that the birds develop independently of one another from that point forward.

However, in order to test hypotheses concerning the effects of social interaction on development, it is necessary to house animals together in groups for extended periods. These designs can be profitably used to explore possible effects of postfledging experience with nestmates or with adult tutors. In such cases, it would be appropriate, unless there is evidence to support the assumption of independence, to regard the number of groups, rather than the number of individuals, as the relevant sample size for statistical purposes (Machlis et al. 1985), although some workers present inferential statistics in such designs using N equal to the number of males. In the case of bird song learning, there is abundant evidence that males, presumably unrelated males in many cases, that are housed together for several months while they are singing do influence one another's songs (reviewed in Nelson 1997). A conservative approach using group-reared birds is illustrated by the study of 48 young chipping sparrows, *Spizella passerina*, and field sparrows, *S. pusilla*, housed in open cages in three rooms (Liu & Kroodsma 1999). Instead

Table 2. Results of paired t tests comparing four variables of vocal development between pairs of nestmates and pairs of non-nestmates that were treated alike

Variable	Paired difference* \pm SD	t_{34}	P	95% CI
Tutors imitated†	-0.07	-0.57	0.57	-0.60–0.36
Phrases imitated†	0.08	0.43	0.67	-0.24–0.54
Mean age	2.10 \pm 21.51	0.58	0.57	-5.28–9.49
% Repertoire shared	-0.06 \pm 0.054	-1.17	0.25	-0.17–0.05

CI = confidence interval.

*Difference between nestmates minus the difference between non-nestmates.

†Descriptive statistics presented in linear scale, backtransformed from log +1. Standard deviation not reported in the linear scale.

of treating each bird as an independent replicate, these authors described their results without the use of inferential statistics.

All experiments involve a series of trade-offs (Wiley 2003), and any putative benefit to pruning a sample size to achieve 'statistical purity' must be weighed against the loss of statistical power and the costs of collecting more nests and possibly causing more disturbance to the local population. When nests are easily found (for example, in populations breeding in artificial nestboxes), there might be little advantage in collecting entire broods as we did with ground-nesting white-crowned sparrows. We conclude that, given the experimental conditions we have used, any genetic or environmental factors shared by nestmates early in life are minor relative to the effects produced by genetic differences that exist between subspecies and the effects produced by postfledging experience with song tutors. In other experimental designs, consideration should still be given to avoiding the use of nestmates or to verifying the assumption that nestmates or animals housed together are independent replicates.

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