INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI®

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A

GENETICAL AND DEVELOPMENTAL ANALYSIS OF EARLY CALL ONTOGENY IN THE CANARY, *SERINUS CANARIA.*

by

JOSEPH F. EMMETT

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1999

UMI Number: 9946159

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

6/1/99
Date

Paul C Mundinger
Chair of Examining Committee, Dr. Paul Mundinger

6/7/99
Date

Richard L. Chappell
Executive Officer, Dr. Richard L. Chappell

Carol A. Simon
Dr. Carol Simon, City College

Dr. Peter Moller, Hunter College

Helen S Cairns
Dr. Helen Cairns, Queens College

Robert F Calhoon
Dr. Robert Calhoon, Queens College

Dr. Donald Bruning, New York Zoological Society

Donald Bruning
Supervising Committee

The City University of New York

Abstract

GENETICAL AND DEVELOPMENTAL ANALYSIS OF EARLY CALL ONTOGENY IN THE CANARY, *SERINUS CANARIA*.

by

Joseph F. Emmett

Adviser: Professor Paul C. Mundinger

Border and Roller canaries have been subjected to differential selection regimes. Border canaries have been bred for external morphology while Rollers have been bred for acoustically pleasing low frequency song tours. This study found that the flight, hunger-location and nestling calls of Roller canaries have sustained a significant reduction in frequency (kHz), suggesting that artificial song selection has affected calls.

Reciprocal cross-fostering experiments between Rollers and Borders revealed that Border canary call ontogeny is influenced by the social environment to a greater extent than is Roller call ontogeny.

Reciprocal hybrids (Roller female x Border male, (RB); and Border female x Roller male, BR) produced calls of intermediate frequencies. Further call analysis for the reciprocal hybrids demonstrated a paternal effect. RB hybrids produced calls with more Border-like frequency values. Conversely, BR hybrids produced calls with more Roller-like frequency values. These results are compatible with the phenomenon of parental genomic imprinting. In genomic imprinting the phenotypic expression of the genotype is dependent on the parental origin of the gene(s).

Individual call stereotypy was identified for both the flight call and the hunger-location call, however, the canary nestling call was found to have variable forms (shapes). Birds demonstrating flight call stereotypy possessed one or two stereotyped patterns while all canaries showed individually distinctive signature hunger-location calls.

Artificial selection on Roller canary song has altered hunger-location call frequency modulation patterns. Roller hunger-location calls are either tonal or begin with an ascending frequency sweep. In contrast, Border canaries (which have not been consciously selected for vocal characters) produce hunger-location calls with initial frequency sweeps which descend.

The hunger-location call appears to be employed as a signal which permits parents to locate and subsequently feed fledged young. This study found that canary fledglings can recognize their mother's vocalization and respond to her with their signature hunger-location calls.

Acknowledgements

Special thanks to Professor Paul C. Mundinger who suggested this project and continually provided critical insights which proved to be invaluable in bringing this study to fruition. Without Dr. Mundinger's expertise, patience and guidance, this study would have been impossible. I am truly grateful to have had such a knowledgeable and talented mentor.

Next, I would like to thank Dr. Carol Simon, Dr. Paul Maderson, Dr. Helen Cairns, Dr. Donald Bruning, Dr. Peter Moller and Dr. Robert Calhoon, for serving on my graduate committee and contributing their professional criticism to this manuscript. Dr. Calhoon's statistical guidance was particularly helpful and very much appreciated.

I would also like to express my appreciation to the following people. To Mr. Ken Page, I would like to say thank you for your welcome companionship on our numerous photocopying "expeditions" to various college libraries. To Mr. and Mrs. Salvatore Mangano, thank you for your constant love, and encouragement as well as for world class cuisine at "Angelina's restaurant." To my parents, Anna and Bertrand C. Emmett, thank you for a lifetime of love, support and understanding. You are the finest parents a son could ever hope for.

Finally, and most importantly, I would like to dedicate this work to my wife, Camille for her patience, devotion and love during the trying times that a study of this size inevitably generates. Thank you for always believing in me and aiding me with your unqualified support. I am truly blessed to have you as my wife.

Table of Contents

List of Tables

List of Illustrations

CHAPTER 1: GENERAL INTRODUCTION:

Aviculturalists have bred the domestic canary, *Serinus canaria*, for at least 470 years (Darwin 1868). Since the 1800's, enthusiasts in the Harz mountains of Germany have bred Roller canaries for their pleasing low frequency song tours, while British fanciers selected Border canaries for posture and form. By examining the calls of these two differentially selected strains, this study sought to illuminate a neglected area of avian vocal development (call ontogeny) and shed some light on the avian song-call dichotomy.

Although there is some debate as to whether dividing avian vocalizations into songs and calls is a real or an artificial distinction, this dichotomy has a history of support in the literature. For example, Lanyon (1960) described the "call notes" of passerines (perching birds) as being similar to non-passerine vocalizations in that both are stereotyped and genetically fixed; vocal learning was considered a quality unique to song. More recently, exceptions have been identified. Mundinger (1970, 1979) has demonstrated that several species of cardueline finches are capable of learning new flight calls. Interestingly, European siskins, *Carduelis spinus*, American goldfinches, *Carduelis tristis*, and American pine siskins, *Carduelis pinus*, imitated both intra and interspecific flight calls without the restriction of a critical learning period often seen in song imitation.

Nowicki (1989) investigated intra-flock call convergence in black-capped chickadees; *Parus atricapillus,* and identified significant vocal convergence within one week of flock creation. Frequency bandwidth convergence was most dramatic for the D-note of the complex "chick-a-dee" call. For birds with the ability to imitate flock mates, call learning should be "open-ended" in order to accommodate turnover in flock membership. Song too can be the product of an "open-ended" process. Unlike the zebra finch, *Taeniopygia guttata*, an age-limited learner, canaries and starlings, *Sturnus vulgaris*, posses the ability to learn new songs in successive years, although the starling's learning capacity is inversely correlated with age (Eens et al. 1992).

Chestnut-sided warblers, *Dendroica pensylvanica,* have a song repertoire which contains two vocal classes; one with accented endings (AE), and one with unaccented endings (UE). These two classes are both considered "song", yet developmentally they are compartmentalized. This was demonstrated in a series of tutoring experiments performed by Byers and Kroodsma (1992). They suggested that AE song is more canalized by a neural template while UE song is shaped more effectively by social interaction.

The boundary between songs and calls is further blurred when considering the repertoire of the rufous-sided towhee, *Pipilo erythrothalamus.* Ewert (1979) observed towhees giving calls which were also used as song syllables. In addition, chaffinches, *Fringilla coelebs*, deafened at three months of age, produce songs with elements similar to the food-begging calls of fledglings (Nottebohm 1970).

Lemon and Herzog (1969) speculate that the songs of cardinals, *Richmondena cardinalis,* and pyrrhuloxias, *Pyrrhuloxia sinuata*, may develop from their species contact calls. These calls contain a percussive sound of broad frequency similar to the sound produced at the beginning or end of many song syllables.

Context may be an important factor in determining whether call notes are incorporated into song. During canary subsong (an early stage of song ontogeny marked by variable song notes), alarm and other call notes are often part of the vocalization (Poulsen 1959); then when crystallization occurs, these calls are dropped from the song. Mundinger (pers. comm.), however, has evidence that a bird (RB-02) tutored with wild canary song learned the song and in addition incorporated background alarm call notes as part of his learned song pattern. This alarm call pattern (within the song) was then modified toward a less call-like form at the time of song crystallization.

Because the song/call dichotomy often appears to be more of a song/call continuum, it may be helpful to consider the operational definitions found in Baker and Bailey (1987) as points of reference: “Song is defined as a vocal display in which one or more sounds are consistently repeated in a specific pattern produced usually by males only and mostly limited to specific periods of the year associated with breeding. Calls, as distinguished from songs, are given by birds of both sexes, of all ages, and at all times of the year."

While these definitions are useful as a frame of reference, they are not without their exceptions. Female oscines occasionally do sing (Poulsen 1959) and aerial alarm calls are given almost exclusively by male chickens, *Gallus gallus,* a non-oscine (Evans et al. 1993). Also, Mundinger (doctoral dissertation) found that some American goldfinch calls were given only during the breeding season, not throughout the year.

This study was also designed to investigate the role of both learning and genetics in canary call ontogeny. The nature/nurture controversy has been debated for many years with much energy and conviction (Barlow 1991). Buffon and Barrington provide historical perspective: while conceding that canaries are able to imitate both titlark and nightingale notes, Buffon (1791) argues that animal behavior "proceeds from nature and not education." Barrington (1791) takes the counterpoint, stating that "notes in birds are no more innate than language is in man."

Currently, two schools of thought, the "ethological" of evolutionary biology (Marler 1984) and the "interactionist" of developmental psychology (Johnston 1988) actively debate the importance of genes and learning in behavioral ontogeny. For some ethologists, the learned-innate dichotomy is real and the behavioral phenotype is seen to contain both innate and acquired aspects, with learning subordinate to instinct. This sentiment is made clear by Tinbergen (in Goodenough et al. 1993): "...learning and many other higher processes are secondary modifications of innate mechanisms, and ...therefore a study of learning processes has to be preceded by a study of the innate foundations of behavior."

For interactionists, the partitioning of behavior into nature and nurture is a bankrupt dichotomy whose time has come and gone; it is a theoretical framework which retards a deeper understanding of behavioral ontogeny. Johnston (1988) argues that when the approach to ontogeny is not interactionist, "experience" is taken to be synonymous with "learning"; for this reason nonobvious contributions to vocal development may be overlooked. An example of a nonobvious contribution is the subtle wing flicks of female cowbirds, *Molothrus ater,* housed with a male whose song is "shaped" by this subtle behavior (West and King 1985).

Gottlieb (1981), another interactionist, identifies three roles for experience in the ontogeny of behavior: maintenance, where experience is critical if a behavior is to be maintained; facilitation, where experience enables the appearance of a particular behavior at the appropriate stage of development; and induction, where a behavior will never be performed unless the necessary experience is provided.

Evidence for the inductive nature of experience is seen in the behavioral ontogeny of mallards, *Anas platyrynchos*, and wood ducks, *Aix sponsa*. These birds (while still in the egg) need to hear the calls of embryos or hatchlings in order to develop response specificity to the maternal call (Gottlieb 1980a; 1980b).

From the interactionist point of view, genes play an important but as yet unspecified role in ontogeny and it is important not to pigeon-hole developmental components into learned and innate categories. This synthetic perspective stands in contrast with Lorenz's belief that development was a process of intercalating instincts and learning (in Barlow 1991).

The ethological perspective concerning avian vocal ontogeny is supported by a large and compelling literature. Chaffinches, *Fringilla coelebs*, raised as Kaspar Hausers (isolates) produce extremely simple songs. Thorpe (1958) concluded that "full chaffinch song is an integration of inborn and learned song patterns, the former constituting the basis for the latter." Chaffinch song was further investigated by Slater and Ince (1982). They determined that the precise structure of individual notes was learned while the temporal pattern of song appeared to be under genetic control.

A similar situation was described in the cardueline finches. Working with canaries and greenfinches, *Chloris chloris*, Guttinger (1977, 1979) reports that a "genetically determined program" sets temporal parameters and controls the general pattern of individual elements in song, while the exact structure of single notes and their syntax are influenced by learning.

Calls, once thought to be completely innate, also appear to have learning and genes as determining elements. Twites, *Acanthus flavirostris*, and house finches, *Carpodacus mexicanus*, are, like canaries, cardueline finches (members of the sub-family Carduelinae). Both species produce flight calls of a general pattern that appear to be species-typical, which infers genetic causality. For twites the general pattern is an abrupt downslur, which is individually modified by learning (Marler and

Mundinger 1975). For house finches the species-specific "V" sonographic pattern of the flight call is plastic enough to become pair-specific through imitation (Mundinger 1979). Other carduelines, such as siskins and goldfinches, also have species-typical call patterns that can be modified slightly by learning while still retaining the overall species typical pattern (Mundinger 1979).

Finally, some species produce a call repertoire which contains both learned and innate calls. Differential call ontogeny was investigated in galahs, *Cacatua roseicapilla,* naturally cross-fostered by Major Mitchell's cockatoos, *C. leadbeateri* (Rowley and Chapman 1986). Galahs, raised by their sympatric congeners, retained galah food begging and alarm calls while learning the Major Mitchell's contact calls. Apparently the food begging and alarm calls rely heavily on innate mechanisms, while contact call structure is significantly influenced by learning.

This study addressed the genetic aspect of canary call ontogeny by analyzing the calls (nestling calls, hunger-location calls and flight calls) of Border canaries, Roller canaries and their reciprocal-cross hybrids. The environmental influence on call ontogeny was assessed by studying the calls of reciprocally cross-fostered Border and Roller canaries.

The calls studied in this thesis develop sequentially (first nestling, then hunger-location and finally the adult flight call), but in this manuscript, I present the adult flight call study first, then the hunger-location call work and finally I treat the earliest to develop nestling call last.

CHAPTER 2: THE CANARY FLIGHT CALL:

INTRODUCTION:

Aviculturalists have bred the domestic canary, *Serinus canaria*, for at least 470 years. Even before 1718, French enthusiasts published a list of some twenty-seven varieties (Darwin 1868). The different canary breeds were selected for different characters; for example, Border canaries from Britain were selected for posture and morphological characteristics while German Roller canaries were selected for song quality.

Avian vocalizations can be altered by artificial selection (Sossinka 1982; Baptista and Abs 1983). Having examined the effects of domestication on song structures in the canary, Guttinger (1985) found wild canary song to be structurally closer to Border song than Roller song. These differences are presumably the result of selective breeding programs, carried out on the Roller canary since the beginning of the 19th century for acoustically pleasing song patterns. These patterns are called tours (a tour is a repetitive sequence of identical syllables). For the Roller canary, breeders recognize nine basic tour patterns (discernable to the trained ear): bass rolls, hollow rolls, bell rolls, water tours, schockles, glucks, and flutes (Guttinger 1985). In addition to selecting for these tours, breeders have also selected against the rough sounding syllables frequently found in wild song. The purpose of this study was to examine how differential selection on Roller and Border song structure influenced the flight calls of these two strains and their reciprocal-cross hybrids.

Avian songs and calls are often presented as two mutually exclusive vocal categories (Spector 1994). Song is traditionally considered a male vocalization associated with territorial defense and or mate attraction. Calls, on the other hand, are produced by both males and females with both sexes calling during all seasons (Baker and Bailey 1987).

But these definitions do not address the issue of genes and learning when making the distinction between songs and calls. This is not to imply that genetics and experience are irrelevant. Rather, the empirical evidence often does not support the commonly held generalization that all calls are innate while imitation is unique to song. Several decades ago the calls of passerines were considered to be similar to non-passerine vocalizations in that both were thought to be stereotyped and genetically fixed (Lanyon 1960). At that time vocal learning was believed to be quality unique to song. Now, however, there are numerous exceptions. Initially, Mundinger (1970, 1979) demonstrated that several species of cardueline finches were capable of learning new flight call patterns, and more recently, Groth (1993) suggests that flight call matching between red crossbill pairs is the result of learning.

This dissertation included a cross-fostering experiment in order to assess the environmental influence on canary flight call development. In addition several hypotheses were tested experimentally.

<u>Hypotheses tested</u>:

1) Songs and calls are part of one vocal communication system and therefore, the song/call dichotomy is arbitrary and utilized for convenience.
2) Genetic background affects flight call development.
3) The social environment affects flight call development.
4) Flight call production is influenced by sex linked factors.
5) Flight calls provide evidence for genomic imprinting at the behavioral level.

MATERIALS AND METHODS:

Sample size:

This study analyzed 3,030 flight calls from 39 domestic canaries (*Serinus canaria*) hatched between 1994 and 1997. The 39 birds represented six genetic or environmental treatments: (1) nine normal-reared Borders, BB (five males, four females), (2) eight normal-reared Rollers, RR (six males, two females), eight reciprocal-cross hybrids, (3) four BR hybrids with a Border mother and Roller father (one male, three females) and (4) four RB hybrids with reciprocal parentage (two males and two females). The final two treatments represent reciprocally cross-fostered Border (CFBB) and Roller (CFRR) canaries: (5) nine cross-fostered Borders (five males, four females) and (6) five cross-fostered Rollers (three males and two females).

The following birds died young and were therefore sexed by using subsong. Birds that produced subsong were considered male while those that did not were considered female: RR-20 male, RR-22 male, BB-22 male, BB-23 female, BB-24 female.

Rearing:

All birds developed their calls in either Hartshorne or IAC soundproof isolation chambers with the father removed prior to hatching (FB-01 and FB-02 are exceptions, their father was removed ten days post-hatch). Mothers were removed when chicks were between 26 and 30 days of age, and all siblings were then maintained as isolated family groups for the duration of the study. Birds cross-fostered in 1996 were reared in isolation booths large enough to accommodate several cages; therefore, it was possible for these birds to hear (but not see) adult birds (both male and female) from the cross-fostering strain as well as same-strain, cross-fostered juveniles from their 1996 cohort. All cross-fostered Borders and Rollers were hatched and reared by a female of the alternative strain.

Individuals were maintained on a 14:10 h light:dark cycle corresponding to the photoperiod for the latitude of New York City and at a temperature of approximately 21°C. Food and water were available *ad libitum.*

Recording:

Observations were conducted through either a one-way glass window, or through a normal window with the observer in a darkened room, and lasted for a minimum of 2 hours/day. The first flight calls were recorded between 30 and 46 days of age and the last were recorded between 50 and 70 days of age when the adult call repertoire is generally in place. After this time most of the male birds were transferred to another study (a song learning study). An average of 6.3 calls/day/individual were analyzed, with an average of 14.9 ± 4.7 days of sampling per individual. In an effort to identify flight call pattern changes over time, calls were analyzed from both the first ten days and last ten days of calling.

Recordings were made using a Radio Shack ultra mini tie clip microphone (cat. No. 33-3003). Since sibling groups remained intact throughout the study, colored plastic leg bands were used to facilitate the individual identification of callers.

Sonographic Analysis:

Recordings of flight calls were analyzed on a Uniscan model 4500 real-time spectrum analyzer using the 10 kHz scale. Frequency values were measured by positioning the on screen cursor at the maximum and minimum frequency points for each call and subsequently recording the frequency values from the digital display. When the vocal elements for some Roller flight calls became noisy (hence more difficult to delimit), frequency measurements were taken at the points where sound energy was concentrated. The fundamental frequency was used for measurements if the call contained harmonics. Call duration was measured using a metric ruler placed against the Uniscan monitor screen: 5.6 cm=1.0 s. Sampled calls were selected for their high signal to noise ratio and spectrogram hard copy was photographed on Kodak 35-mm linograph paper.

Statistical analysis:

There were six parameters measured and subjected to statistical analysis: 1) maximum frequency of the call, 2) minimum frequency of the call, 3) frequency range of the call, 4) call duration, 5) number of elements/call, 6) call rate (no. elements/s). Bonferroni tests, regression analysis, one-way ANOVAs and two-way ANOVAs were used to test for significant differences between the treatments. The standard deviation was used as a measure of data dispersion about the mean.

RESULTS:

Qualitative analysis:

The flight call pattern consists of 1 to 8 elements, with multiple elements being produced rapidly creating a trilling quality. An element is defined as a continuous trace on a sonagram temporally distinct from adjacent elements (Eales 1985). These calls are given in a flight context as birds flit from perch to perch, but may also be given when poised for flight or upon completion of flight. The calls are given with the bill either open or closed and are produced antiphonally. After analyzing 3,167 flight calls (produced by birds with siblings) I have found no evidence of cage mates calling contemporaneously. The only cases of simultaneous calling were seen when several flight cages were maintained in the same soundproof booth. The vocal overlap occurred when birds in different cages called at the same time (this overlap could be seen when viewing the Uniscan). Since birds that called simultaneously could not see each other it appears that vocal turn taking may be visually mediated.

Figures 1-12 illustrate representative sonagrams of canary flight calls. During the 1st ten days of flight call production, canaries produce calls with considerable structural variability. Within this period 26 of the 39 birds studied produced flight calls which lacked stereotypy (Table 1). (Close matching of call patterns provided evidence of stereotypy). The vocal plasticity evident during the 1st ten days of call ontogeny was much reduced during the last ten days of sampling. At this stage of development (although calls can still be produced with variability) 33 of 39 birds produced flight calls illustrating individual stereotypy (Table 2). These data suggest that eventually juvenile canaries produce flight calls with only one or two stereotyped patterns.

Both normal reared and cross-fostered Roller canaries produce flight calls containing elements of shorter duration and lower frequency than both normal reared and cross-fostered Border canaries. Furthermore, the temporal interval between elements is shorter in Roller flight calls than in Border flight calls. These two factors (shorter elements and shorter intervals) underlie the Roller canary's tendency to produce flight calls at a faster trill-delivery rate than Border canaries.

While Border canary element patterns sometimes demonstrate structural complexity (Figures 1 and 2), most Roller canary flight calls are simply a series of low frequency fundamentals (often in the shape of chevrons) at the base of noisy harmonics (Figures 3 and 4: however, see RR-23 and RR-24 for exceptions, Fig. 4). A chevron shaped element is characteristic of Roller flight calls. Border canaries will use the pattern as well, however, they deliver it at a higher frequency (e.g. BB-01, Fig. 1; and BB-05, Fig. 2). Interestingly, unusually low pitched Roller-like chevrons were produced during the first ten days of call ontogeny by the cross-fostered borders BB-23 and BB-24 (Figure 9).

Analysis of the reciprocal hybrids revealed that RB canaries produce flight call elements more closely resembling Border elements than Roller elements, however, the RB calls are delivered at a lower frequency than Border calls. BR hybrids give flight calls at frequencies (in kHz) below RB birds. These BR flight calls are often structurally similar to Roller flight calls (however, BR-1 and BR-3 did produce some Border-like elements, Figures 5 and 6). These reciprocal-cross hybrid results suggest that the paternal genetic contribution may influence the phenotype of canary flight calls. The flight calls produced by BR-18 clearly demonstrate the importance of genes as causal elements in flight call production. This particular hybrid was raised as an isolate (it had no siblings) therefore its only social interaction was with its Border mother, yet its flight call pattern is decidedly Roller-like (Figures 5 and 6).

Quantitative analysis:

1a. Maximum frequency for the 1st ten days of sampling:

A two-way ANOVA revealed that there is a highly significant difference in maximum frequency between the six treatments (F(5,1481) = 319.72, *P*<.0001: see Table 3 and Fig. 13) as well as between the sexes (F(1,1481) = 45.93, *P*<.0001; Fig. 14). Within Rollers, however, Bonferroni tests did not reveal a significant difference between Roller males and females whether the birds were cross-fostered or normal-reared (these tests were performed at the alpha = 0.05 level of significance). In addition, the maximum frequency of normal-reared Roller flight calls was not found to be significantly different from the calls of cross-fostered Rollers (as alpha is > 0.05, Bonferroni).

Border canaries produce flight calls with the highest maximum frequencies while Roller canary calls are delivered at the lowest maximum frequencies (Fig. 13). Considering that German Roller canaries have undergone strong artificial selection for pleasing low frequency songs, there may be a common causal link between their songs and calls. Border canaries have not been subjected to song selection. Flight calls for cross-fostered Borders, however, are significantly lower in frequency than normal-reared Borders (alpha = 0.01, Bonferroni) providing evidence for greater plasticity in Border call production as compared to Roller calls, and an important role for the social environment in its call ontogeny.

The reciprocal hybrid treatments (RB and BR) were found to be intermediate to Borders and Rollers, with RB birds calling at a higher mean frequency than BR individuals. However, this difference is not significant within sexes and between these two (RB and BR) hybrid treatments (as alpha is > 0.05, Bonferroni; Table 3).

Figure 14 demonstrates that there is a significant sex by treatments interaction for maximum frequency (two-way ANOVA, F(5,1481) = 13.0, *P*<.0001). This interaction reveals that male canaries give flight calls at higher maximum frequencies than females and the difference in maximum frequency between males and females was significantly different among the six treatments. It should be noted that normal-reared Border males delivered flight calls at lower maximum frequencies than normal-reared Border females, however this difference was not significant (as alpha is > 0.05, Bonferroni).

1b. Maximum frequency for the last 10 days of sampling:

A two-way analysis of variance performed on all birds pooled for the last ten days demonstrated no significant difference between the sexes in maximum frequency production ($\bar{x}$=3.52 kHz for males, 3.51 kHz for females; F(1,1525) = 0.08, *P*=.78). The frequency difference between breeds (Table 3) and the sex by breeds interaction, however, were both highly significant (F(5,1525) = 822.50, *P*<.0001; and F(5,1525) = 14.50, *P*<.0001). Figure 15 illustrates the interaction and illuminates an interesting development involving the RB hybrids who show a marked increase in maximum frequency over their 1st 10 days value. At this stage RB hybrids call at a maximum frequency much closer to Borders than earlier, while BR hybrids are producing flight calls with maximum frequencies closer to Rollers for both 10-day periods. (compare Figs. 14 and 15). Since RB hybrids had Roller mothers yet their call increased in frequency and their genomes came from both sexes, these results suggest that for the maximum frequency of flight calls in the last 10 day period, the paternal genetic contribution has a marked influence on this parameter, an effect which may have been masked by the maternal social contribution in the earliest 10 day period of development

Regression analysis performed on hybrid flight calls revealed that the maximum frequency of BR flight calls tended to decrease over time (when the 1st and last 10 days of sampling are compared) while the maximum frequency of RB flight calls increased. This trend caused the RB calls to become more Border-like in maximum frequency and BR calls to become more Roller-like in maximum frequency. Regression coefficients for BR and RB hybrids were compared and found to be significantly different (ANOVA, F(2,967) = 361.13, $P<.0001$).

Another interesting development illustrated in figure 15 is that cross-fostered females produce flight calls at a higher mean maximum frequency than cross-fostered males. In all other treatments the male call had the higher mean maximum frequency. The reason for this frequency reversal is unclear to me and may represent a chance effect.

Similar regression analyses were made for the four other treatments (BB, CFBB, RR, and CFRR). Of the nine normal reared Borders studied, four birds demonstrated a significant increase in maximum frequency over time (for three birds $P<.0001$, one bird $P=.0006$); four birds did not show a significant correlation; and one Border showed a significant decrease in maximum frequency over time.

Regression analysis for the cross-fostered Borders revealed that seven birds showed a significant increase in maximum frequency over time ($P<.001$) while two birds did not demonstrate a significant correlation between maximum frequency and time. Interestingly, it appears that the Border ontogenetic program, which results in frequency increase over time, may be active in the development of RB flight calls. All four RB hybrids studied showed a similar pattern of frequency increase. Once again the paternal pattern is demonstrated in both male and female RB hybrids.

The regression analysis for normal reared Rollers showed that maximum frequency for three birds decreased significantly over the duration of the study ($P<.001$) while five birds showed no significant correlation between maximum frequency and age in days. Similar analyses for the cross-fostered Rollers identified one bird with a significant decrease in maximum frequency over time ($P=.01$), one bird with a significant increase ($P=.003$) and three Rollers which did not show a significant correlation between maximum frequency and time.

Apparently, the developmental program (active in Rollers) which favors a decrease in maximum frequency or produces no significant change over time is also important during the development of BR hybrid flight calls. BR hybrids show a similar pattern of either decreasing maximum frequency or no significant change in maximum frequency over time. (N.B. An exception is the cross-fostered Roller, RR-05 which showed a significant increase in maximum frequency over time

P=.003). This result provides further evidence for the importance of the paternal genetic contribution to the phenotype.

2a. Minimum frequency for the 1st ten days of sampling:

A two-way ANOVA revealed that there is a highly significant difference in minimum frequency between the six treatments; F(5,1481) = 228.66; $P<.0001$, see Table 3 and Figure 16.

Interestingly, the minimum frequency parameter demonstrates the same ranking sequence (BB to CFRR) as maximum frequency when the six treatments are considered in order of decreasing mean frequency (Fig. 16). The difference in minimum frequency between the sexes (for all birds) is highly significant (two-way ANOVA, F(1,1481) = 14.27; $P<.001$) as is the sex by treatments interaction (two-way ANOVA, F(5,1481) = 6.49; $P<.0001$; Fig 17).

As was demonstrated with maximum frequency, the mean minimum frequency for BR hybrid flight calls is lower in frequency than RB hybrid flight calls, once again suggesting the salience of the paternal genetic contribution to the behavioral phenotype.

Also of interest, at this stage in call development, cross-fostered Borders are producing calls with mean minimum frequencies closer to normal reared Rollers than to normal-reared Borders; in fact, the difference between cross-fostered Border females and normal reared Roller females is not significant (as alpha is > 0.05, Bonferroni). Conversely, cross-fostered Rollers are giving flight calls with mean minimum frequencies below even normal reared Rollers. It should be noted, however, that the difference between normal reared Roller males and cross-fostered Roller males is not significant (as alpha is > 0.05, Bonferroni). Once again, Border canaries are revealed as a strain whose call parameters are influenced by their social environment, whereas Roller canaries appear to be so developmentally canalized that cross-fostered individuals (those with exposure to Border vocalizations) produce flight calls with the lowest mean minimum frequencies of all six treatments.

2b. Minimum frequency last ten days of sampling:

As in the first ten day period, when the calls given in the final ten days of sampling were analyzed a two-way ANOVA for all birds revealed that there is a highly significant difference in minimum frequency between the six treatments; F(5,1525) = 377.38, *P*<.0001, see Table 3. The difference in minimum frequency between the sexes, however, is not significant when all birds are analyzed together (two-way ANOVA, F(1,1525) = 2.29, *P*=.13). But further analysis revealed a significant sex by treatments interaction illustrated in Figure 18 (two-way ANOVA, F(5,1525) = 3.15 *P*<.01).

At this stage of call ontogeny the reciprocal hybrids continue to demonstrate the importance of the paternal genetic contribution to the canary flight call phenotype, with BR calls reaching lower frequencies than RB calls. In addition, both male and female BR hybrids show a drop in mean minimum frequency during the last 10 days as compared to the first ten days of recording, this trend is also seen in the normal-reared Rollers. The RB hybrids, however, reversed this: for these birds, both males and females show an increase in mean minimum frequency for the last ten days as compared to the first ten days. A similar minimum frequency increase is also seen for cross-fostered Borders (both males and females) and male normal-reared Borders (normal-reared Border females showed no difference in mean minimum frequency between the first and last ten days of recording). Paralleling the BR hybrids, both cross-fostered and normal-reared Rollers showed a decrease in mean minimum frequency during the last ten days and these two treatment groups produced flight calls with nearly identical minimum frequency values.

The minimum frequency data for the last ten days revealed that males call at higher minimum frequency levels than females (however, for RB hybrids the minimum frequency difference between males and females is not significant (as alpha is > 0.05, Bonferroni). This sex difference is probably not due to size since males are generally larger, yet have slightly higher- pitched calls.

3a. Frequency range first ten days of sampling:

During the first ten days of call production the six treatments were found to be significantly different for the frequency range parameter (two-way ANOVA, F(5,1481) = 98.07, *P*<.0001; Table 3).

The difference in frequency range between the sexes (for all birds) is highly significant (two-way ANOVA, F(1,1481) = 24.90, *P*<.0001) with females having less range than males, as is the sex by treatments interaction (two-way ANOVA, F(5,1481)

= 10.38, *P*<.0001). Figure 19 illustrates this sex by treatments interaction. At this stage of flight call ontogeny, the two hybrid treatments (BR and RB) are once again producing flight calls with parameter values (i.e. frequency range) between the normal reared Borders and the normal reared Rollers (birds with the broadest and narrowest frequency ranges respectively). Interestingly, the sex by treatments interaction is most pronounced for the hybrids, with the hybrid males producing flight calls of frequency range values well above females ($\bar{x}$ frequency range for males: BR= 1.88 kHz, RB=1.93 kHz; females: BR= 1.39 kHz, RB= 1.34 kHz). It is also worth noting that the frequency range difference between the reciprocal cross hybrid females is not significant, and neither was it for males (as alpha is > 0.05, Bonferroni). This changed in the last ten days of sampling (see below).

Cross-fostering narrowed the frequency range of cross-fostered Border calls, revealing the importance of the social environment in Border canary flight call ontogeny (it should be noted, however, that the frequency range of normal reared Border males vs. cross-fostered Border males, was not significant (as alpha is > 0.05, Bonferroni) while the frequency range of normal reared Border females vs. cross-fostered Border females, was significant at alpha = 0.05, Bonferroni). Roller canaries, however, did not demonstrate a significant cross-fostering effect for frequency range (as alpha is > 0.05, Bonferroni) providing further evidence of no, or very little, social effect on flight call ontogeny in Roller canaries.

3b. Frequency range last ten days of sampling:

Once again, as in the first ten day period, a two-way ANOVA demonstrated that there is a highly significant difference in frequency range between the six treatments; F(5,1525) = 241.27, $P<.0001$, Table 3). When compared to the first ten days of calls, the last ten days revealed a general increase in frequency range. Normal reared Rollers are an exception, showing a small mean frequency range decrease (from $\bar{x}$ = 1.09 kHz to x = 1.05 kHz).

The difference in frequency range between the sexes (for all birds) at this stage of ontogeny is no longer significant (two-way ANOVA, F(1,1525) = 0.04, $P=.85$); however, the sex by treatments interaction is highly significant (two-way ANOVA, F(5,1525) = 12.80, $P<.0001$). Figure 20 shows this sex by treatments interaction and illustrates the important changes in hybrid flight call frequency ranges. BR hybrid females are now significantly different from RB hybrid females (alpha = 0.01, Bonferroni). Similarly, BR hybrid males now produce calls significantly lower in frequency range than RB hybrid males (alpha = 0.01, Bonferroni). During this period of flight call development RB hybrids demonstrate frequency ranges closer to Borders while BR hybrid frequency ranges are more Roller-like.

The largest frequency increases are seen in the RB hybrids where RB females increase their call range by 0.74 kHz and RB males broaden their range by 0.62 kHz. The frequency increase for the RB males results in the largest frequency range of all the treatments ($\bar{x}$=2.55 kHz compare Figs. 19 and 20). This frequency range for the RB males is 0.31 kHz greater than the normal reared Border males (significant difference, alpha = 0.01, Bonferroni) and reflects the minimum frequency data where RB males produce flight calls with mean minimum frequencies 0.53 kHz below normal reared Border males. During this period, BR males demonstrate a decrease in mean frequency range which is consistent with the decreasing range trend seen in normal-reared Roller males.

Only cross-fostered Border males and cross-fostered Roller females demonstrated a significant cross-fostering effect when compared to their normal-reared counterparts on the frequency range parameter during the last ten days of sampling (alpha = 0.01, Bonferroni).

4a. Call duration first ten days of sampling:

Flight call duration (in seconds) is significantly different between the six treatments (two-way ANOVA, F(5,1481) = *P*<.0001, Table 4). The difference in flight call duration between the sexes (for all birds) is not significant (two-way ANOVA, F(1,1481) = 3.46, *P* =.063), however, the sex by treatments interaction is significant (two-way ANOVA, F(5,1481) = 5.09, *P*=.0001). Figure 21 illustrates this sex by treatments interaction. During these first ten days of flight call development, both normal reared Rollers and cross-fostered Rollers produce flight calls which are not significantly different from each other in duration (as alpha is > 0.05, Bonferroni). Border canaries produce calls of longer duration than Rollers, with cross-fostered Borders producing calls of longest duration ($\bar{x}$=0.224 sec for CFBB females and 0.240 sec for CFBB males).

4b. Duration last ten days of sampling:

The difference in flight call duration, between the six treatments, continues to be highly significant during this sampling period (two-way-ANOVA, F(5,1525) = 57.44, $P<.0001$; Table 4).

During the last ten days of calling, canaries tend to give flight calls of somewhat longer duration, with cross-fostered Borders and normal reared Rollers producing the longest and shortest calls respectively. Cross-fostered Borders continue to produce flight calls much longer in duration than normal reared Borders, reflecting the large variability within this parameter. It is interesting to note that Roller canaries, which have been artificially selected for longer duration song tours (in addition to low-pitched sounds), produce flight calls of the shortest duration.

The duration of hybrid canary flight calls is intermediate to Border and Roller flight calls, with males giving longer calls than females; however, the difference between treatments (BR vs. RB) is not significant for the hybrids (as alpha is > 0.05, Bonferroni).

5a. Number of elements/call first ten days of sampling:

A two-way ANOVA revealed that the number elements/flight call is significantly different between the six treatments; F(5,1481) = 57.96, $P<.0001$, see Table 4. The difference in number of elements/call between the sexes (for all birds) is not significant (two-way ANOVA, F(1,1481) = 0.85, $P=.2263$); however, the sex by treatments interaction is highly significant (two-way ANOVA, F(5,1481) = 7.31, P

<.0001). Figure 23 illustrates this interaction and shows that it is most pronounced for the reciprocal hybrids. Female BR hybrids produce calls with significantly more elements than female RB hybrids (alpha = 0.01, Bonferroni); however, the difference between the reciprocal males lacks significance (as alpha is > 0.05, Bonferroni).

Cross-fostered Borders (which give flight calls of longest duration) produce flight calls with the greatest number of elements. However, because cross-fostered Borders produce calls with an even greater mean number of elements per call than normal reared Rollers, there may be a great deal of individual variation within this parameter (Fig. 23).

Both normal reared Rollers and cross-fostered Rollers produce calls that are not significantly different in number of elements (as alpha is > 0.05, Bonferroni).

<u>5b Number of elements/call last ten days of sampling:</u>

As in the first ten-day period, when the calls given in the final ten days of sampling were analyzed two-way analysis of variance for all birds revealed that there is a highly significant difference in number of elements/flight call between the six treatments; F(5,1525) = 17.73, *P* <.0001, see Table 4.

The difference in number of elements/call between the sexes (for all birds) is highly significant (two-way ANOVA; F(1,1525) = 10.74, *P* <.0001) as is the sex by treatments interaction (two-way ANOVA, F(5,1525), *P* =.01). Figure 24 illustrates the interaction and demonstrates that cross-fostered Borders continue to give flight calls containing the greatest number of elements. At this stage of call ontogeny most males produce flight calls with a greater number of elements per call than females. As a possible exception, males of both Border treatments (BB and CFBB) produce slightly fewer elements/call than females. However, this difference between Border males and females (whether normal-reared or cross-fostered) is not significant (as alpha is > 0.05, Bonferroni).

In the last ten days of calling, normal reared Rollers show a decrease in the number of elements/call, while cross-fostered Rollers demonstrate an increase in the number of elements/call. Both Roller treatments, however, continue to lack significant differences between each other in number of elements/call, once again demonstrating the absence of a cross-fostering effect in Roller flight call vocalizations (as alpha is > 0.05, Bonferroni).

When compared with the first ten days of calling, the last ten days show that the reciprocal hybrids (BR and RB) both display a decrease in the number of elements/call. However, the relationship within the sexes and between hybrid treatments is consistent with the first ten days of calling (i.e. the difference in number of elements/call between males is not significant (as alpha is > 0.05, Bonferroni) and the difference between females is significant (alpha = 0.05, Bonferroni).

6a. Call rate (number of elements/sec) first ten days of sampling:

During the first ten day sampling period the six treatments produced flight calls at significantly different rates (two-way ANOVA, F(5,1481) = 125.04, *P*<.0001, see Table 4).

The difference in call rate between the sexes (for all birds) is significant (two-way ANOVA, F(1,1481) = 7.81, *P*=.0052) as is the sex by treatments interaction (two-way ANOVA, F(5,1481), *P*=.0105). Figure 25 illustrates this interaction and shows that normal reared Rollers produce flight calls with elements delivered at the fastest rate, while normal reared Borders give flight calls at the slowest delivery rate.

Cross-fostered Roller flight calls are delivered at a slightly lower rate than normal reared Rollers; however, the difference is not significant (as alpha is > 0.05, Bonferroni). Normal reared Border males are not significantly different from cross-fostered Border males (as alpha is > 0.05, Bonferroni), however, Border females show a significant cross-fostering effect (alpha = 0.01, Bonferroni).

The flight call rate of the hybrids falls between the call rate of Rollers and Borders. In addition, the difference within sexes and between hybrid treatments is not significant (as alpha is > 0.05, Bonferroni).

<u>6b. Call rate (number of elements/sec) last ten days of sampling</u>:

As was seen during the first ten days of sampling two-way ANOVA for the last ten days demonstrated that there is a highly significant difference in call rate between the six treatments; F(5,1525) = 186.98, *P*<.0001, Table 4.

The difference in call rate between the sexes (for all birds) is not significant during this period (two-way ANOVA, F(1,1525) = 0.77, *P*=.38), however, the sex by treatments interaction is highly significant (two-way ANOVA, F(5,1525) = 13.76, *P*<.0001). Figure 26 shows this interaction and demonstrates that Roller canaries continue to produce flight calls with the fastest rate of delivery. (N.B. During the last ten days, cross-fostered Rollers demonstrated a significant difference in call rate between males and females that was not evident during the first ten days of call ontogeny (alpha = 0.01, Bonferroni).

Hybrid canaries continue to deliver flight calls at rates between Roller and Border canaries; however, during the last ten days of call production there is a significant difference in call rate between BR females and RB females (alpha = 0.01). Males, on the other hand, continue to give flight calls at delivery rates that are not significantly different (for BR males vs. RB males, alpha is > 0.05, Bonferroni).

Discussion

The domestic canary, *Serinus canaria*, has undergone artificial selection for the past 470 years (Darwin 1868). While British Border canaries have not been selected for song (they have been selected for morphological characters), German Rollers produce songs of low frequency which have been selected by aviculturalists since the beginning of the 19th century (Guttinger 1985). Because the validity of dividing avian vocalizations into songs and calls is currently debated, this study sought to determine if the flight calls of Roller canaries have been affected by selection pressure on their songs and if Border canary flight calls resemble or are different from Roller flight calls.

Presumably, if the song/call dichotomy were simply a division of convenience, one would expect to observe a significant difference between Roller calls and Border calls, since Roller calls would have changed along with their song under artificial selection. Under this scenario, songs and calls are affected by the same genetic mechanism, (i.e. they may be different expressions of the same vocal system) and Roller flight calls should then have changed in association with strong artificial selection on song. If the song/call distinction is real, however, in that they represent two independent communication systems, then Rollers and Borders may illustrate similar call parameters.

In order to illuminate the contribution of genes and environment to flight call ontogeny; the study also employed both a genetic breeding experiment (reciprocal Border x Roller crosses) and cross-fostering experiment.

The results clearly show that the two highly inbred canary strains (Rollers and Borders) produce flight calls which are significantly different from each other in qualitative structure and in all quantitative parameters studied (maximum frequency, minimum frequency, frequency range, duration, number of elements and call rate). This supports the hypothesis of one vocal communication system and suggests that the song/call dichotomy arbitrary and is utilized for convenience.

Discussion of qualitative analysis:

Flight call production, during the first ten days of call ontogeny, is characterized by a good deal of pattern variability produced by individual birds. This is reduced as the bird matures and increases the production of one or a few more stereotyped patterns.

Oscine song vocalizations also develop through stages of decreasing note variability. Subsong, which may be analogous to a baby's babble, is the earliest stage, and here birds produce vocalizations of a highly variable nature. In canaries, subsong appears roughly coincident with the first adult calls, and when the HVC (High Vocal Center, a forebrain nucleus which controls song production) first appears as a discrete nucleus in the brain (Konishi and Nottebohm 1969; Nottebohm 1991).

This study provides evidence that canary flight calls may develop through a similar period of vocal plasticity (I call it sub-call) prior to developing one or two stereotyped patterns. A similar number of individually distinctive flight call patterns (2-3) has been reported for another cardueline, the American goldfinch, *Carduelis tristis* (Mundinger 1970). Mundinger suggests that individually stereotyped flight call patterns permit individual recognition, thereby aiding reproductive pairs in coordinating their activities.

Although an increase in canary flight call stereotypy was identified during the last ten days of sampling, several birds continued to give calls which were variable in structure. It may be that selection for a stereotyped flight call has been relaxed by domestication. Breeders select breeding pairs and these pairs will often breed in small flight cages. Mulligan (1969) reports that canary courtship feeding calls, which are given by mates in close proximity, are highly variable in structure. Apparently there is little need to encode individual identification information in a call given at close range. Visual cues may be sufficient for identifying mates. Alternatively, birds with variable flight calls may develop some degree of stereotypy at a later stage of ontogeny, or they may have multiple social bonds.

Earlier studies described the call notes of passerines as being similar to non-passerine vocalizations in that both are stereotyped and genetically fixed; vocal learning was considered a quality unique to song (Lanyon 1960). Support for this evaluation is provided by Guttinger and Nicolai (1973) who performed experiments with 15 species of African estrildid finches in the genera *Lagonostica* and *Estrilda*. The authors reported that all 122 individuals cross-fostered by Bengalese finches developed their social calls independent of imitation.

More recent work has provided evidence for call learning in some passerines. In the first such studies, Mundinger (1970, 1979) demonstrated that several species of cardueline finches are capable of learning and imitating new flight calls. Similarly, Nowicki (1989) has investigated intra-flock call convergence in black capped chickadees, *Parus atricapillus* and identified significant vocal convergence within one week of flock formation. Male Zebra finches, *Taeniopygia guttata*, produce distance calls, which are strongly altered by cross-fostering with Bengalese finches, *Lonchura striata* (Zann 1985). Zann reports that 60% of foster-reared males lacked the noise element in their distance calls. Apparently, environmental input from the father is necessary for the production of a normal distance call, which would contain this noise element. Call learning in passerines has also been demonstrated in the absence of imitation. Robertson (1996) found that the eastern silvereye, *Zosterops lateralis,* produces individually distinct contact calls, which are recognizable to their mates.

Although imitative learning of flight calls may be possible for canaries it was not evident nor was it thoroughly investigated at this stage of ontogeny. Flight call patterns were either variable or demonstrated individual stereotypy. However, a cross-fostered Border did produce a Roller flight call pattern and there was some pattern sharing among siblings, suggesting that vocal imitation is possible and should be experimentally analyzed. The cross-fostered Borders provide evidence that frequency parameters may be learned. These birds produce Border element patterns but give the call at reduced frequency levels. Apparently, Borders are sensitive to their developmental environment and 1) can modify the frequency envelope of innate element patterns, or 2) their sensory-motor system is tuned by the acoustic experience of hearing their mother and this is reflected in the reduced frequency of their call. By contrast, cross-fostered Rollers produced flight calls very similar to the calls of normal-reared Rollers in both frequency and pattern, suggesting that the social-acoustic environment does not detectably affect the ontogeny of Roller flight calls.

To an experienced listener, the tonal quality of Roller flight calls often has a harsh quality due to the noise content of the vocalization. Conversely, Border canaries tend to produce flight calls with a brighter, sharper tone due to a lack of these noisy elements. Lade and Thorpe (1964) report that crosses between different dove species in the genus *Streptopelia* produce hybrids whose calls resemble the calls of one of the parental species in tonal quality. The reciprocal-cross canary hybrids present a similar picture when element structure and tonal quality are considered. RB hybrids give flight calls with Border-like element structure and timbre, while BR vocalizations are often noisy with Roller-like elements. Although some hybrid calls may vary from these characterizations, the generalizations are important and suggest the salience of the paternal genome.

Discussion of quantitative analysis:

Border canaries produce flight calls with significantly higher maximum frequencies than Roller canaries ($\bar{x}$= 4.55 kHz for Borders and 2.30 kHz for Rollers). A similar frequency pattern is seen when comparisons are made between Border and Roller songs (Guttinger 1985; Mundinger 1999). In addition, wild canary songs contain more Border-like patterns than Roller-like patterns (Guttinger 1985; Mundinger 1999).

The low pitch of Roller flight calls is not merely a function of size: Roller canaries are smaller than Borders in body mass yet produce vocalizations of lower frequency. More commonly, birds with greater body mass produce vocalizations with lower emphasized frequencies (Wallschlager 1980; Ryan and Brenowitz 1985). While the inverse relationship between body mass and vocal frequency is widespread in birds across species, within a species Ballintijn and Ten Cate (1997) found no significant correlation between body weight and fundamental frequency in the collared dove, *Sreptopelia decaocto.* But Clayton (1990) reciprocally cross-fostered two subspecies of zebra finch, *Taeniopygia guttata guttata,* and *T. g. castanotis* and found both subspecies learned song syllables from their foster fathers but delivered them at frequencies typical of their own subspecies. The larger subspecies, *castanotis,* sang imitated syllables at lower frequencies while the smaller, *guttata* produced songs at higher frequencies. So the difference found within the canary species is perhaps significant and unusual. Baptista (1996) suggests that studies of single neuron responses in song control centers may be useful in determining whether a vocalization is neurally constrained or constrained by a caller's physical size.

Further evidence for neural control of call frequency can be seen when the calls of males and females are compared. Male canaries are generally larger yet produce slightly higher pitched flight calls than females. Although syringeal and motor constraints can limit vocal performance (Podos 1996) these are not likely to be the cause of low frequency Roller canary flight calls because the canary syrinx can generate highly variable, high frequency note patterns during subsong (Guttinger 1985). Interestingly, canaries start producing flight calls ($\bar{x}$=39 days of age) at about the same time they are heard to give their initial bouts of subsong and this is roughly coincident with the maturation of telencephalic song nuclei which are small in nestling oscines (Nottebohm 1980). Roller subsong then exhibits a reduction in pitch.

The mean minimum frequency for Roller canary flight calls is significantly lower than for Border canary flight calls ($\bar{x}$= 1.24 kHz for Rollers and 2.42 kHz for Borders). This might suggest different constraints on sound production in these two strains. However, Mundinger (1995) has reported that Border canaries have retained the wild type ability to produce songs with low-pitched patterns, and echoing this I found that some cross-fostered Borders were able to produce flight calls with minimum frequencies similar to normal reared Rollers. Again, there appears to be neural control involved with vocal generation rather than a syringeal constraint limiting flight call production.

The frequency range parameter is of course dependent upon maximum and minimum frequency data. Not suprisingly, Border canaries demonstrate a significantly wider frequency range than Roller canaries ($\bar{x}$= 2.14 kHz for Borders and 1.07 kHz for Rollers). This difference serves to emphasize the highly derived nature of Roller flight calls. Canary flight calls are generally given in the context of movement. (Wild canaries will often give the call when a flock takes flight. Mundinger pers. comm.) As such, they may be useful as contact calls promoting flock cohesion. Vocalizations with broad frequency ranges (as seen in Border canaries) are easier to locate (Marler 1955, Redondo and Arias De Reyna 1988) than calls with narrow frequency ranges like those produced by Rollers. Apparently, artificial selection on song has narrowed the frequency range of Roller flight calls and domestication has relaxed the selection pressure for contact calls of broad frequency.

As might be expected, mean frequency values for hybrids (maximum frequency, minimum frequency, and frequency range) fall between the frequency means for Borders and Rollers (RB males are an exception, in the last ten days of calling the frequency range for these hybrid males is significantly higher than Border males). This strong genetic effect on vocalization parameters has also been identified for intraspecific hybrids of two subspecies of zebra finch, *Taeniopygia guttata guttata* and *T. g. castanotis*. Clayton (1990) found that these hybrids produced songs with fundamental frequencies and frequencies of maximum volume, intermediate to the two parental subspecies. But Baptista (1996) attributed these differences to size differences within this species. In addition, the distress calls of chicken, *Gallus domesticus,* x ring-necked pheasant, *Phasianus colchicus,* hybrids have been analyzed for heritability of frequency (McGrath et al.1972). It was found that the distress calls of hybrids was intermediate between chickens and pheasants. Congruent with the effect of body size on vocal production, larger hybrids gave calls of lower frequency. The authors conclude that inheritance of the frequency of the principal call component may be polygenic.

Guttinger (1979) has also demonstrated the moderating effects of hybridization in a study with canary x greenfinch, *Chloris chloris*, hybrids. He reported that the highest frequency of tours for hybrid songs was intermediate between both parental species.

Although the flight call frequency values for canary hybrids fall between Rollers and Borders, the frequency pattern produced by the reciprocal hybrids does not demonstrate a purely additive effect. This can be seen most clearly when considering the mean maximum frequency of flight calls for the last ten days of calling. There is a clear separation in mean maximum frequency between RB and BR hybrids. RB hybrids exposed earlier only to maternal Roller vocalizations, nevertheless produce flight calls with maximum frequencies closer to Borders. On the other hand BR hybrids, exposed earlier only to maternal Border vocalizations, give flight calls closer to Rollers in maximum frequency. Apparently, the paternal genomic contribution is most important in determining the maximum frequency phenotype of canary flight calls.

The "differential modification of maternal and paternal genetic contributions to the zygote resulting in the differential expression of parental alleles" is known as genomic imprinting (Monk 1988). These call ontogeny data suggest that the gene(s) controlling flight call frequency may be maternally imprinted (i.e. the maternal gene expression is reduced or eliminated). While genomic imprinting has been reported for mammals (Li et.al. 1993), it has yet to be described in birds. If the suggestion of genomic imprinting is true, this study represents the first time that possible genomic imprinting has been identified in a vertebrate class other than mammals.

Imprinting has been identified in polygamous mammals and is thought to arise from parent-offspring conflict for limited resources (Haig and Westoby 1989). In mice, alleles which promote embryonic growth at the expense of the mother's fitness may be imprinted by the mother (Haig and Graham 1991). Although wild passerines do engage in extra-pair copulations (polygamy), current imprinting theory would not explain the phenomenon of imprinted vocalizations.

Although imprinting in birds has not been reported, the molecular mechanism most often associated with imprinting, DNA methylation, does play a role in avian genetics (Wilks et al. 1982, the chicken vitellogenin gene; Haigh et al. 1982, the chicken globin gene). DNA methylation is "the addition of a methyl group (CH_3) to the 5-position of the cytidine ring in genomic DNA by DNA methyl transferase" (Rainier and Feinberg 1994). Studies with transgenic animals (animals whose genome includes a successfully integrated foreign gene) show that the expressed gene is often hypomethylated (Swain et al. 1987).

Rarely are single genes linked to a behavior; however, a single imprinted gene has been found to influence maternal behavior in mice. The paternal allele of the *Mest* gene is unmethylated and expressed; whereas the maternal allele is silent and methylated in a CpG-rich region. Without a functioning paternal copy of this gene, female mice fail to retrieve their pups and often display impaired placentophagia (Lefebvre et al. 1998).

The imprinting of genes involved with the expression of a behavioral phenotype has also been described in females with Turner's syndrome. Individuals with the syndrome lack one of the two X chromosomes. Females with a paternally derived X chromosome were found to have superior verbal and social skills when compared with females possessing a maternally derived X chromosome. This suggests sex-linked imprinting on the X chromosome. Presumably, there is a locus involved with cognitive function, which is maternally imprinted and normally expressed only from the paternal X chromosome (Skuse et al. 1997). Normal (XX) females also have only one functional X chromosome per cell (dosage compensation), however, this is not imprinting because X inactivation (creation of the Barr body) is a random event which is not dependent on the X chromosome's parental origin (i.e. in some cells the paternal X is inactivated, while for others the maternal X is not transcribed).

In birds, where there is no dosage compensation (Lyon 1994), females are the heterogametic sex (WZ), while males are the homogametic sex (ZZ). The frequency pattern seen in the reciprocal hybrids is not compatible with a sex-linked explanation unless imprinting is involved. If the call frequency trait was sex-linked (without imprinting) then BR females (WZ) would be expected to produce flight calls of low pitch while RB females (WZ) would produce calls of high pitch. Males (ZZ), on the other hand, would be expected to give flight calls with no frequency difference between the BR and RB hybrids: this pattern was not seen. Figure 15 shows that RB hybrids (both males and females) produced flight calls with maximum frequencies closer to Borders while BR hybrids (both males and females) gave calls closer to Rollers in maximum frequency. The hybrid frequency pattern is therefore more compatible with the genomic imprinting hypothesis than the sex-linkage (without imprinting) hypothesis. However, these data are not able to definitively differentiate between sex-linked imprinting or autosomal imprinting. Compare figures 27-33 which illustrate predicted autosomal and sex-linked inheritance patterns both with and without imprinting.

Cross-fostered Rollers produce flight calls with frequency parameters very similar to normal reared Roller canaries, whereas cross-fostered Borders produce flight calls at significantly lower frequencies than normal reared Borders. This suggests that the effect of the rearing hen is less important for flight call ontogeny in Roller canaries than in Border canaries.

A similar pattern can be seen when the songs of these two canary strains are compared. Cross-fostered Roller canary song learning is less, or is not affected by the rearing parents, but Border canary song learning is demonstrably affected by the song of the foster father (Mundinger 1995). In addition, Border canaries reared in isolation produced songs abnormal in structure, suggesting that external models are needed for normal Border song ontogeny. Conversely, Roller isolates produced songs with many normal appearing tours (e.g. flutes) created independently of learning. (A flute is one of several different generic tour patterns which Roller breeders select for). Apparently, flutes are heritable syllable types (Mundinger 1988). Furthermore, Mundinger (1995; 1999) provides evidence that canary song pitch (high for Borders, low for Rollers) is not due to hearing constraints. Several cross-fostered Border canaries learned Roller tours from live tutors and two cross-fostered Roller males apparently imitated a few Border song tours in modified (low-pitched) form.

Roller canary flight calls tend to be shorter in duration than Border flight calls; however, normal reared Rollers produce more elements per call (normal reared Roller females are an exception. During the last ten days, they produce fewer elements/call than normal reared Border females; however, the difference is not significant; as alpha is > 0.05, Bonferroni). Consequently, Roller canaries (both normal reared and cross-fostered) deliver flight calls at a faster rate (elements/sec) than Border canaries (both normal reared and cross-fostered). The call rate for hybrids is intermediate to Borders and Rollers, with BR hybrids calling at a somewhat faster rate than RB hybrids (again suggesting a heritable paternal effect).

Intrageneric hybrids with call rhythms intermediate between the parental species have been reported for doves in the genus *Streptopelia* (Lade and Thorpe 1964). In passerines, (where vocal learning is well documented), canary x greenfinch hybrids tutored with canary song, produce songs with greenfinch rhythm (Guttinger 1979). Thus providing further evidence for the heritability of vocal rate patterns.

Roller canaries have undergone strong artificial selection for specific song syllable categories such as the base roll, hollow roll, and bell roll all of which have very short syllables (less than 50 ms) and can be delivered rapidly (Guttinger 1985). It appears that the call rate of Roller canary flight calls has also been increased by the apparent selection on the roll tours of their songs.

Taken as a whole these data provide evidence that canary flight calls can be significantly altered by artificial selection on song, suggesting that songs and flight calls may share some common genetic mechanisms. The results also show that imprinted genes may influence the frequency parameters of canary flight calls.

CHAPTER 3: THE CANARY HUNGER-LOCATION CALL:

INTRODUCTION:

The process of domestication is analogous to speciation (Darwin 1868). During this process, humans artificially isolate gene pools based upon desired character traits (Baptista 1996). This study sought to compare the juvenile hunger-location calls of two highly inbred strains of domestic canaries, *Serinus canaria*, which have been subjected to different selection regimes. The German Roller canary has been selected for low frequency song trills, while the British Border canary has been bred for external morphology and posture.

Although there have been many studies illuminating oscine song development, (Guttinger et al. 1978; Guttinger 1979; Nelson and Marler 1993; Podos 1996) oscine call development has received less attention (but see Mundinger 1970, 1979; Chaiken 1990).

This study was designed to test following hypotheses:

1.) Differential selection on Roller and Border canaries has affected the hunger-location calls of these strains as well as the hunger-location calls of their reciprocal hybrids.

2.) In light of Mundinger's (1970, 1979) discovery of call learning in cardueline finches, it is predicted that cross-fostering will affect the structure of Border and Roller canary hunger-location calls.

3.) Canary hunger-location calls possess sound features which make them eminently locatable. Certain features of sound (broad frequency range, abrupt transients and frequency modulation) enhance a listener's ability to accurately locate a caller's position (Marler 1955; Rooke and Knight 1977); and presumably the hunger-location call is one for which locatability is important.

4.) Canary fledglings are able to recognize and specifically respond to maternal vocalizations with signature hunger-location calls. Parent-offspring recognition is important in species which nest colonially or semi-colonially (Medvin et al. 1993; Lefevre 1998). Because canaries nest in semi-colonial aggregations (Mundinger pers. comm.), they would presumably benefit from an efficient parent-chick recognition system.

MATERIALS AND METHODS:

This study analyzed 1,806 hunger-location calls from 42 domestic canaries hatched between 1993 and 1997. The 42 birds represented six treatments: (1) nine normal-reared Borders, (2) eight normal reared Rollers, ten reciprocal hybrids, (3) five RB hybrids and (4) five BR hybrids. Two additional treatments represent reciprocally cross-fostered Border and Roller canaries: (5) ten cross-fostered Border and (6) five cross-fostered Roller canaries. Rearing conditions are described in the materials and methods section of the flight call chapter (this thesis).

The first hunger-location calls were recorded between 16 and 23 days of age and the last calls were recorded between 21 and 27 days of age. An average of 7.3 calls/day/individual were analyzed, with an average of 5.5 ± 1.7 days of sampling per individual.

Hunger-location calls were recorded individually by isolating the mother and the fledgling to be recorded. Three Roller siblings (RR-22, RR-23 and RR-24) were an exception. These birds were recorded early in the study before I began isolating fledglings for individual recording. Consequently, their hunger-location calls were determined by evaluating sonographs containing all three birds, searching for stereotypy, and then sorting. To test for call stereotypy, twenty-four naïve observers were asked to match the hunger-location calls of thirteen different birds (3 Borders, 5 rollers, 4 RB hybrids and 1 BR hybrid) given on two consecutive days. Sonagrams of the calls were presented in two randomly mixed matrices and observers were tasked with matching calls produced by the same bird on the two different days.

Sonographic Analysis:

Calls were analyzed on a Uniscan model 4500 real-time spectrum analyzer using the 10.0 kHz scale. Film speed was set at 20.0 mm/second. Frequency in kHz was measured using a metric ruler (2.70 mm = 1.0 kHz) on 35.0 mm prints of sonagrams. There were four quantitative parameters measured and subjected to statistical analysis: 1) maximum frequency of the call, 2) minimum frequency of the call, 3) frequency range of the call, and 4) call element duration.

Maternal Recognition Study: Playback tests were used to assess whether canary fledglings are able to recognize their mother's vocalizations. Hunger-location calls given in response to maternal calls provided evidence of parental recognition by offspring.

The twenty-three chicks tested (7 Borders, 5 Rollers, 5 BR hybrids and 6 RB hybrids) were placed in soundproof "test chambers" and allowed to settle down for approximately fifteen minutes. Five recognition tests were then conducted for each fledgling over a period of two to three days. No more than three tests were performed on any given day; and same day tests were separated by a minimum of five minutes. Each test included playbacks of the maternal flight call, the flight call of a same strain female unfamiliar to the young , and the flight calls of two unfamiliar alternative strain females. The presentation was randomized with two minutes separating each playback. Vocal response was monitored for one minute of pretest, ten seconds of playback and a one minute post test portion.

RESULTS:

Qualitative analysis:

Canaries deliver hunger-location calls for approximately eight days ($\bar{x}=19.6 \pm 1.8$ to 24.7 ± 1.5 days of age) and initial call production is coincident with fledging ($x=19.7 \pm 1.3$ days of age). The hunger-location call is given by juveniles both prior to and during bouts of feeding. Maternal approach and or maternal vocalizations can elicit hunger-location calls, or they may be given "spontaneously." While producing the call, fledglings may actively follow the mother or remain stationary, sometimes fluttering their wings during call production.

Figures 34-36 illustrate representative sonograms of canary hunger-location calls. The call is given repetitively and usually consists of a single note (for exceptions see the binote calls of BB-18, BR-18 and BR-03). Harmonic structure was observed in the hunger-location calls of 15 birds, while the second voice phenomenon was seen in the calls of 8 individuals.

The hunger-location calls of all canaries studied were individualistic and stereotyped within the individual. However, there is marked pattern variability during the first days and last days of hunger-location call production and even "stereotyped" patterns may differ slightly (e.g. when begging intensely vs. begging weakly). Twenty-four naïve observers inspected sonograms and confirmed the functional stereotypy of hunger-location calls. Twenty-two of the observers correctly matched 100% of the calls produced by the same bird on two consecutive days, the two remaining observers successfully matched the calls for 11 of 13 birds.

Sonographic analysis revealed strain-typical patterns within the breeds and differences between breeds. Border canary hunger-location calls begin with descending frequency sweeps and often end with frequency modulation (Fig. 34A). By contrast, Roller canary hunger-location calls begin with ascending or tonal note patterns and there is usually little frequency modulation (Fig. 34B). In general, cross-fostering did not change these strain-typical patterns, however, RR-06 (a cross-fostered Roller) did show a frequency modulation in the terminal portion of its call (Fig. 35B). Cross-fostered Borders continued to produce hunger-location calls with an initial frequency decrease while cross-fostered Rollers produced calls which were tonal or displayed an initial increase in frequency (Figure 35).

Analysis for the reciprocal hybrids revealed that BR hybrids produce hunger-location calls which are more Roller-like in pattern while RB hybrid calls tend to be more Border-like in pattern (Figure 36; however, see RB-05 for an exception). Note that this observation suggests that the paternal genomic contribution may be important as RB hybrids had Border fathers and BR hybrids had Roller ones.

Quantitative Analysis:

1.) Maximum frequency parameter:

An analysis of variance revealed a highly significant difference between the six treatments in hunger-location call maximum frequency ($F(5,1800) = 755.23$, $P<.0001$; see Table 5 and Fig 37).

Border canaries produce hunger-location calls with the highest maximum frequencies ($\bar{x} = 5.77 \pm .54$ kHz), whereas Roller canaries, selected for low frequency song, produce calls with the lowest maximum frequencies observed for all six treatments ($\bar{x} = 3.82 \pm .33$ kHz). See figure 4.

While the reciprocal hybrids (RB and BR) produce hunger location calls with maximum frequencies intermediate to Border and Roller canaries, they are significantly different from each other (RB $\bar{x}$ = 4.83 ±.48 kHz, for BR $\bar{x}$ = 4.12 ±.44 kHz; alpha = 0.01, Bonferroni). RB hybrids deliver hunger-location calls with maximum frequencies closer to Border canaries, while BR hybrids give calls with maximum frequencies closer to Rollers. This pattern (i.e. the salience of the paternal genome) was also seen for canary nestling calls and canary flight calls.

Cross-fostering appears to have had a small but significant effect on the maximum frequency of Roller canary hunger-location calls ($\bar{x}$ = 4.01 ± .32 kHz for CFRR vs. 3.82 ±.33 kHz for RR; alpha = 0.01, Bonferroni). Border canaries, however, seem to show a greater sensitivity to their developmental environment and demonstrate a more pronounced cross-fostering effect ($\bar{x}$ = 4.69 ± .51 for CFBB vs. 5.77±.54 kHz for BB; alpha = 0.01, Bonferroni).

2.) Minimum frequency parameter:

An ANOVA revealed that there is a highly significant difference in minimum frequency between the six treatments (F(5,1800) = 306.93, $P<.0001$; see Table 5 and Fig.38).

Border canary hunger-location calls are delivered with the highest mean minimum frequency ($\bar{x}$ = 3.74 ± .34 kHz) while Roller canaries give hunger-location calls displaying the lowest mean minimum frequency ($\bar{x}$ = 2.90 ±.25). This difference is significant at alpha = 0.01 (Bonferroni).

As seen with the maximum frequency parameter, RB hybrids produce hunger-location calls with mean minimum frequencies closer to Borders ($\bar{x}$ = 3.63 ± .44 kHz for RB) and BR vocalizations are somewhat closer to Rollers in minimum frequency ($\bar{x}$ = 3.35 ± .30 kHz for BR). The reciprocal hybrids are significantly different from each other for the minimum frequency parameter (alpha = 0.01, Bonferroni).

Once again, Border canaries demonstrate a sensitivity to their social environment which manifests itself in a significant cross-fostering effect ($\bar{x}$ = 3.14± .38 kHz for CFBB; alpha = 0.01, Bonferroni). In contrast, the cross-fostering of Roller canaries has no significant effect on the mean minimum frequency of their hunger-location calls (RR $\bar{x}$ = 2.90±.27 kHz and CFRR $\bar{x}$ = 2.94 ±.31 kHz; as alpha is > 0.05, Bonferroni).

3.) Frequency range parameter:

The six treatments were found to produce hunger-location calls with significantly different frequency ranges (ANOVA, F(5,1800) = 282.10, *P*<.0001; see table 5 and Fig. 39).

Border canaries produce hunger-location calls with the broadest frequency range (BB $\bar{x}$ = 2.03±.68 kHz) while both, Roller canaries and BR hybrids deliver calls of the narrowest frequency ranges (RR $\bar{x}$ = .92 ±.44 kHz and BR $\bar{x}$ = .78 ±.31). This difference between normal-reared Rollers and BR hybrids is significant (alpha = 0.05, Bonferroni). RB hybrids prove once again to be more similar to Border canaries (RB $\bar{x}$ = 1.20 ± .41 kHz) and BR hybrids are shown to be closer to Roller canaries in call frequency.

Cross-fostering appears to have had an effect on both Border and Roller canary hunger-location call frequency ranges. Cross-fostered Borders produce hunger-location calls within a significantly narrower frequency range than normal-reared Borders (CFBB $\bar{x}$ = 1.55 ±.35 kHz; BB $\bar{x}$ = 2.03 ±.68; alpha = .01, Bonferroni) and cross-fostered Rollers produce hunger-location calls with slightly broader frequency ranges than normal- reared Rollers (CFRR $\bar{x}$ = 1.07±.35 kHz; RR $\bar{x}$ = 0.92±.44; alpha = 0.01, Bonferroni).

4.) Duration parameter:

An analysis of variance revealed that the duration of hunger-location call elements was significantly different between the six treatments (F(5,1800) = 77.33, $P<.0001$; see Table 5 and Figure 40). However, Roller canaries produced hunger-location calls which were not of significantly longer duration than Border canaries (BB $\bar{x}$ = .21 ± .06 seconds vs. RR $\bar{x}$ = .21 ±.08 seconds; as alpha is > 0.05, Bonferroni).

The reciprocal hybrids produced calls of significantly different duration from each other (RB $\bar{x}$ = .22 ± .07 seconds vs. BR $\bar{x}$ = .17 ± .03 seconds; alpha = 0.01, Bonferroni).

The cross-fostered birds produced calls significantly different in duration from normal reared members of the same strain (CFBB $\bar{x}$ = .23 ± .05 seconds, CFRR $\bar{x}$ = .29 ± .06 seconds; BB vs. CFBB, alpha = 0.01, Bonferroni; RR vs. CFRR, alpha = 0.01, Bonferroni).

Maternal Recognition Tests:

Chi-square analysis revealed that canary fledglings produce significantly more hunger-location vocalizations upon hearing maternal calls as opposed to hearing another mother's vocalization (chi-square = 200.88, d.f. = 1, alpha < 0.001). However, fledglings did not discriminate between same strain unfamiliar females and alternative strain unfamiliar females (chi-square = 0.176, d.f. = 1, alpha > 0.90; see Table 6).

Discussion:

The results show that strong artificial selection on Roller canary song has dramatically affected the pattern of Roller canary hunger-location calls. Roller hunger-location calls are either tonal or begin with an ascending frequency sweep. By contrast, Border canaries (selected for external morphology) produce hunger-location calls of broader frequency range containing an initial descending frequency sweep. This broad frequency range may represent the wild-type pattern of canary hunger-location calls. Sounds with broad frequency ranges are easier to locate (Marler 1955). This broad frequency range could provide canary parents with a mechanism for efficiently locating and subsequently feeding fledglings. Roller juveniles, producing hunger-location calls with narrower frequency ranges would potentially be more difficult to locate.

Although Roller canaries are smaller birds than Border canaries, Roller hunger-location calls are produced at lower frequencies. This suggests that the frequency parameters of Roller canary hunger-location calls are not merely a function of body mass. In many species of birds, including oscines, frequency varies inversely with body size (Wallschlager 1980; Baptista 1996). It is possible that artificial song selection has altered the Roller syrinx in such a way as to lower the mean frequency of its vocalizations. Male flammulated owls, *Otus flammeolus*, are half the mass of male western screech-owls, *O. kennicotti*, yet their calls are five to six halftones lower. The low frequency of their calls results from heavy tympaniform membranes which are covered with papillae and rugae. These syringeal modifications serve to damp tympaniform vibrations effecting a concomitant drop in frequency (Miller 1947). Although syringeal morphology may play an important role in the production of low frequency Roller calls, it is likely that other organizational levels also influence Roller vocal production. Indeed, the fact that Roller hunger-location call patterns are not simply lower frequency Border patterns suggests that song selection in Rollers may have had an important effect at the neuronal level.

While cross-fostering did not change strain-typical call patterns, cross-fostered Borders did show a significant drop in both maximum and minimum call frequency. Perhaps Border canaries are more sensitive to their developmental environment than are Roller canaries, whose call frequencies are somewhat less affected by cross-fostering. Echoing these results, much of Border canary song structure is learned, and low frequency tours characteristic of Roller songs can develop independent of learning (Poulsen 1959; Mundinger 1988, 1999).

Hunger-location calls of the reciprocal hybrids again suggest the importance of the paternal genomic contribution. RB hybrids (reared exclusively by Roller hens) had Border fathers and produced hunger-location calls which were more Border-like in both pattern and frequency as compared to BR hybrids. Conversely, BR hybrids (reared exclusively by Border hens) produced hunger-location calls which were more Roller-like in pattern and frequency, and they had Roller fathers. These data are compatible with the phenomenon of parental genomic imprinting. In genomic imprinting there is "a differential modification of the maternal and paternal contributions to the zygote, resulting in the differential expression of parental alleles" (Monk 1988). Parental genomic imprinting may also influence the development of canary nestling calls, and flight calls (see Nestling Call and Flight Call chapters, this thesis).

The duration of hunger-location calls for the reciprocal hybrids did not demonstrate the parental pattern seen in the frequency parameters. While RB birds were similar to Borders in call duration, BR canaries produced calls of significantly shorter duration than either Roller or RB hybrids. These results are not clear to me and may represent the chance effects of a highly variable parameter.

Canary hunger-location calls contain additional features which enhance locatability. The calls are repetitive and individual notes are temporally brief with abrupt onsets. By contrast, sounds with gradual amplitude transients are often more difficult to locate (Marler 1955; Rooke and Knight 1977). The frequency modulation present in hunger-location calls also aids the listener in positioning the caller with greater accuracy. Conversely, tonal vocalizations have been described as ventriloquial and are difficult to locate. Marler has reported that several European passerines employ tonal "seeep" alarm calls which may function to reduce the risk of predation (Marler 1955; Thorpe 1961).

In addition to containing features which enhance locatability, hunger-location calls encode information which facilitates individual identification. These vocalizations are stereotyped and each bird has its own unique vocal signature. Significantly, call stereotypy is in place when juveniles fledge. Hunger-location call ontogeny is therefore compatible with Beecher's hypothesis of "*type 1*" kin recognition (Beecher 1982). In this type of kin recognition, parents learn to recognize offspring when contextual clues provide certainty of relatedness (e.g. chicks in a nest should be the offspring of the nest builders). Subsequently, offspring could be identified and fed when contextual clues of parentage are lacking (e.g. when chicks fledge and disperse into surrounding vegetation).

Beecher has proposed a multilocus mechanism which could potentially generate a vocal signature pattern (Beecher 1982). In this mechanism several independent loci would possess multiple alleles. The number of distinct hunger-location patterns would then be dependent upon the number of loci involved with call phenotype and the number of different alleles present at these loci.

Prior to delivering hunger-location calls, canary chicks produce nestling vocalizations which are highly variable in nature. When still in the nest a variable hunger-location call may be given too, but its ontogeny quickly leads to a more stereotyped signature pattern. Upon fledging, individually stereotyped hunger-location calls could help ensure that parents avoid feeding unrelated fledglings and instead concentrate provisioning efforts on their own brood. Because wild canaries nest semi-colonially (Mundinger pers. comm.), feeding alien young is a potential risk and would presumably be selected against.

Stereotyped fledgling calls are widespread in many species of colonially nesting birds. The colonially nesting bank swallow, *Riparia riparia*, is able to identify its chicks only when they acquire signature calls at 15 to 17 days of age. This time frame coincides with chicks leaving the nest burrow to fledge and consequently running the risk of parental misidentification when young congregate in creches. Parallel results have also been described for European bee-eaters, *Merops apiaster*, which nest in burrows as well (Lessells et al. 1991).

Interestingly, rough-winged swallow, *Stelgidopteryx ruficollis*, juveniles do not develop signature calls. These birds are non-colonial and their young do not congregate in creches; consequently parents appear unable to use fledgling calls as a criterion for recognition (Beecher 1982).

Signature fledgling calls have also been reported for several species of alcids (common murres, *Uria aalgae*, Tschanz 1959; ancient murrlets, *Synthliboramphus antiquus*, Jones et al.1987; thick-billed murres, *Uria lomvia*, Lefebvre et al.1998). These are species which nest in dense colonies, where the young intermingle and therefore the risk of misidentification by parents is high.

Individually stereotyped juvenile vocalizations are not found exclusively in birds. Among mammals big brown bats, *Eptesicus fuscus*, produce isolation calls which permit mothers to locate and nurse their pups from amongst the hundreds in a colony (Rasmuson et al. 1991). The cries of human infants were also found to have an individual signature that proved "remarkably robust to degradation over distance" (Gustafson et al. 1984; Gustafson et al. 1994).

Parent-offspring interactions can be mediated by individually distinctive vocalizations in non-colonial species as well. Smith and Merkt (1980) report that song sparrow, *Melospiza melodia*, fledglings give individually distinct food-begging calls (also called hunger-location calls in this thesis). Since males and females feed select members of the brood while excluding others , these distinctive calls may serve to maintain specific parent-young feeding units. Female black-headed grosbeaks, *Pheucticus melanocephalus*, showed a significant approach response to the "phee-oo" call of their young (Ritchison 1983); and non-colonial

Florida scrub jays, *Aphelocoma coerulescens*, respond more strongly to the stereotyped calls of non-offspring than to calls of their own young (Barg and Mumme 1994). The authors interpret these puzzling results in light of the scrub jay's highly territorial social organization, where parents respond aggressively to intruders.

Parental recognition of young is often only one half of the communication relationship between parents and offspring. This study showed that canary chicks are sensitive to their developmental environment and do learn to respond to maternal vocalizations. Responding preferentially to parental vocalizations should provide for efficient food transfer as well as limiting unwanted attention from predators attracted to juvenile vocalizations.

Vocal recognition of parents by young has been reported for several species of non-oscine, colonially nesting birds (laughing gulls, *Larus atricilla*, Beer 1969; common terns, *Sterna hirundo*, Stevenson et al. 1970; gannets, *Sula bassana*, and Snares crested penguins, *Eudyptes robustus*, Proffit 1991).

Because young birds in dense colonies are unlikely to be fed by neighboring adults (indeed they may be attacked by non parental adults), Ritchison (1983) suggests that chicks who can successfully recognize their parent's calls will have selective advantage.

The recognition of parental vocalizations by non-colonial altricial species has received less attention. Young black-headed grosbeaks respond preferentially to the songs of their parents; however, they also exhibit significant vocal response to the songs of strange males and females. This seemingly indiscriminate behavior may be advantageous because some juvenile grosbeaks employ an "adoption" strategy as a means of increasing feeding opportunities (Ritchison 1983).

Nelson and Marler (1993) found that hand-reared white-crowned sparrow, *Zonotrichia leucophrys nuttalli*, fledglings gave significantly more "fledgling-location chips" to white crown sparrow song than to the songs of four other oscine species (song sparrow, *Melospiza melodia*; savannah sparrow, *Passerculus sandwichensis*; American goldfinch, *Spinus tristis*; and the yellowthroat, *Geothlypis trichas*). The authors concluded that the selective responsiveness to conspecific song, demonstrated by naïve juveniles, is due to an "innately specified auditory template".

Perhaps other juvenile oscines, such as canaries, are also predisposed to focus on species-specific vocalizations. Canary chicks could then presumably learn their parent's vocalizations and respond appropriately to parental vocalizations with individually stereotyped hunger-location calls.

CHAPTER 4: THE CANARY NESTLING CALL:

INTRODUCTION:

The canary nestling call is the earliest vocalization in this passerine's repertoire. As such, it may be the most primitive call, and the call least likely to be influenced by the developmental environment. Earlier studies of oscine song development have shown that song experience prior to twenty days of age has no effect on the song development of either white-crowned sparrows, *Zonotrichia leucophrys,* or song sparrows, *Melospiza melodia* (Marler 1970; Slater 1983; Marler and Peters 1987). So one might surmise that a call that is given only early in life (approximately 1 – 18 days of age) would not be affected by experience either.

The British Border canary has been revealed as an oscine whose song ontogeny is strongly influenced by learning. In contrast the German Roller canary is capable of producing normal Roller song patterns independent of experience (Mundinger 1995). Paralleling these results, I found that both the flight calls and hunger-location calls of Border canaries were significantly influenced by cross-fostering. In contrast, cross-fostering had less impact on Roller canary flight call and hunger-location call development.

This study also included a cross-fostering experiment in order to assess the environmental influence on canary nestling call development. The genetic aspect of nestling call ontogeny was addressed by following the call development of two parental strains with very different songs, and their reciprocal Border x Roller hybrids.

The structure of nestling vocalizations was also analyzed to determine if these noisy vocalizations posses design features which would make them more difficult to locate. Nestling vocalizations enable hungry chicks to compete successfully with siblings when the parents feed the brood (Cotton et al. 1996; Ottoson et al. 1997). However, these repetitive calls may also attract the unwanted attention of predators. Presumably, nestling calls would be selected to include design features which would reduce the likelihood of nest-site discovery by predators.

Hypotheses tested:

1) Genetic background affects nestling call ontogeny.

2) Early developmental experience has no effect on canary nestling call ontogeny.

3) Canary nestling vocalizations possess design features which reduce locatability.

MATERIALS and METHODS:

Sample size:

This study analyzed 1,495 nestling calls from 42 domestic canaries hatched between 1993 and 1997. The 42 birds represented six treatments: (1) nine normal-reared Borders, from four nest cups; (2) eight normal-reared Rollers, from three nest cups; (3) five RB hybrids, from three nest cups; and (4) five BR hybrids, from two nest cups. The final two treatments represent reciprocally cross-fostered Border and Roller canaries: (5) ten cross-fostered Borders, from four nest cups; and (6) five cross-fostered Rollers, from two nest cups. Rearing conditions and sonographic analyses are described in the materials and methods section of chapters 2 and 3 of this thesis.

Initially, recordings of individual nestlings were attempted using out of chamber facilities; however, the young were then often reluctant to call. Consequently, nestling recordings were made of the entire brood (1-4 birds) in the nest cup simultaneously. This approach was also helpful in reducing nest chamber disturbance. Such disturbance increases the risk of having a mother desert the nest.

The first nestling calls analyzed were produced by birds between 7 and 17 days of age and the last calls analyzed were recorded between 15 and 21 days of age. An average of 12.8 calls/day per nest cup (1-4 individuals/cup) were analyzed, with an average of 6.4± 2.2 days of sampling per nest cup.

RESULTS:

Qualitative analysis:

Canaries produce nestling calls for approximately eighteen days (from hatch to $\bar{x}$ = 18.5 ±1.6 days of age). Calls given during the first week of life are of low amplitude, short duration and are often delivered with the bill closed. Nestling vocalizations are usually associated with parental food delivery. When the chicks are somewhat older these calls are given in response to parental vocalizations and or a parent's landing on the nest rim. But during the first week of nestling call ontogeny, chicks are often silent during feeding. Only after being fed do these young chicks vocalize. As chicks mature, nestling calls increase in amplitude as well as duration, and are given both prior to and during feeding.

Figures 41-46 illustrate representative sonograms of nestling calls from eighteen different nest cups. The call consists of a single note and is given repetitively. Unlike canary hunger-location calls (given by fledglings), canary nestling calls are individually variable and lack individual stereotypy.

Nestling calls for all six treatment groups began with increasing frequency sweeps. Chevrons and frequency modulated plateaus appear to be call patterns favored by nestling canaries (see Figures 41-43).

Sonographic analysis did not reveal discernable strain-typical call patterns within the breeds. This result stands in sharp contrast to the strain-typical patterns seen in fledgling hunger-location calls, which replace nestling vocalizations in the juvenile call repertoire. Apparently, artificial selection on Roller song has had little effect on the acoustic form of Roller canary nestling calls.

Quantitative Analysis:

1.) Maximum Frequency Parameter:

An analysis of variance did reveal, however, a highly significant difference between the six treatments in nestling call maximum frequency (ANOVA, F(5,1489) = 41.22, $P < .0001$; see Table 7 and Fig. 47).

Border canaries delivered nestling vocalizations with the highest maximum frequencies ($\bar{x}$ = 7.25 ± .85 kHz); while Roller canaries, selected for low frequency song tours, produced calls with significantly lower maximum frequencies. (RR $\bar{x}$ = 6.40±1.04 kHz; alpha = 0 .01, Bonferroni).

The reciprocal hybrids produced nestling vocalizations with maximum frequencies which were significantly different from each other (alpha = 0.01, Bonferroni). RB hybrid vocalizations displayed maximum frequencies which were more Border-like ($\bar{x}$ = 6.90 ±.97 kHz), while BR hybrids produced calls with more Roller-like frequencies($\bar{x}$ = 6.38 ± .72 kHz). This trend (Border-like RB calls and Roller-like BR calls) was found to be a recurring theme throughout canary call ontogeny (i.e. nestling call, hunger-location call and flight call ontogeny) and suggests the importance of the paternal genomic contribution.

Border nestlings raised by Roller hens called at a lower mean maximum frequency than normal-reared Borders, however, this difference was not significant (CFBB $\bar{x}$ = 7.04±.79 kHz vs. BB $\bar{x}$ = 7.25±.85 kHz; as alpha is > 0.05, Bonferroni). Unexpectedly, I found that Roller nestlings also showed a cross-fostering effect and this was significant (CFRR $\bar{x}$ = 6.87±.75 kHz vs. RR $\bar{x}$ = 6.4±1.04 kHz, alpha = 0.01, Bonferroni). Interestingly, the difference in mean maximum frequency between cross-fostered Rollers and cross-fostered Borders was not significant (as alpha is > 0.05, Bonferroni).

2.) Minimum Frequency Parameter:

An analysis of variance revealed that there is a highly significant difference in minimum frequency for nestling calls between the six treatments (ANOVA, F(5,1489) = 18.66, $P < .0001$; see Table 7 and Fig. 48).

RB hybrids and Border canaries delivered nestling calls with the highest mean minimum frequencies (RB $\bar{x}$ = 5.30 ± .86 kHz; BB $\bar{x}$ = 5.25 ±.67 kHz). However, the difference between these two treatments is not significant (as alpha is > 0.05, Bonferroni). BR hybrids and Roller canaries produced nestling calls with the lowest mean minimum frequencies (BR $\bar{x}$ = 4.67 ± .58 kHz; RR $\bar{x}$ = 4.80 ± 1.12 kHz). This frequency difference also lacks significance (as alpha is > 0.05, Bonferroni). Once again the reciprocal hybrids reveal the important influence of the paternal genetic contribution.

Cross-fostered Border canaries showed a small drop in mean minimum frequency, however, this result was not significant. (CFBB $\bar{x}$ = 5.04 ± .77 kHz vs.BB $\bar{x}$ = 5.25 ± .67 kHz; as alpha is > 0.05, Bonferroni); cross-fostered Roller canaries also failed to demonstrate a significant cross-fostering effect (CFRR $\bar{x}$ = 4.92 ±.58 kHz vs. RR $\bar{x}$ = 4.80 ± 1.12 kHz; as alpha is > 0.05, Bonferroni).

3.) Frequency Range Parameter:

An analysis of variance revealed significant differences in frequency range between the six treatments (ANOVA, F(5,1489) = 16.77, $P < .0001$; see Table 7 and Fig. 49).

The two highly inbred canary strains (Borders and Rollers) produced nestling calls with significantly different frequency ranges (alpha = 0.01, Bonferroni). Border canaries produced vocalizations with the broadest frequency range ($\bar{x}$ = 2.00 ±.88 kHz), while Roller canaries gave nestling calls with the narrowest frequency range ($\bar{x}$ = 1.59±.71 kHz).

The reciprocal hybrids (RB and BR) delivered nestling calls with frequency ranges intermediate to Borders and Rollers. BR birds produced calls with slightly broader frequency ranges than RB birds, however, this difference was not significant (BR $\bar{x}$ = 1.71±.71 kHz, RB $\bar{x}$ = 1.60±.86 kHz; as alpha is > 0.05, Bonferroni).

Roller canaries did show a significant cross-fostering effect (CFRR $\bar{x}$ = 1.96± .78 kHz; alpha = 0.05, Bonferroni), while cross-fostered Border canaries did not demonstrate a significant difference in frequency range when compared with normal-reared Borders (CFBB $\bar{x}$ = 2.00±.85 kHz vs. BB $\bar{x}$ = 2.00±.88 kHz; as alpha is > 0.05, Bonferroni).

4.) Duration Parameter:

The six treatments were found to produce nestling call elements with significantly different durations (ANOVA, F(5,1489) = 34.11, $P < .001$; see Table 7 and Fig. 50).

Border canary nestling calls are significantly longer in duration than Roller nestling vocalizations (BB $\bar{x}$ = .19± .07 seconds; RR $\bar{x}$ = .13±.06 seconds; alpha = 0.01, Bonferroni).

The reciprocal hybrids are once again intermediate to Borders and Rollers and are not significantly different from each other (BR $\bar{x}$ = .16±.05 seconds; RB $\bar{x}$ = .15± .06; as alpha is > 0.05, Bonferroni).

Whereas cross-fostering appears to have had a significant effect on Roller nestling call durations (CFRR $\bar{x} = .17 \pm .05$ seconds vs. RR $\bar{x} = .13 \pm .06$ kHz; alpha = 0.01, Bonferroni), Border canaries did not demonstrate a significant cross-fostering effect (CFBB $\bar{x} = .19 \pm .08$ seconds vs. BB $\bar{x} = 19 \pm .07$ kHz., as alpha is > 0.05, Bonferroni).

DISCUSSION:

Genetic Background:

The results show that Roller canary nestling vocalizations are delivered at significantly lower frequencies than the nestling calls of Border canaries. Apparently, artificial selection for low frequency song tours has also lowered the nestling call frequencies in Roller canaries, even at this earliest stage of call ontogeny. Border canaries have never been subjected to artificial song selection and therefore produce calls of a more wild-type nature (Guttinger 1985).

The reciprocal hybrids produce nestling calls with maximum and minimum frequencies closer to their paternal canary strain. This trend (i.e. RB birds calling at Border-like frequencies and BR birds calling at Roller-like frequencies) is also seen in both canary hunger-location calls and canary flight calls. A possible explanation for these results is the phenomenon of parental genomic imprinting. Genomic imprinting is “a reversible modification of DNA that causes differential expression of maternally and paternally inherited homologous chromosomes or genes” (Rainier and Feinberg 1994). This phenomenon was looked at more closely in the flight call chapter of this thesis. The fact that evidence for genomic imprinting has been found in the developmental analysis of all three calls studied (nestling, hunger-location and flight call) suggests that all three vocalizations may share a common underlying neural mechanism which is affected by genomic imprinting.

As was seen with flight calls and hunger-location calls, the duration of nestling calls did not demonstrate the parental pattern seen in the frequency parameters. This suggests that canary call duration is controlled by a mechanism other than genomic imprinting. A mechanism that has yet to be identified.

The Effect of Early Experience on Nestling Call Ontogeny:

The hypothesis that early experience has no affect on nestling call ontogeny was not supported. Cross-fostering does appear to influence the frequency parameters of the nestling call for both Border and Roller canaries. This result is interesting in light of earlier studies of oscine song development where song experience had no effect on "song acquisition or development prior to 20 days of age" (Marler 1970; Slater 1983; Marler and Peters 1987), and studies of other calls in this thesis where Roller hunger-location calls and flight calls are not affected or are only minimally affected by cross-fostering. Apparently, canaries of both strains used in this study can be sensitive to their developmental environment at an early age, but that in Rollers this effect is ephemeral and decreases over time. Why might this be so?

Gottlieb (1981) found that the early experience of hearing ones own "nestling calls" (while in the egg) apparently tunes the perceptual system of mallard, *Anas* *platyrynchos*, ducklings. Upon hearing these vocalizations, the duckling develops response specificity to the maternal call. Recognizing a maternal call because the subject heard its own call in the egg is a truly "nonobvious" (in the interactionist sense) consequence of vocal development. An altricial passerine nestling like a canary may be developmentally analogous to a precocial duckling still in its shell. If so, then perhaps the finding that cross-fostering affects Roller and Border nestling call frequencies (recall that the mean maximum frequencies of CFRR and CFBB nestling calls were not significantly different from each other) is somehow tracking a similar phenomenon; i.e. the perceptual system of developing passerines may be tuned by its mother's calls. But how this hypothesized perceptual tuning affects motor output is unclear. Adult maternal vocalizations which influence nestling call frequencies would also seem to qualify as a "nonobvious" contributor to vocal development.

Other Results:

Canary nestling calls demonstrate a good deal of pattern variability. Because similar nestling call patterns (i.e. variable chevrons) have been reported for other representative passerines (e.g. short-toed tree creepers, *Certhia brachydactyla*, Thielcke 1965; and the twite, *Acanthus flavirostris*, Marler and Mundinger 1975) these "chevron-shaped" nestling calls may represent a primitive, evolutionarily conserved pattern. By contrast, Monk (1997) has found that the nestling calls of western bluebirds, *Sialia mexicana*, are highly distinctive. However, since these bluebird nestling calls were recorded toward the end of the nestling period (fourteen to nineteen days of age), it may be that the bluebird chicks were beginning to develop signature fledgling calls (see hunger-location call chapter this thesis). Upon leaving the nest, (fledging) young birds would presumably benefit from individually stereotyped juvenile calls designed to aid parents in identifying offspring. Nestlings, however, should not be under selection pressure for “signature” calls since the nest-site would provide certainty of parentage. Canary nestling vocalizations should therefore have functions other than individual identification.

Because very young canary chicks are silent during feeding and often call immediately after being fed, the call may serve to stimulate the female to feed, as opposed to incubate only or it may serve a thermoregulatory function. Evidence for this latter interpretation comes from observing call reduction, which is concomitant with maternal brooding.

Evans (1992, 1994) has found that neonatal American white pelicans, *Pelicanus erythrorhynchos*, produce “squawk” vocalizations which elicit parental brooding behavior designed to ameliorate the shivering of offspring. Among mammals, the ultrasound vocalizations of neonatal rodents are also believed to elicit brooding warmth from a parent (Allin and Banks 1970).

Nestling vocalizations are also thought to provide parents with an honest signal of a nestling’s nutritional needs. Trivers (1974) has theorized that parents and offspring are in conflict over care allocation. Chicks are selected to demand more effort than parents can provide and parents are selected to conserve efforts in order to ensure future reproductive success. The evolutionary stable strategy of honest signaling has helped to resolve this conflict (Godfray 1991, 1995).

Cotton et al. (1996) have identified honest signaling in the begging calls of starling, *Sturnus vulgaris,* chicks. After manipulating the feeding levels of nest mates, the authors found that begging behavior of hungry nest mates did not increase the begging effort of unmanipulated chicks. In line with these results, I observed that recently fed canary nestlings (with full crops) begged with less intensity (if at all).

Presumably, there is a selective cost for dishonest begging. Indiscriminate begging could potentially reduce nestling fitness by depleting energy reserves that might otherwise be sequestered into new tissue. Although begging does require energy expenditure, recent studies have shown that the energetic cost of begging in nestling passerines may be quite low (McCarty 1996).

Bachman and Chappell (1998) used open-circuit respirometry to measure the energy costs of begging in nestling house wrens, *Troglodytes aedon.* They found that over a twenty-four hour period the energy allocated to begging was only 0.02 % of the energy budget in three-day old chicks, and only 0.22 % of the energy budget for ten-day old chicks.

Because begging appears to be energetically inexpensive and is used by siblings to compete successfully with each other for limited parental resources (Ottoson et al. 1997), chicks might be expected to maximize their fitness by calling vigorously and continuously. Indeed, pied flycatcher, *Ficedula hypoleuca*, chicks that beg more often are able to increase parental feeding rates over the course of the entire nestling period (Ottoson et al. 1997). Given these findings, what factor might be instrumental in limiting indiscriminate begging behavior?

Marler and Mundinger (1975) provided useful insight into this question. Cardueline finches beg regularly between feedings; however, nestling twites only call during bouts of feeding. Because the twite is a ground-nesting species, the authors conclude that predation may be selecting against spontaneous calling.

Predation may have also played a role in shaping the frequency parameters of nestling vocalizations. Certain design features of passerine nestling vocalizations (broad frequency range, frequency modulation and repetitive notes) make them easy to locate (Marler 1955; Rooke and Knight 1977). The benefit of noisy, locatable nestling calls is increased parental attention, the cost is unwanted attention from predators.

Redondo and Arias De Reyna (1988) suggest that predation risk is minimized by producing nestling calls with higher frequencies. Higher frequency sounds are attenuated more quickly than low frequency sounds (Wiley and Richards 1978). This is because they suffer greater atmospheric absorption and are more easily scattered by environmental obstructions than are sounds with longer wavelengths. Nestling calls with higher emphasized frequencies could then presumably serve as a mechanism to reduce predation.

Nestling vocalizations are usually produced at higher frequencies than other calls in an avian call system (e.g. canary nestling calls are delivered at higher frequencies than the hunger-location calls of canary fledglings). This is due in part to the inverse relationship between signal frequency and the body mass of the caller (Wallschlager 1980). However, hole-nesting species (subject to less predation pressure than open-nesting species; Lack 1968) tend to produce begging calls with "lower frequency values, on average, than open-nesting ones of similar adult weight (Redondo and Arias De Reyna 1988).

Genetic and environmental factors, therefore, appear to influence the frequency parameters of passerine nestling vocalizations, and these factors may include neural circuits, body mass, predation pressure and exposure to maternal vocalizations.

CHAPTER 5: GENERAL SUMMARY:

This study was both a descriptive study of canary call ontogeny and an experimental analysis of the effect of genetic background on call ontogeny. In addition, it attempted to shed some light on the long debated song-call dichotomy. The results showed that genetic background does affect call ontogeny and that Roller canary calls have been significantly altered by strong artificial selection on Roller song. Border canaries (which were not artificially selected for song) produce calls which are significantly different from Rollers. These Border calls may represent the more wild-type condition.

The Roller results point to an integrated avian communication system in which calls are one expression and songs another, of the same vocal system. Song ontogeny in domestic canaries appears to be influenced by sex linked factors, some of which may demonstrate genomic imprinting (Mundinger pers. comm.). This call study has clearly identified the possible influence of genomic imprinting in the development of canary flight calls, hunger-location calls, and nestling calls when the frequency parameters are followed. These findings are the first steps toward determining the genetic system underlying the canary vocal communication system.

Song learning is well documented in the domestic canary (Marler and Waser 1977; Mundinger 1995, 1999), and this study showed that frequency parameters of canary calls can also be influenced by the bird's developmental environment. Once again songs and calls appear to share an acoustical environmental effect on developmental characteristics. The finding that canary songs and calls are different expressions of the same vocal system should provide workers with fresh approaches to the study of avian neurobiology (e.g. Does the HVC, which develops coincident with both, flight calls and subsong, influence call production as well as song development?).

This study also illuminated another potential benefit for researchers interested in studying avian communication. Because call data can be collected from both male and female birds, investigators performing breeding studies of call parameters can potentially double their sample sizes as compared to song parameters. This may be helpful in behavioral studies, where small sample sizes are sometimes a concern.

SUMMARIES of the THREE CALL CHAPTERS:

I) Flight Calls:

Results of testing the hypotheses:

1. The data support the hypothesis that songs and calls are part of one vocal communication system. Rollers produce flight calls whose parameters have been affected by artificial song selection.
2. The hypothesis that genetic background affects flight call development was accepted. The two inbred strains of canaries (Rollers and Borders) exhibit different flight calls development patterns.
3. The hypothesis that the social environment affects flight call development was accepted. It should be noted, however, that this environmental influence was most pronounced for Border canaries.

4. The frequency (kHz) pattern observed in the calls of the reciprocal hybrids (RB and BR) was more compatible with a genomic imprinting hypothesis than a sex-linkage hypothesis without imprinting. However, the data are not able to definitively differentiate between sex-linked imprinting or autosomal imprinting.

Summary:

1.) The canary flight call is a complex (trilled) call and is first produced at approximately 39 days of age. Initially, flight calls are highly variable (sub-call). Older birds (approximately 50 to 60 days of age) demonstrate less variable call patterns and may produce 1 – 2 stereotyped calls.

2.) Roller canary flight calls appear to have been affected by artificial song selection. Rollers are smaller birds than Border canaries, yet they produce flight calls with significantly lower frequencies than Borders and the form of the call is different.

3.) There appears to be a mixed social effect on flight call development. When compared with cross-fostered Roller canaries, cross-fostered Border canaries display a greater sensitivity to their rearing environment. This sensitivity manifests itself in flight calls (produced by cross-fostered Borders) with lower frequencies than normal-reared Borders. Border call patterns (shapes), however, do not appear to be appreciably affected by cross-fostering. Cross-fostered Rollers were found to produce flight calls with frequency (kHz) parameters which were less influenced by the social environment. Consequently, Roller flight call development may be more developmentally canalized than Border flight call development, or at that point in development the Roller brain may be more constrained than the Border brain.

4.) Flight call analysis for the reciprocal hybrids showed that frequency and call rate parameters demonstrated a paternal effect. RB hybrids (with Border fathers, and raised by Roller mothers) produced flight calls with more Border-like parameter values. Conversely, BR hybrids (with Roller fathers, and raised by Border mothers) produced flight calls with more Roller-like parameter values. These results are compatible with the phenomenon of parental genomic imprinting. In genomic imprinting the phenotypic expression of the genotype is dependent on the parental origin of the gene(s). Flight call duration does not appear to be a parameter influenced by genomic imprinting.

II.) Hunger-location Calls:

Results of testing the hypotheses:

1. The hypothesis that differential selection on Roller and Border canaries has affected the hunger-location calls of these strains was accepted (for details see below).
2. The hypothesis that cross-fostering will affect the structure of Border and Roller canary hunger-location calls was accepted. However, while both the maximum and minimum frequency parameters of cross-fostered Border canary calls were

significantly affected, only the maximum frequency parameter for cross-fostered Rollers demonstrated a significant difference from normal-reared Rollers.

3. The hypothesis that canary normal hunger-location calls possess sound features which make them eminently locatable was accepted. All calls possessed abrupt transients, however, when compared with Rollers, Border calls displayed broader frequency ranges and greater frequency modulation.
4. The hypothesis that canary fledglings are able to recognize and respond specifically to maternal vocalizations was accepted.

Summary:

1.) Canary hunger-location calls are delivered for approximately eight days, with initial production coinciding with fledging (approximately 18 days of age). The call is given repetitively and consists of one to two notes.

2.) The hunger-location call is individualistic and stereotyped within the individual.

3.) Hunger-location calls appear to be employed as a signal which permits parents to locate and subsequently feed fledged young. This study found that canary fledglings can recognize their mother's vocalization and respond to her with their signature hunger-location calls.

4.) Artificial selection on Roller canary song has lowered Roller hunger-location call frequencies and altered hunger-location call forms (shapes). Roller hunger-location calls are either tonal or begin with an ascending frequency sweep. In contrast, Border canaries (which have not been consciously selected for vocal characters) produce hunger-location calls with higher frequencies, broader frequency ranges and initial frequency sweeps which descend.

5.) As was observed for the flight call, cross-fostering provided evidence for a mixed social effect on the frequencies of canary hunger-location calls. Cross-fostered Borders (reared by Roller hens) demonstrated a significant drop in maximum frequency when compared to normal-reared Borders. Cross-fostered Rollers (reared by Border hens) demonstrated a small but significant increase in mean maximum hunger-location call frequency, when compared with normal-reared Rollers. With respect to minimum frequency, the mean minimum frequency of cross-fostered Border hunger-location calls was significantly lower than normal-reared Borders; however, the mean minimum frequencies of cross-fostered Roller calls were not significantly different from normal-reared Rollers.

6.) Echoing the flight call results, RB hybrid (had Border fathers) hunger-location calls were generally more Border-like in pattern and frequency, while BR hybrids (had Roller fathers) produced hunger-location calls which were more Roller-like in nature. These results provide further evidence for the salience of the paternal genomic contribution to call ontogeny. However, as with flight calls, call duration did not appear to demonstrate a paternal effect.

III.) Nestling Calls:

Results of testing the hypotheses:

1. The hypothesis that genetic background affects nestling call ontogeny was accepted. The two inbred strains of canaries (Borders and Rollers) exhibit different nestling call development patterns.
2. The hypothesis that early developmental experience has no effect on canary nestling call ontogeny was rejected. Cross-fostering significantly affected the maximum frequency of Roller canary nestling calls.
3. The hypothesis that canary nestling calls posses designs features which reduce locatability, and thereby reduce predation, was accepted (see below).

Summary:

1.) Canary nestling calls are individually variable in structure and are produced for approximately 18 days (if calls are first given on the day of hatch). These single note calls are delivered repetitively and at higher frequencies (in kHz) than either flight calls or hunger-location calls. This high frequency delivery may reflect the effect of sensitivity to maternal vocalizations and, or may serve as an anti-predator device.

2.) Even at this early stage of vocal ontogeny, selection for low frequency Roller song has apparently affected the frequencies of Roller canary nestling vocalizations.

Rollers produce nestling calls at significantly lower frequencies than the larger and heavier British Border canaries.

3.) Cross-fostered Borders and cross-fostered Rollers both showed a significant cross-fostering effect for nestling call maximum frequency. This result was unexpected given the primitive nature of this call and the fact that both Roller flight call development, and to some degree hunger-location call development seem less affected by the social environment than is call development in Border canaries. It is suggested that this result may point to a "non-obvious" maternal call effect on nestling call development, and that Roller call development may be less canalized at this earliest stage in their post-hatch life as compared to latter stages in call ontogeny.

4.) The reciprocal cross hybrids produced nestling calls with frequency measures closer to the mean values of their father's strain. This finding, although not as striking as the paternal effect on hunger-location and flight calls, is consistent with the frequency data for flight calls and hunger-location calls and may be compatible with a genomic imprinting interpretation for a general frequency trait. Call duration, however, did not demonstrate a paternal effect. This finding (i.e. the lack of a paternal effect on duration) is also consistent with the call duration results for canary flight calls and hunger-location calls.

TABLES

Table 1: Flight call stereotypy for the first 10 days of sampling.

Treatment	No. of birds demonstrating one stereotyped call pattern.	No. of birds demonstrating two stereotyped call patterns.	No. of birds demonstrating variable call patterns.	Totals
BB	0	0	9	9
RR	2	0	6	8
BR	1	0	3	4
RB	2	0	2	4
CFBB	3	1	5	9
CFRR	4	0	1	5
Totals	12	1	26	39

Table 2: Flight call stereotypy for the last 10 days of sampling.

Treatment	No. of birds demonstrating one stereotyped call pattern.	No. of birds demonstrating two stereotyped call patterns.	No. of birds demonstrating variable call patterns.	Totals
BB	5	1	3	9
RR	5	2	1	8
BR	4	0	0	4
RB	2	2	0	4
CFBB	6	1	2	9
CFRR	5	0	0	5
Totals	27	6	6	39

Table 3: Canary flight calls: Mean values for maximum frequency (kHz), minimum frequency (kHz) and frequency range (kHz) for all treatment groups.

a.) First 10 days of sampling.

Treatment	Males (N)	Females (N)	Max. Freq.	s.d.	Min. Freq.	s.d.	Freq. Range	s.d.
BB	5	6	4.48	.72	2.39	.61	2.10	.67
RR	6	2	2.41	.49	1.32	.28	1.09	.38
BR	1	3	2.87	.65	1.41	.35	1.46	.54
RB	2	2	3.48	.84	1.76	.49	1.72	.64
CFBB	5	4	3.38	.79	1.57	.47	1.81	.64
CFRR	3	2	2.30	.47	1.14	.27	1.16	.38

b.) Last 10 days of sampling.

Treatment	Max. Freq.	s.d.	Min. Freq.	s.d.	Freq. Range	s.d.
BB	4.62	.57	2.44	.52	2.18	.58
RR	2.21	.39	1.17	.25	1.05	.32
BR	2.88	.68	1.32	.37	1.56	.56
RB	4.21	.60	1.95	.50	2.27	.60
CFBB	3.93	.69	1.95	.61	1.98	.48
CFRR	2.37	.49	1.06	.18	1.31	.47

Table 4: Canary flight calls: Mean values for call duration (sec.), number of elements/call, and call rate (number of elements/sec.), for all treatment groups.

a.) First 10 days of sampling.

Treatment	Males (N)	Females (N)	Durat.	s.d.	Number of elements/call	s.d.	Call Rate	s.d.
BB	5	4	.18	.06	2.62	.73	15.0	.24
RR	6	2	.17	.06	3.21	.95	19.6	.26
BR	1	3	.19	.06	3.24	.95	17.8	.22
RB	2	2	.18	.06	2.93	.90	17.0	.24
CFBB	5	4	.23	.07	3.63	.93	16.0	.22
CFRR	3	2	.17	.05	3.08	.89	18.7	.23

b.) Last 10 days of sampling.

Treatment	Durat.	s.d.	Number of elements/call	s.d.	Call Rate	s.d.
BB	.21	.08	2.92	1.02	14.2	.25
RR	.16	.05	3.08	.85	19.2	.26
BR	.18	.05	3.07	.82	17.3	.21
RB	.17	.05	2.73	.75	16.2	.21
CFBB	.25	.08	3.52	.93	14.4	.26
CFRR	.17	.06	3.25	.93	19.5	.34

Table 5: Mean values for maximum frequency (kHz), minimum frequency(kHz), frequency range (kHz) and duration (sec.) of hunger-location calls for all treatment groups.

Treatment	Males (N)	Females (N)	Max. Freq.	s.d.	Min Freq	s.d.	Freq. Range	s.d.	Duration	s.d.
BB	5	5	5.77	.54	3.74	.34	2.03	.68	.21	.06
RR	6	2	3.82	.33	2.90	.25	.92	.44	.21	.08
BR	1	4	4.12	.44	3.35	.30	.78	.32	.17	.03
RB	3	2	4.83	.48	3.63	.44	1.20	.40	.22	.07
CFBB	5	4	4.69	.51	3.14	.38	1.55	.42	.23	.05
CFRR	3	2	4.01	.32	2.94	.31	1.06	.35	.29	.07
Totals	23	19								

Table 6: Fledgling response to playbacks of maternal vocalizations. Values indicate the total number of hunger-location calls given by each bird, for all five recognition tests. Birds with duplicate identifications were reared in different years (*) 1994, (!) 1995.

Test Subject	Response to biological mother	Response to same strain female	Response to alternative strain female (1)	Response to alternative strain female (2)
BB-01	10	0	0	0
BB-02	3	0	0	0
BB-03	32	3	0	25
BB-04	5	7	3	1
BB-05	6	5	17	10
BB-07	0	0	2	0
BB-08	10	5	0	8
RR-01	2	0	0	0
RR-02	0	0	0	0
RR-03	12	6	2	11
RR-10	13	0	0	0
RR-25	15	5	0	0
RB-03	7	2	0	1
RB-04*	4	0	0	0
RB-05*	10	0	0	0
RB-02	1	0	1	0
RB-04!	22	2	0	2
RB-05!	23	0	0	4
BR-18	23	17	0	3
BR-01	1	0	0	0
BR-02	0	0	0	0
BR-03	0	0	0	0
BR-04	45	11	14	14
Totals	244	63	39	79

Table 7: Mean values for maximum frequency (kHz), minimum frequency (kHz), frequency range (kHz) and duration (sec.) of nestling calls for all treatment groups.

Treatment	Number of Nest Cups	Max. Freq.	s.d.	Min. Freq.	s.d.	Freq. Range	s.d	Duration	s.d.
BB	4	7.25	.85	5.25	.67	2.00	.88	.19	.07
RR	3	6.40	1.04	4.80	1.12	1.59	.71	.14	.06
BR	2	6.38	.72	4.67	.58	1.71	.71	.16	.05
RB	3	6.91	.97	5.30	.86	1.60	.86	.15	.06
CFBB	4	7.04	.79	5.04	.77	2.00	.85	.19	.08
CFRR	2	6.87	.75	4.92	.58	1.96	.78	.16	.05

ILLUSTRATIONS

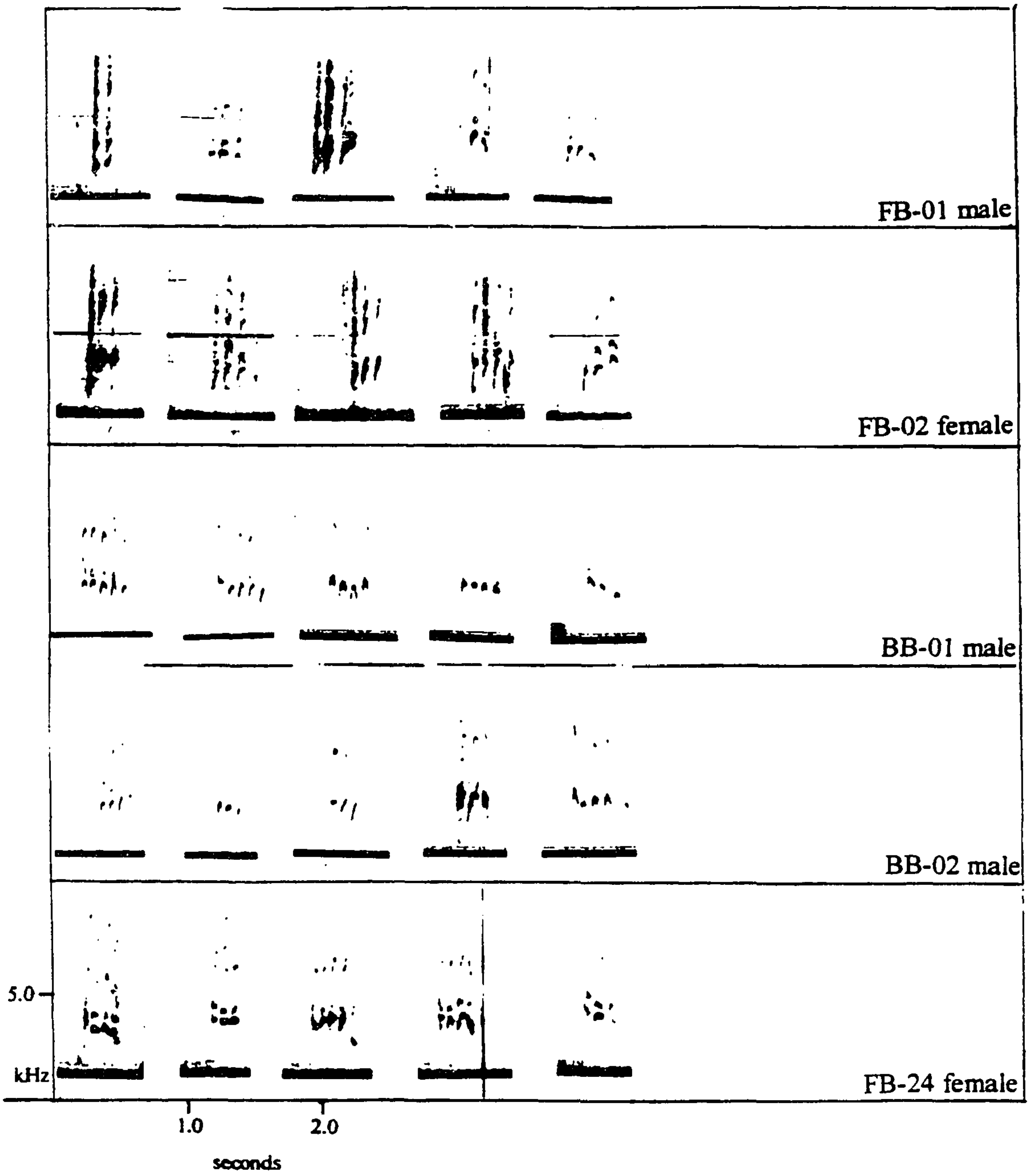

Fig. 1 : Sonograms of flight calls given by normal-reared Borders during the 1st ten days of sampling. Identities of birds are shown next to each sonogram.

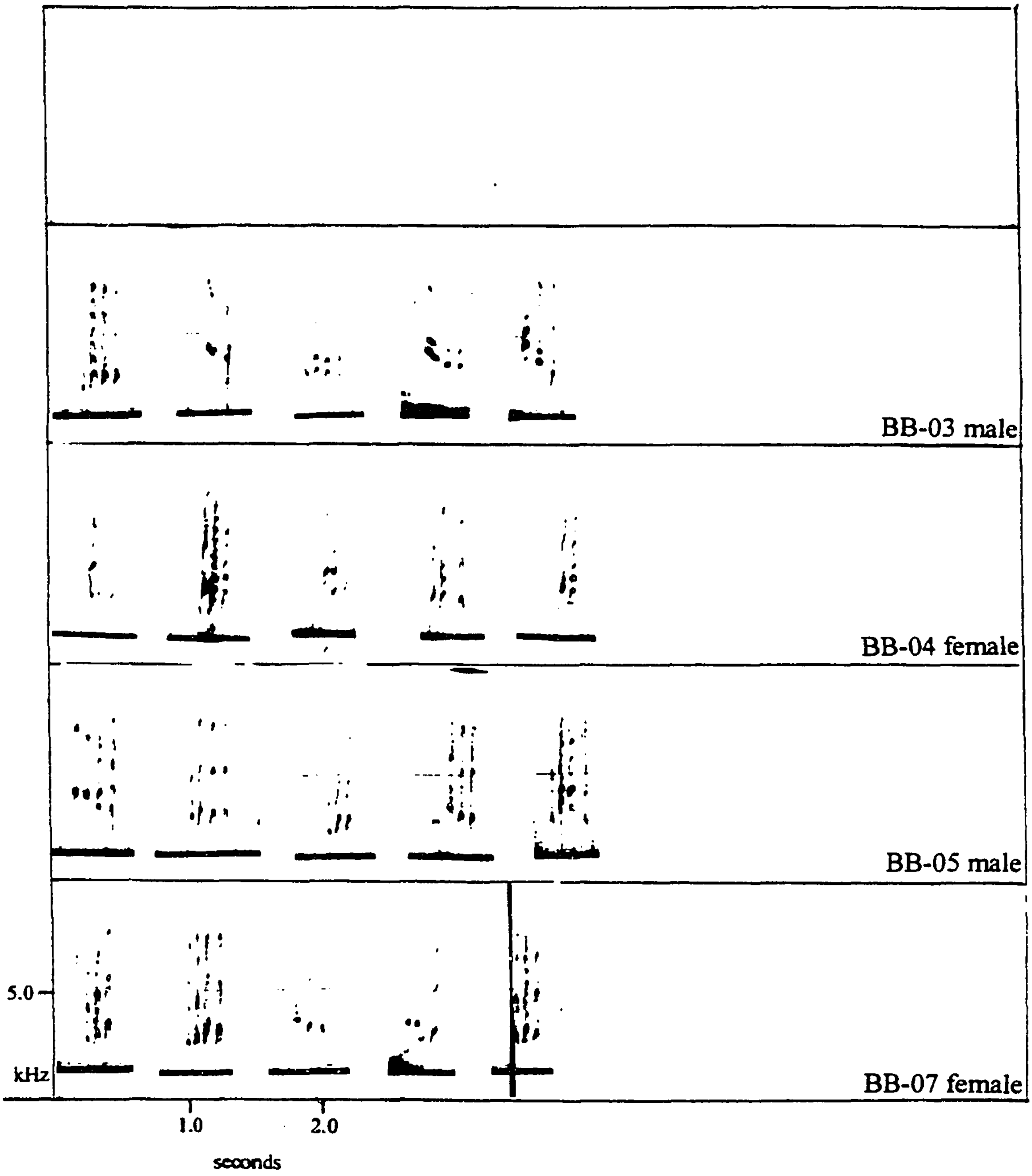

Fig. 1 : Continued.

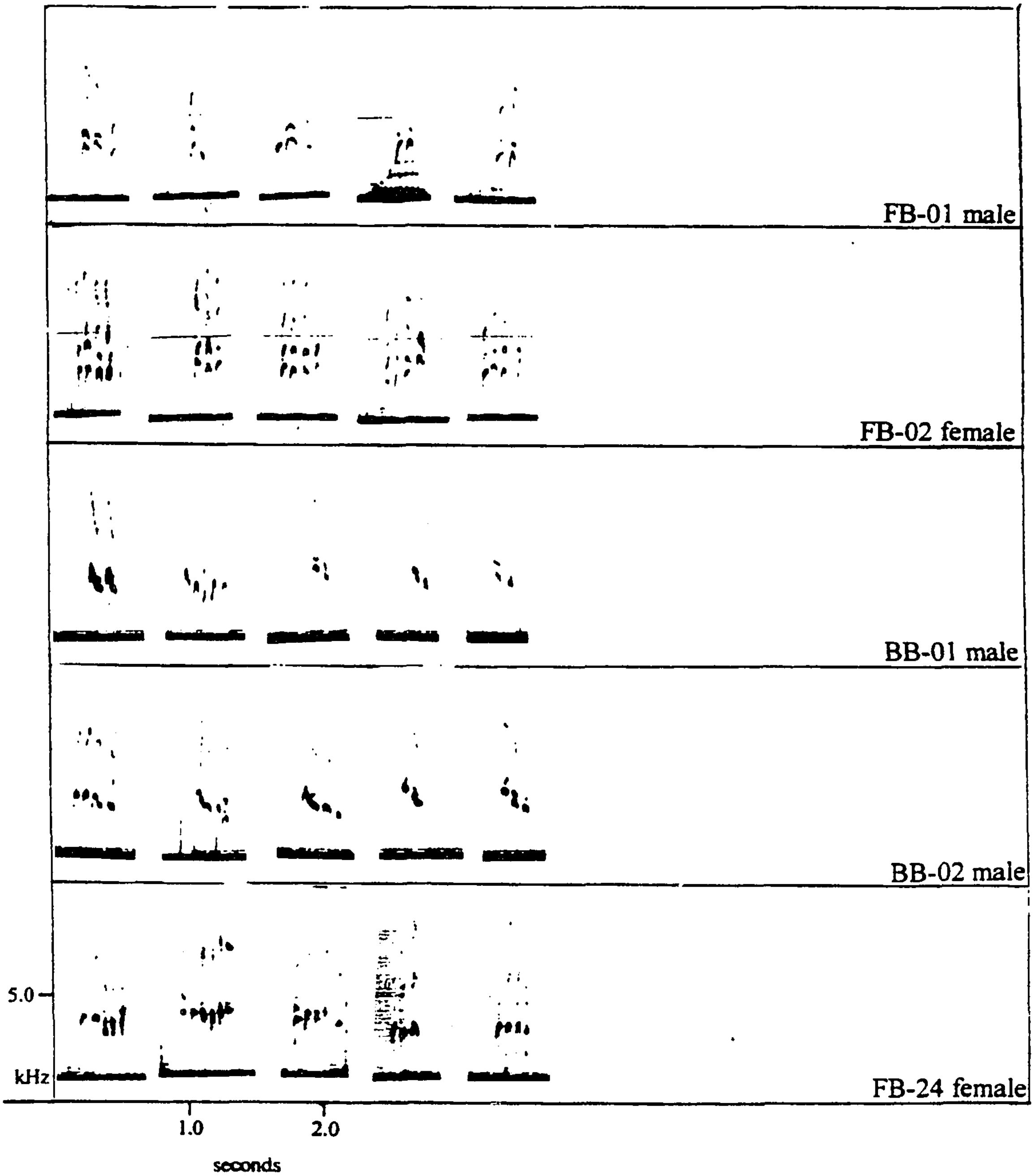

Fig. 2 : Sonograms of flight calls given by normal-reared Borders during the last ten days of sampling. Identities of birds are shown next to each sonogram.

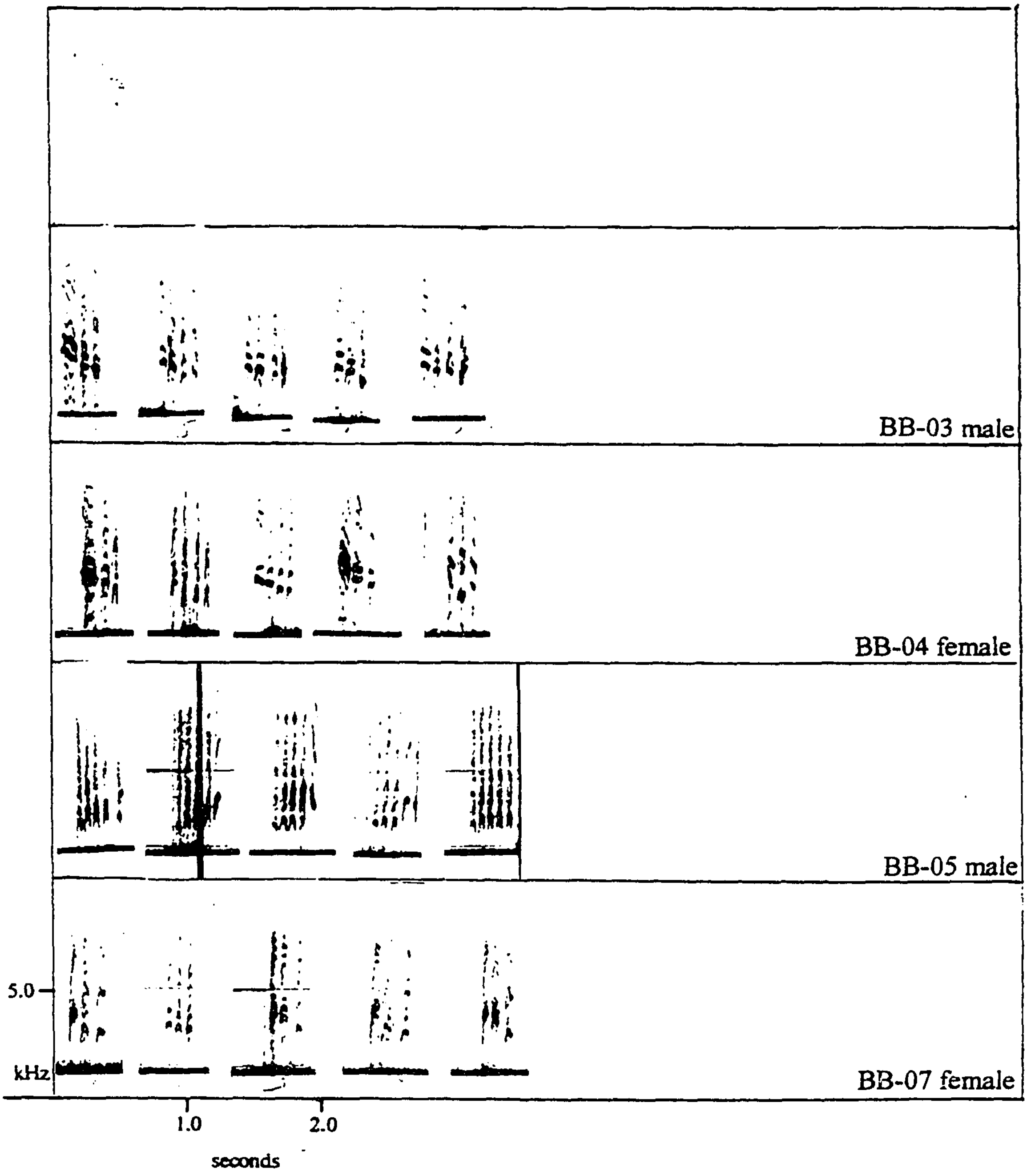

Fig. 2: Continued.

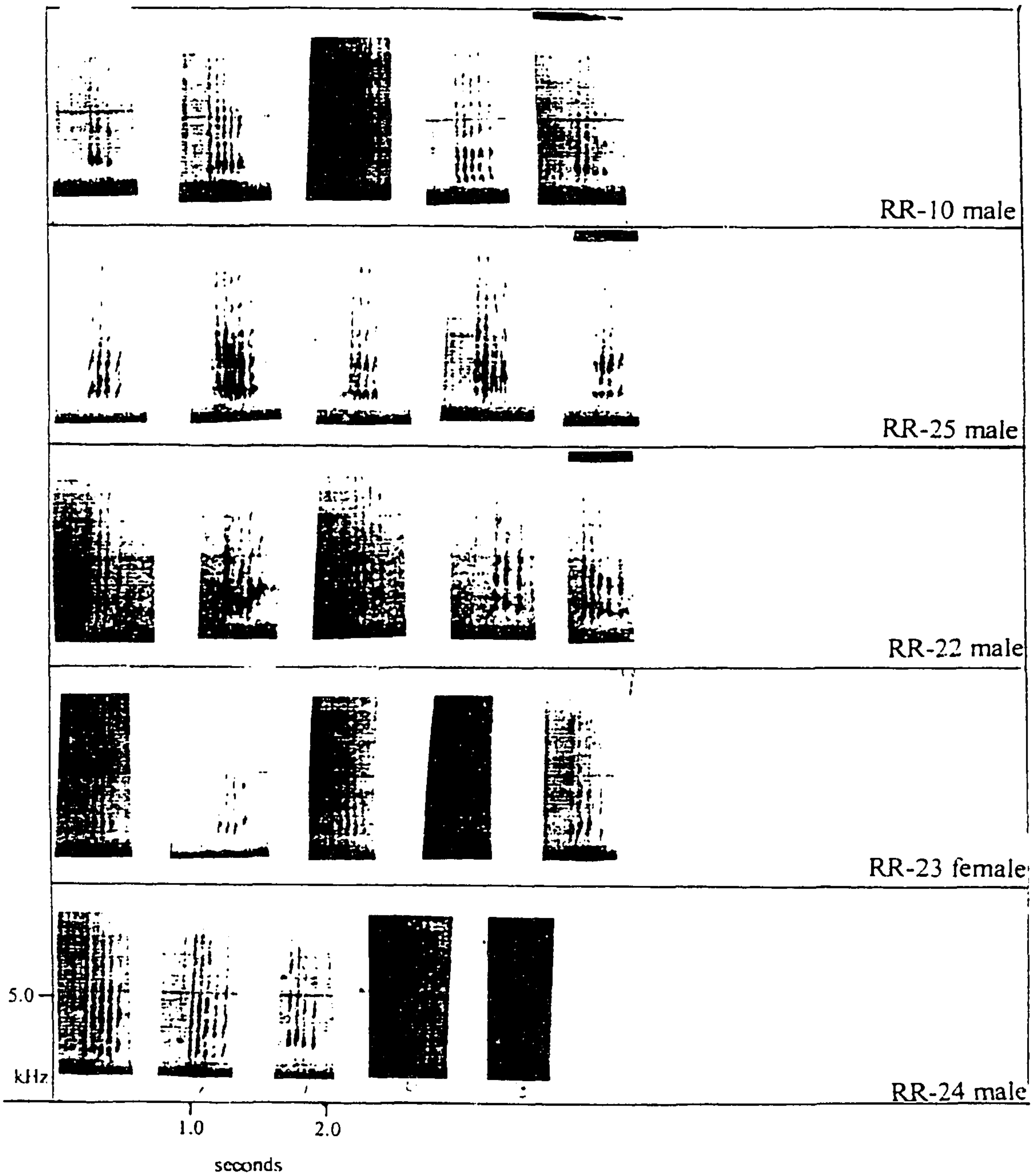

Fig. 3 : Sonograms of flight calls given by normal-reared Rollers during the 1st ten days of sampling. Identities of birds are shown next to each sonogram.

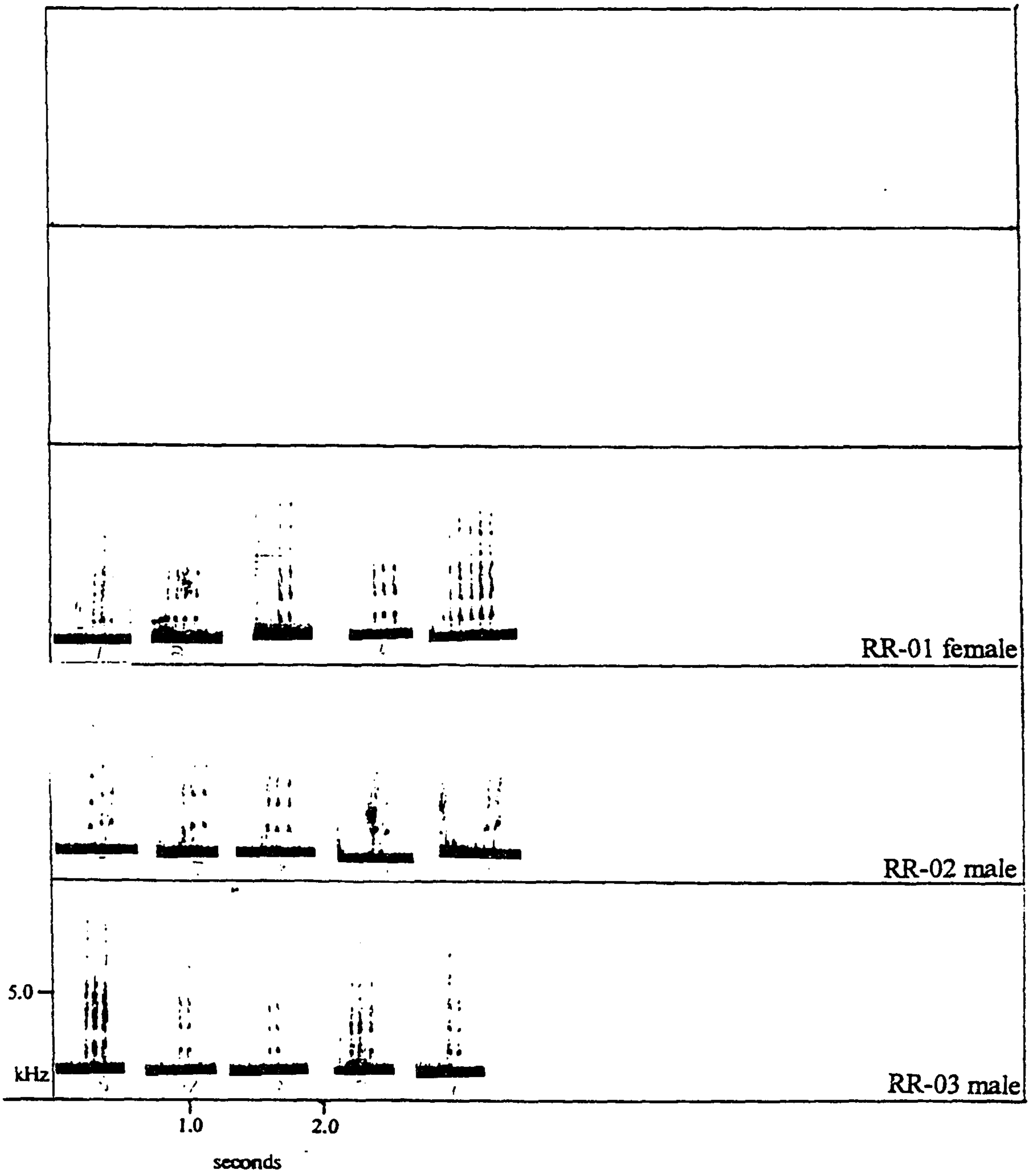

Fig. 3 : Continued.

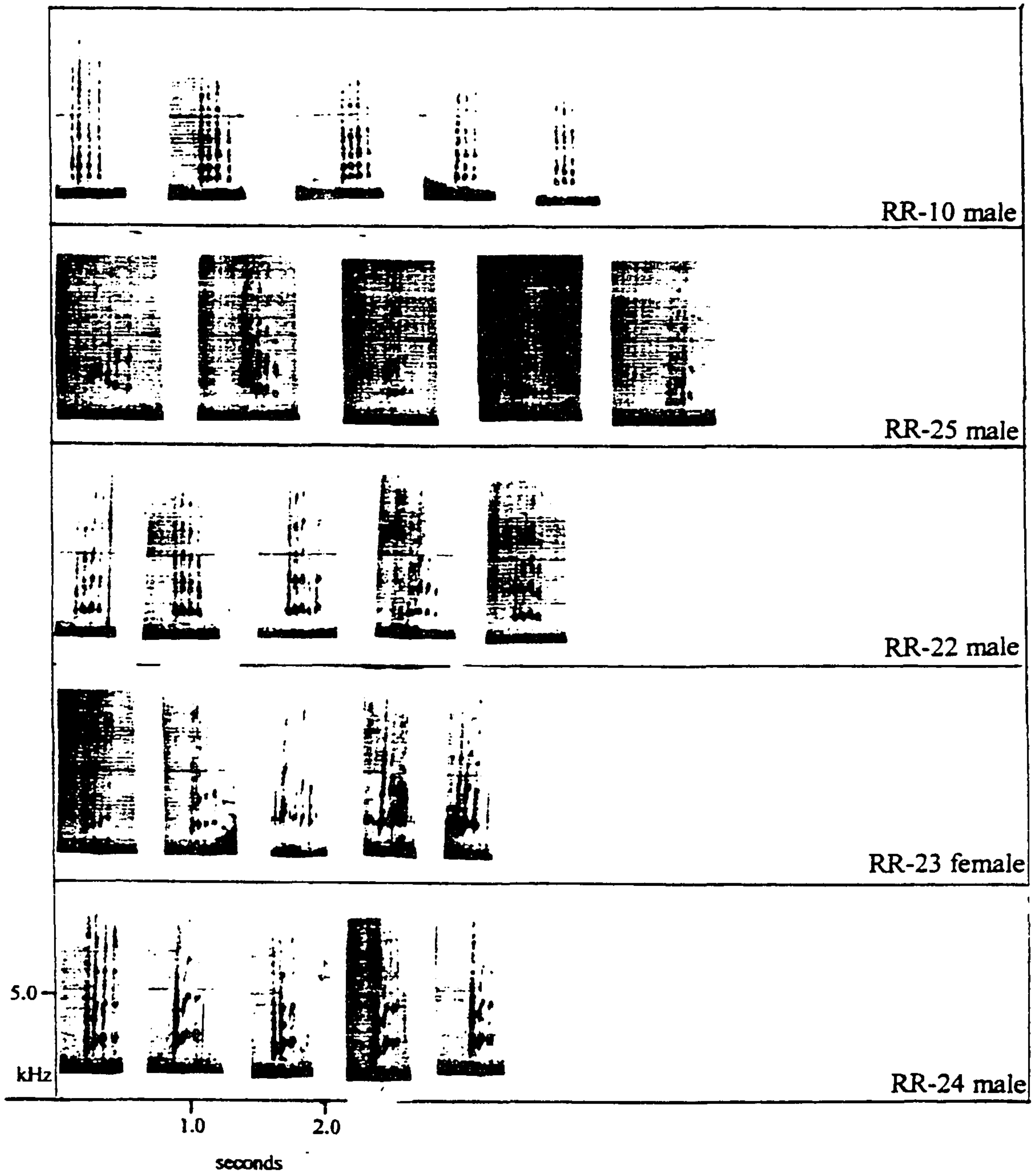

Fig. 4: Sonograms of flight calls given by normal-reared Rollers during the last ten days of sampling. Identities of birds are shown next to each sonogram.

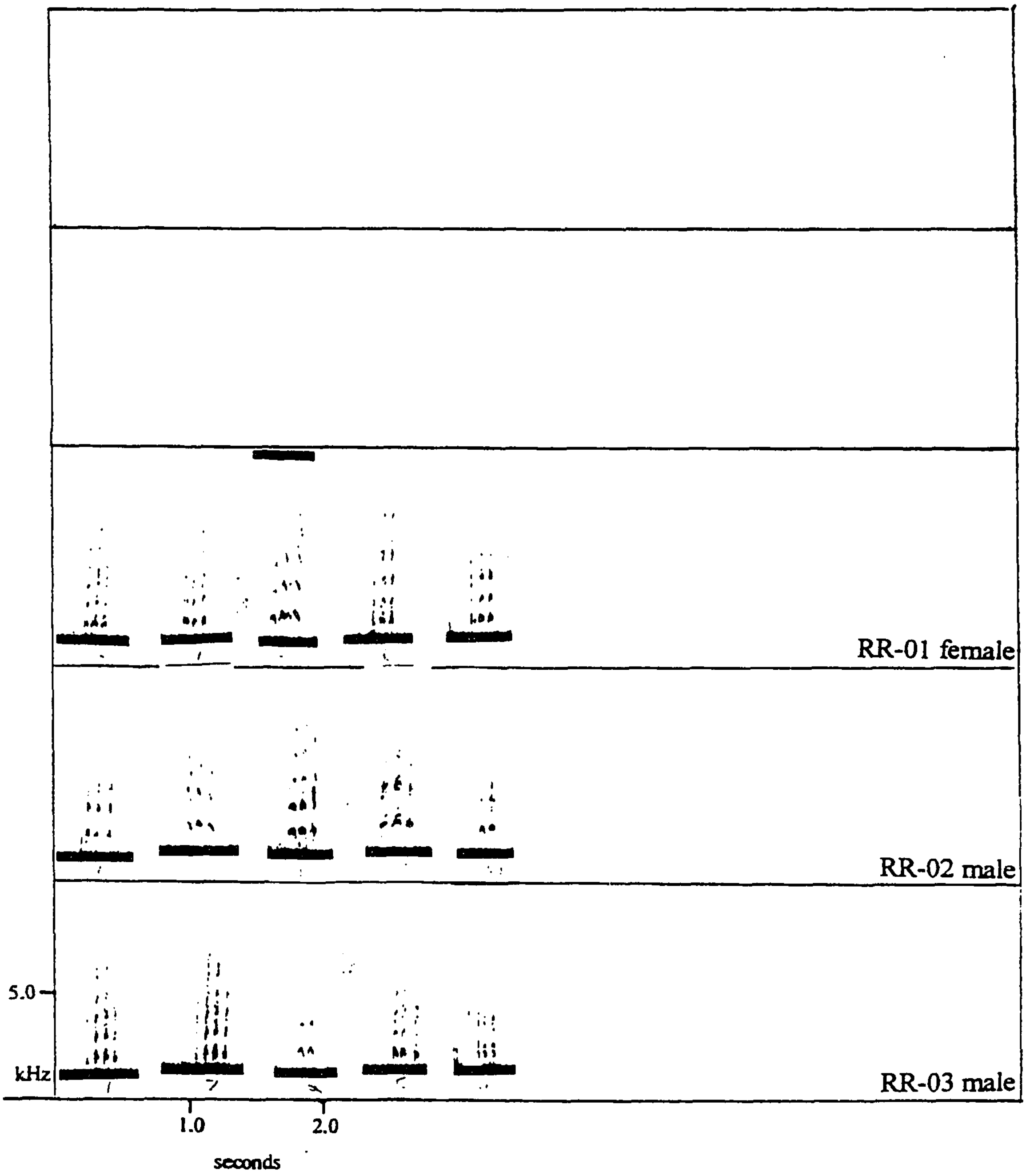

Fig. 4: Continued.

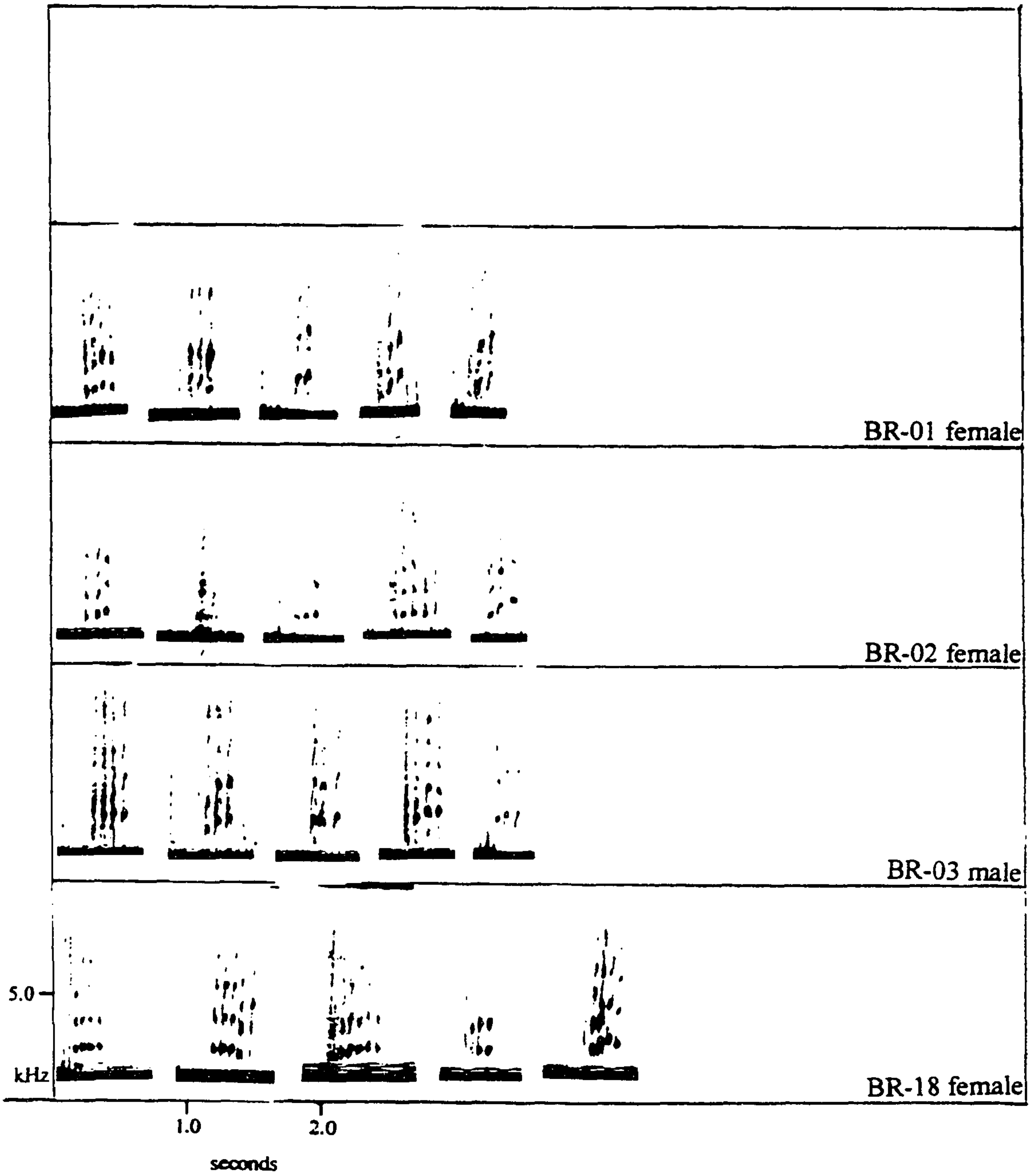

Fig. 5: Sonograms of flight calls given BR hybrids during the 1st ten days of sampling. Identities of birds are shown next to each sonogram.

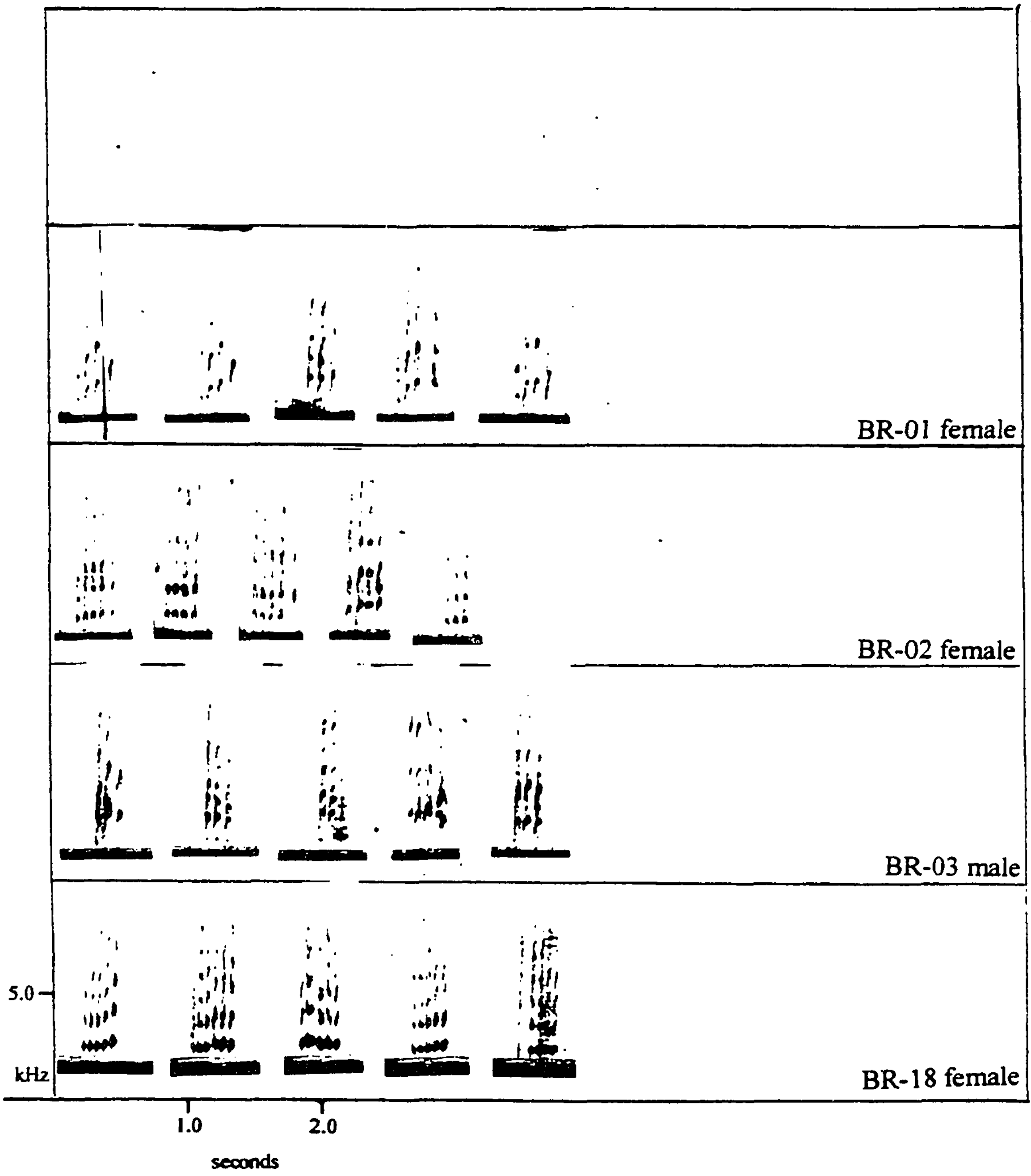

Fig. 6: Sonograms of flight calls given BR hybrids during the last ten days of sampling. Identities of birds are shown next to each sonogram.

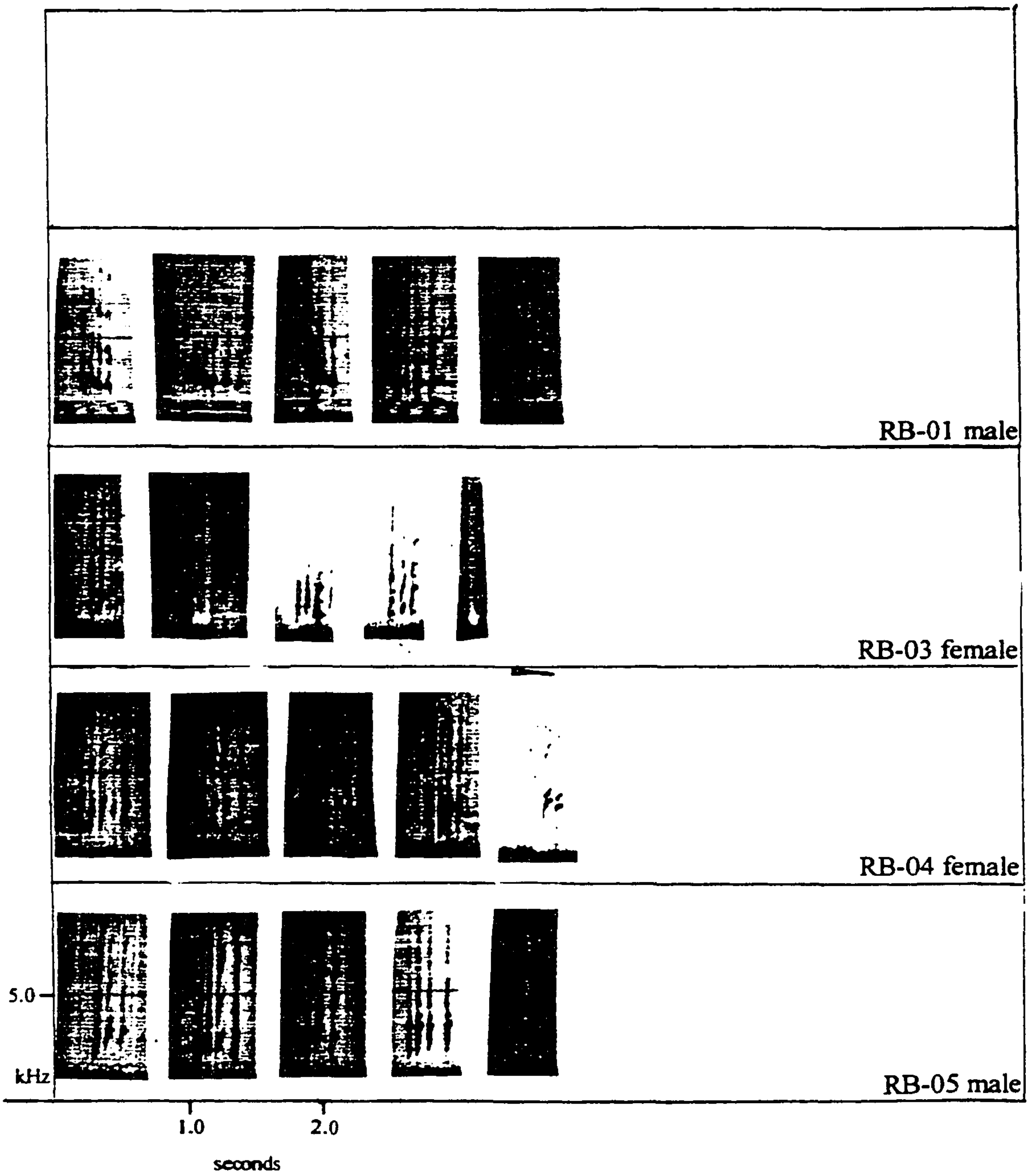

Fig. 7: Sonograms of flight calls given RB hybrids during the 1st ten days of sampling. Identities of birds are shown next to each sonogram.

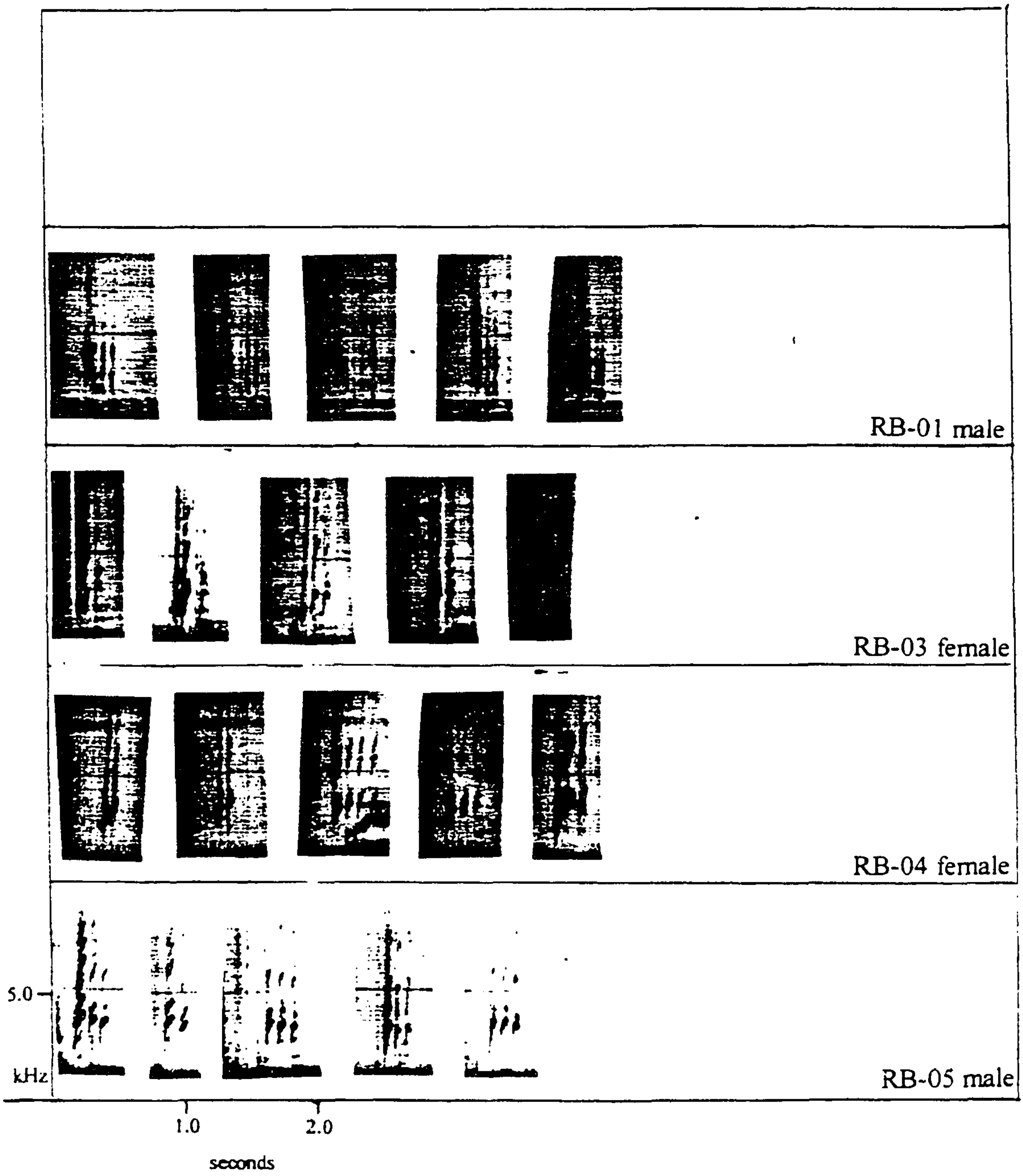

Fig. 8: Sonograms of flight calls given RB hybrids during the last ten days of sampling. Identities of birds are shown next to each sonogram.

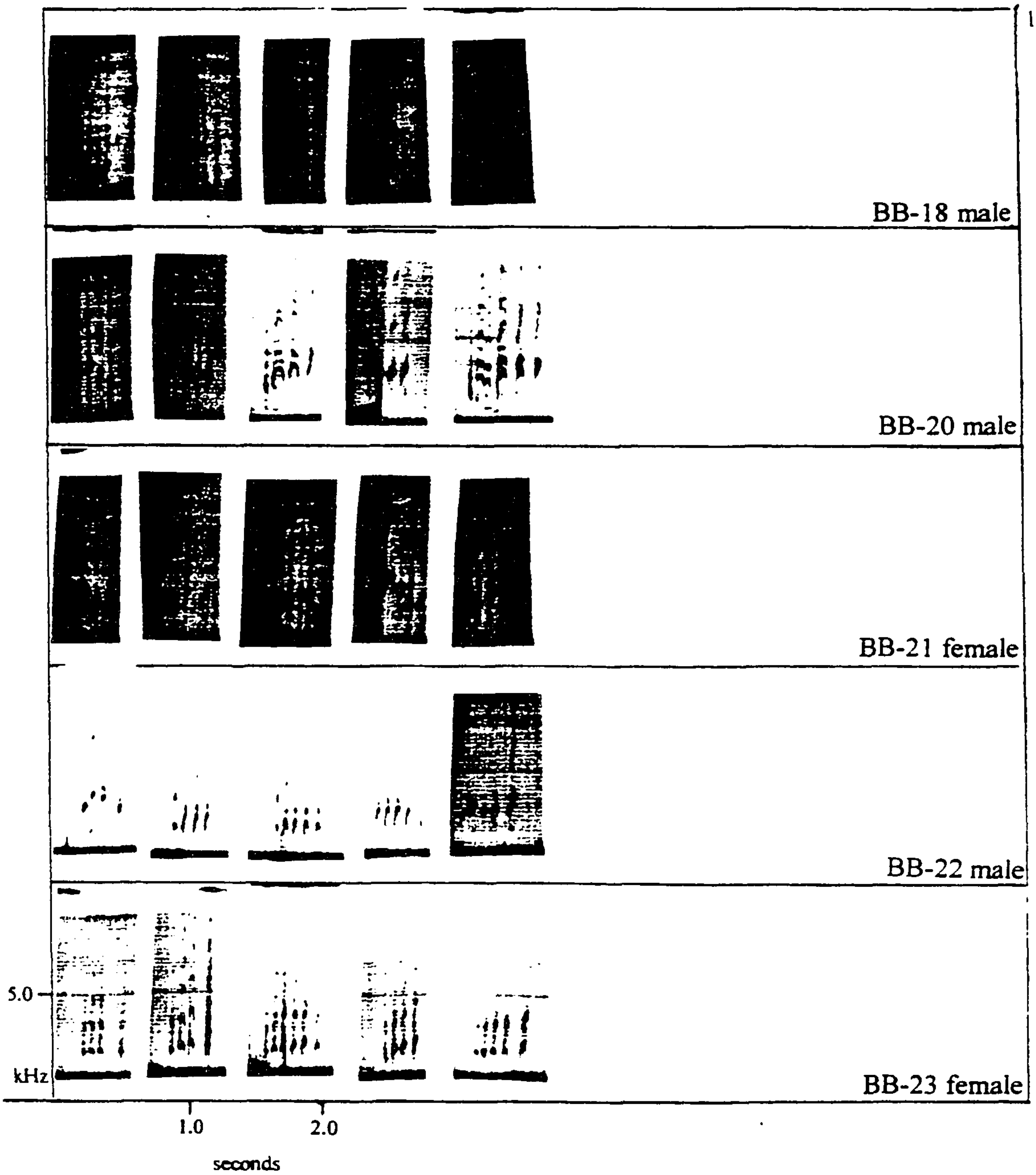

Fig.9 : Sonograms of flight calls given by cross-fostered Borders during the 1st ten days of sampling. Identities of birds are shown next to each sonogram.

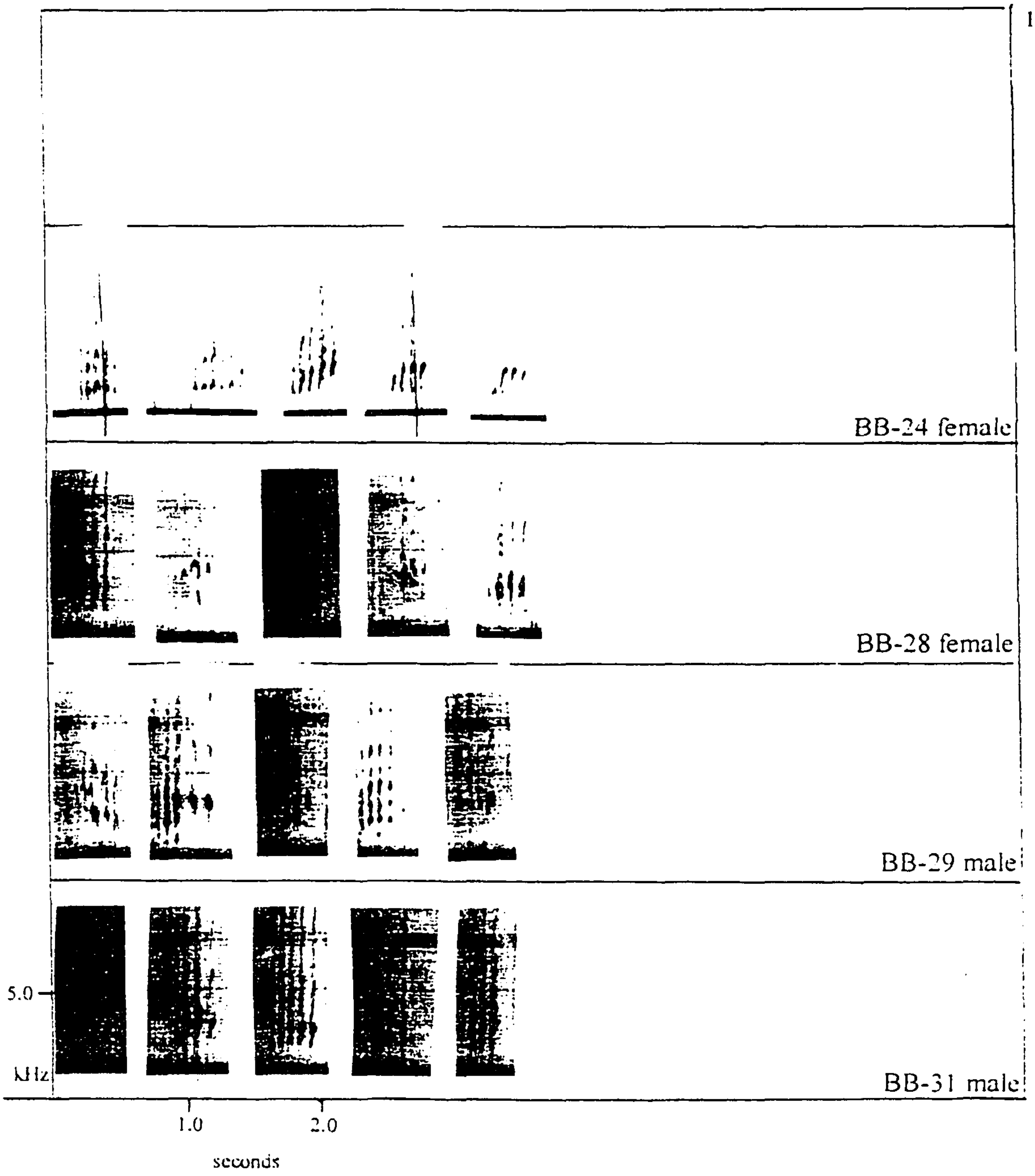

Fig. 9: Continued.

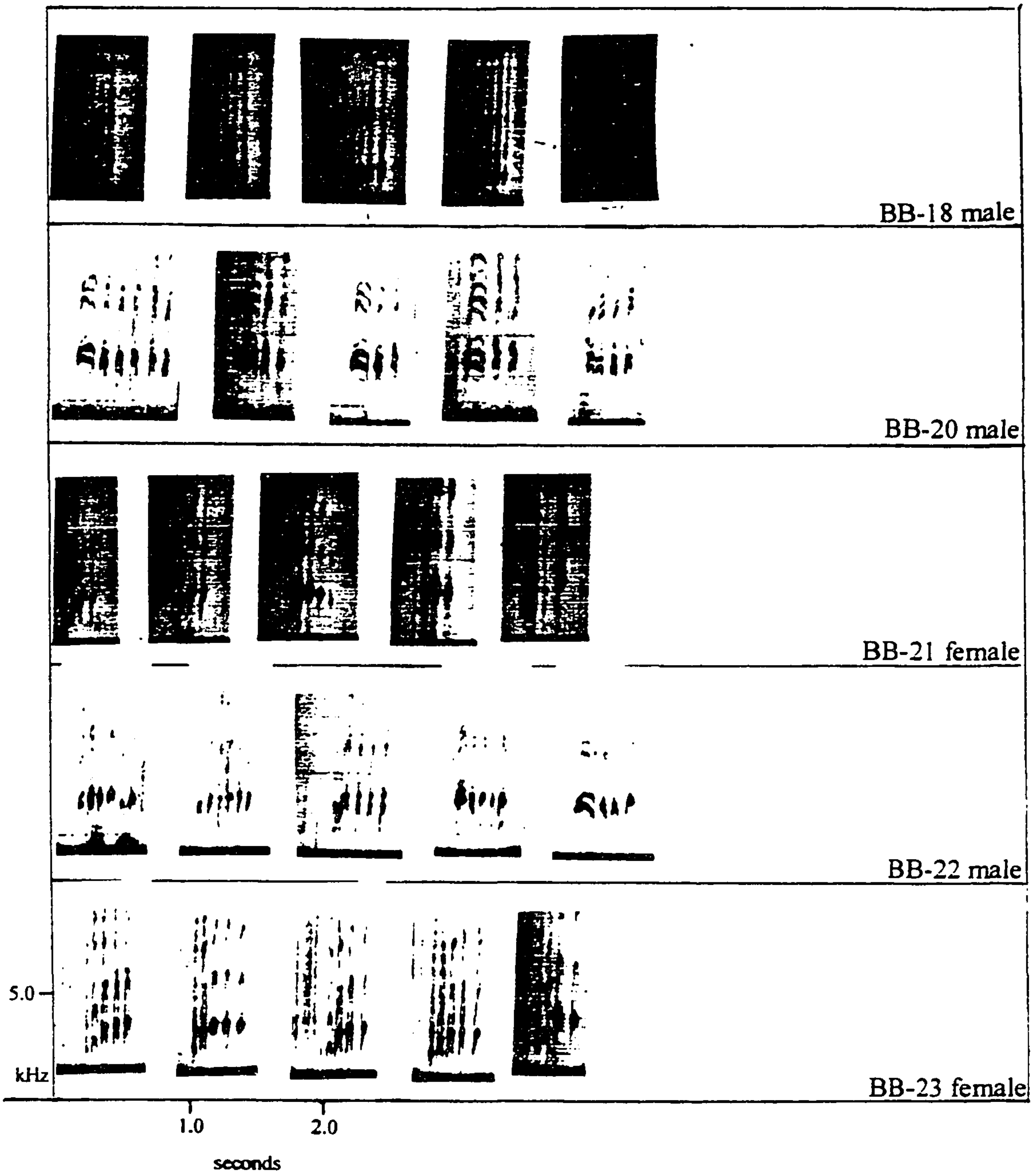

Fig.10: Sonograms of flight calls given by cross-fostered Borders during the last ten days of sampling. Identities of birds are shown next to each sonogram.

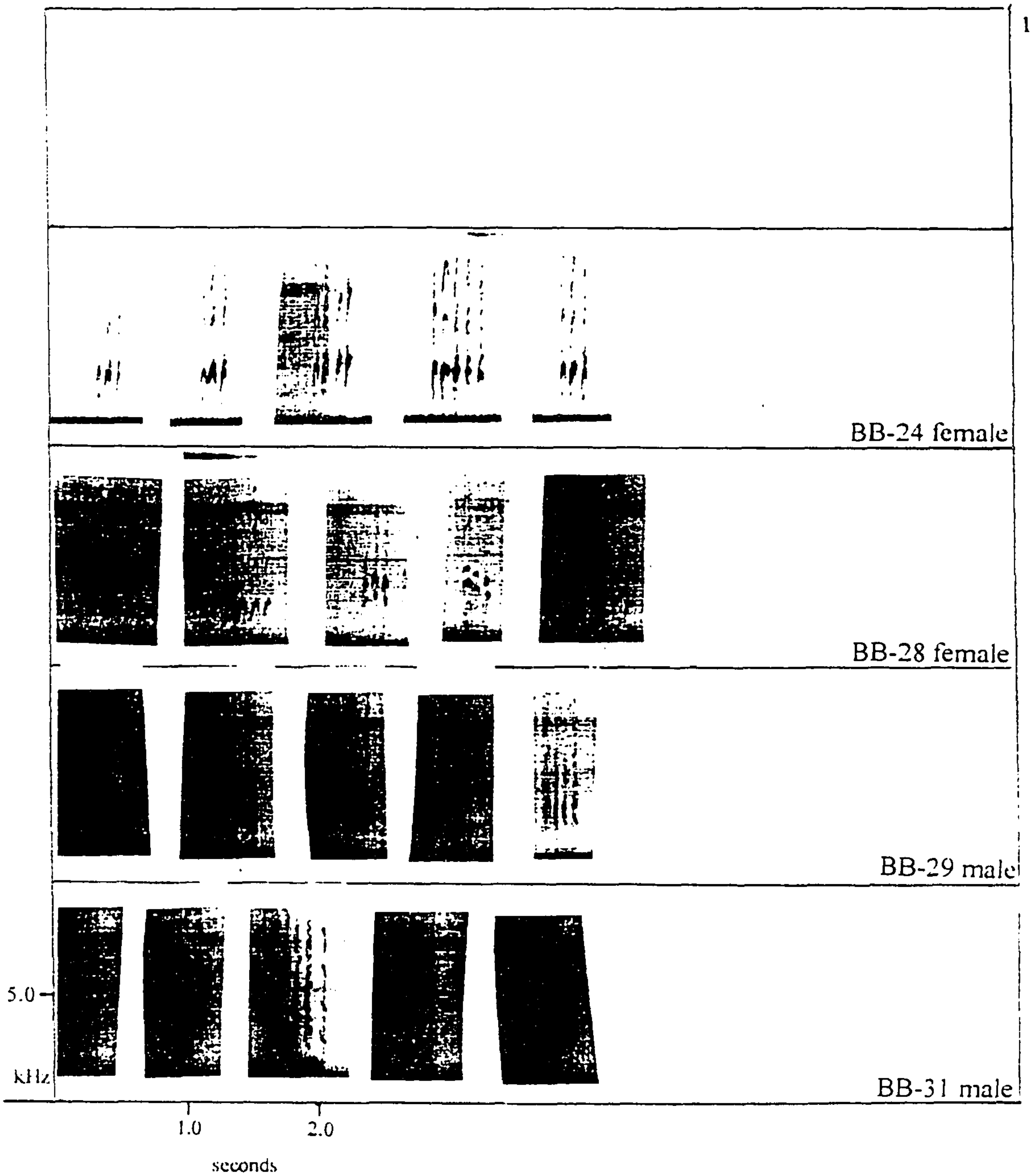

Fig.10:Continued.

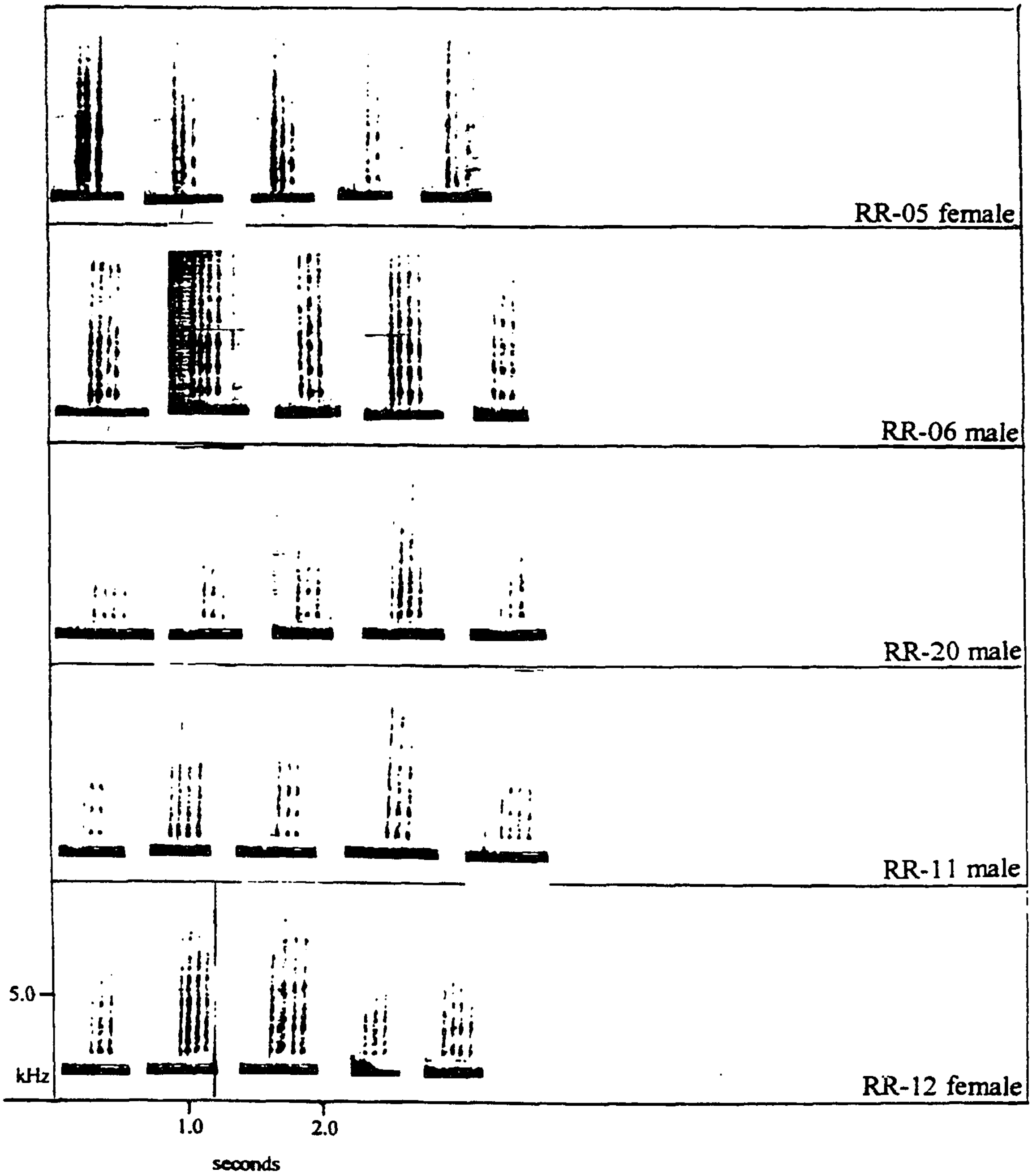

Fig.11:Sonograms of flight calls given by cross-fostered Rollers during the 1st ten days of sampling. Identities of birds are shown next to each sonogram.

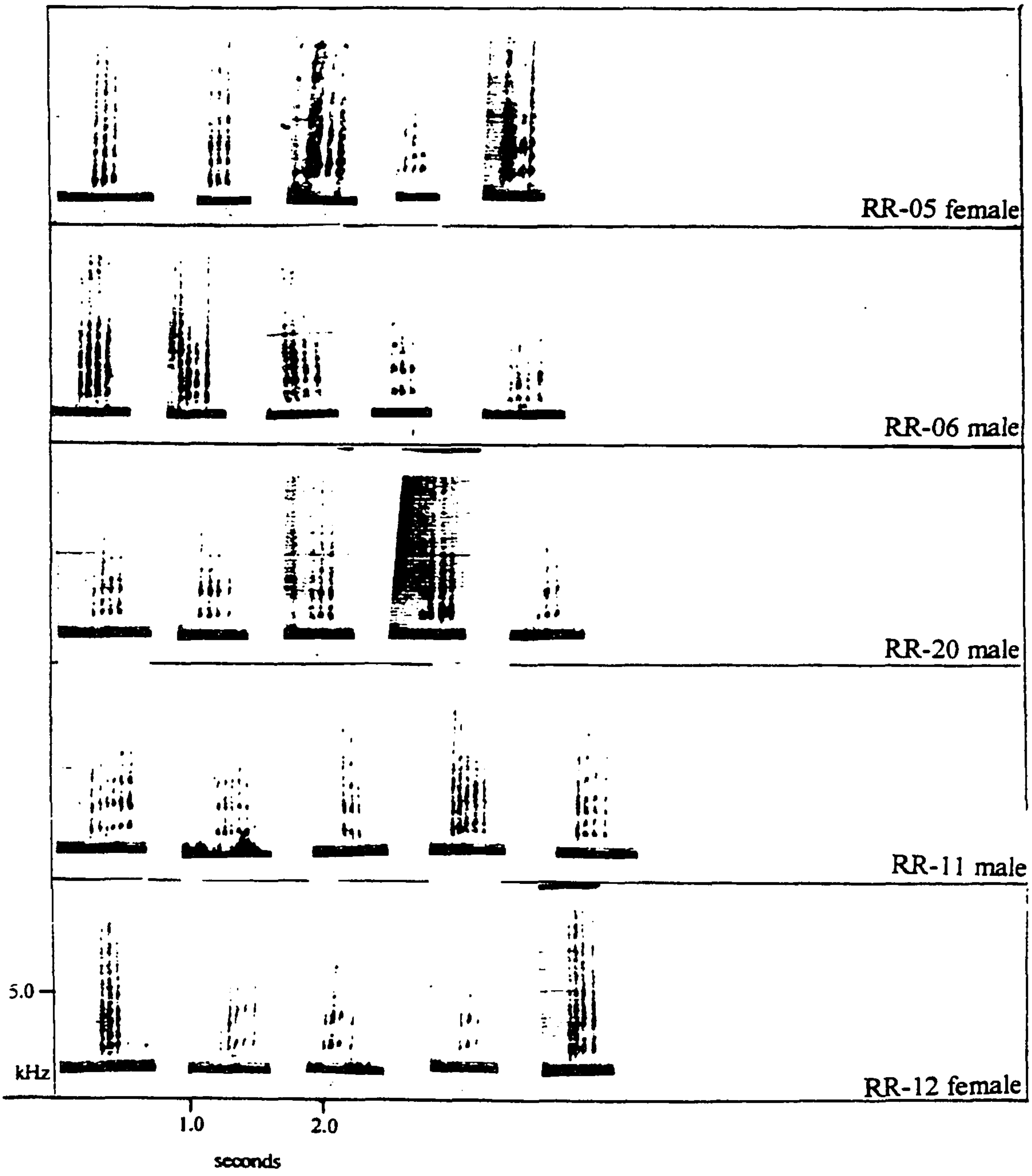

Fig.12:Sonograms of flight calls given by cross-fostered Rollers during the last ten days of sampling. Identities of birds are shown next to each sonogram.

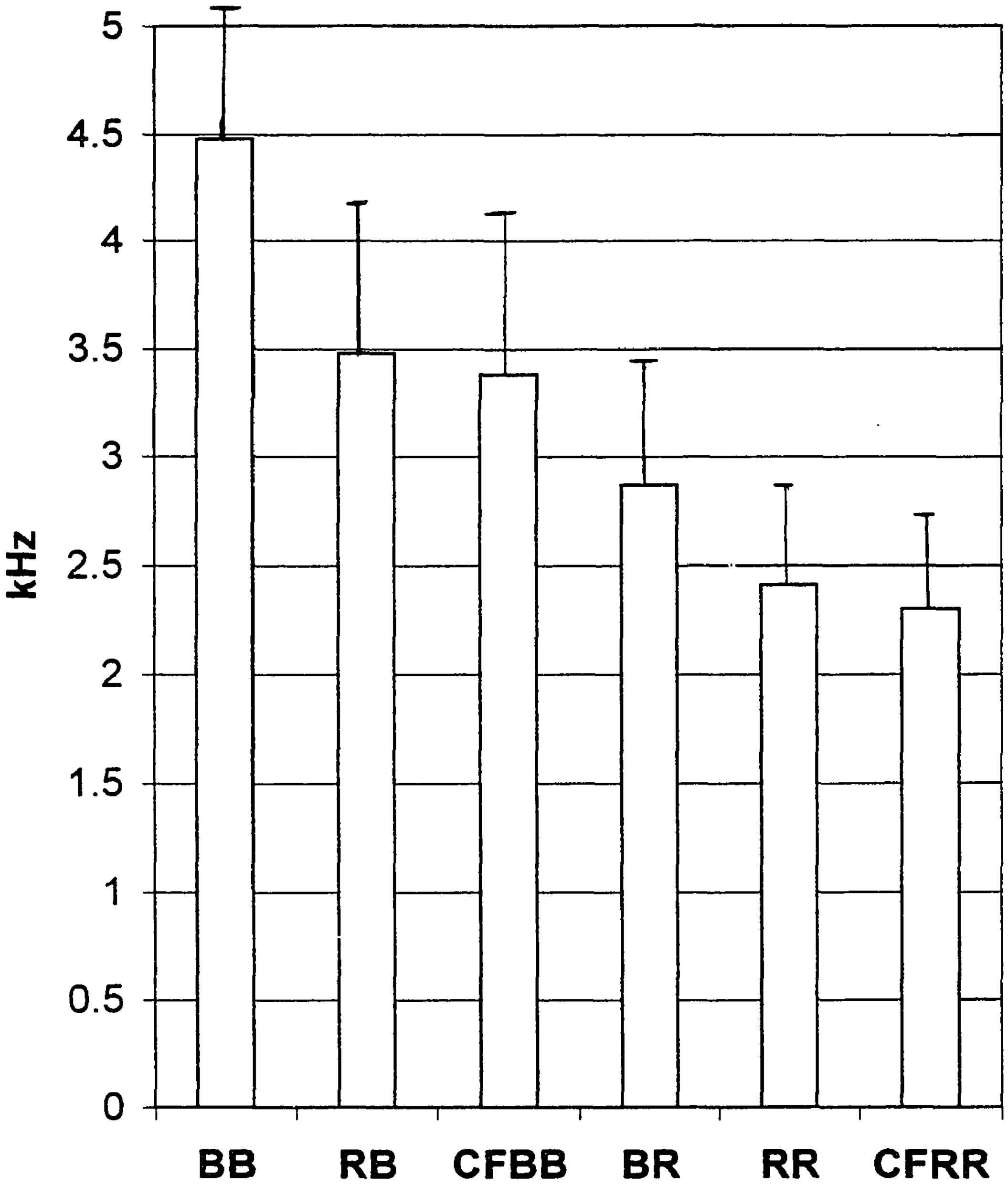

Fig. 13: Mean maximum frequency of flight calls for all six treatments during the first ten days of sampling.

]

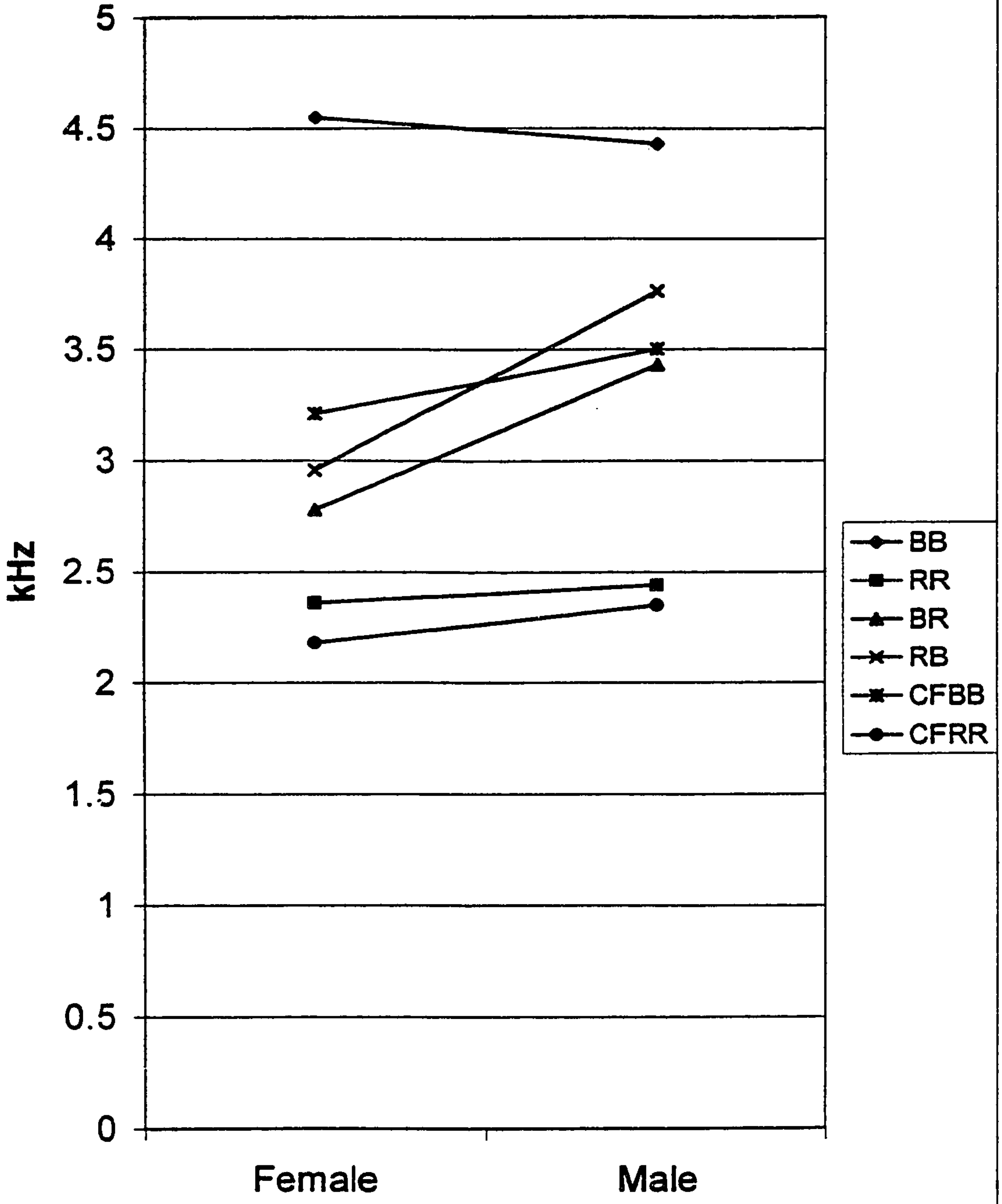

Fig. 14: Maximum frequency for flight calls, sex by treatments interaction. First ten days of sampling.

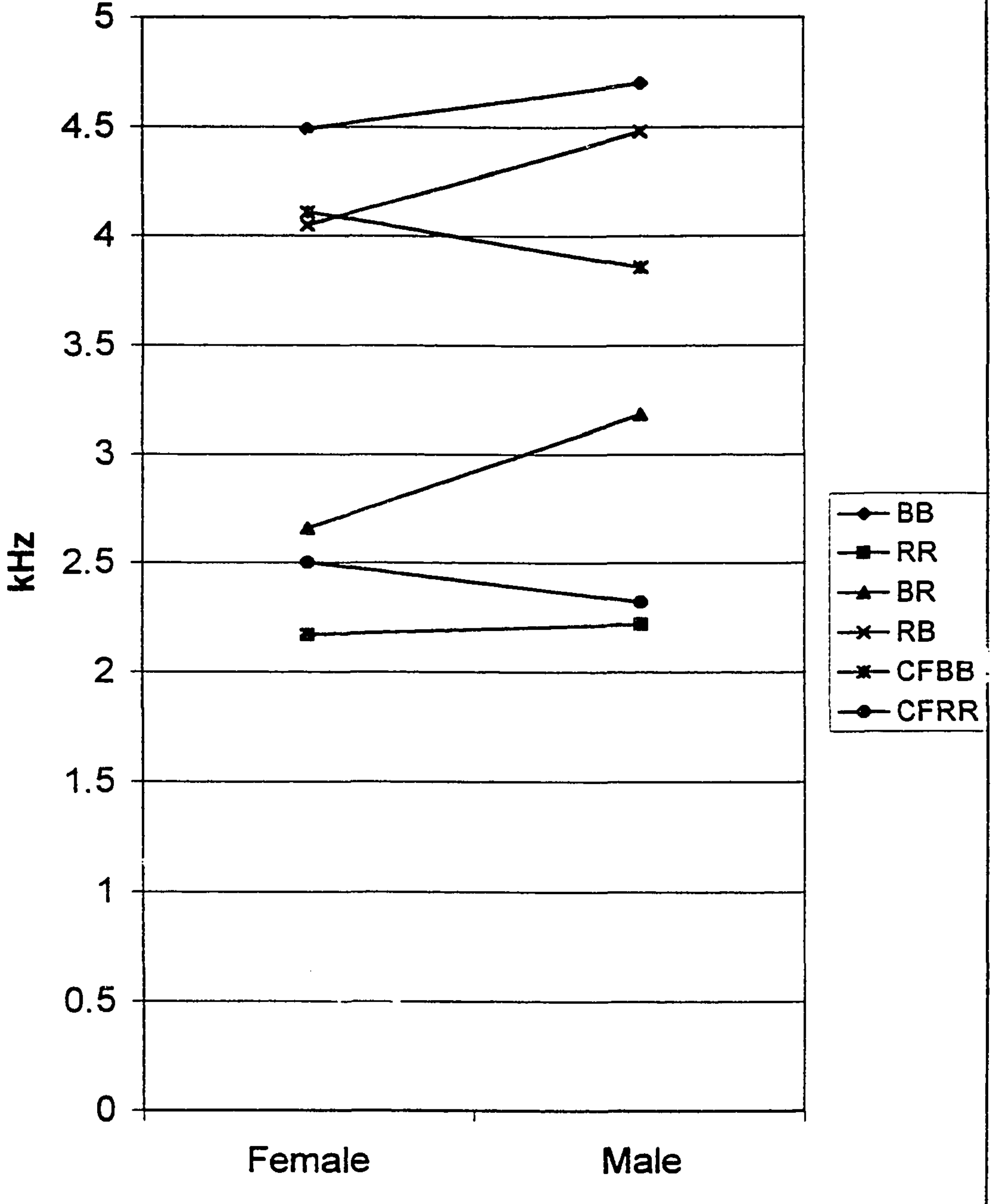

Fig. 15: Maximum frequency for flight calls, sex by treatments interaction. Last ten days of sampling.

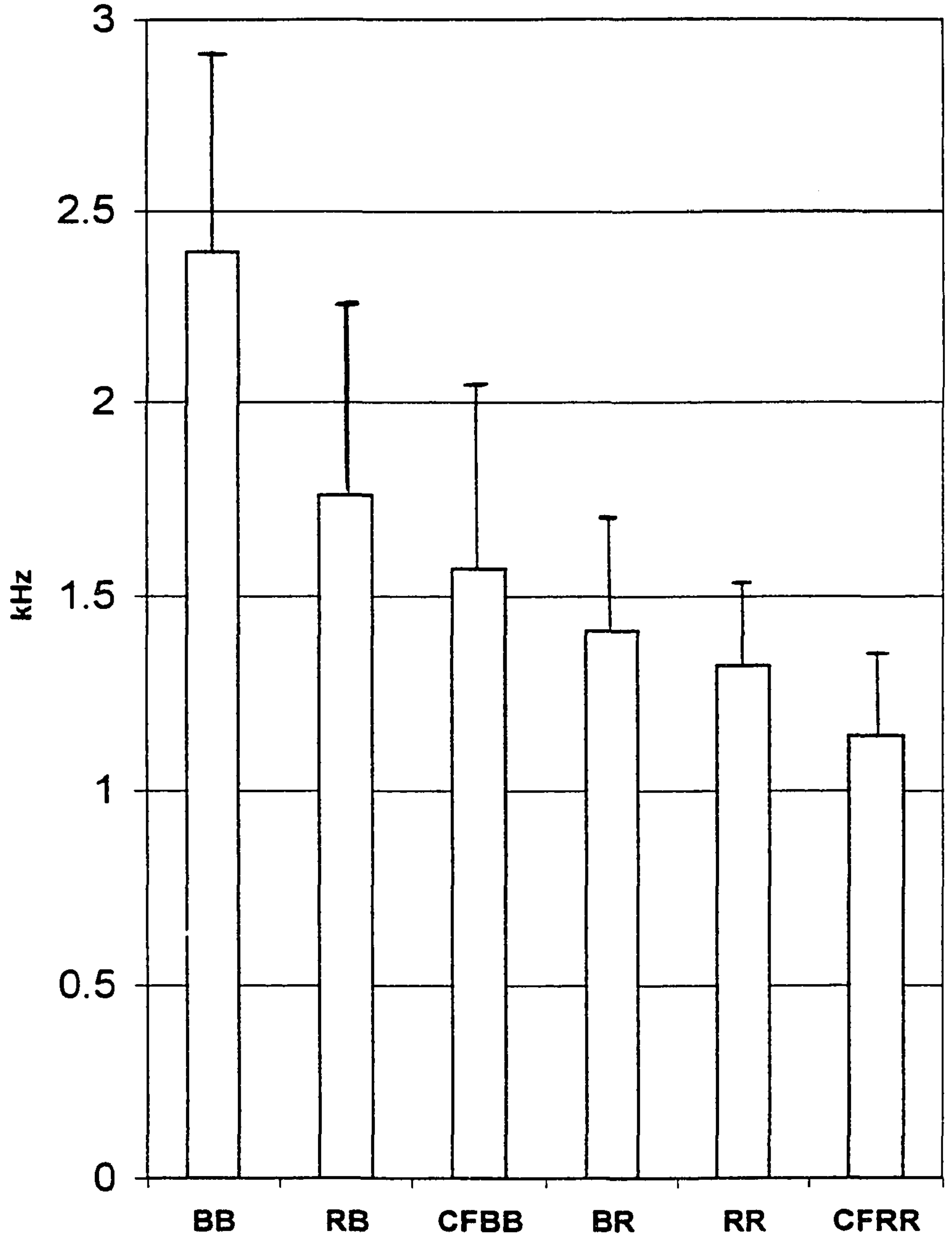

Fig.16: Mean minimum frequency of flight calls for all six treatments during the first ten days of sampling.

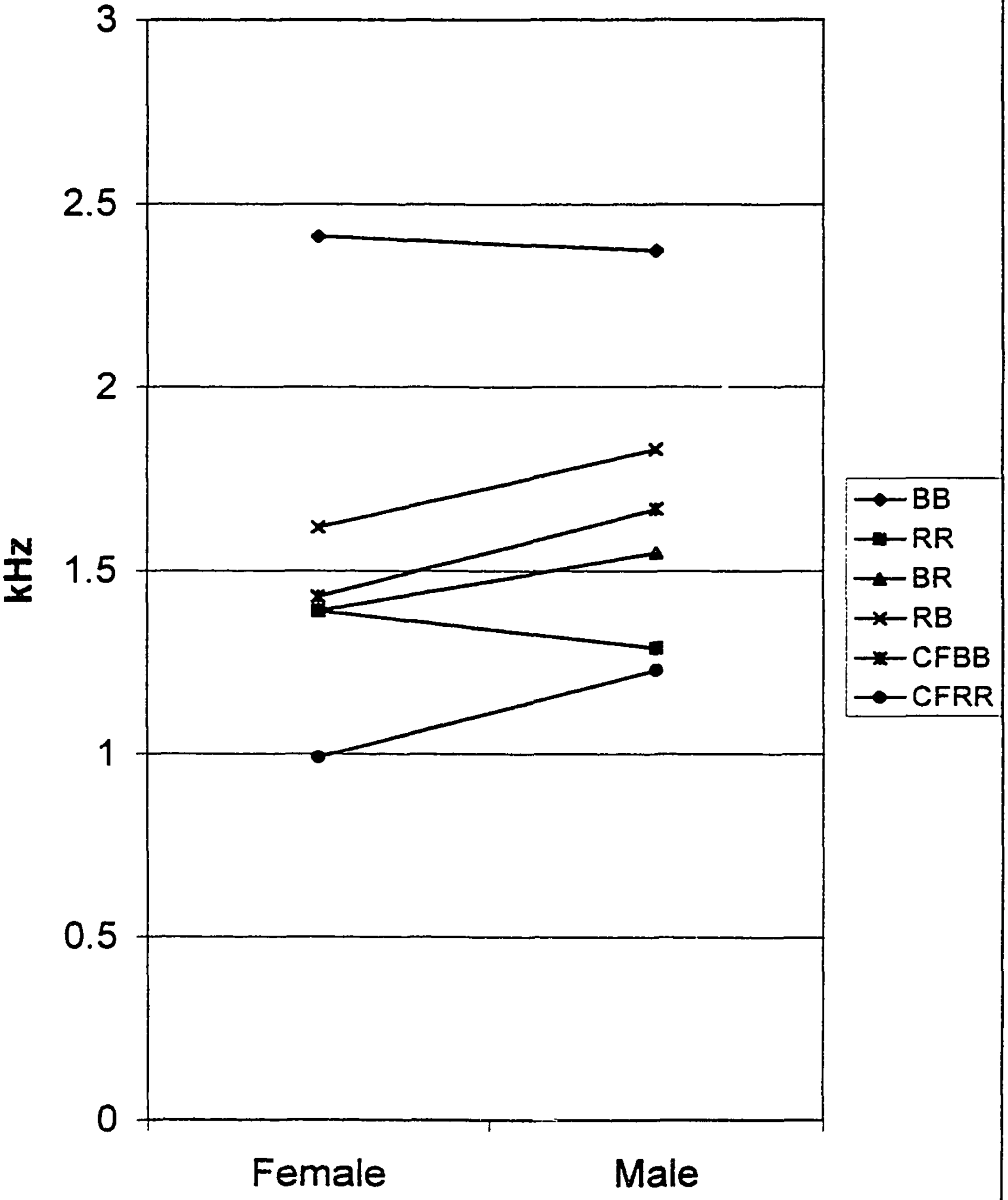

Fig. 17: Minimum frequency of flight calls, sex by treatments interaction. First ten days of sampling.

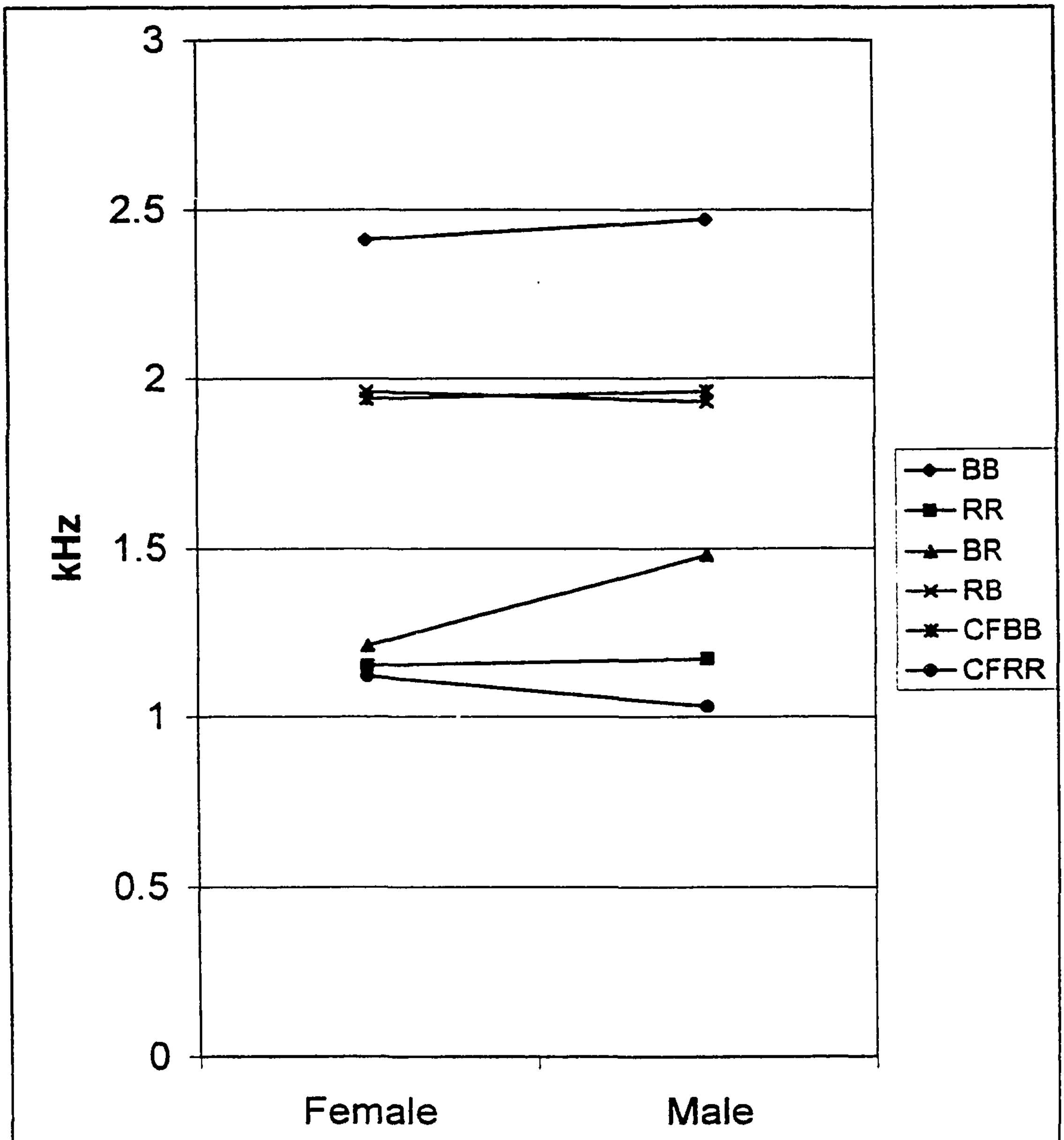

Fig. 18: Minimum frequency of flight calls, sex by treatments interaction. Last ten days of sampling.

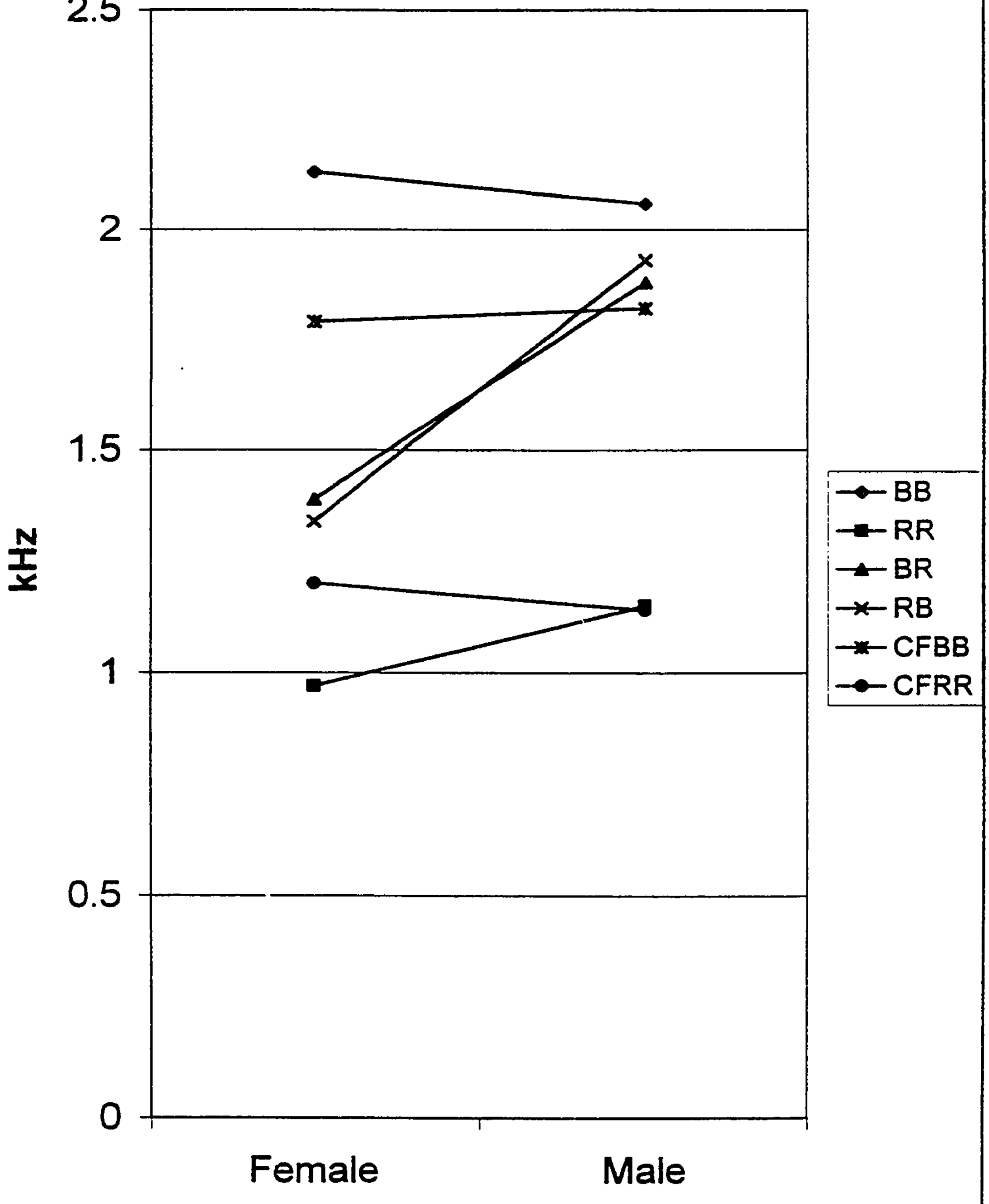

Fig. 19: Frequency range of flight calls, sex by treatments interaction. First ten days of sampling.

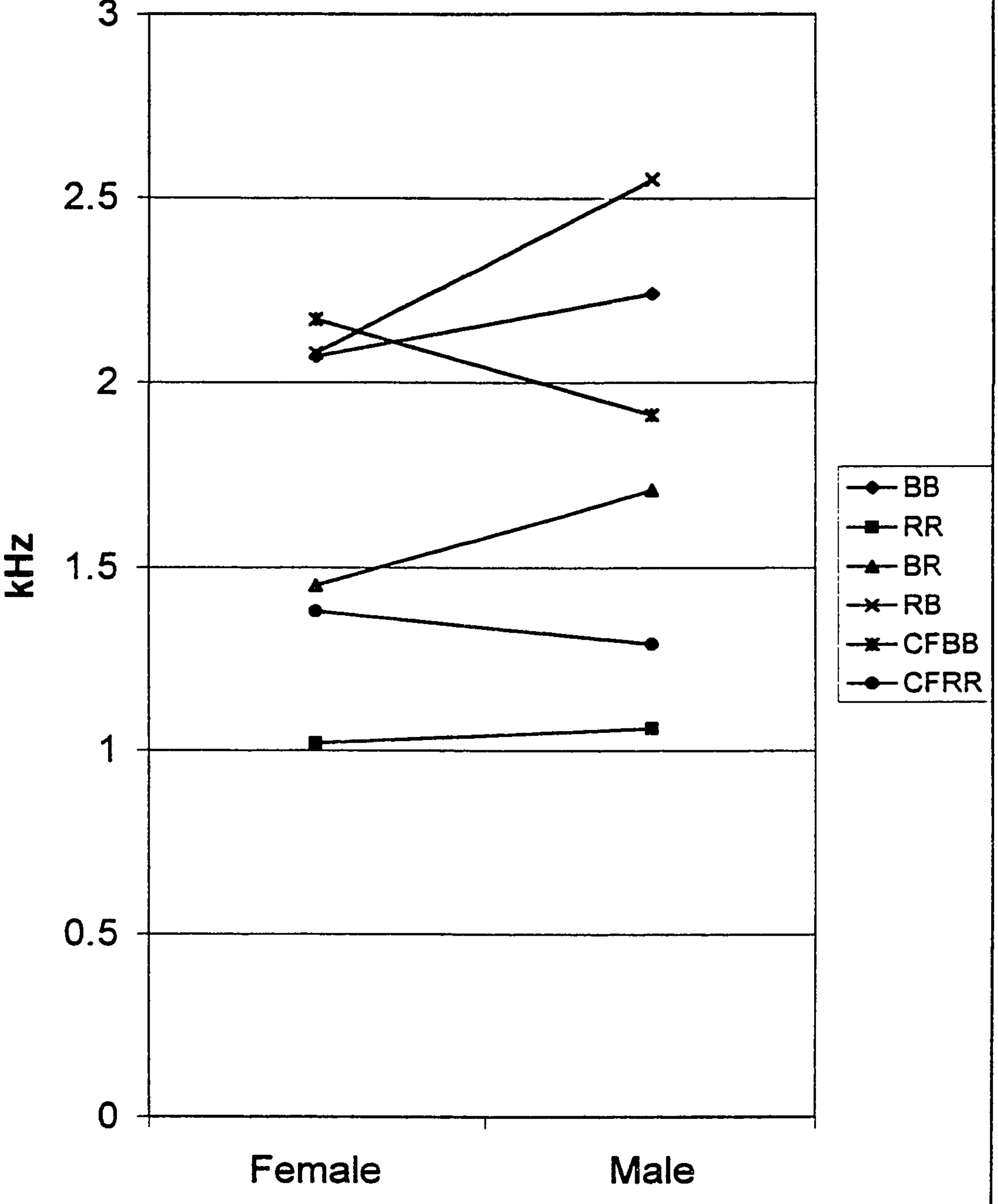

Fig. 20: Frequency range of flight calls, sex by treatments interaction. Last ten days of sampling.

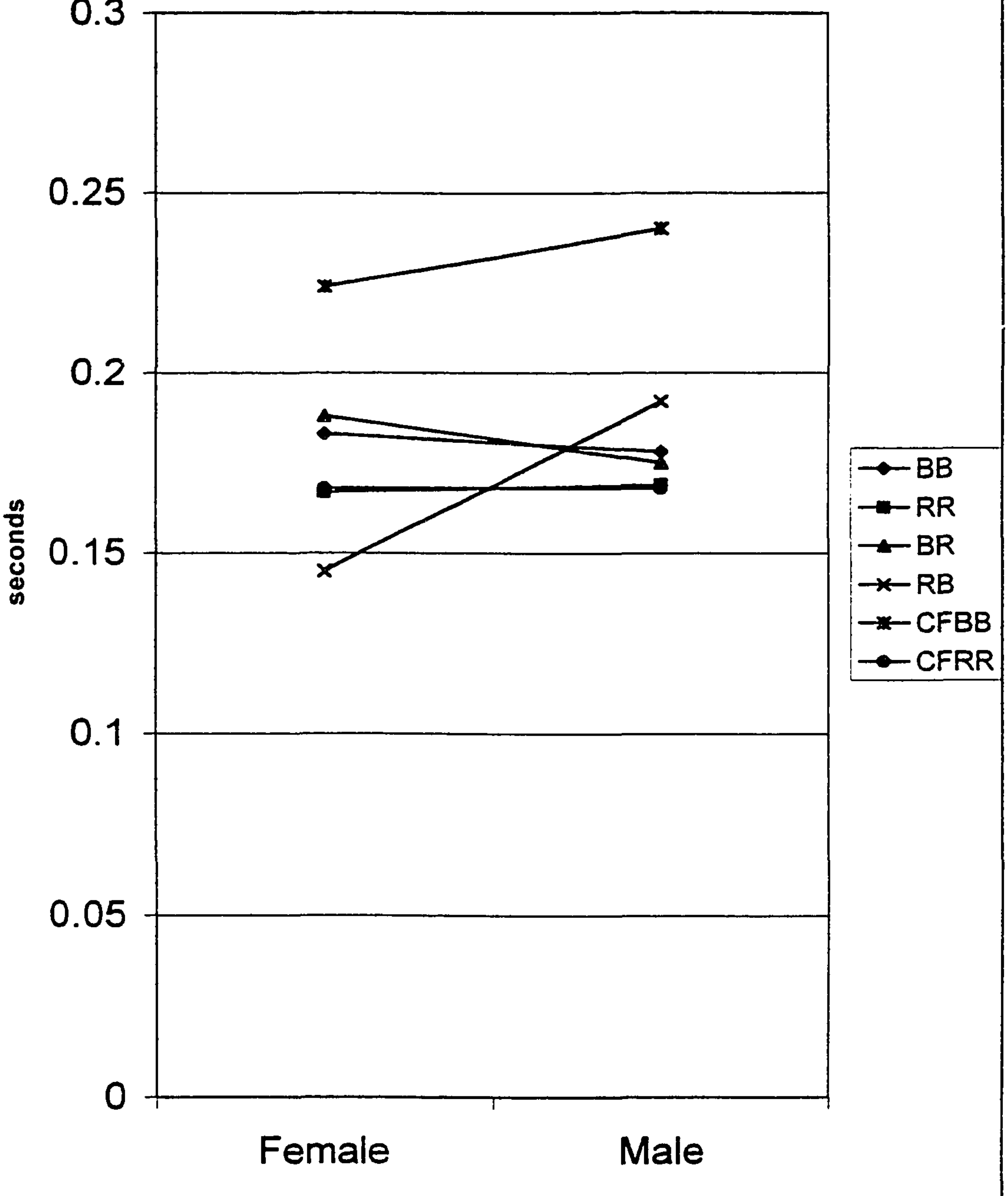

Fig. 21: Duration of flight calls, sex by treatments interaction. first ten days of sampling.

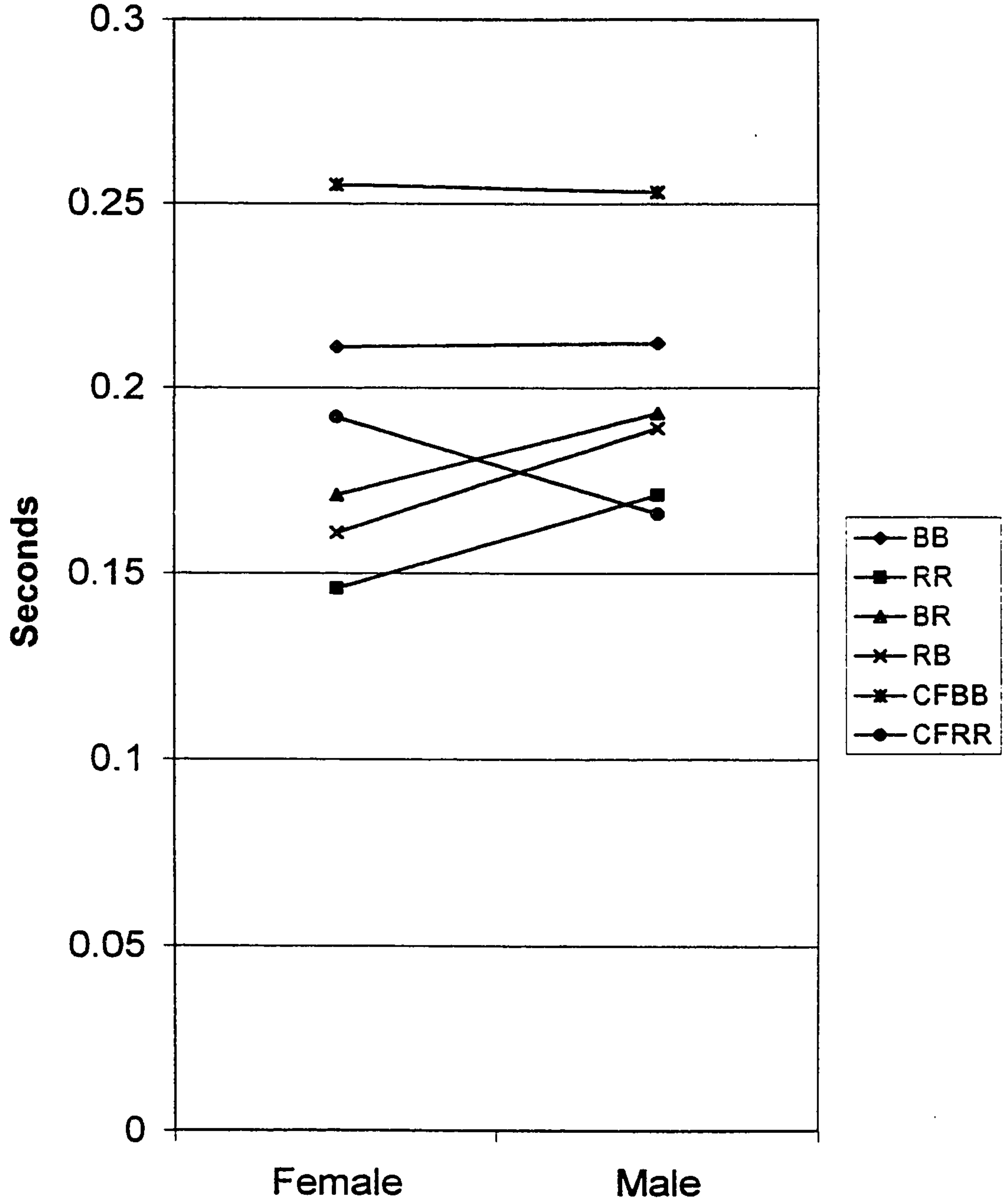

Fig. 22: Duration of flight calls, sex by treatments interaction. Last ten days of sampling.

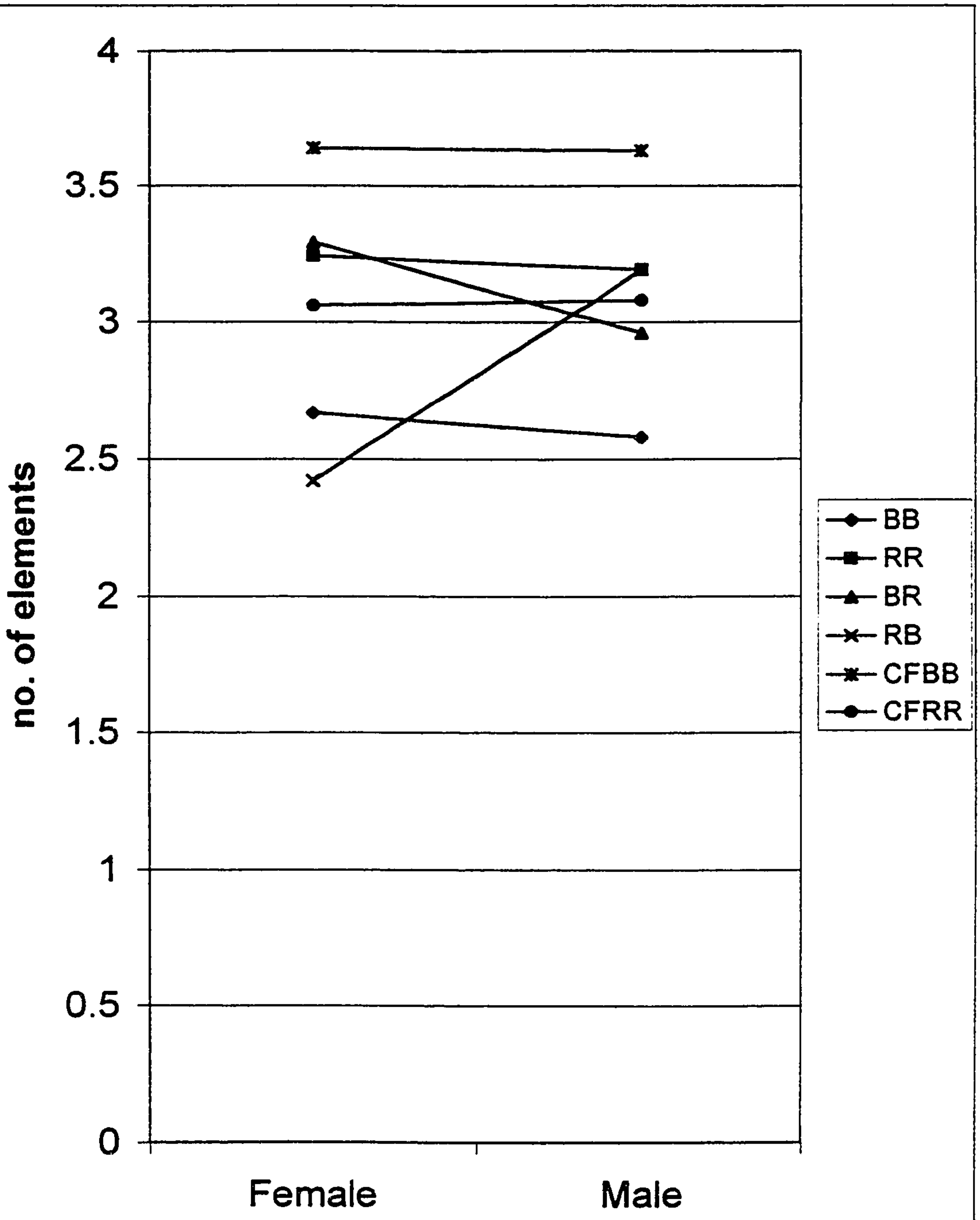

Fig. 23: Number of elements per flight call, sex by treatments interaction. First ten days of sampling.

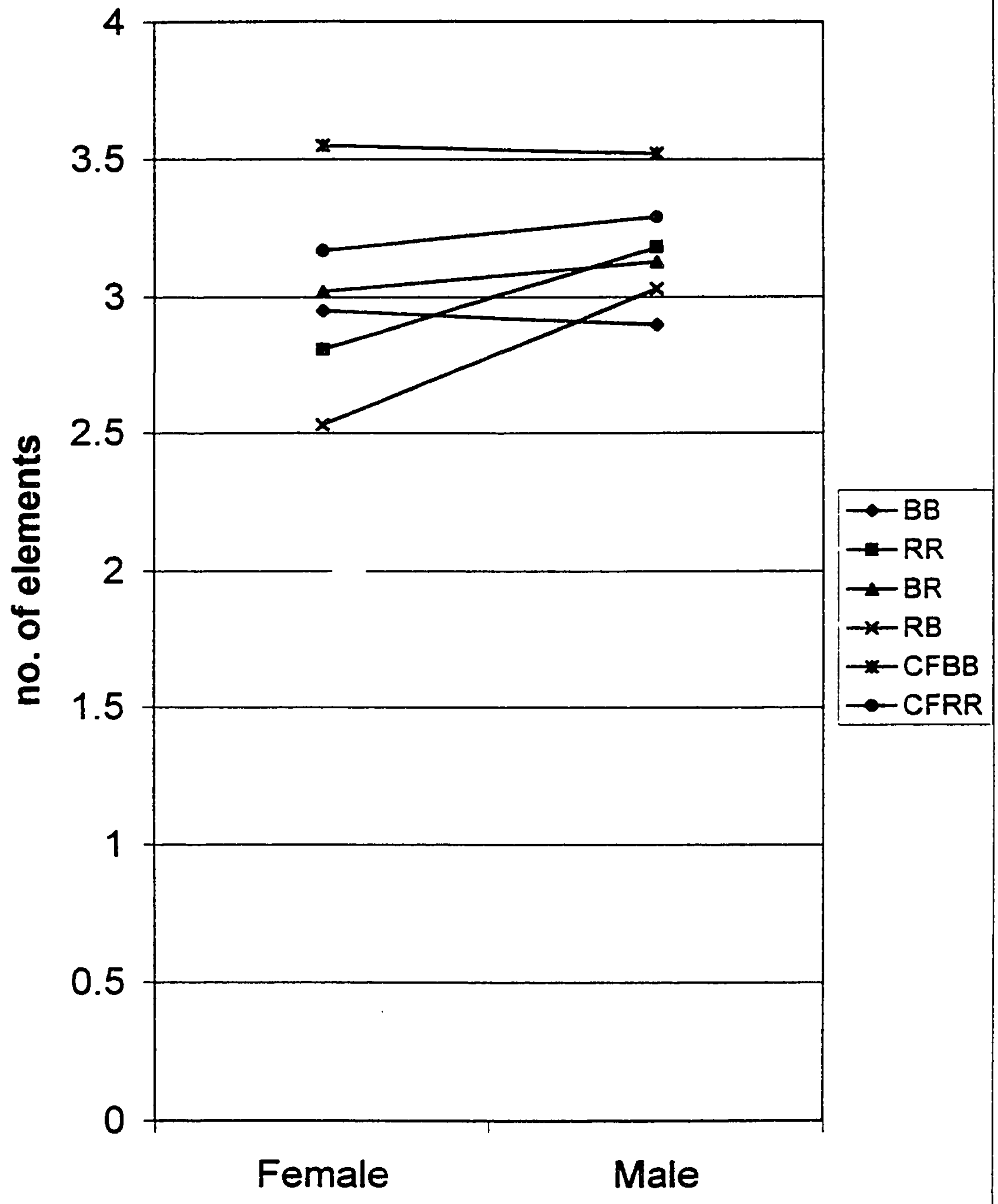

Fig. 24: Number of elements per flight call, sex by treatments interaction. Last ten days of sampling.

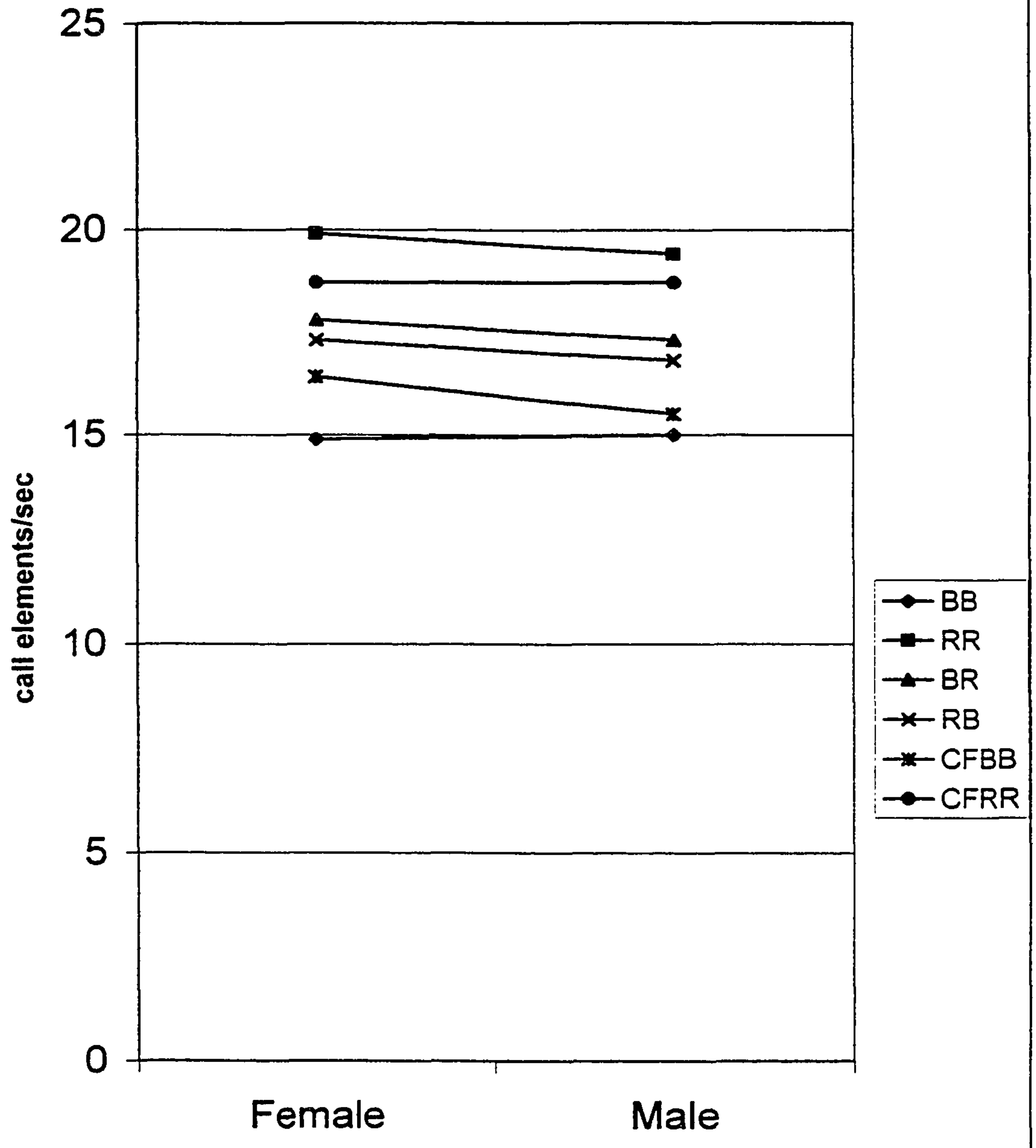

Fig. 25: Flight call rate, sex by treatments interaction. First ten days of sampling.

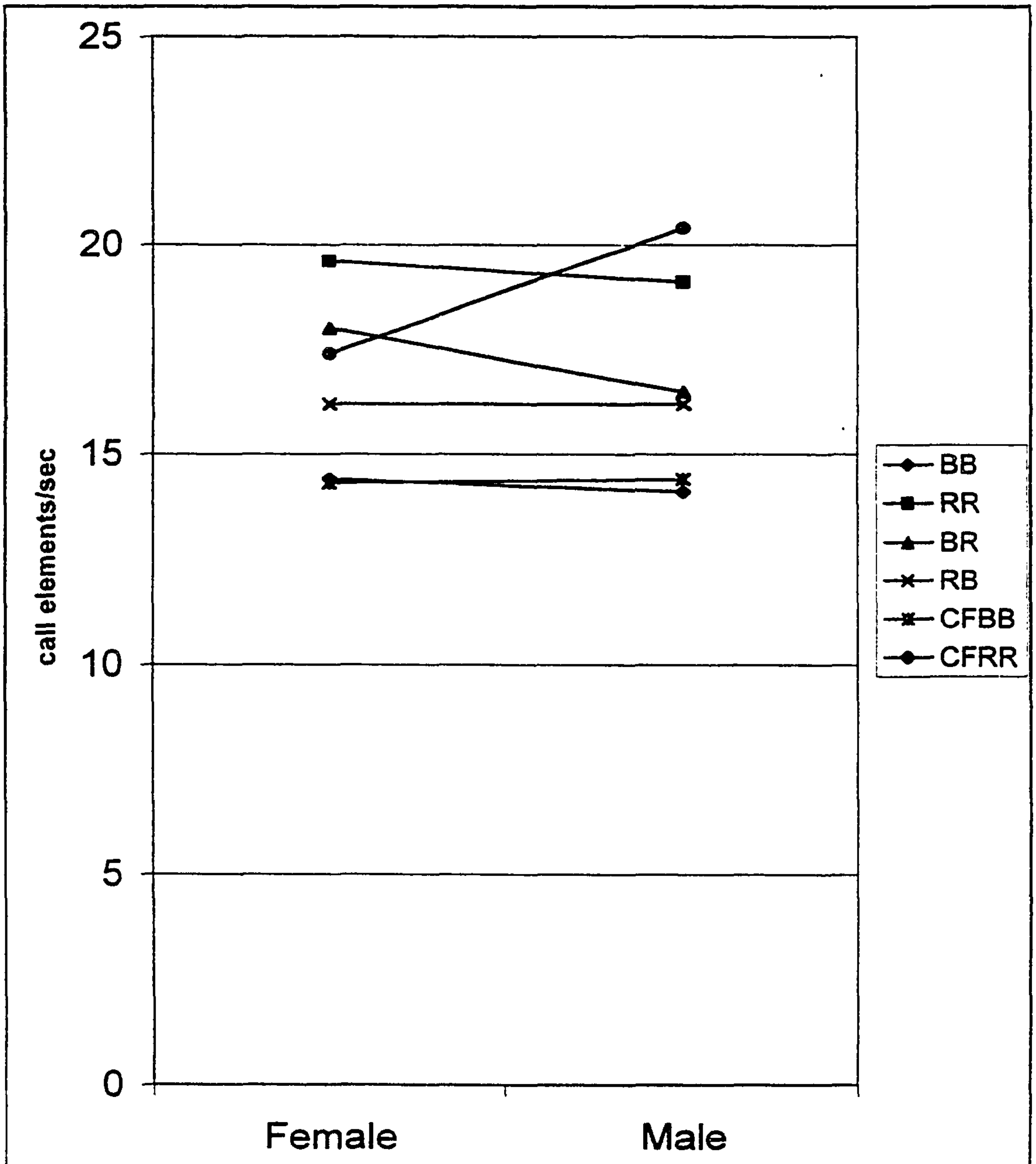

Fig. 26: Flight call rate, sex by treatments interaction. Last ten days of sampling.

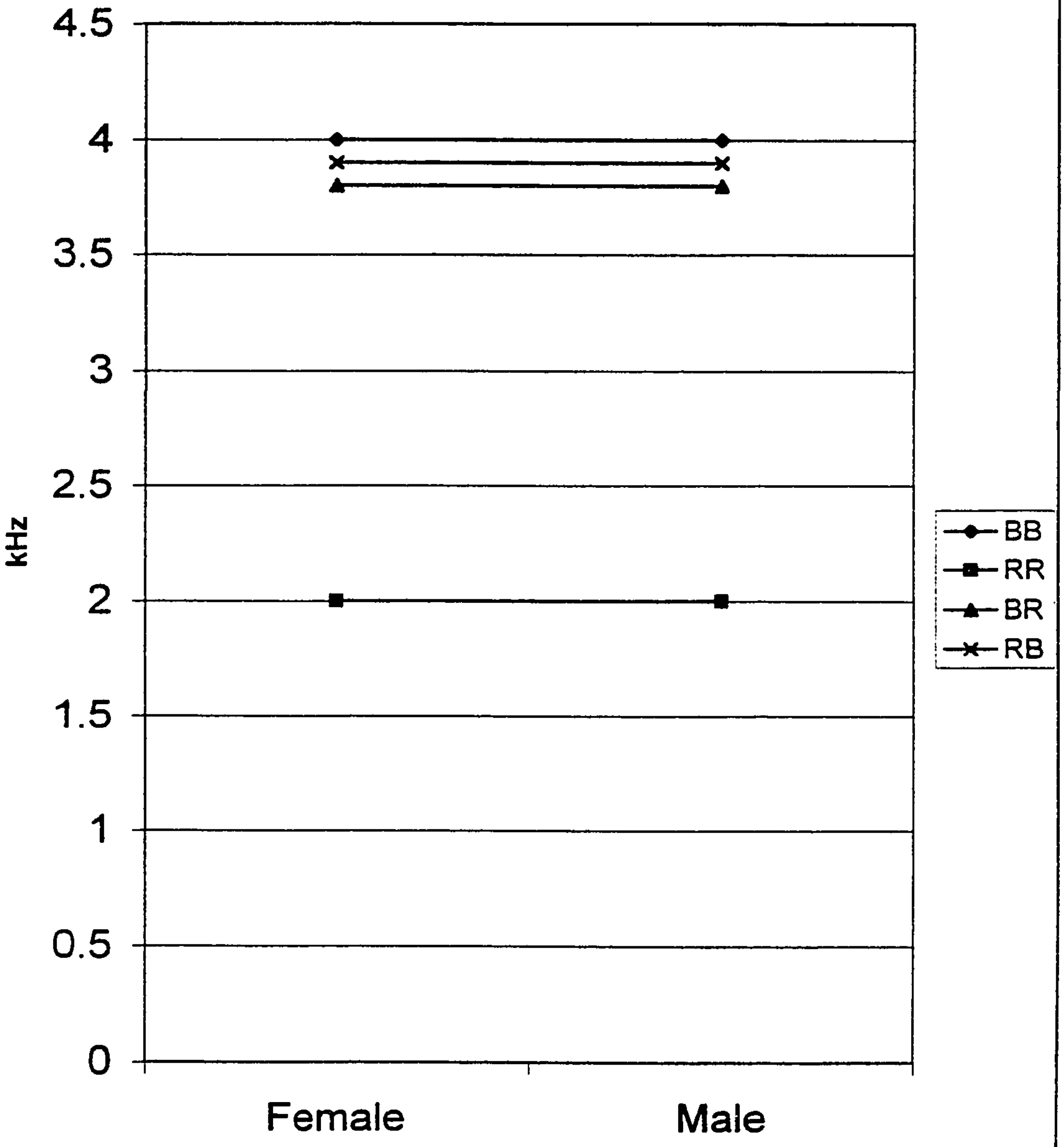

Fig.27:Predicted sex by treatments interaction if the maximum frequency parameter displays autosomal inheritance without imprinting (assuming the Border gene(s) are dominant).

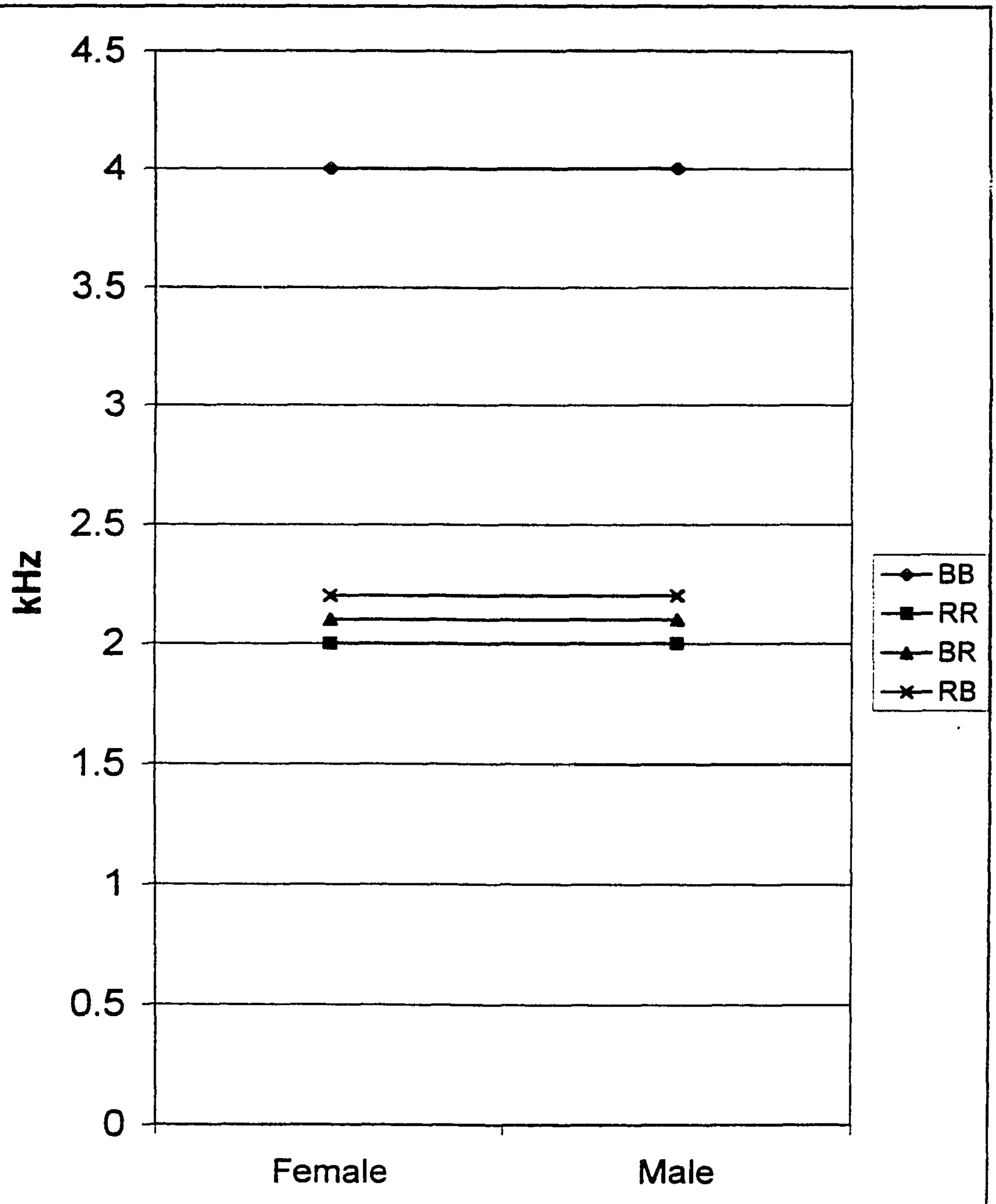

Fig.28:Predicted sex by treatments interaction if the maximum frequency parameter displays autosomal inheritance without imprinting (assuming the Roller gene(s) are dominant).

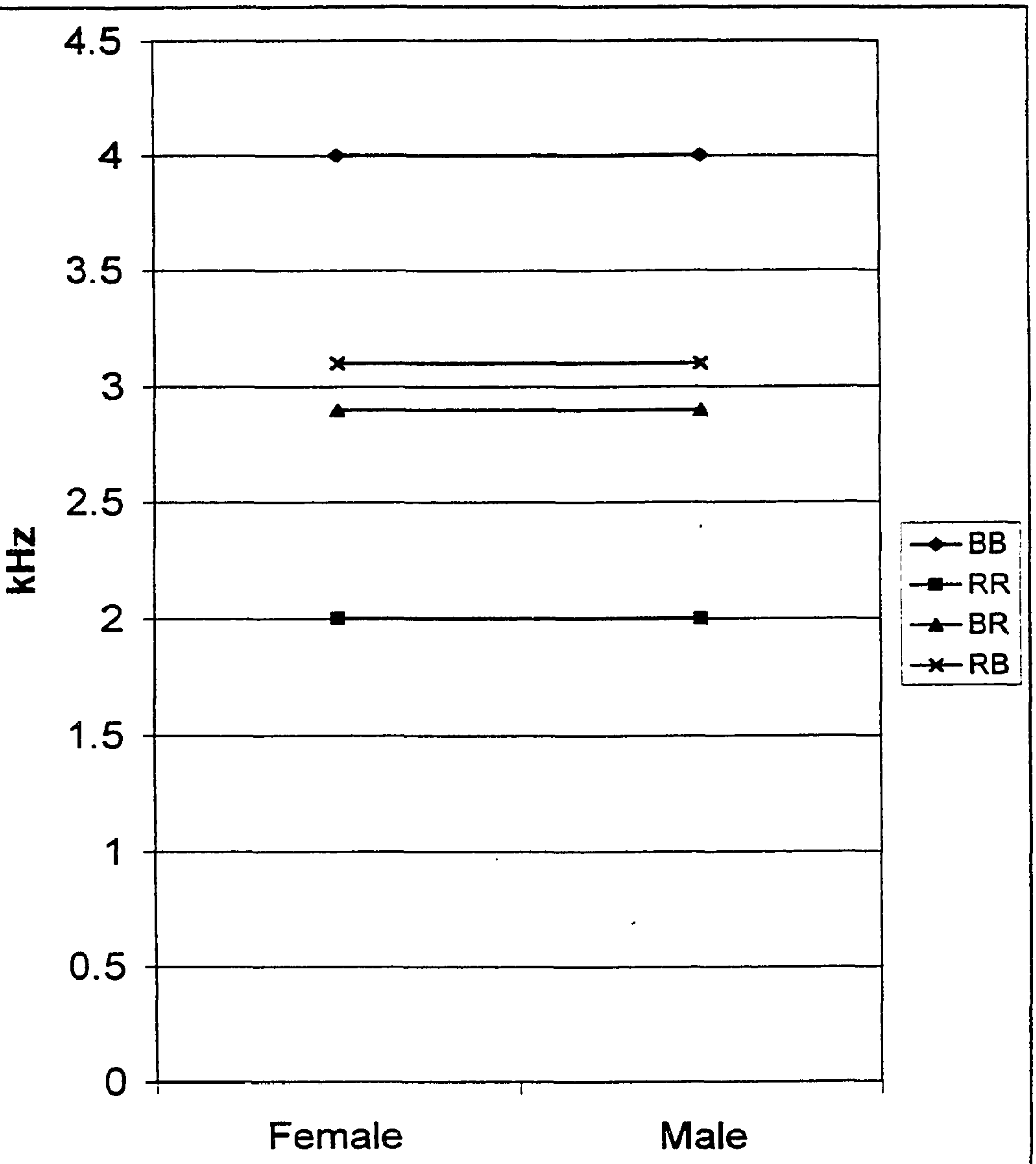

Fig.29: Predicted sex by treatments interaction if the maximum frequency parameter displays autosomal inheritance without imprinting and the gene(s) which control the trait display incomplete dominance.

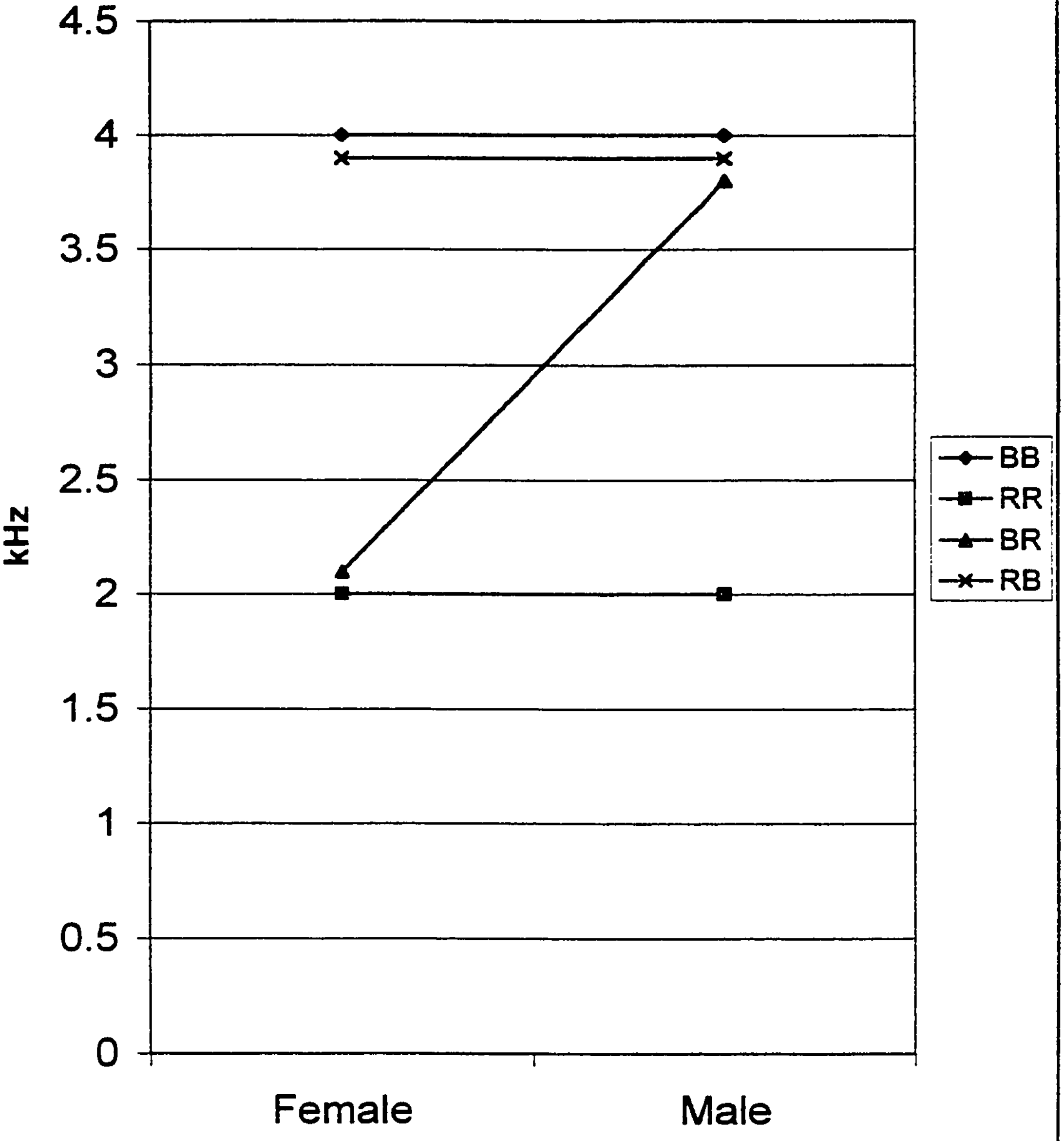

Fig.30:Predicted sex by treatments interaction if the maximum frequency parameter displays sex-linked inheritance without imprinting (assuming the Border gene(s) on the Z chromosome are dominant).

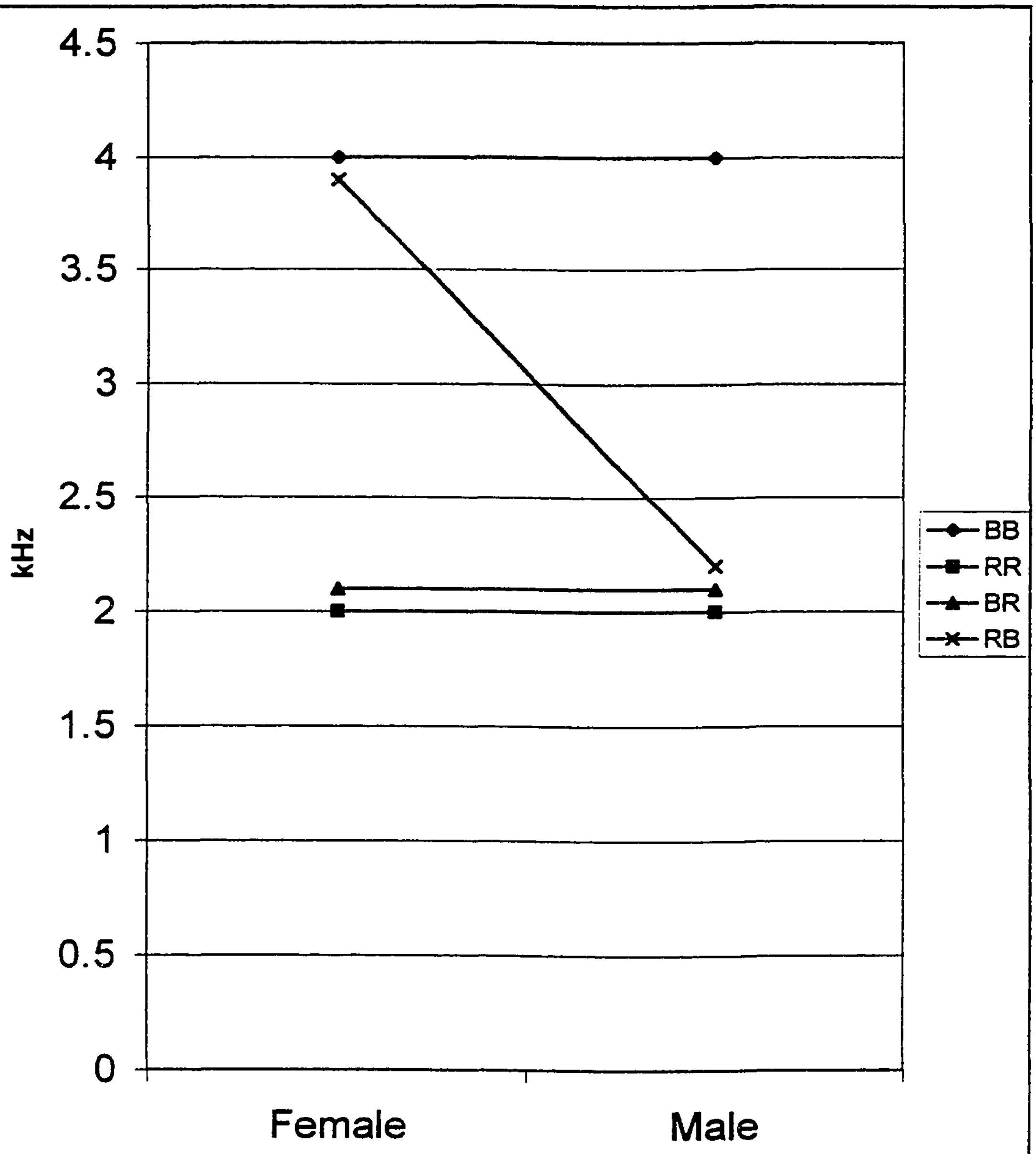

Fig.31:Predicted sex by treatments interaction if the maximum frequency parameter diaplays sex-linked inheritance without imprinting (assuming the Roller gene(s) on the Z chromosome are dominant).

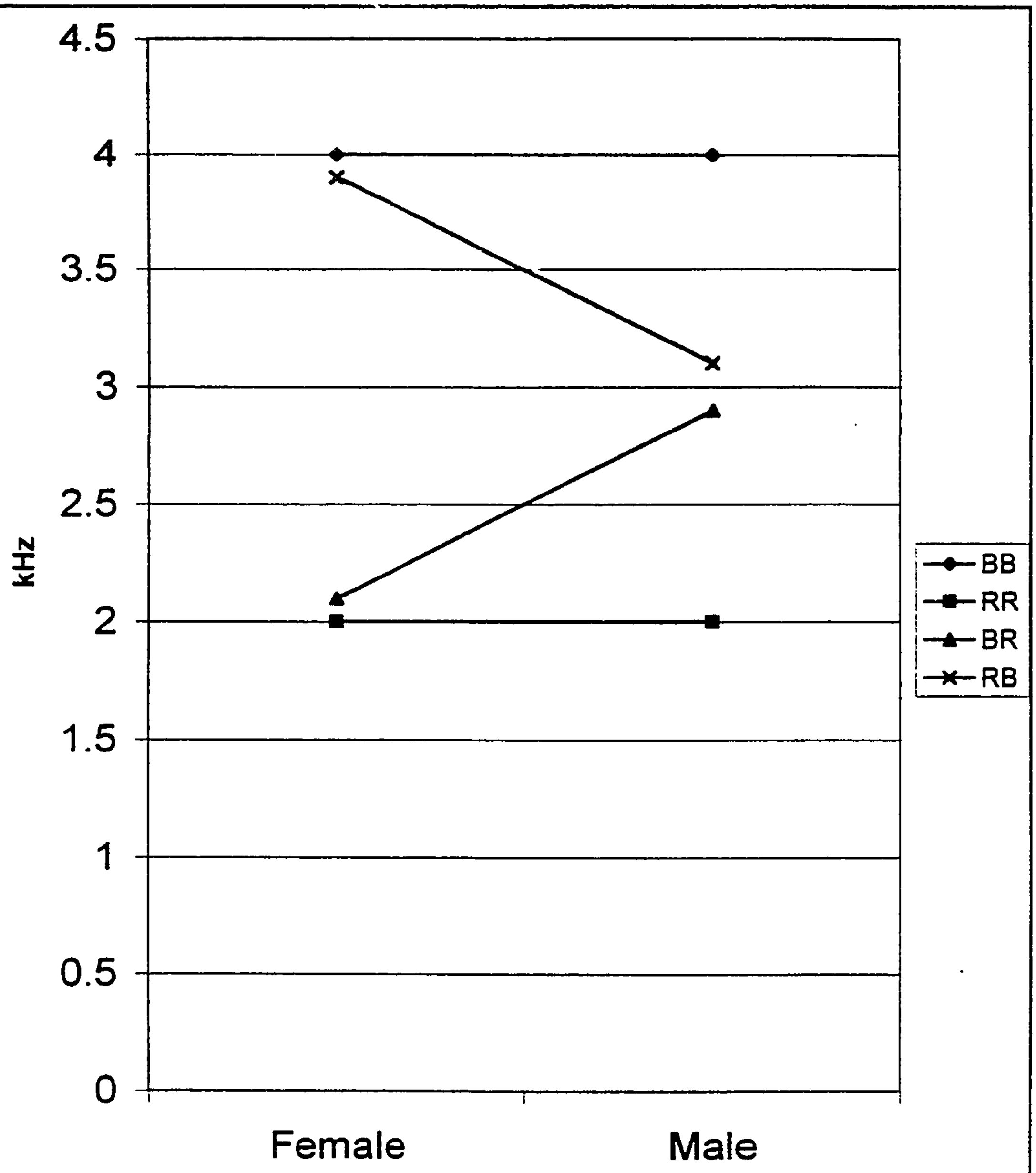

Fig.32:Predicted sex by treatments interaction if the maximum frequency parameter displays sex-linked inheritance without imprinting and the gene(s) which control the trait display incomplete dominance.

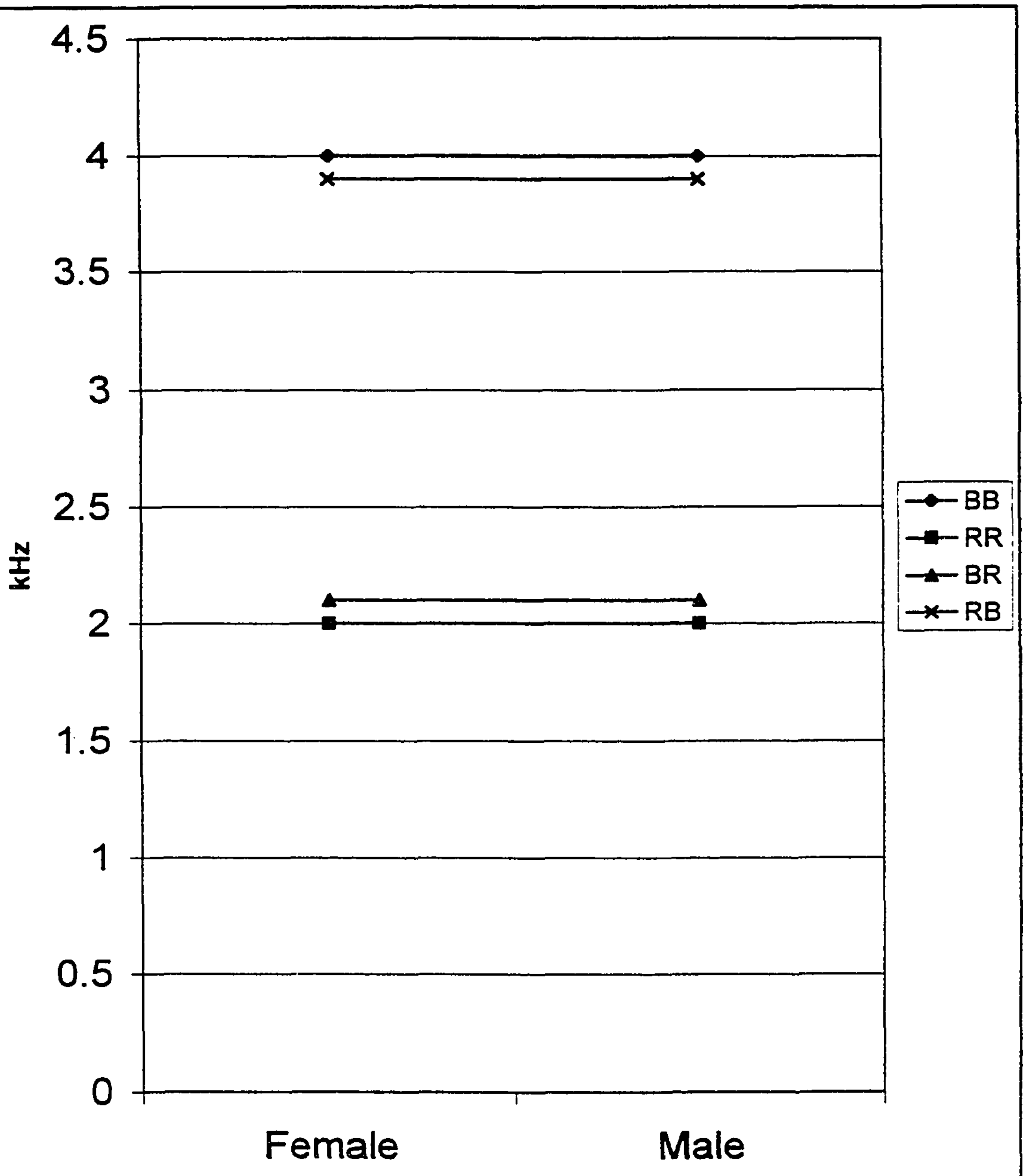

Fig.33:Predicted sex by treatments interaction if the maximum frequency parameter displays maternal genomic imprinting (this result would be seen for both autosomal and sex-linked inheritance).

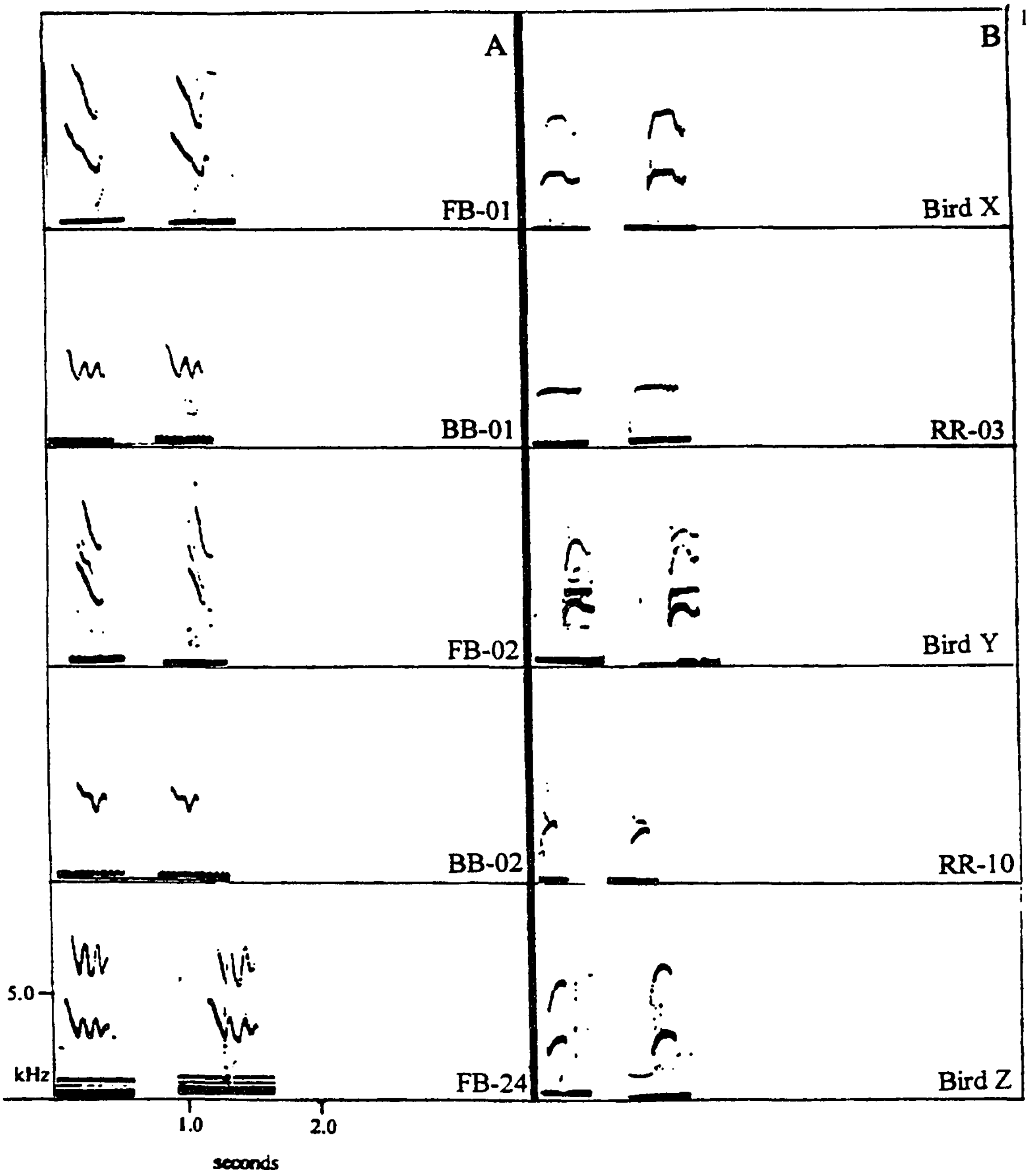

Fig.34: Individually stereotyped hunger-location calls given by (A) five normal-reared Border canaries and (B) five normal reared Roller canaries. Birds X, Y and Z are the siblings, RR-22, RR-23 and RR-24; however the individual identity of each caller is not known.

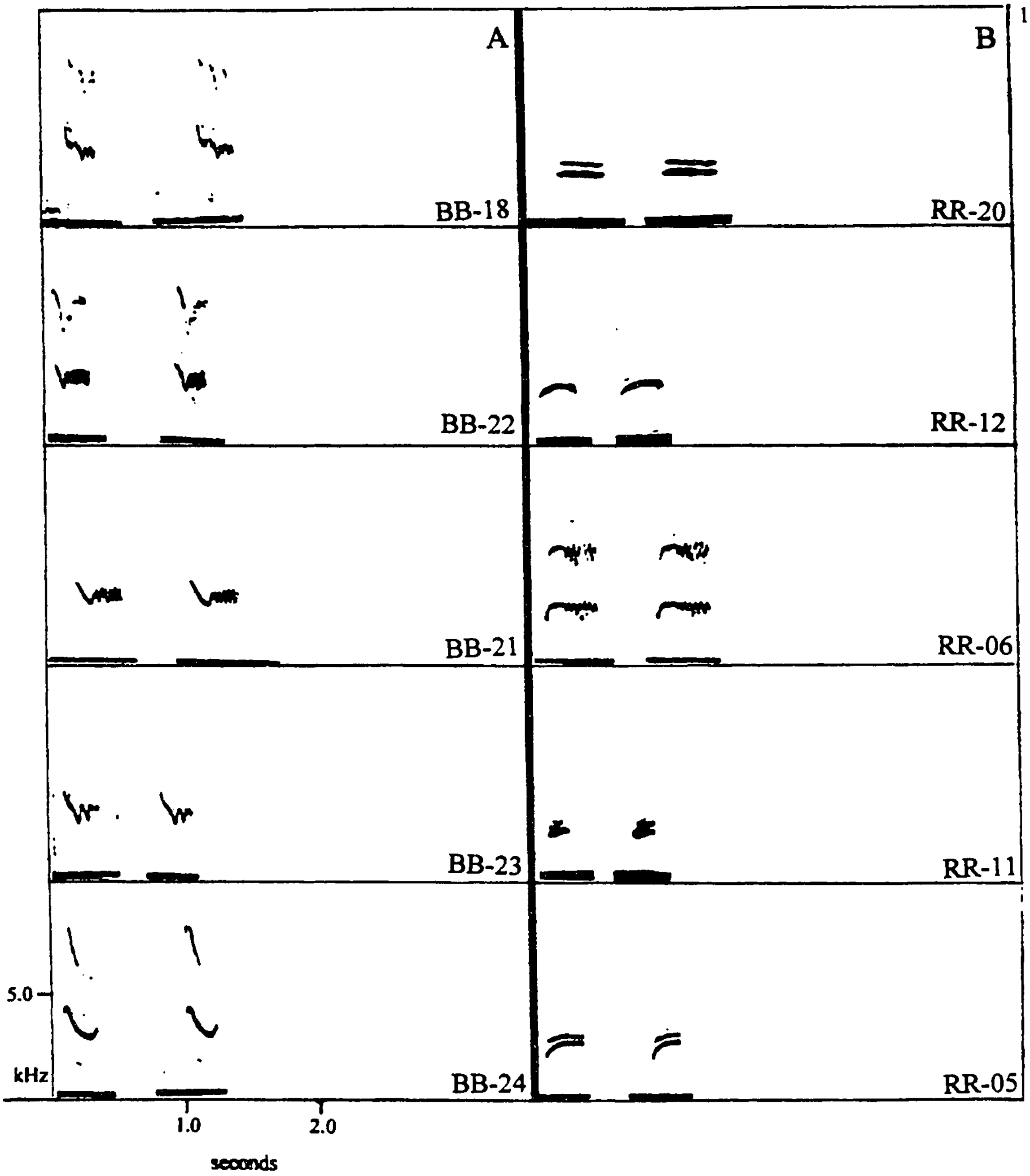

Fig.35:Individually stereotyped hunger-location calls given by (A) five cross-fostered Border canaries and (B) five cross-fostered Roller canaries.

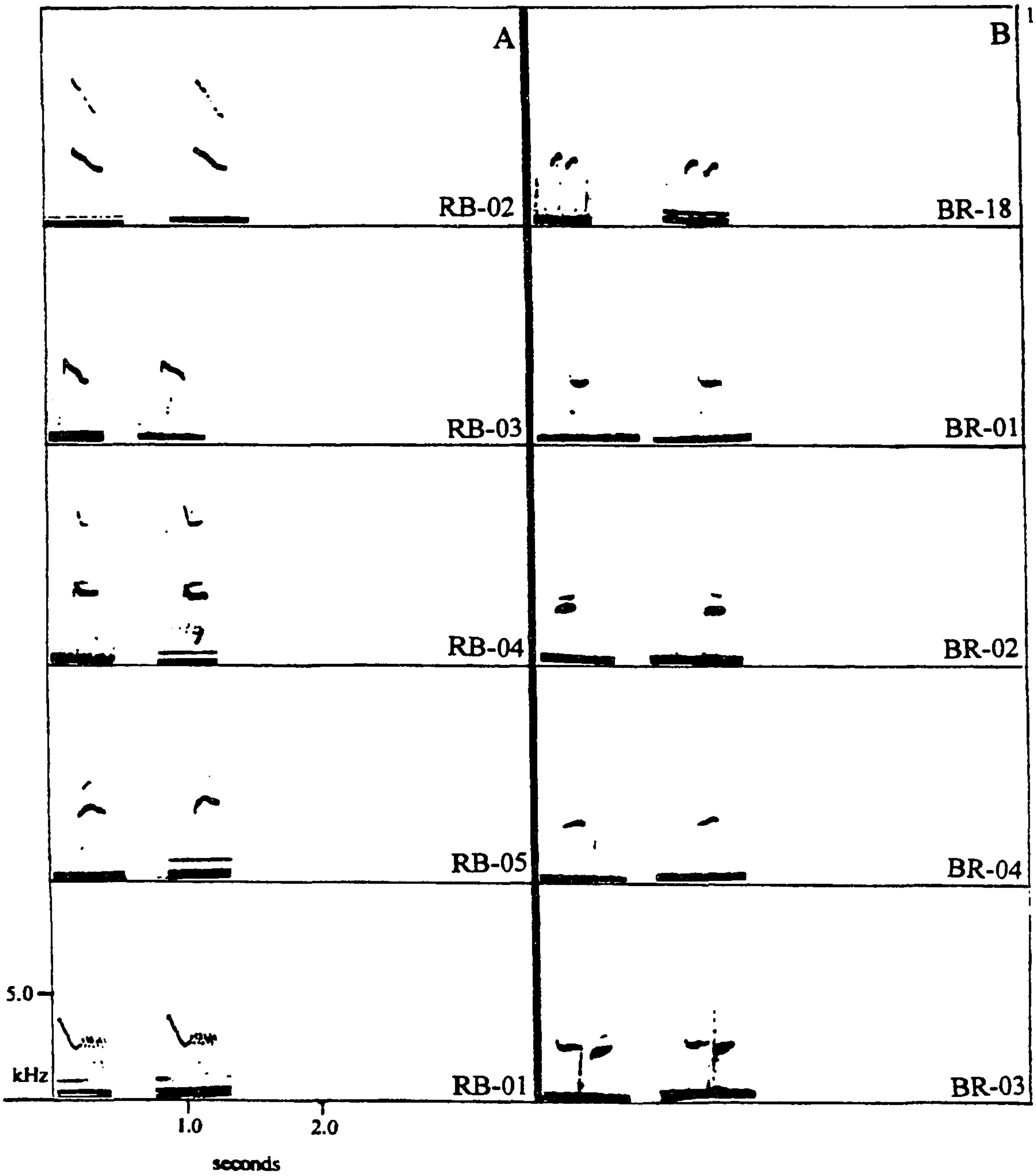

Fig.36: Individually stereotyped hunger-location calls given by (A) five RB hybrid canaries and (B) five BR hybrid canaries.

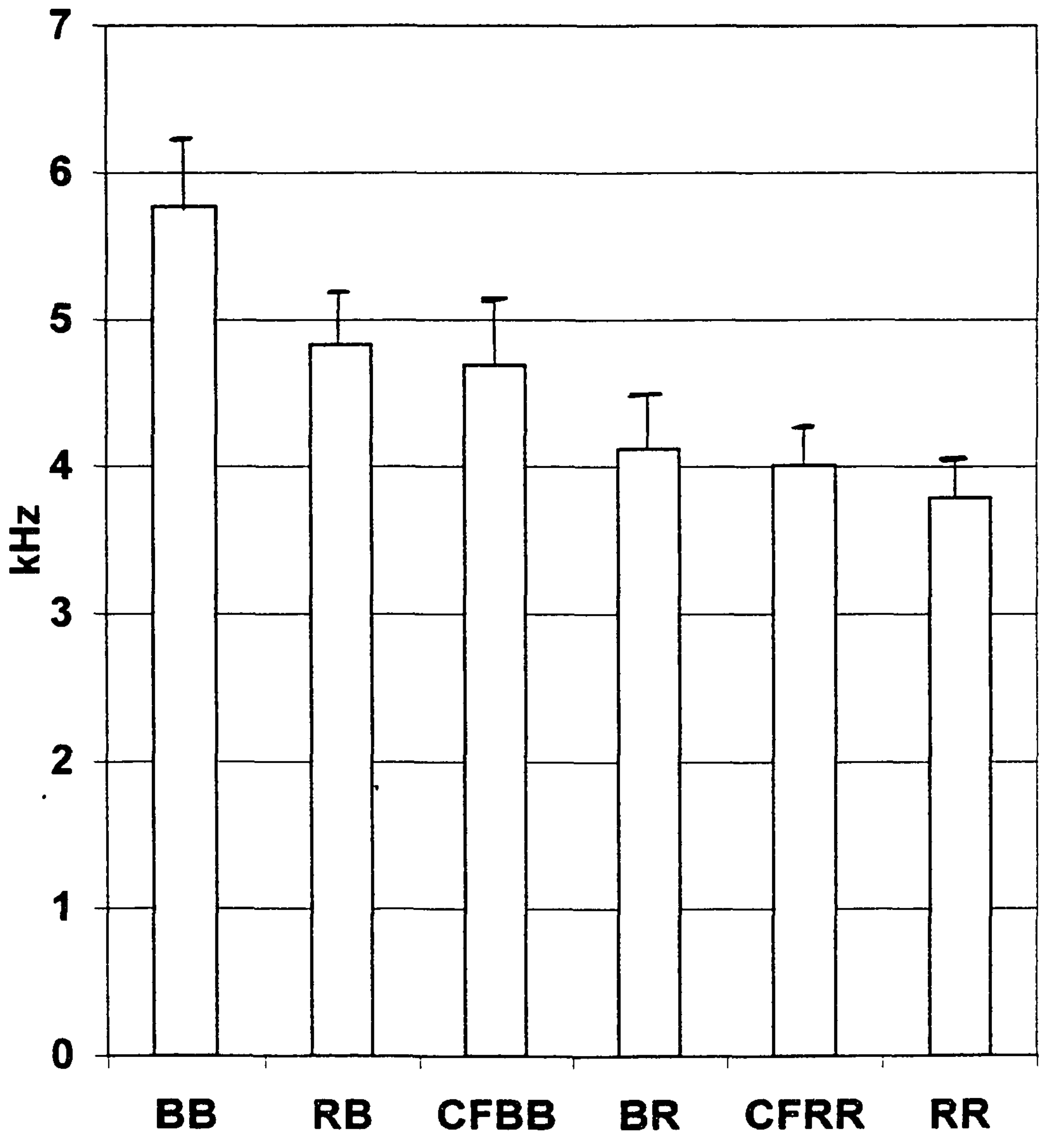

Fig.37: Mean maximum frequency of hunger-location calls for all six treatments.

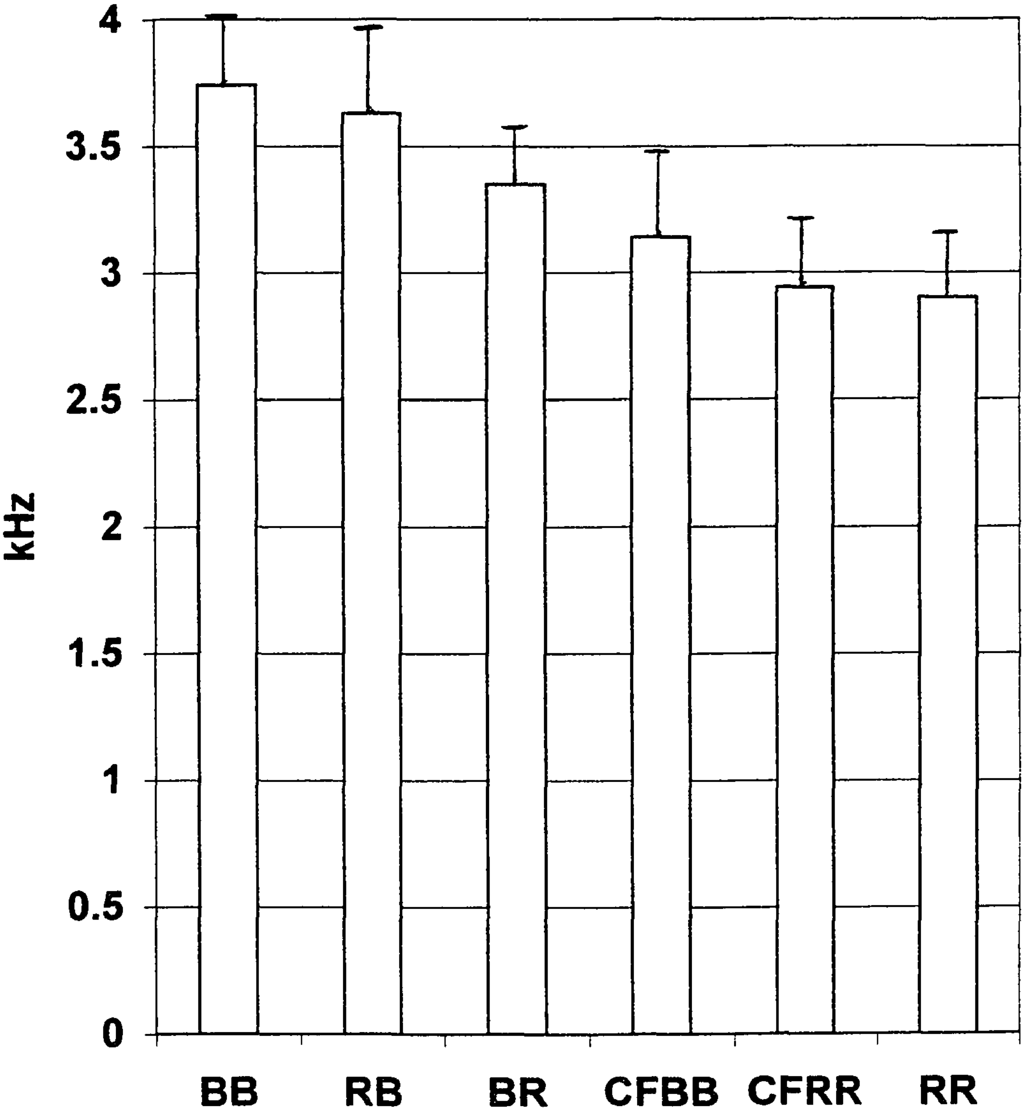

Fig. 38: Mean minimum frequency of hunger-location calls for all six treatments.

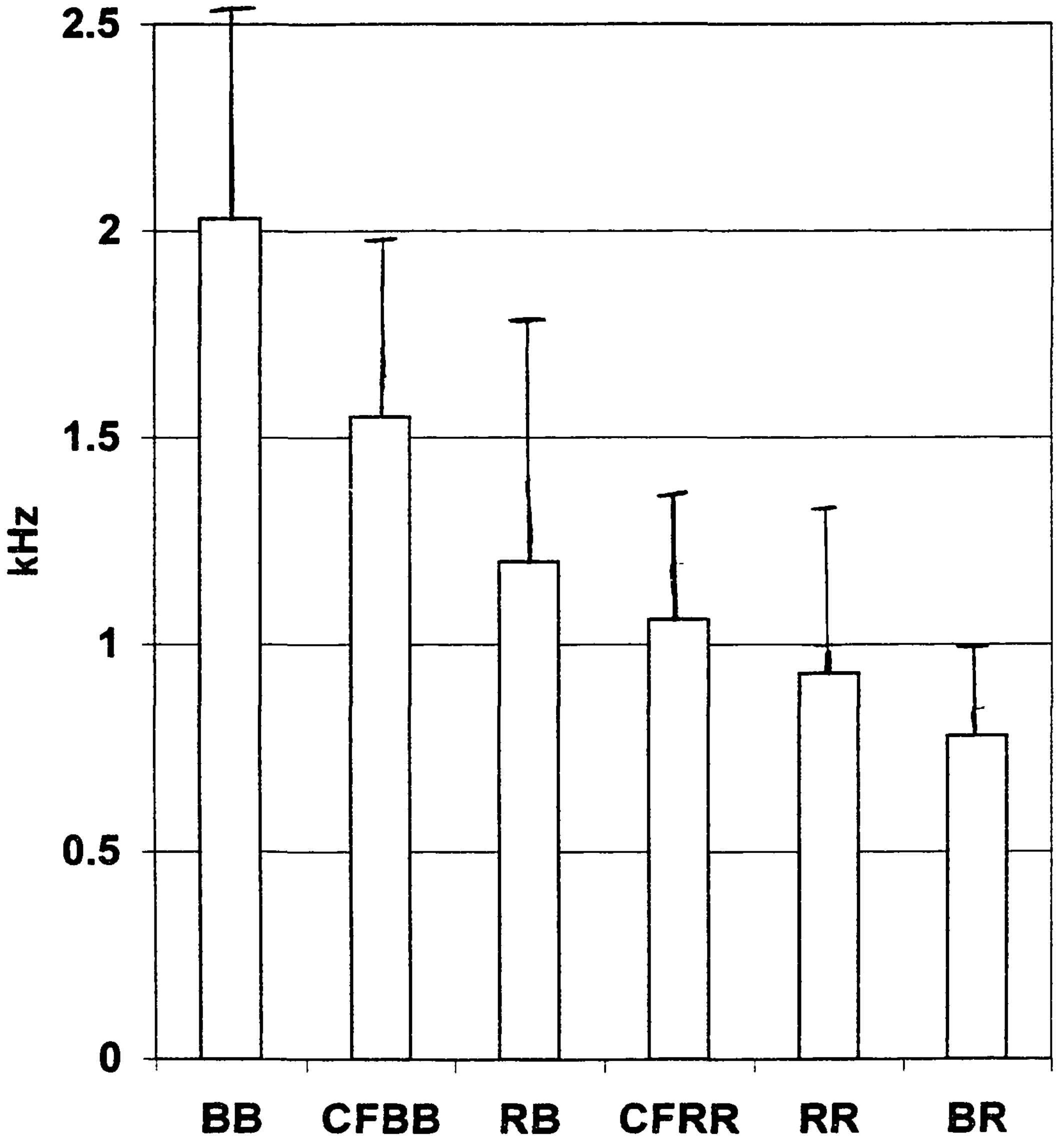

Fig.39: Mean frequency range of hunger-location calls for all six treatments.

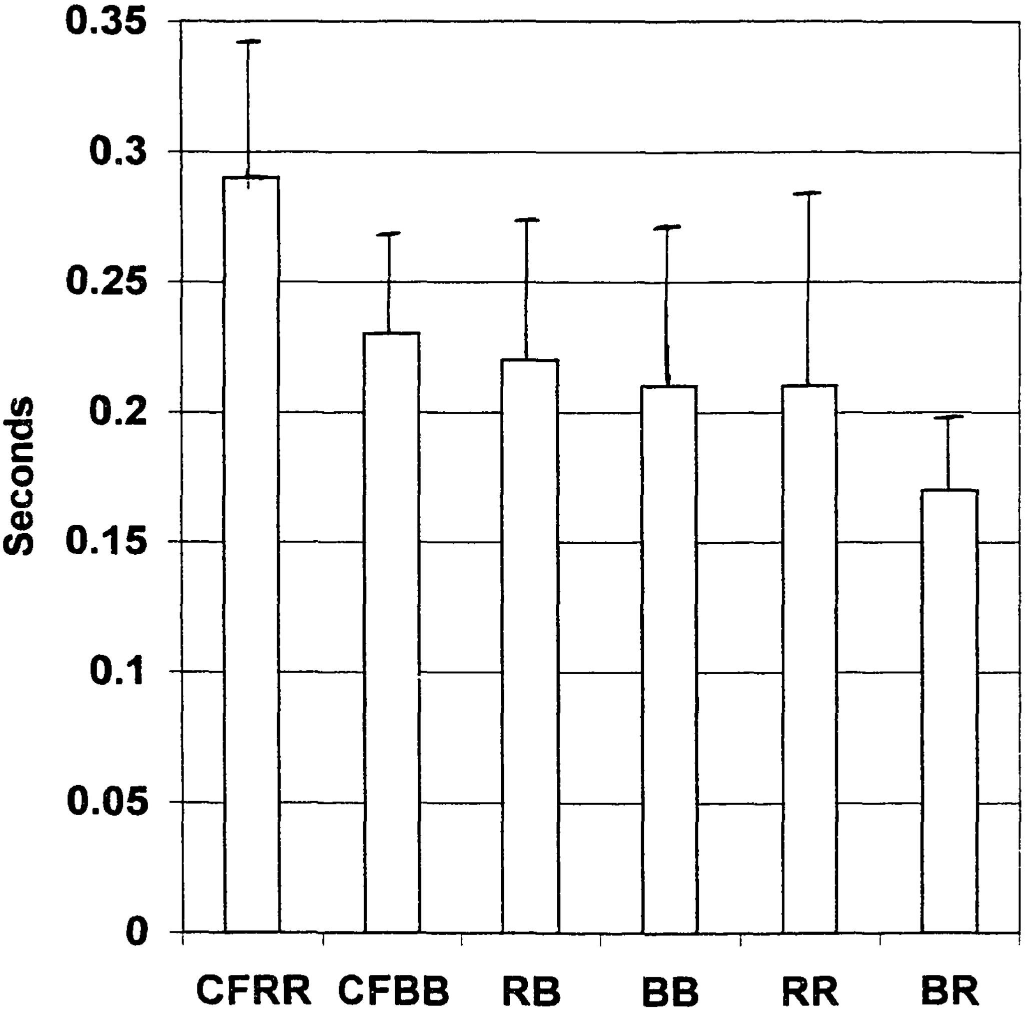

Fig. 40: Mean duration of hunger-location calls for all six treatments.

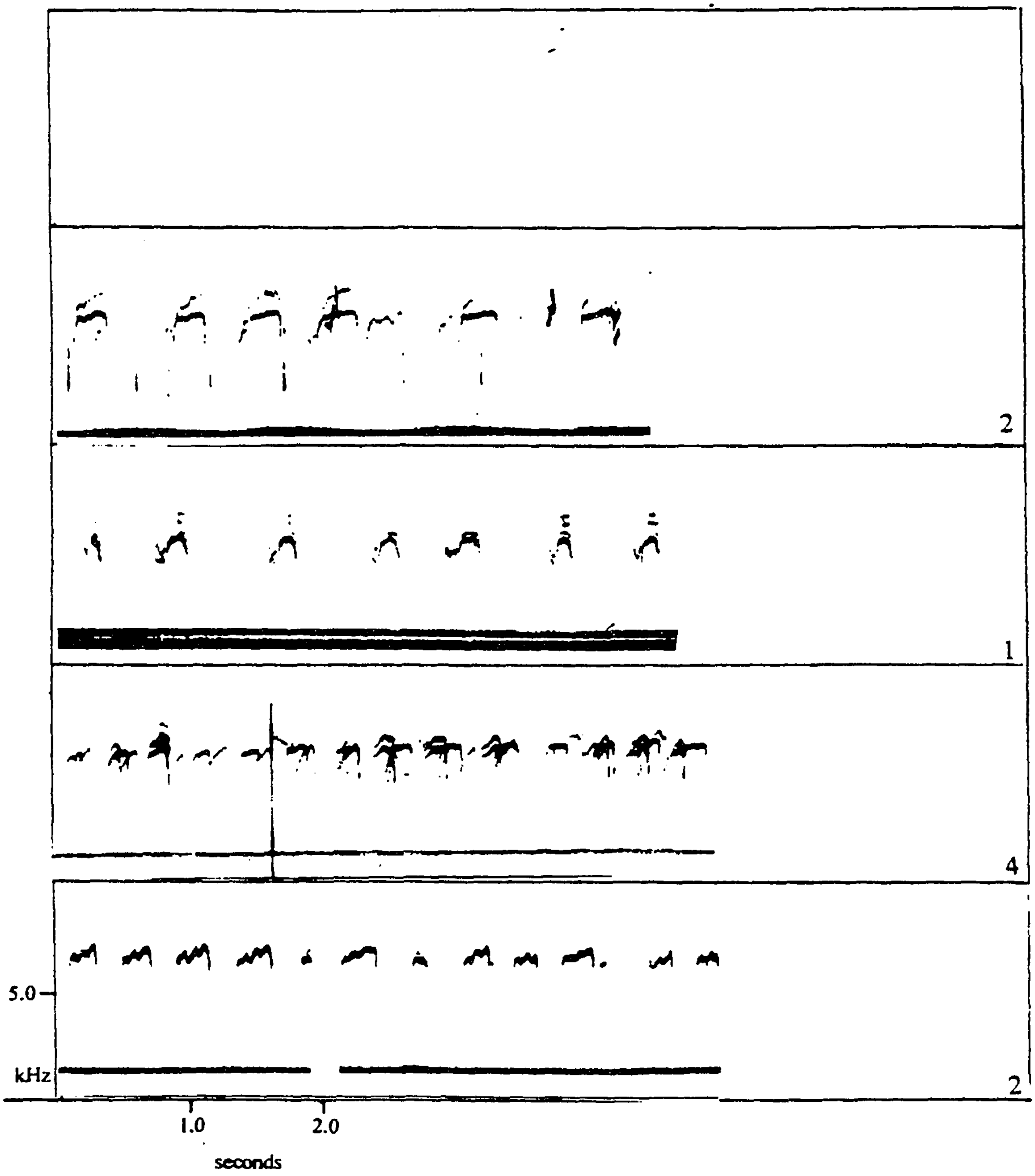

Fig. 41: Normal-reared Border canary nestling calls from four different nest cups. Numbers indicate the number of chicks in each nest.

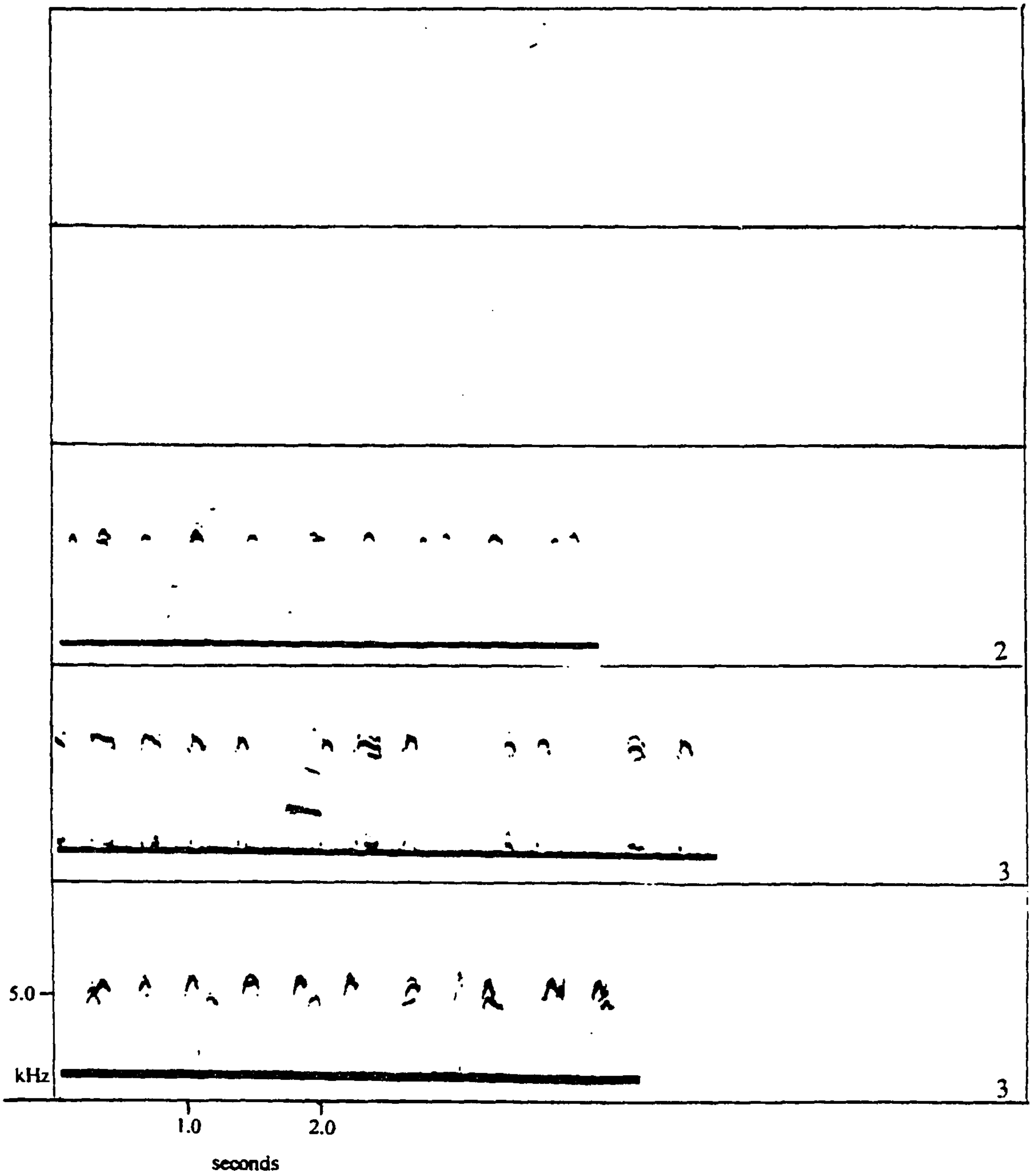

Fig. 42: Normal-reared Roller canary nestling calls from three different nest cups. Numbers indicate the number of chicks in each nest.

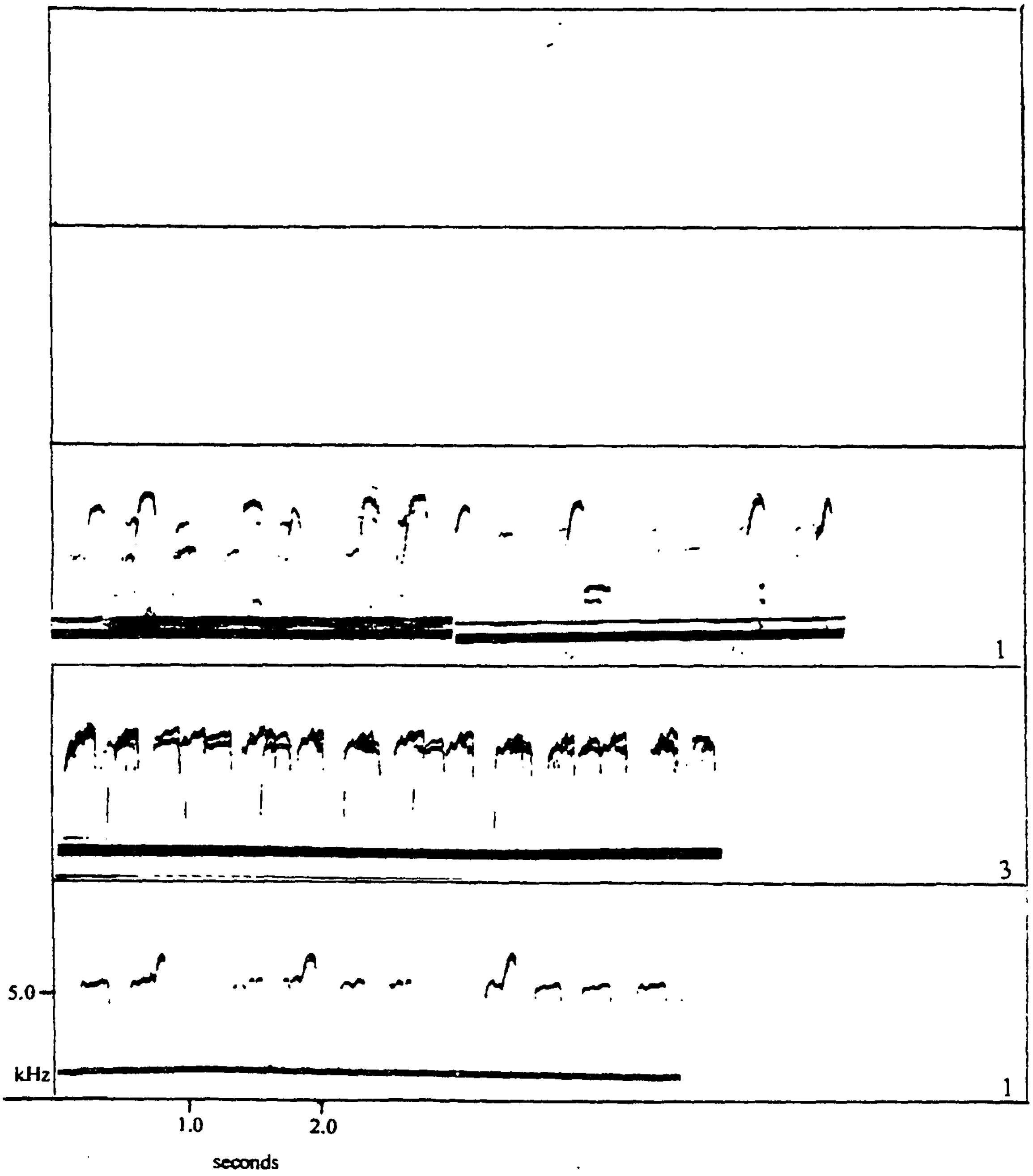

Fig.43: RB hybrid canary nestling calls from three different nest cups. Numbers indicate the number of chicks in each nest

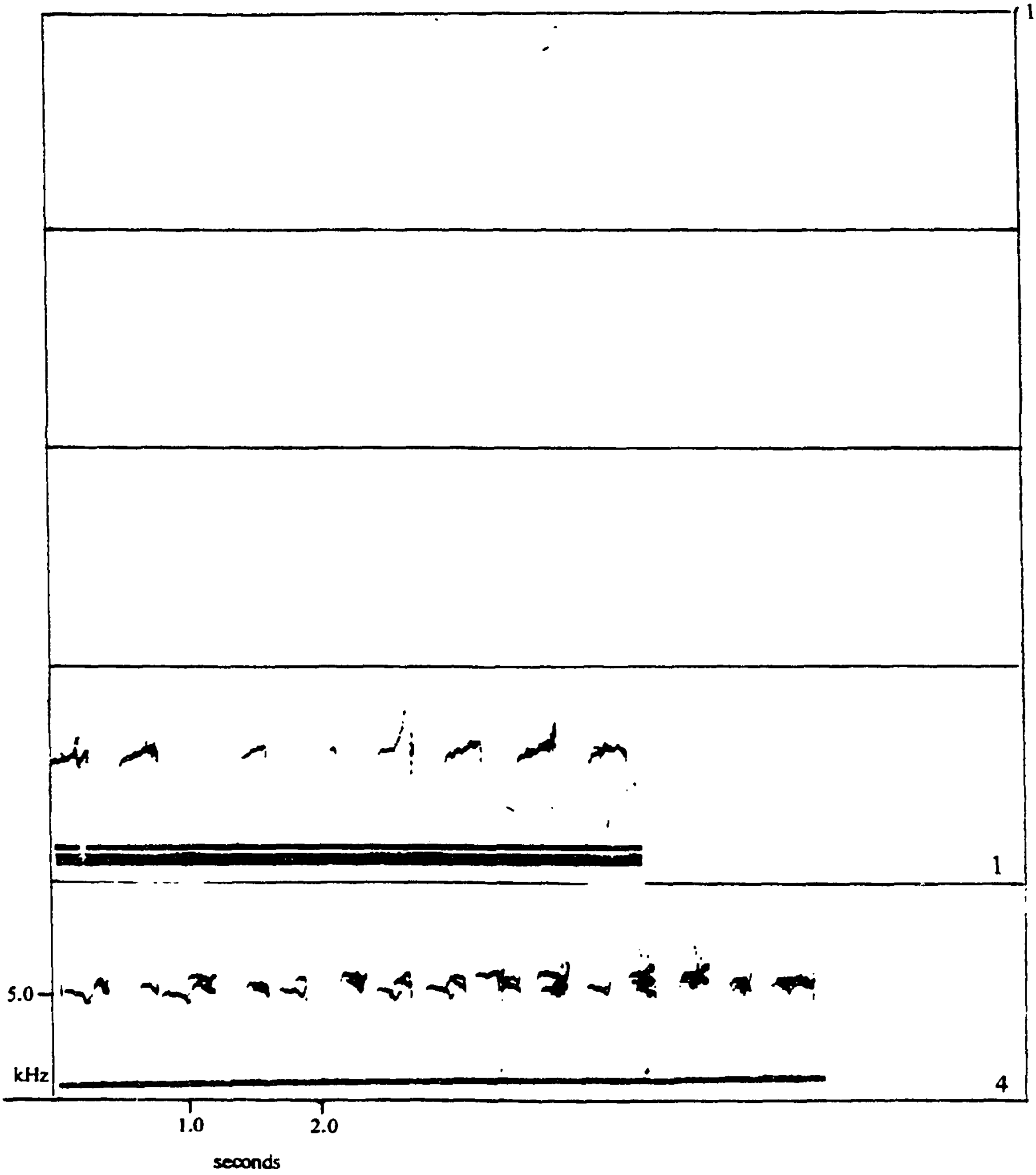

Fig.44: BR hybrid canary nestling calls from two different nest cups. Numbers indicate the number of chicks in each nest

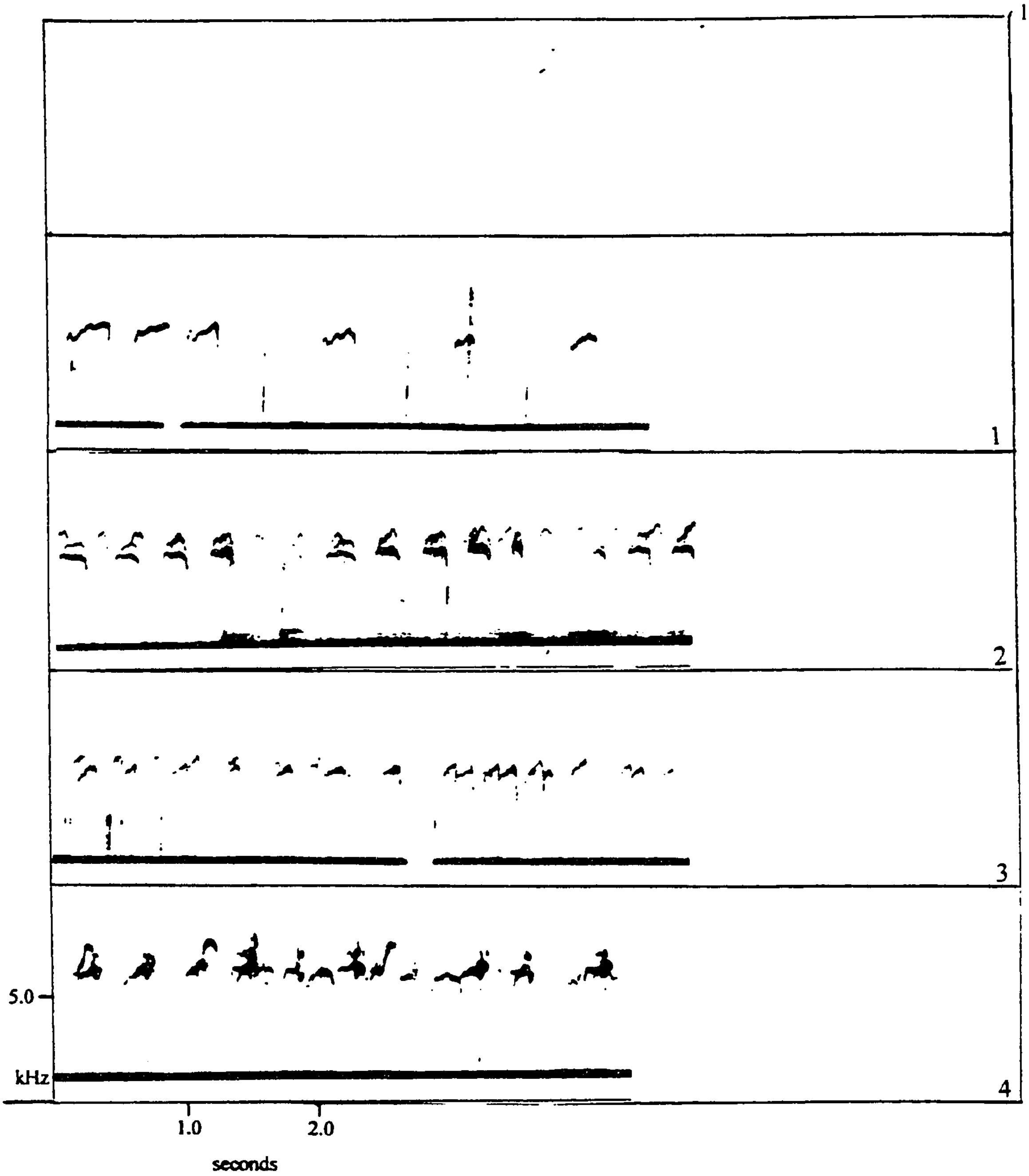

Fig. 45: Cross-fostered Border canary nestling calls from four different nest cups. Numbers indicate the number of chicks in each nest.

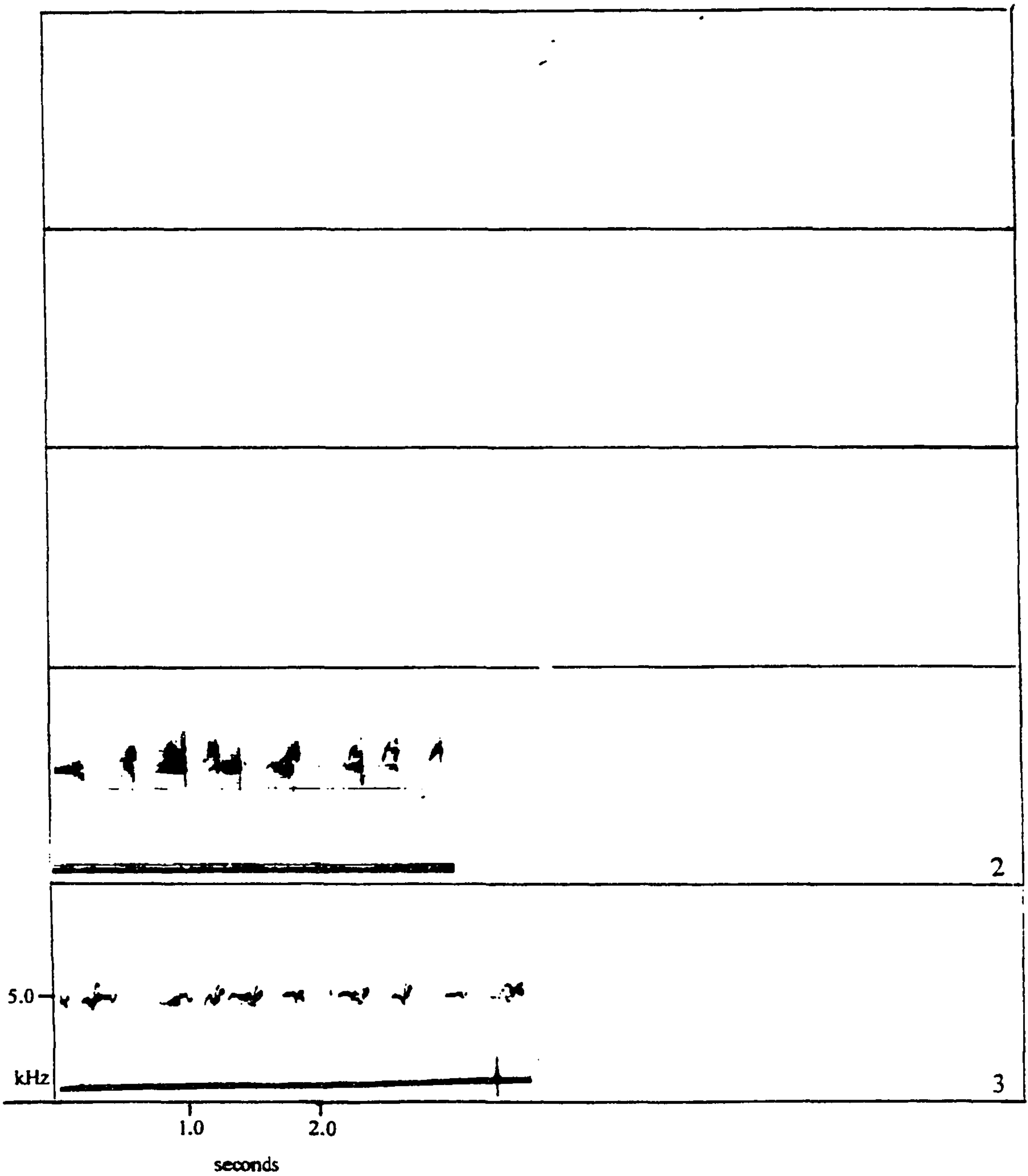

Fig. 46: Cross-fostered Roller canary nestling calls from two different nest cups. Numbers indicate the number of chicks in each nest.

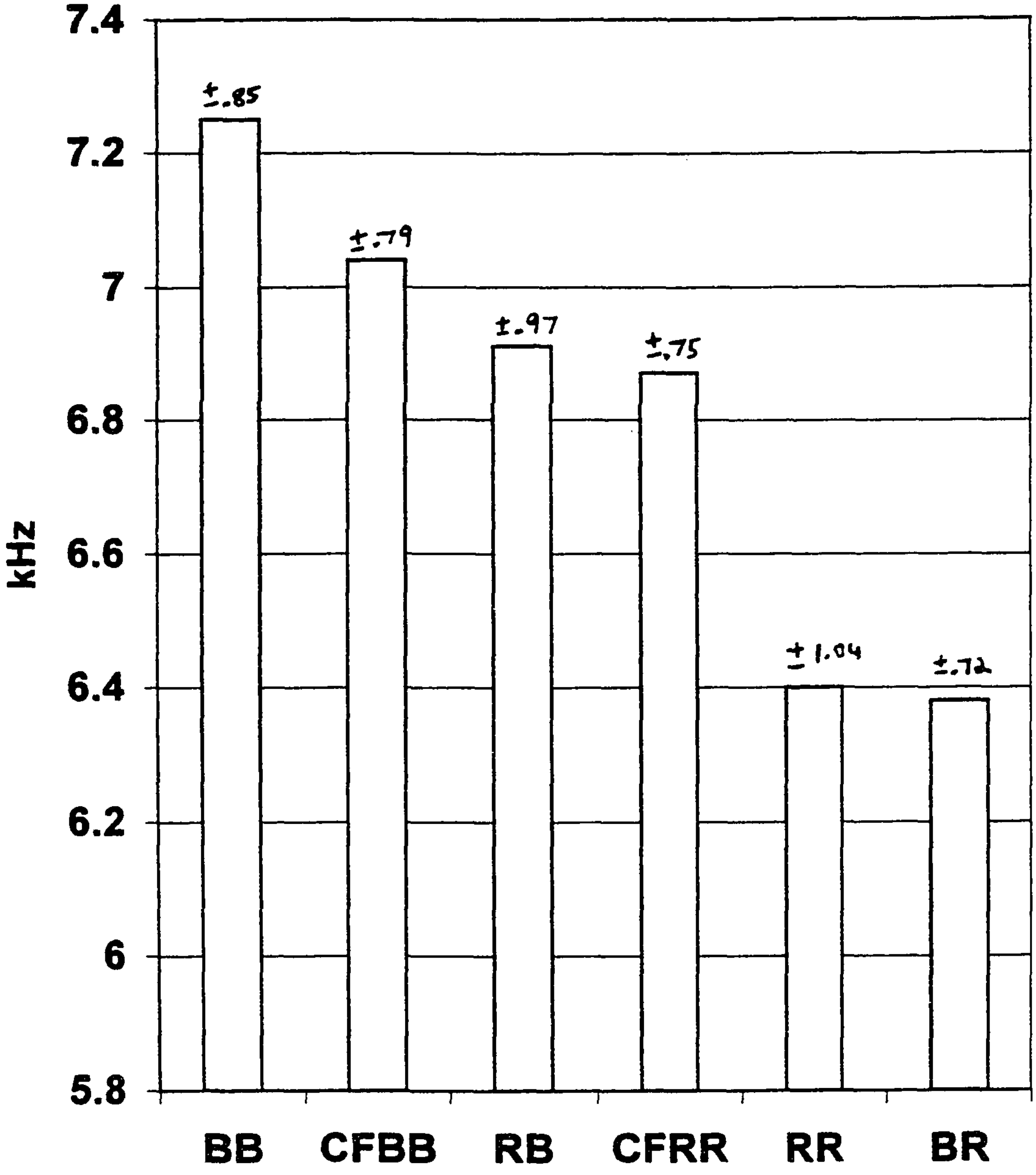

Fig.47: Mean maximum frequency of nestling calls for all six treatments.

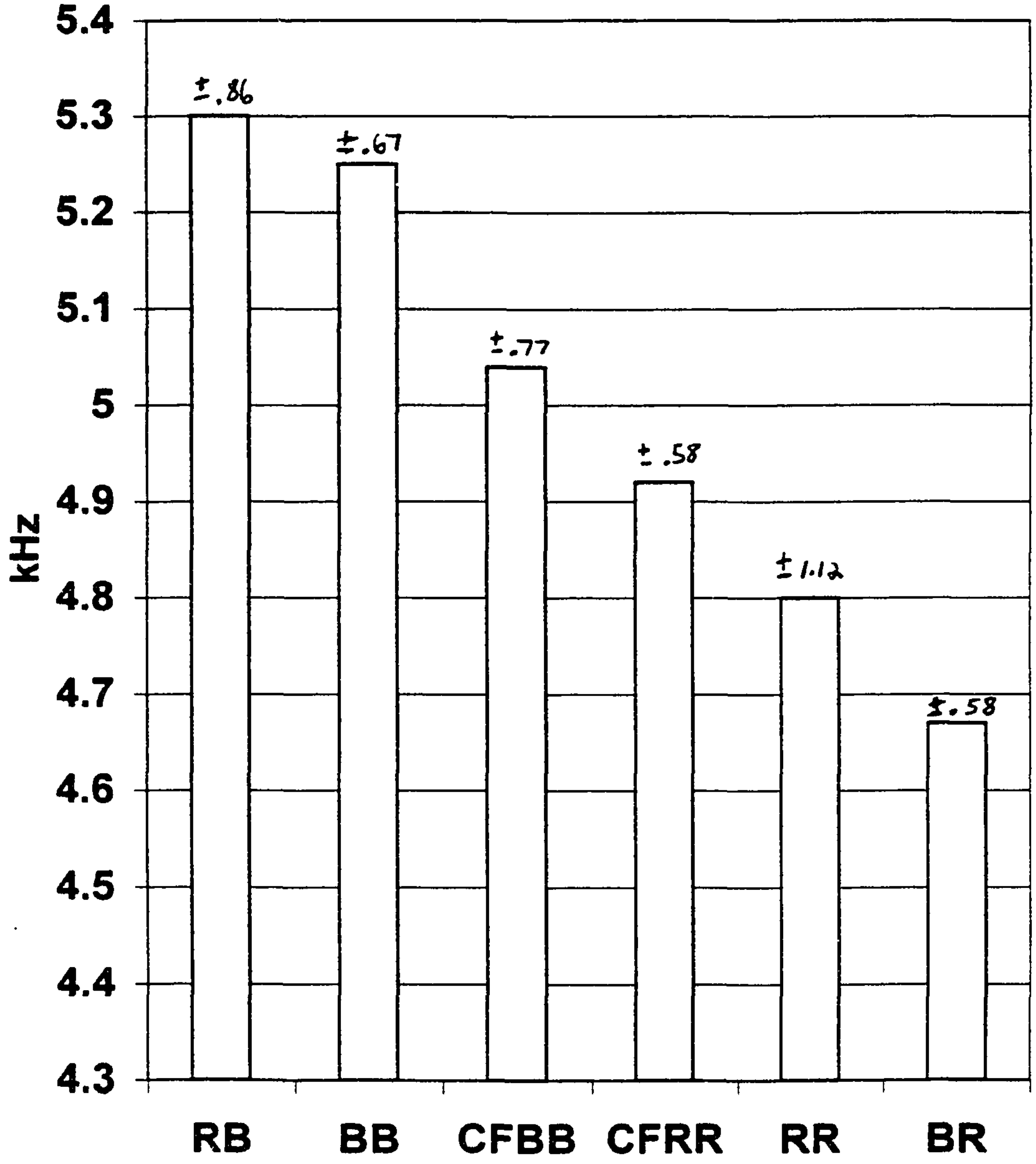

Fig.48: Mean minimum frequency of nestling calls for all six treatments.

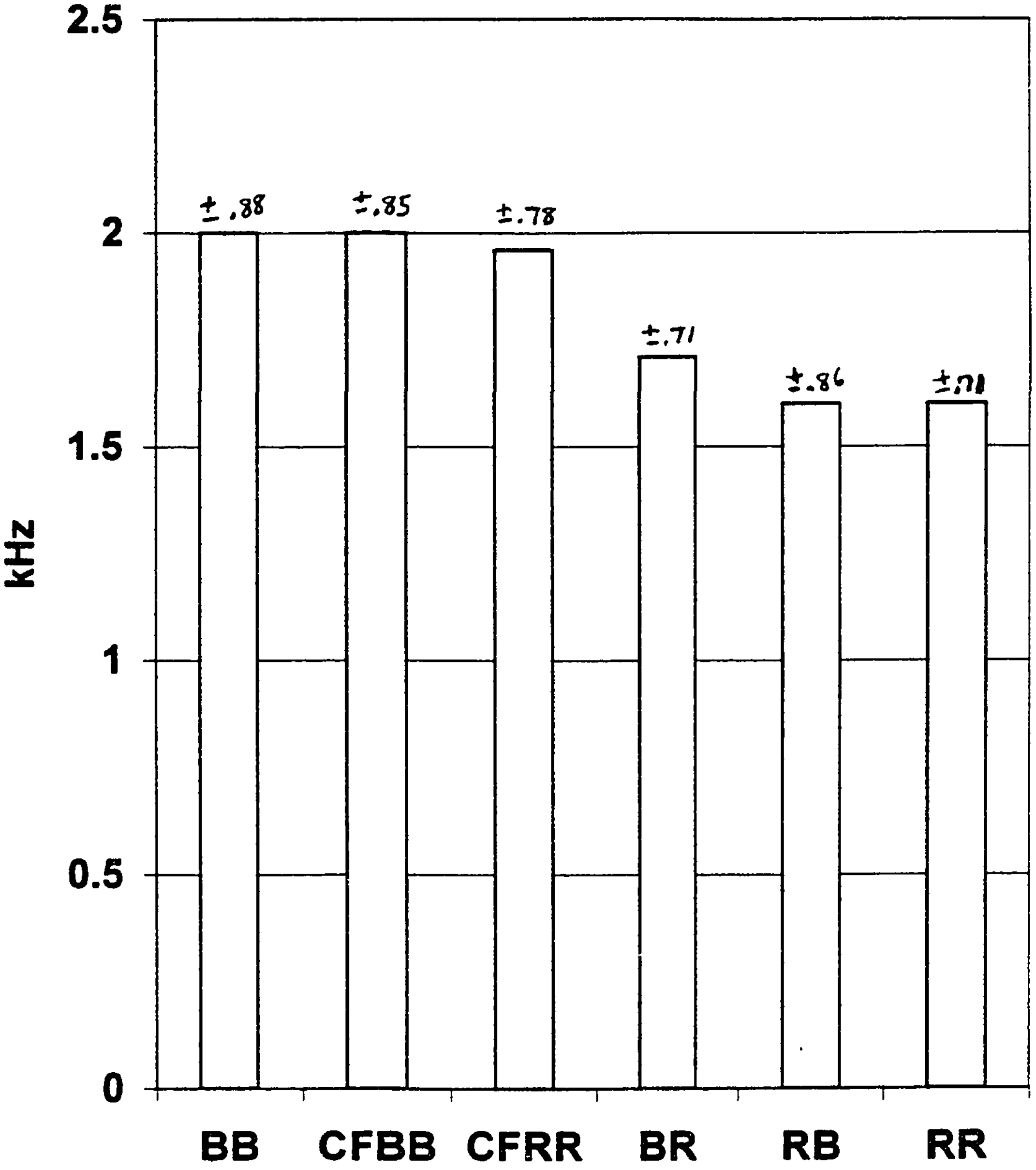

Fig.49: Mean frequency range of nestling calls for all six treatments.

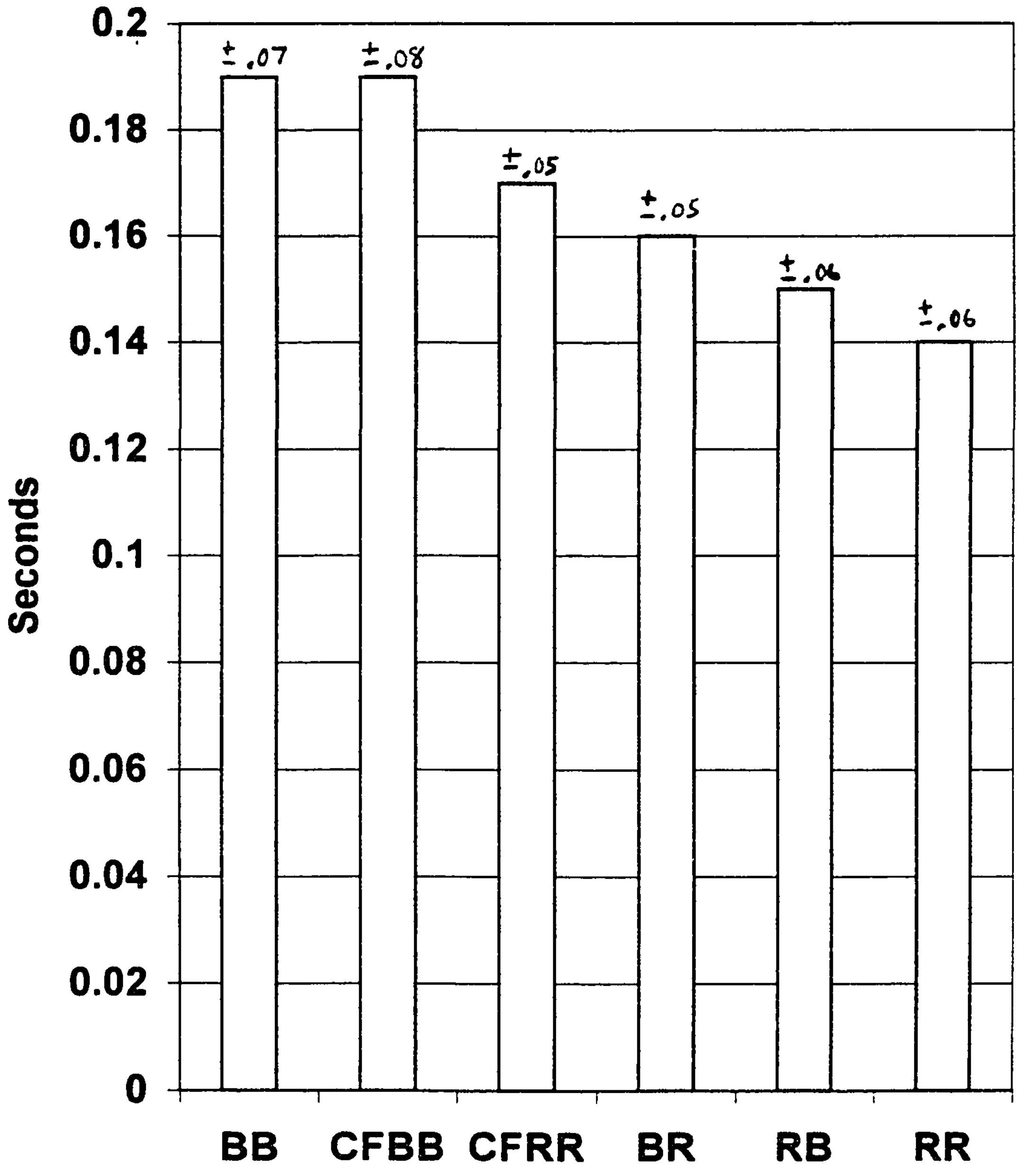

Fig. 50: Mean duration of nestling calls for all six treatments.

LITERATURE CITED:

Allin, J. T. and Banks, E.M. 1970. Effects of temperature on ultrasound production by infant albino rats. *Devel. Psychobiol.* 4: 149-156.

Bachman, G. C. and Chappell, M. A. 1998. The energetic cost of begging behaviour in nestling house wrens. *Anim. Behav.* 55: 1607-1618.

Baker, J.A. and Bailey, E.D. 1987. Ontogeny of the separation call in northern bobwhite (*Coinus virginianus*). *Can. J. Zool.* 65: 1016-1020.

Ballintijn, M.R. and Ten Cate, C. 1997. Vocal development and its differentiation in a non-songbird: the collared dove (*Streptopelia decaocto*). *Behaviour* 134: 595-621.

Baptista, L.F. 1996. Nature and nurture in development. In: *Ecology and Evolution of Acoustic Communication in Birds.* (Kroodsma, D.E. and Miller, E.H., Eds.) Cornell Univ. Press pp. 39-60.

Baptista, L.F. and Abs, M. 1983. Vocalizations. In: *Behavior and physiology of the pigeon* (Abs, M., Ed.). Academic Press, New York. pp. 309-325.

Barg, J.B. and Mumme, R.L. 1994. Parental recognition of juvenile begging calls in the Florida scrub jay. *Auk* 111: 459-464.

Barlow, G.W. 1991. Nature-nurture and the debates surrounding ethology and sociobiology. *Amer. Zool.* 31: 286-296.

Barrington, D. 1798. Experiments and observations on the singing of birds. In: *The History of Singing Birds* (Buffon, G. Ed.). Arno Press: N. Y., pp. 164-192.

Beecher, M.D. 1982. Signature systems and kin recognition. *Amer. Zool.* 22: 477-490.

Beecher, M.D., Beecher, I.M. and Hahn, S. 1981. Parent-offspring recognition in bank swallows (*Riparia riparia*): II. Development and acoustic basis. *Anim. Behav.* 29: 95-101.

Beer, C.G. 1969. Laughing gull chicks: recognition of their parents' voices. *Science* 166: 1030-1032.

Buffon, G. 1798. *The History of Singing Birds.* Arno Press: N.Y., pp. 75-126.

Byers, B.E. and Kroodsma, D.E. 1992. Development of two song categories by chestnut-sided warblers. *Anim. Behav.* 45: 799-810.

Chaiken, M. 1990. The ontogeny of antiphonal calling in European starlings. *Developmental psychobiol.*. 23: 233-246.

Clayton, N.S. 1990 Subspecies recognition and song learning in zebra finches. *Anim. Behav.* 40: 1009-1017.

Cotton, P.A., Kacelnik, A. and Wright, J. 1996. Chick begging as a signal: Are nestlings honest? *Behav. Ecol.* 7: 178-182.

Darwin, C. 1868. *The Variation of Animals and Plants under Domestication.* J. Murray, London.

Eens, M., Rianne, P. and Verheyen, R.F. 1992. Song learning in captive European starlings. *Anim. Behav.* 44: 1131-1143.

Evans, C.S., Evans, L. and Marler, P. 1993. On the meaning of alarm calls: functional reference in an avian vocal system. *Anim. Behav.* 46: 23-38.

Evans, E.M. 1992. Embryonic and neonatal vocal elicitation of parental brooding and feeding responses in American white pelicans. *Anim. Behav.* 44: 667-675.

Evans, E.M. 1994. Cold-induced shivering in young American white pelicans: honest signalling of offspring need for warmth in a functionally integrated thermoregulatory system. *Behaviour* 129: 13-

Ewert, D.N. 1979. Development of song of a rufous-sided towhee raised in acoustic isolation. *Condor* 81: 313-316.

Godfray, H.C.J. 1991. Signalling of need by offspring to their parents. *Nature*, Lond. 352: 328-330.

Godfray, H.C.J. 1995. Signalling of need between parent and young: Parent-offspring conflict and sibling rivalry. *Am. Nat.* 146: 1-24.

Goodenough, J., McGuire, B. and Wallace, R.A. 1993. In: *Perspectives on Animal Behavior.* John Wiley and Sons, New York.

Gottlieb, G. 1980a. Development of species identification in ducklings: VI. Specific embryonic experience required to maintain species-typical perception in Peking ducklings. *Journal of Comparative and Physiological Psychology* 94: 579-587.

Gottlieb, G. 1980b. Development of species identity in ducklings: VII. Highly specific early experience fosters species-specific perception in wood ducklings. *Journal of Comparative and Physiological Psychology.* 94: 1019-1027.

Gottlieb, G. 1981. Roles of early experience in species-specific perceptual development. In: *Development of Perception*, vol. 1. (Aslin, R.N., Alberts, J.R. and Peterson, M.R. Eds.) Academic Press.

Groth, J.G. 1993. Call matching and positive assortative mating in red crossbills. *Auk* 110: 398-401.

Gustafson, G.E., Green, J.A. and Tomic T. 1984. Acoustic correlates of individuality in the cries of human infants. *Develop. Psychobiol.* 17:311-324.

Gustafson, G.E., Green, J.A., and Cleland, J.W. 1994. Robustness of individual identity in the cries of human infants. *Develop. Psychobiol.* 27: 1-9.

Guttinger, H.R. 1977. Variable and constant structures in greenfinch songs (*Chloris chloris)* in different locations. *Behaviour* 60: 304-318.

Guttinger, H.R. 1979. The integration of learnt and genetically programmed behavior: A study of hierarchical organization in songs of canaries, greenfinches and their hybrids. *Z. Tierpsychologie* 49: 285-303.

Guttinger, H.R. 1985. Consequences of domestication on the song structures in the canary. *Behavior* 94: 254-278.

Guttinger, H.R., and Nicolai, J. 1973. Struktur und Funktion der Rufe bei Prachtfinken (Estrildidae). *Z. Tierpsychol.* 33: 319-334.

Guttinger, H.R., Wolffgramm, J. and Thim, F. 1978. The relationship between species specific song programs and individual learning in songbirds. *Behaviour*, 65: 241-262.

Haig, D. and Graham, C. 1991. Genomic imprinting and the strange case of the insulin-like growth factor II receptor. Cell. 64: 1045-1046.

Haig, D. and Westoby, M. 1989. Parent-specific gene expression and the triploid endosperm. Am. Nat. 134: 147-155.

Haigh, L.S., Owens, B.B., Hellewell, S. and Ingram, V.M. 1982. DNA methylation in chicken alpha-globin gene expression. *Proc. Natl. Acad. Sci. USA* 79: 5332-5336.

Johnston, T.D. 1988. Developmental explanation and the ontogeny of birdsong: nature/nurture redux. *Behav. Brain Sci.* 11: 617-663.

Jones, I.L., Falls, J.B. and Gaston, A.J. 1987. Vocal recognition between parents and young of ancient murrelets, *Synthliboramphus antiquus* (Aves: Alcidae). *Anim. Behav.* 35: 1405-1415.

Konishi, M. and Nottebohm. F. 1969. Experimental studies in the ontogeny of avian vocalizations. In: *Bird Vocalizations* (Hinde, R.A., Ed.) Cambridge Univ. Press, London. Pp. 29-48.

Lack, D. 1968. *Ecological Adaptations for Breeding in Birds.* London: Metheun.

Lade, B.I. and Thorpe, W.H. 1964. Dove songs as innately coded patterns of specific behaviour. *Nature* 202: 366-368.

Lanyon, W.E. 1960. In: *Animal Sounds and Communication.* (Lanyon, W.E. and Tavolga, W.N., Eds.) p 326.

Lefebvre, L., Viville, S. Barton, S.C., Ishino, F., Keverne, E.B. and Surani, M.A. 1998. Abnormal maternal behavior and growth retardation associated with loss of the imprinted gene *Mest. Nature Genetics* 20: 163-169.

Lefevre, K., Montgomerie, R. and Gaston, A.J. 1998. Parent-offspring recognition in thick-billed murres (Aves: Alcidae) *Anim. Behav.* 55: 925-938.

Lemon, R.E. and Herzog, A. 1969. The vocal behavior of cardinals and pyrrhuloxias in Texas. *Condor* 71: 1-15.

Lessells, C.M., Coulthard, N.D., Hodgson, P.J. and Krebs, J.R. 1991. Chick recognition in European bee-eaters: acoustic playback experiments. *Anim. Behav.* 42: 1031-1033.

Li, E.,Beard, C. and Jaenisch, R. 1993. Role for DNA methylation in genomic imprinting. *Nature* 366: 362-365.

Lyon, M.F. 1994. Evolution of mammalian sex-chromosomes. In: *The Differences Between the Sexes.* (Short, R.V. and Balaban, E. Eds.) Cambridge Univ. Press. Pp. 381-396.

Marler, P. 1955. *Characteristics of some animal calls.* Nature, London., 176: 6-8.

Marler, P. 1970. A comparative approach to vocal learning: song development in white-crowned sparrows. *J. comp. physiol. Psychol. (Monogr.)* 71: 1-25.

Marler, P. and Mundinger, P.C. 1975. Vocalizations, social organization and breeding biology of the twite *Acanthus flavirostris. Ibis* 117: 1-17.

Marler, P. and Peters, S. 1987. A sensitive period for song acquisition in the song sparrow, *Melospiza melodia*: a case of age-limited learning. *Ethology* 76: 89-100.

Marler, P. and Waser M.S. 1977. Role of auditory feedback in canary song development. *J. Comp. Phsy. Psych.* 91: 8-16.

McCarty, J.P. 1996. The energetic cost of begging in nestling passerines. *Auk* 113: 178-188.

McGrath, T.A., Shalter, M.D. and Schleidt, W.M. 1972. Analysis of chicken x pheasant hybrids. *Nature* 237: 47-48.

Medvin, M.B., Stoddard, P.K. and Beecher, M.D. 1993. Signals for parent-offspring recognition: A comparative analysis of the begging calls of cliff swallows and barn swallows. *Anim. Behav.* 45: 841-850.

Miller, A.H. 1947. The structural basis of the voice of the flammulated owl. *Auk* 64: 133-135.

Monk, D.S. 1997 Individual, brood and sex variation in begging calls of western bluebirds. *Wilson Bulletin* 109: 328-332.

Monk, M. 1988. Genomic imprinting. *Genes and Development.* 2: 921-925.

Mulligan, J.A. and Olsen, K.C. 1969. Communication in canary courtship calls. In: *Bird Vocalizations* (Hinde R.A. Ed.) Cambridge University Press, pp. 165-184.

Mundinger, P.C. 1970 Vocal imitation and individual recognition of finch calls. *Science* 168: 480-482.

Mundinger, P.C. 1979 Call learning in the carduelinae: Ethological and systematic considerations. *Systematic Zoology* 28: 270-283.

Mundinger, P.C. 1988. Conceptual errors, different perspectives, and genetic analysis of song ontogeny. *Behav. Brain Sci.* 11: 643-644.

Mundinger, P.C. 1995. Behaviour-genetic analysis of canary song: inter-strain differences in sensory learning, and epigenetic rules. *Anim. Behav.* 50: 1491-1511.

Mundinger, P.C. 1999. Genetics of song learning: innate mechanisms and other neurobiological considerations. In: *Neural Mechanisms of Communication.* (Konishi, M. and Hauser, M. Eds.) M.I.T. Press: Cambridge, Ma. Pp. 369-389.

Nelson, D.A. and Marler, P. 1993. Innate recognition of song in white-crowned sparrows: a role in selective vocal learning? *Anim. Behav.* 46: 806-808.

Nottebohm, F. 1970. Ontogeny of bird song. *Science* 167: 950-956.

Nottebohm, F. 1980. Brain pathways for vocal learning in birds: a review of the first 10 years. In: *Progress in Psychobiology and Physiological Psychology,* vol. 9 (Sprage, J.M.S. and Epstein, A.N.E., Eds.) Academic Press, New York. pp85-124.

Nottebohm, F. 1991. Reassessing the mechanisms and origins of vocal learning in birds. *Trends in Neurosciences* 14: 206-211.

Nowicki, S. 1989. Vocal plasticity in captive black-capped chickadees: the acoustic basis and rate of call convergence. *Anim. Behav.* 37: 64-73.

Ottoson, V., Backman, J. and Smith, H.G. 1997. Begging affects parental effort in the pied flycatcher, *Ficedula hypoleuca. Behav. Ecol. Sociobiol.* 41381-384.

Podos, J. 1996. Motor constraints on vocal development in a song bird. *Anim. Behav.* 51: 1061-1070.

Poulsen, H. 1959. Song learning in the domestic canary. *Z. Tierpsychol.* 16: 173-178.

Proffit, F.M. and McLean, I.G. 1991. Recognition of parents' calls snares crested penguin. *Bird Behav.* 9: 103-113.

Ranier, S. and Feinberg, A.P. 1994. Genomic imprinting, DNA methylation, and cancer. *J. Natl. Cancer Institute* 86: 753-759

Rasmuson, T.M. and Barclay R.M.R. 1991. Individual variation in the isolation calls of newborn big brown bats (*Eptesicus fuscus*): Is variation genetic? *Can. J. Zool.* 70: 698-702.

Redondo, T. and Arias De Reyna, L. 1988. Locatability of begging calls in nestling altricial birds. *Anim. Behav.* 36: 653-661.

Ritchison, G. 1983. The function of singing in female black-headed grosbeaks (*Pheuticus melanocephalus*): family group maintenance. *Auk* 100: 105-116.

Robertson, B.C. 1996. Vocal mate recognition in a monogamous, flock-forming bird, the silvereye, *Zosterops lateralis. Anim. Behav.* 51: 303-311.

Rooke, I.J. and Knight, T.A. 1977. Alarm calls of honeyeaters with reference to locating sources of sound. *Emu* 77: 193-198.

Rowley, I. and Chapman, G. 1986. Cross-fostering, imprinting and learning in two sympatric species of cockatoo. *Behaviour* 96: 1-16.

Ryan, M.J. and Brenowitz, E.A. 1985. The role of body size, phylogeny, and ambient noise in the evolution of bird song. *Am. Nat.* 126: 87-100.

Skuse, D.H., James, R.S., Bishop, D.V.M., Coppin, B., Dalton, P., Aamodt-leeper, G., Bacarese-Hamilton, M., Creswell, C., McGurk, R. and Jacobs, P.A. 1997. Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature* 387: 705-708.

Slater, P.J.B. 1983. Bird song learning: Theme and variations. In: *Perspectives in Ornithology* (Brush A.H. and Clark, G.A., Jr., Eds.) Cambridge University Press. pp. 475-499.

Slater, P.J.B. and Ince, S.A. 1982. Song development in chaffinches: what is learnt and when? *Ibis* 124: 21-26.

Smith, J.N.M. and Merkt, J.R. 1980. Development and stability of single-parent units in the song sparrow. *Can. J. Zool.* 58: 1869-1875.

Sossinka, R. 1982. Domestication in birds. In: *Avian biology* (Farner,D.S., King, J.R. and Parkes, K.C. Eds.) Academic Press, New York. pp. 373-403.

Spector, D.A. 1994. Definition in biology: The case of "bird song". *J. Theor. Biol.* 168: 373-381.

Stevenson, J.G., Hutchison, R.E., Hutchison, J.B., Bertram, B.C.R., Thorpe, W.H. 1970. Individual recognition by auditory cues in the common term, *Sterna sandvicensis. Nature* 226: 562-563.

Swain, J.L., Stewart, T.A. and Leder, P. 1987. Parental legacy determines methylation and expression of an autosomal transgene: A molecular mechanism for parental imprinting. *Cell* 50: 719-727.

Thielke, G. 1965. Die Ontogenese der Bettellaute von Garten-und Waldbaumlaufer (*Certhia brachydactyla* Brehm und *C. familiaris* L.) *Zool. Anz.* 174: 237-241.

Thorpe, W.H. 1958. The learning of song patterns by birds, with especial reference to the song of the chaffinch, *Fringilla coelebs. Ibis* 100: 535-570.

Thorpe, W.H. 1961. *Bird song.* Cambridge University Press.

Trivers, R.L. 1974. Parent-offspring conflict. *Am. Zool.* 14: 249-264.

Tschanz, B. 1959. Zur Brutbiologie der Trottellumme (*Uria aalge aalge* Pont.). *Behaviour* 14: 1-100.

Wallschlager, D. 1980. Correlation of song frequency and body weight in passerine birds. *Experiencia* 36: 412.

West, M.J. and King, A.J. 1985. Social guidance of vocal learning by female cowbirds: validating its functional significance. *Z. Tierpsychol.* 70: 225-235.

Wiley, R.H. and Richards, D.G. 1978. Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. *Behav. Ecol. Sociobiol.* 3: 69-94.

Wilks, A.F., Cozens, P.J., Mattaj, I.W. and Jost, J.P. 1982. Estrogen induces a demethylation at the 5′end region of the chicken vitellogenin gene. *Proc. Acad, Sci. USA* 79: 4252-4255.

Zann, R. 1985. Ontogeny of the zebra finch distance call. I. Effects of cross-fostering to Bengalese finches. *Z. Tierpsychol.* 68: 1-23.

Zann, R. 1990. Song and call learning in wild zebra finches in south-east Australia. *Anim. Behav.* 40: 811-828.