

Role of gender, season, and familiarity in discrimination of conspecific song by zebra finches (*Taeniopygia guttata*)

(birdsong/neuroethology/operant/seasonality/sexual dimorphism)

JEFFREY CYNX AND FERNANDO NOTTEBOHM

Rockefeller University Field Research Center for Ecology and Ethology, Tyrrel Road, Millbrook, NY 12545

Contributed by Fernando Nottebohm, October 21, 1991

ABSTRACT Operant conditioning techniques were used to assess how gender and song familiarity affect song discrimination in adult zebra finches (*Taeniopygia guttata*). Twenty-five males and females, divided into five cohorts, were trained to discriminate between conspecific songs at different times of year. Males discriminating between their own and another song from their own aviary reached criterion in the fewest number of trials, followed by males discriminating between two songs from their own aviary, then by males discriminating between songs they had not heard before. Females discriminating between two songs from their own aviary required more trials than males to reach criterion, but, unlike in males, song familiarity did not have a significant effect on song discrimination by females. The number of trials required to reach criterion was greater in winter than in summer, suggesting a photoperiodic effect in what has been regarded as a nonphotoperiodic species. Gender, season, and familiarity appear to affect how zebra finches discriminate between conspecific songs.

Members of the same species must give *different* responses to *different stimuli* for us to know that these stimuli are perceived as different. Stimuli that elicit different responses are said to be *discriminated*. For example, male songbirds involved in territorial defense ignore the song of an established neighbor but respond vigorously to that of a newcomer (1, 2). Likewise, the readiness with which female songbirds give solicitation displays to song playbacks depends on what songs are used (3–5). These responses to song occur naturally. They are determined by the stimulus properties and by the respondent's age, gender, territorial status, experience, and reproductive condition.

Naturally occurring responses to song that differ, for example, between males and females, do not tell us whether all members of a species can discriminate equally well between the same pair of songs. To answer such a question the birds to be compared must undergo *discrimination training*. During this training all individuals learn to produce a particular response to one of two stimuli, which thereby acquires a meaning it did not have before. The response must be such that all individuals compared produce it readily regardless of gender and reproductive condition. Birds have been trained in this manner to discriminate between different calls, songs, or pieces of songs (6–10). Discriminations that require many trials before reaching a learning criterion are said to be *difficult*; those mastered in fewer trials are said to be *easier*.

The use here of terms such as *difficult* and *easy* is descriptive and presupposes nothing about the processes involved. The performance of an animal in discrimination training is not likely to reflect a unitary process, but one governed by

variables as diverse as motivation, attention, perception, and memory. In addition, discrimination training is affected by the relative ease with which a particular stimulus can be associated with a particular response (11). Whereas an animal's ability to discriminate between two stimuli indicates that it can tell them apart, its inability to discriminate between them can involve variables other than perception. However, if two stimuli are readily discriminated by members of one group, but not of another, and if this situation persists during other discrimination paradigms, then one wonders whether members of both groups perceive the stimulus differences equally well. The comparative study of perception is still in its infancy, and operant training using a diversity of stimuli and responses is just one of many approaches to map the perceptual world of animals and its ability to govern different behaviors.

Earlier anatomical work showed that the brain pathways for the acquisition and production of learned song can differ markedly between male and female songbirds (12, 13). Subsequent physiological observations suggested that male and female zebra finches process differently the songs they hear and therefore might also perceive them differently (14, 15). The present report provides evidence that gender, season, and stimulus familiarity affect the relative ease with which adult zebra finches can discriminate between conspecific songs.

METHODS

Subjects. We trained and tested 15 male and 10 female zebra finches (*Taeniopygia guttata*). All birds were adults (older than 120 days). They were kept in aviaries under full-spectrum fluorescent illumination that paralleled the natural photoperiod for Millbrook, New York. Year-round temperature in the aviaries was between 21°C and 27°C. A bird that lived in any one aviary was presumed to know or recognize the songs in that aviary, and males were presumed to have acquired their song by imitating the songs of other males in that aviary (16). At the start of the experiment, birds were removed from their aviaries and housed in individual cages in the same room. All birds were experimentally naive regarding the discrimination task. Birds were food-deprived 4–6 daylight hr before each experimental session. The aviary diet consisted of dry finch seed along with a mixture of soaked seed and ground hard-boiled eggs with shell. Dry seed was used during experimental sessions to reinforce discrimination behavior. Water and grit were available to the birds at all times.

Behavioral and Acoustical Apparatus. Birds were trained singly in six identical operant stations. The test cages were closed horizontal cylinders of 1.2-cm wire mesh, each measuring 20 cm in diameter and 33 cm in length. One end of each cylinder was mounted on a 20 × 20 cm aluminum panel that held two food dispensers (with goal lights) 10 cm apart. Only the right food dispenser was used in experiments reported in

this article. A speaker (10 cm in diameter) was centered above the pair of food dispensers.

Perch hopping was the operant behavior. An aluminum observation perch was placed parallel to the operant panel, 17 cm in front of the speaker. It contained a 2.5-cm infrared beam detector zone. Hopping elsewhere on the perch had no experimental consequences. A similarly equipped response perch was placed at the entrance to the food dispenser. Earlier research suggested that the general activity levels of birds (noncontingent perch hopping) can interfere with this particular operant response (17). We therefore placed a wooden perch 27 cm back from the aluminum panel to allow the bird to engage in perch hopping activity free of experimental consequences.

The test cages were placed inside sound attenuation chambers with internal dimensions of 40 cm wide \times 36 cm high \times 59 cm long. All inside surfaces of the chambers were lined with 1.27-cm embossed acoustic foam. Twenty-five watt light bulbs mounted in the ceiling fixtures provided illumination. Behavior was observed via one-way glass windows and speaker monitors. Stimulus presentation, experimental contingencies, and data collection were controlled on line by a microcomputer using software written for this purpose. Three 24-bit parallel interface boards in the computer were wired through six separate optically isolated relay racks and then wired to equipment for the experimental chambers. Three two-channel, 12-bit analog boards digitized and produced the stimuli at a sampling rate of 20 kHz. Six separate output lines went to three two-channel power amplifiers, then to separate bandpass filters (0.2–10 kHz), and finally to the six speakers.

Stimuli and Procedures. The two stimuli to be discriminated were single, stereotyped zebra finch songs [also termed song motifs (18, 19)] of comparable duration (± 170 ms) and containing a variety of syllable types. Mean average length of stimuli was 840 ms (range = 680–1080 ms). The initial recordings were made with a cassette recorder, digitized, and

stored on the computer's hard disk. The overall loudness level of each stimulus was measured with a sound level meter and then set to 70 dB(A) sound pressure level, as measured at the point in front of the speaker where a bird heard the sound.

The 25 birds were grouped into five cohorts of three males and two females each. The birds in each cohort were trained to discriminate between the same two songs (Fig. 1), and the majority of birds in a cohort was trained at the same time (the females in two cohorts were trained 1 year later than the males). Each bird in a cohort had a unique relation to the two stimuli for that cohort. One bird, a male, was trained to discriminate between his own song and that of another bird in its own aviary. Two other birds, one male and one female, were trained to discriminate between the same two stimuli as the first pair. We assumed that these birds knew the songs because they were from the same aviary as the birds who produced the songs. The last two birds, also one male and one female, were from other aviaries or were raised separately. They were therefore discriminating between two unknown but conspecific songs. The five cohorts were trained at various times of the year. One cohort was trained during 14.5 (May), one during 12 (September), one during 10.5 (January), and two during 9.5 (December) hr of daylight.

The finches were first taught that hopping to the center of the observation perch operated the food dispenser. This shaping procedure taught them how to operate the equipment. When they learned this, they were placed on a *go/no-go* procedure, and data collection for the discrimination experiment began. A bird perching in the detector zone on the observation perch initiated a trial. The computer randomly selected a *go* or *no-go* stimulus with equal probability. A *go* response consisted of moving to the response perch in front of the feeder within the 3-s *go* period. A *go* response to the *go* stimulus during the *go* period produced access to food. The same response to the *no-go* stimulus turned off the house light for 15 s, signaling to the bird that it had made an error.

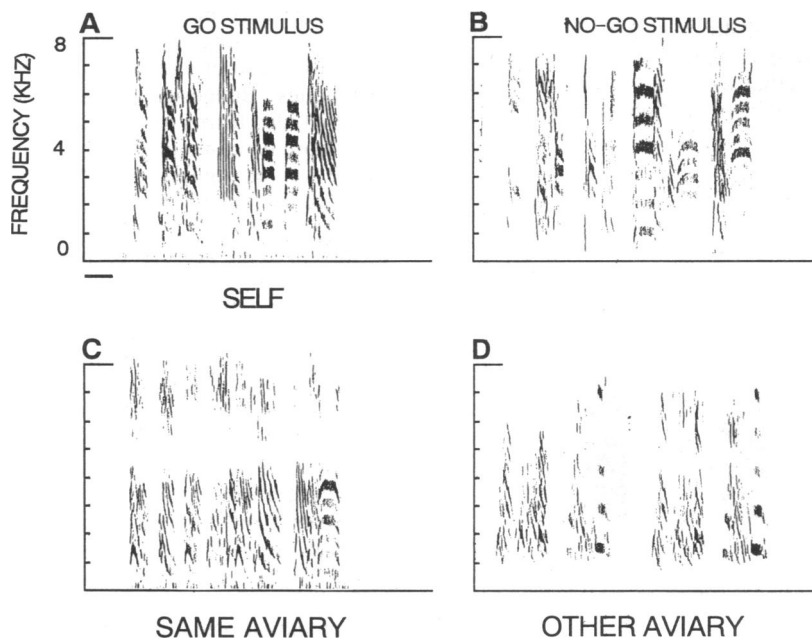


FIG. 1. Stimuli and songs of one cohort (10.5 hr of daylight). Each cohort included three males, whose songs are shown here (A, C, and D). One of the songs (A) was the *go* stimulus. The *no-go* stimulus (B) was the song of a bird that was not used in this cohort but was from the same aviary as A and C. The *go* and *no-go* stimuli were the same for all the birds in the cohort. Thus one male ("self") was presented with his own song and another song from his aviary, another male ("same aviary") was presented with two songs that were from his own aviary, and the third male ("other aviary") was presented with two songs that were not from his aviary. In addition, the cohort included two females, who do not sing, but were matched to the "same aviary" and "other aviary" males in stimulus condition. All songs were recorded from males as they courted females (18, 19). The time frequency display has an ordinate that ranges from 0 to 8 kHz (Kay sonograph, wide-band filter). (Bar below A = 100 ms.)

A *no-go* response always resulted in the end of the trial after 3 s.

DATA ANALYSIS AND RESULTS

The acquisition criterion was set at a mean of 75% correct—halfway between chance and errorless performance—for 100 trials. Group differences were tested with planned comparisons and considered significant only when they exceeded the $P < 0.05$ level.

All birds acquired the discriminations. Birds reached criterion in one to eight consecutive daily sessions. An initial stepwise regression showed that a bird's gender, its previous exposure to the song stimuli, and the photoperiod corresponding to the time when each cohort was trained accounted for >90% of the variance. Two other variables, the age of birds and the differences in duration between song stimuli used with each cohort, accounted for <1% of the variance. The latter two variables were therefore excluded from further analysis.

A multiple regression analysis, supplemented with planned comparisons, showed that the gender of a bird produced an overall significant effect, $F(1, 13) = 18.34$; $P < 0.01$ (Fig. 2), but only between males and females discriminating between songs from their own aviary, $P < 0.05$. There was no significant difference between males and females discriminating between songs from another aviary.

There was also an overall significant effect concerning a bird's relation to song (self, own aviary, and other aviary), $F(2, 13) = 7.11$; $P < 0.05$ (Fig. 2). This effect was significant only for males. Males discriminating between their own song and another aviary song required significantly fewer trials to reach criterion than males discriminating between two songs from their own aviary. Likewise, males discriminating between two songs from their own aviary required significantly fewer trials than males discriminating between two songs from another aviary, both $P < 0.05$.

Finally, there was an unexpected significant difference between cohorts, $F(1, 13) = 42.40$; $P < 0.01$ (Fig. 3). The cohort trained during 14.5 hr of daylight required significantly fewer trials to reach criterion than any other cohort, $P < 0.05$, and the cohort trained on 12.0 hr of daylight required signif-

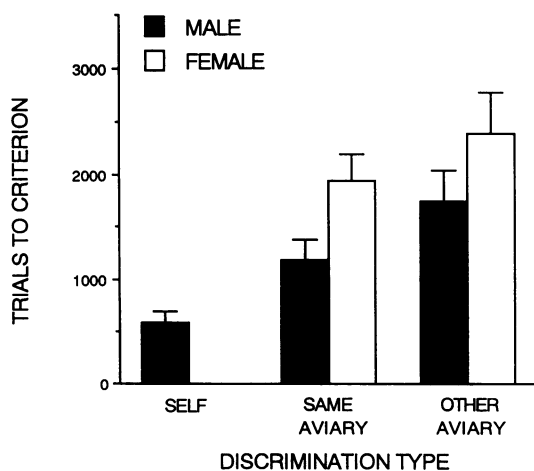


FIG. 2. Mean trials to criterion for each of the five stimulus conditions. Each bar on the abscissa represents the mean + SEM for five birds, one from each cohort. Filled bars represent males, and open bars represent females (females do not sing, so they could not be tested with their own song). The three male groups differed significantly among themselves, but the difference between the two female groups was not significant. Males in the "same aviary" condition required significantly fewer trials than the corresponding females.

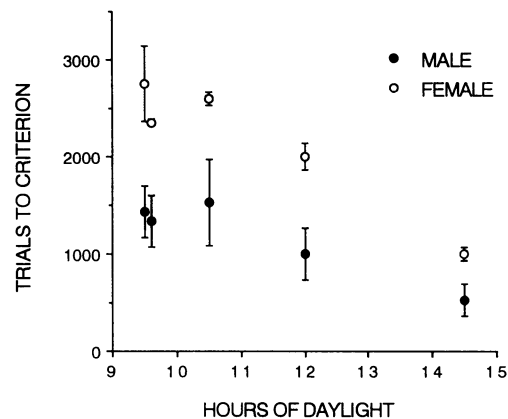


FIG. 3. Mean number of trials \pm SEM needed for the three males (filled circles, averaging "self," "same aviary," and "other aviary" conditions) and two females (open circles, averaging "same aviary" and "other aviary" conditions) in each cohort to reach criterion. Each cohort is plotted according to the number of daylight hours available during training. All birds were on a natural photoperiod.

icantly fewer trials than one cohort trained on 9.5 hr of daylight, $P < 0.01$. Both males and females showed a seasonal effect. None of the interactions between the three factors was significant.

DISCUSSION

Familiarity and Own Song Advantage. Male zebra finches discriminating between their own song and the song of another bird in their own aviary acquired the discrimination in the fewest number of trials, followed by males discriminating between two songs drawn from their own aviary. Males discriminating between songs from another aviary required the greatest number of trials. These behavioral results argue for a familiarity advantage in males. This advantage may be related to the fact that males sing and acquire their songs by vocal learning (20). Our results fall in line with those of another study that used different operant techniques. Budgerigars (*Melopsittacus undulatus*), a species that also learns its vocalizations, find their own contact calls more discriminable than those of their cage mates (21). There was no significant difference in the number of trials required by female finches learning to discriminate between the songs of birds from their own or another aviary. This may be related to the fact that females do not sing and do not learn any vocalization (22).

The male zebra finches discriminating between their own song and another song from their own aviary reached criterion in as few as 200 trials. We do not know how much of the training effort was necessary for the birds to master the training paradigm, memorize the song stimuli, and associate each stimulus with a *go* or *no-go* response. However, such an acquisition rate is, to our knowledge, more rapid than any reported for songbirds using other natural or arbitrary auditory stimuli and operant techniques (9, 23–25). This suggests that the use of conspecific song, and particularly use of a bird's own song, can provide an experimenter with a rapid and reliable assessment of whether anatomical or physiological manipulations alter song discriminations (26–29). We do not know whether the bird's own song is easier to discriminate because it is very familiar or because the bird has learned to produce it and therefore might process it differently.

There are some similarities between our results and those obtained with electrophysiological methods. Brain nuclei that are part of the song motor pathway respond to auditory stimulation (15, 30). Maximal responses in males often occur

when the bird hears a playback of its own song (31, 32). However, physiological studies do not reveal whether the neural activity has behavioral correlates. Field studies suggest some correlates. For example, behavioral responses to song playbacks to chaffinches and song sparrows in reproductive condition are maximal when the song played is the bird's own (33, 34). In other cases, countersinging birds use songs that best resemble the stimulus song (35). These examples suggest that a bird's own song, encoded in its song system, helps it to discriminate between conspecific songs and influences the nature and strength of a response. Preliminary observations offer partial support for this interpretation. Male zebra finches with lesions in their song system fail to discriminate easily between their own song and that of a conspecific (26, 28, 29).

Gender Differences. Females required more trials than males to discriminate between songs from their own aviary. This could have come about because of differences in how the two sexes process song or because of social factors.

Most of the nuclei of the song system are larger in male than in female zebra finches (12) and certain connections that exist in males appear to be absent in females (13, 14). Moreover, parts of the motor pathway that respond to sounds in male zebra finches do not respond in females (14, 15). If these anatomical and physiological differences affect the way in which the two sexes attend to and perceive conspecific song, then this could be the basis for gender differences in song discrimination.

We do not know if the song system of female songbirds responds selectively to conspecific song, as has been described for males (31, 32). Electrophysiological recordings from the song system of females have been limited to responses to arbitrary stimuli (14). Our results (and results presented in ref. 36) suggest that these female data would be particularly interesting to have. Does the ability of females to discriminate between conspecific songs require, as in males (26, 28, 29), the presence of the nuclei used by males for song production?

Juvenile male and female zebra finches may interact differently with adult males, and this social factor may favor the recollection of aviary songs by males but not by females. Adult female zebra finches remember and respond preferentially to their father's or mate's song (37, 38), and therefore females might find discriminations that included the song of their father or mate particularly easy to master.

In this article, we assumed that the ability to produce the operant response should not differ between the sexes. This assumption seems supported by the fact that males and females required a comparable number of trials to discriminate between the songs of males from another aviary.

Seasonal Effects on Song Discriminations. Zebra finches are characterized as opportunistic breeders (39, 40). The term implies that the reproductive biology of zebra finches is not affected by seasonal changes in day length. Our study provides evidence that song discrimination in male and female zebra finches is influenced by season. We have found a similar seasonal effect in other song discrimination experiments (28). Another report suggests that the seasonal effect on song discrimination results from hormonal variables and that it is specific to zebra finch song (27).

Conclusion. The prominence of the song system of birds for neuroethological studies of learning has been based primarily on experiments that focused on song as an acquired motor skill. However, recent evidence suggests that the production and perception of song may be closely related (15, 31, 32, 41). The results presented here are a first attempt to identify the kinds of song stimuli that are readily discriminated and the variables that affect the process of discrimination. All mem-

bers of a species may not attend to or perceive song in the same manner. Instead, these functions may differ between individuals depending on their gender, song, and time of year.

We thank Barbara Petri, Mary Lou Sotanski, and Gabor Tomasz for their laboratory assistance, Marta Nottebohm and Heather Williams for helpful editorial comments, and Gregory Ball for raising two of the zebra finches. This research was supported by National Institutes of Health Grant DC 00182, the Marilyn M. Simpson Charitable Trust, the Whitehall Foundation, and the Mary Flagler Cary Charitable Trust.

- Weeden, J. S. & Falls, J. B. (1959) *Auk* **76**, 343–351.
- Falls, J. B. (1982) *Acoustic Communication in Birds* (Academic, New York), Vol. 2, pp. 237–278.
- King, A. P. & West, M. J. (1977) *Science* **195**, 1002–1004.
- Searcy, W. A. & Marler, P. (1981) *Science* **213**, 926–928.
- West, M. J. & King, A. P. (1988) *Nature (London)* **334**, 244–246.
- Sinnott, J. M. (1980) *J. Acoust. Soc. Am.* **68**, 494–497.
- Shy, E., McGregor, P. K. & Krebs, J. R. (1986) *Behav. Proc.* **13**, 1–12.
- Weary, D. M. (1989) *J. Comp. Psychol.* **103**, 320–325.
- Cynx, J., Williams, H. & Nottebohm, F. (1990) *J. Comp. Psychol.* **104**, 303–308.
- Dooling, R. J., Park, T. J., Brown, S. D. & Okanoya, K. (1991) *J. Comp. Psychol.* **101**, 367–381.
- Konorski, J. (1967) *Integrative Activity of the Brain: An Interdisciplinary Approach* (Univ. of Chicago Press, Chicago).
- Nottebohm, F. & Arnold, A. P. (1976) *Science* **194**, 211–213.
- Konishi, M. & Akutagawa, E. (1985) *Nature (London)* **315**, 145–147.
- Williams, H. (1985) *Behav. Neural Biol.* **44**, 470–484.
- Williams, H. & Nottebohm, F. (1985) *Science* **229**, 279–282.
- Williams, H. (1990) *Anim. Behav.* **39**, 745–757.
- Stevenson, J. G. (1967) *Anim. Behav.* **15**, 427–432.
- Price, P. H. (1979) *J. Comp. Physiol. Psychol.* **93**, 260–277.
- Sossinka, R. & Böhner, J. (1980) *Z. Tierpsychol.* **53**, 123–132.
- Immelmann, K. (1969) in *Bird Vocalisations*, ed. Hinde, R. A. (Cambridge Univ. Press, London), pp. 61–74.
- Brown, S. D., Dooling, R. J. & O'Grady, K. (1988) *J. Comp. Psychol.* **102**, 236–247.
- Simpson, H. B. & Vicario, D. S. (1990) *J. Neurosci.* **10**, 1541–1556.
- Hulse, S. H., Cynx, J. & Humpal, J. (1984) *J. Exp. Psychol. Gen.* **113**, 38–54.
- Park, T. J. & Dooling, R. J. (1985) *J. Comp. Psychol.* **99**, 391–402.
- Okanoya, K. & Dooling, R. J. (1986) *J. Acoust. Soc. Am.* **83**, 1690–1693.
- Cynx, J. & Nottebohm, F. (1989) *Assoc. Res. Otolaryngol. Abstr.* **12**, 264.
- Cynx, J. & Nottebohm, F. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1376–1378.
- Cynx, J., Williams, H. & Nottebohm, F. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1372–1375.
- Cynx, J., Scharff, C. & Nottebohm, F. (1991) *Soc. Neurosci. Abstr.* **17**, 1051.
- Katz, L. C. & Gurney, M. E. (1979) *Brain. Res.* **221**, 192–197.
- Margoliash, D. (1983) *J. Neurosci.* **3**, 1039–1057.
- Margoliash, D. (1986) *J. Neurosci.* **6**, 1643–1661.
- McArthur, P. (1986) *Anim. Behav.* **34**, 199–207.
- Hinde, R. A. (1958) *Anim. Behav.* **6**, 211–218.
- Falls, J. B., Horn, A. G. & Dickinson, T. E. (1988) *Anim. Behav.* **36**, 579–585.
- Brenowitz, E. A. (1991) *Science* **251**, 303–305.
- Miller, D. B. (1979) *Anim. Behav.* **27**, 376–380.
- Miller, D. B. (1979) *Nature (London)* **280**, 389–391.
- Immelmann, K. (1971) in *Avian Biology*, eds. Farner, D. S. & King, J. R. (Academic, New York), Vol. 1, pp. 341–389.
- Marshall, A. J. & Serventy, D. L. (1958) *J. Exp. Biol.* **35**, 666–670.
- Nottebohm, F., Alvarez-Buylla, A., Cynx, J., Kirn, J., Ling, C.-Y., Nottebohm, M. E., Suter, R., Tolles, A. & Williams, H. (1990) *Philos. Trans. R. Soc. London Ser. B* **329**, 115–124.