Development of a contact call in black-capped chickadees (*Poecile atricapillus*) hand-reared in different acoustic environments

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The *tseet* contact call, common to black-capped (*Poecile atricapillus*) and mountain chickadees (*P. gambeli*), is the most frequently produced vocalization of each species. Previous work has characterized the *tseet* call of black-capped and mountain chickadees from different geographic locations in terms of nine acoustic features. In the current study, using similar methods, the *tseet* call of black-capped chickadees that were hand reared with either conspecifics, heterospecifics (mountain chickadees), or in isolation from adult chickadees are described. Analysis of call features examined which acoustic features were most affected by rearing environment, and revealed that starting frequency and the slope of the descending portion of the *tseet* call differed between black-capped chickadees reared with either conspecific or heterospecific adults. Birds reared in isolation from adults differed from the other hand-reared groups on almost every acoustic feature. Chickadee *tseet* calls are more individualized when they are reared with adult conspecifics or heterospecifics compared to chickadees that are reared in isolation from adults. The current results suggest a role of learning in this commonly used contact call. © *2011 Acoustical Society of America.*

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I. INTRODUCTION

Songbird vocalizations fall into one of two categories, calls or songs. Songs are given mainly by males during breeding season and function in territorial defense and serve as a sexually selected trait (Catchpole and Slater, 2008). Songs are typically long, acoustically complex, and learned from a tutor during a sensitive period (Doupe and Kuhl, 1999). Conversely, calls are typically shorter, simpler and produced year round by both sexes. Calls serve specific functional roles, such as predator deterrence, raising alarm within a flock, and coordination of flock movement (Smith, 1991; Marler, 2004). Although calls were once thought to be innate, there is evidence to suggest that normal production of some calls of a few species also require experience hearing adult vocalizations (for example, chickadees, Hughes *et al.*, 1998; for a general overview see Vicario *et al.*, 2002; Marler and Slabbekoorn, 2004). Because calls are utilized by both males and females in year-round daily life, the extent to which songbird calls are learned should be more thoroughly examined.

The *tseet* call is a contact call common to black-capped (*Poecile atricapillus*) and mountain chickadees (*P. gambeli*). The *tseet* call is the most commonly given call by black-capped (Odum, 1942) and possibly by mountain chickadees (pers. obs.), although not yet quantified. *Tseet* calls are low amplitude and therefore are used for communication when birds are at short distances from each other (Ficken *et al.*, 1978). The function of *tseet* calls is likely to maintain group or pair cohesion while foraging (Smith, 1991).

We have previously described the *tseet* call in three populations of adult, wild-caught chickadees (sympatric and allopatric black-capped chickadees and sympatric mountain chickadees) using nine acoustic features in descriptive statistics, potential for individual coding (PIC) and linear discriminant analysis (LDA; Guillette *et al.*, 2010). *Tseet* calls were analyzed and classified at three levels: (1) by the individual that produced the call, (2) by the group that produced the call. In the latter case, groups could be defined by species and geography (e.g., sympatric black-capped chickadee, allopatric black-capped chickadee or mountain chickadee) or (3) by species, geography and sex (e.g., male sympatric black-capped chickadee, female sympatric black-capped chickadee, etc.).

Analyses at these three levels revealed marked differences between *tseet* calls produced by the various groups and

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individuals. For instance, the *tseet* calls produced by male black-capped chickadees in allopatry were more highly individualized compared to *tseet* calls produced by their female counterparts. This trend was not witnessed in the population of sympatric black-capped chickadees or mountain chickadees. Sympatric black-capped and mountain chickadees are often found feeding in mixed species flocks during the winter months (pers. obs.). In these two populations (sympatric black-capped and mountain chickadees), the *tseet* call of both males and females was highly individualized. Another finding was that the *tseet* call produced by sympatric chickadees was less accurately classified by the LDA model (76% of all cases) compared to the *tseet* call produced by allopatric black-capped (91%) or mountain chickadees (96%).

To understand how these acoustic differences might arise during vocal development, either via learning and/or genetic factors, we analyzed *tseet* calls produced by allopatric black-capped chickadees that were reared in one of three distinct acoustic environments. Young birds were brought into the lab approximately 10–12 days post hatch and reared in colony rooms with either adult black-capped chickadees, adult mountain chickadees, or no adult chickadees present. Hand-reared birds' vocalizations were recorded at one year of age. As we had done previously (e.g., Guillette *et al.*, 2010), nine acoustic features were used to describe the *tseet* calls through summary statistics, the PIC and classification by LDA. We added parametric (MANOVAs) and non-parametric (Kruskal-Wallis and Mann-Whitney U) statistics in the current study to test for differences in the nine acoustic features, and differences in the individuality of the tseet call produced by birds in the three distinct rearing conditions.

If learning plays a role in the development of *tseet* calls then we expect to find that the LDA model should be able to differentially classify calls produced by birds reared in different environments (e.g., comparable in correct classification to the LDAs used previously to classify adult wild-caught birds; Guillette *et al.*, 2010). If learning does not play a role in the development of *tseet* calls, then the model should not differentiate among the calls from different rearing conditions, and instead should classify the *tseet* call of birds reared in the different environments as being produced by the same group of birds.

II. METHODS

A. Subjects

Twenty-five black-capped chickadees were taken from seven nest sites around Edmonton, Alberta, Canada at approximately 10–12 days post hatch (around June 1st) in 2003–2005, and in 2008. Individuals were hand-reared until independence, and then continued to live in one of three randomly assigned conditions: (1) in a colony room with individually housed, adult, black-capped chickadees (adults were wild caught in either Edmonton, Alberta or Kananaskis Valley, Alberta, Canada for hatchlings caught in 2003–2005, or with adults originating from only Edmonton, Alberta for hatchlings caught in 2008), (2) in a colony room with individually housed, adult mountain chickadees (originating from Kananaskis, Alberta), or (3) in a sound attenuating chamber with no adults present; however, these hatchlings could hear and see each other. For the details of the rearing condition, year and location caught, see Table I.

TABLE I. The rearing condition, year, location and number of individual retrieved from different nest sites around Edmonton, Alberta, Canada. BC reared = reared with black-capped chickadees, MO reared = reared with mountain chickadees, Isolate = reared in isolation from adults. Whitemud Ravine and the Edmonton River Valley are located in Edmonton, Alberta, Canada. Tofield, Alberta is located 70 km southeast of Edmonton. The lower case letters (a)– (d) represent different nests within the same general area.

Rearing Condition	Year	Number	Location/Nest
BC reared males	2003	3	Whitemud Ravine, a
	2005	1	Edmonton River Valley, c
		1	Edmonton River Valley, d
BC reared females	2004	1	Edmtonton River Valley, b
	2005	1	Edmonton River Valley, c
		2	Edmonton River Valley, d
	2008	1	Tofield, Alberta
MO reared males	2003	1	Whitemud Ravine, a
		1	Whitemud Ravine, b
	2004	2	Edmtonton River Valley, b
	2005	1	Edmonton River Valley, d
MO reared females	2003	1	Whitemud Ravine, a
	2005	3	Edmonton River Valley, c
		1	Edmonton River Valley, d
Isolate males	2008	2	Tofield, Alberta
Isolate females	2008	1	Tofield, Alberta
		2	Whitemud Ravine, c

Hatchlings were hand fed a mixture of dry food (Mazuri Small Bird Maintenance Diet; Mazuri, St. Louis, MO), wheat germ, water, and small amounts of pureed vegetable (e.g., spinach, carrots, etc.). The hatchlings were housed in the same cage until they showed evidence of independent feeding (approximately 30–35 days of age). They were then housed in individual cages and weaned off the mixture onto dry food (approximately 40–45 days of age). After weaning was complete, the hatchlings were treated in an identical manner as the adult-caught birds, described below.

Each adult bird was housed individually at the University of Alberta in Jupiter Parakeet cages (30 × 40 × 40 cm; Rolf C. Hagen, Inc., Montreal, Canada), which allowed for auditory and visual, but not physical contact among birds. Each bird had free access to dry food, water (vitamin supplemented on alternating days; Prime vitamin supplement, Hagen, Inc., Montreal, Canada), grit and cuttlebone. Each bird was given three to five sunflower seeds daily. Each bird also received one mealworm or superworm three times a week and a mixture of greens and eggs twice a week. Colony rooms were maintained on a light/dark cycle that mimicked the natural light cycle for Edmonton, Alberta, Canada. Sex was determined by DNA analysis (Griffiths *et al.*, 1998).

B. Recordings

During recording sessions, birds were removed from the colony room and recorded individually in a sound attenuating chamber (1.7 × 0.8 × 0.58 m), Industrial Acoustics Corporation, Bronx, NY) using an AKG C 1000S condenser microphone connected to a Marantz PMD 670 digital recorder (frequency response: 10–20 000 Hz; Marantz Electronics, Eindhoven, Netherlands). Digitized files (44 100 Hz) were downloaded to an Intel based PC and analyzed with SIGNAL 4.0 (Engineering Design, Berkley, CA). Each bird was recorded during several five to fifteen minute sessions over a four week period between its 12th and 13th month. Birds were returned to the colony room after each recording session.

C. Acoustic analysis

Ten high quality calls were randomly selected from several recording sessions for each bird. Several spectral and temporal measures were taken on each call (see below). Please refer to Guillette *et al.* (2010) for details of the acoustic analysis.

The three temporal measures were: (1) the ascending duration (AD), which is measured from the start of the call to the point where the ascending portion stops, (2) the descending duration (DD) is measured from the highest frequency of the call, where the call begins to descend in frequency, until the end of the call, and (3) the total duration (TD) of the call. The three frequency measures were: (1) the start frequency (SF), the (2) high frequency (HF) and (3) end frequency (EF) of the call. Three additional features of the calls were: the (1) maximum or peak (i.e., loudest) frequency (PF) that was measured in a spectrum window (modal window size = 4096 points), the (2) rate of rise for the ascending slope (FMasc) was calculated using the formula (HF-SF)/AD, and the (3) rate of decay in the descending slope (FMdesc) was calculated using the formula (EF-HF)/DD; see Fig. 1.

D. Potential for individual coding (PIC)

To determine which acoustic features may provide cues of identity in the *tseet* call, we examined the potential for individual coding (PIC) (Charrier *et al.*, 2004). In common with the analysis performed in Guillette *et al.* (2010) we calculated the PIC value: the ratio of the coefficient of variation between individuals within a particular group (e.g., black-capped reared chickadees) (CV_b) to the average of the coefficients of variation within an individual (CV_w), for that same group. Here, $CV_b = (SD/X) \times 100$, where SD is the standard deviation of the group (e.g., black-capped reared chickadees) and X is the group mean for an acoustic feature (e.g., PF) and CV_w is the average of the coefficients of variation for each individual in that group. Acoustic features with a PIC value greater than one are potentially used for individual recognition since the inter-individual variability (for that specific acoustic feature) is greater than the intra-individual variability (Robisson *et al.*, 1993).

E. Linear discriminant analyses

We employed forward stepwise linear discriminant analyses (LDA; PASW Statistics 18.0) to classify each *tseet* call by the individual or group of chickadees that produced it. Several different LDAs were performed. Each LDA could use the nine different acoustic features, outlined in the acoustic analysis section above, as independent variables to classify *tseet* calls into groups according to which chickadee produced the call. Analyzing classification errors in the LDA's predictions helps identify subtle similarities and differences between the *tseet* calls produced by the different individuals, rearing groups and sexes. For details of rationale and interpretation of LDA, see Guillette *et al.* (2010).

A separate forward, stepwise LDA was conducted for each group of birds (males reared with black-capped chickadees, females reared with black-capped chickadees, males reared with mountain chickadees, females reared with mountain chickadees, and isolate chickadees) to classify *tseet* calls in terms of which individual produced it. Male and female isolates were not analyzed separately for individual identity because there were only two and three individuals, respectively. The purpose of these LDAs was to determine the ability of the model to correctly classify each *tseet* call in terms of which individual produced it and to determine the correspondence between features identified in the PIC and LDA. In this way, we used two complimentary statistical techniques for assessing individualization of *tseet* calls.

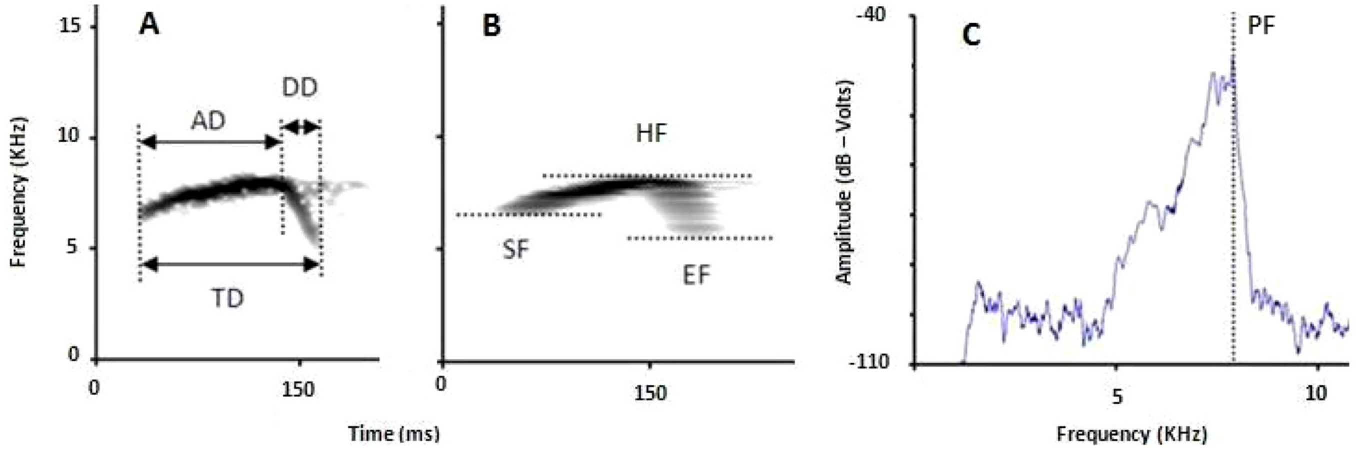

FIG. 1. (Color online) Sound spectrogram and spectrum illustrating note-type features measured in *tseet* calls. Panel (a) frequency (*y* axis) by time (*x* axis) sound spectrogram (FFT window = 256 points) of a *tseet* call. Vertical lines represent boundaries for ascending duration (AD), descending duration (DD), and total note duration (TD). Panel (b) frequency (*y* axis) by time (*x* axis) sound spectrogram (FFT window = 1024 points) of a *tseet* call. Horizontal lines illustrate the start frequency (SF), high frequency (HF), and end frequency (EF). Panel (c) relative amplitude (*y* axis) by frequency (*x* axis) sound spectrum (window size = 4096 points) of a *tseet* call. The vertical line illustrates the frequency at max amplitude (peak frequency, PF).

III. RESULTS

A. Classification of *tseet* call by individuals

Tables II and III show the means, standard deviations, coefficients of variation within and between individuals, as well as the potential for individual coding (PIC). Most PIC values are greater than one (i.e., for each group, and sex). However, this is not true for isolate male chickadees where four of nine acoustic features have a PIC value lower than one. A Kruskal-Wallis ANOVA by ranks was conducted to test for differences among the PIC values for the nine acoustic features for males reared in the three different environments and revealed a significant effect $H(2, \mathrm{N} = 27) = 17.99$, $p < 0.001$. Therefore, Mann-Whitney U tests were performed to compare differences in acoustic features among rearing groups. Males reared in isolation from adults had

TABLE II. Means, standard deviations, between-bird coefficients of vatiation ($\mathrm{CV_b}$), within-bird coefficients of variation ($\mathrm{CV_w}$), and PIC for each feature measured in *tseet* calls. ∑PIC is the sum of the PIC values for each sex, in each rearing environment. The Individual *F* value is the value returned from univariate ANOVAs that test for differences in each acoustic feature between individuals. Significant values (Bonferroni corrected for multiple comparisons) are starred.

Male Black-cap Reared		TD	AD	DD	SF	HF	EF	PF	FMasc	FMdesc
n = 5	X	69.68	30.08	13.88	4233.76	5451.69	4410.86	5222.42	48.24	– 69.46
	SD	23.06	26.02	13.67	1320.30	1249.70	557.70	1178.10	37.32	97.54
	CVb	33.09	86.49	98.52	31.19	22.92	12.64	22.56	77.36	– 140.44
∑ PIC	CVw	21.80	43.69	92.11	6.42	6.33	8.77	10.69	42.80	– 92.35
19.92	PIC	1.52	1.98	1.07	4.86	3.62	1.44	2.11	1.81	1.52
	Individual *F*	4.55*	16.5*	5.79*	247.87*	81.60*	3.71	21.66*	26.73*	2.55
Female Black-cap Reared		TD	AD	DD	SF	HF	EF	PF	FMasc	FMdesc
n = 5	X	51.61	27.87	12.56	5713.52	6953.45	5733.92	6465.40	49.14	– 84.50
	SD	25.82	25.16	25.17	1536.10	1147.35	1713.79	1132.55	41.25	119.28
	CVb	50.02	90.27	200.46	26.89	16.50	29.89	17.52	83.95	– 141.15
∑ PIC	CVw	29.56	73.27	94.48	9.70	3.71	7.93	4.48	69.78	– 86.93
22.77	PIC	1.69	1.23	2.12	2.77	4.45	3.77	3.91	1.20	1.62
	Individual *F*	5.88*	7.61*	3.09	46.62*	189.44*	175.71*	114.75*	13.22*	6.36*
Male Mountain Reared		TD	AD	DD	SF	HF	EF	PF	FMasc	FMdesc
n = 5	X	61.02	33.07	15.64	5047.82	6092.81	5027.15	5766.93	47.26	– 110.12
	SD	20.54	29.43	26.69	981.81	928.96	1071.01	995.32	43.24	93.65
	CVb	33.67	89.00	170.69	19.45	15.25	21.30	17.26	91.50	– 85.05
∑ PIC	CVw	17.10	75.15	78.51	14.52	12.37	15.70	13.02	74.77	– 73.60
12.96	PIC	1.97	1.18	2.17	1.34	1.23	1.36	1.33	1.22	1.16
	Individual *F*	9.62*	0.92	1.65	4.72*	2.46	6.38*	2.04	4.39*	3.85
Female Mountain Reared		TD	AD	DD	SF	HF	EF	PF	FMasc	FMdesc
n = 5	X	56.81	39.05	9.37	3966.14	6433.51	5050.42	5868.31	104.33	– 108.64
	SD	18.88	28.52	14.03	1202.81	404.89	757.98	269.44	110.33	119.47
	CVb	33.24	73.03	149.73	30.33	6.29	15.01	4.59	105.75	– 109.97
∑ PIC	CVw	19.95	54.37	79.01	21.51	3.58	9.17	3.15	57.39	– 62.98
14.76	PIC	1.67	1.34	1.89	1.41	1.76	1.64	1.46	1.84	1.75
	Individual *F*	7.02*	19.95*	2.25	11.30*	23.56*	20.40*	9.11*	5.85*	5.80*
Male Isolate		TD	AD	DD	SF	HF	EF	PF	FMasc	FMdesc
n = 2	X	68.13	15.19	25.28	6101.24	7035.97	5293.07	6391.86	65.62	– 105.76
	SD	14.07	13.86	26.22	901.37	1055.98	1006.69	861.72	35.26	112.70
	CVb	20.65	91.24	103.74	14.77	15.01	19.02	13.48	53.74	– 106.56
∑ PIC	CVw	20.38	85.02	106.58	14.17	14.56	19.23	13.80	54.78	– 96.55
9.18	PIC	1.01	1.07	0.97	1.04	1.03	0.99	0.98	0.98	1.10
	Individual *F*	0.05	4.40	0.01	1.76	0.32	0.25	0.01	0.22	0.12
Female Isolate		TD	AD	DD	SF	HF	EF	PF	FMasc	FMdesc
n = 3	X	56.87	7.40	22.71	7147.72	7756.07	5827.41	7341.03	48.46	– 128.94
	SD	5.20	7.52	14.59	354.76	330.11	566.71	317.24	51.48	85.46
	CVb	9.14	101.58	64.25	4.96	4.26	9.72	4.32	106.24	– 66.28
∑ PIC	CVw	9.03	98.57	47.36	3.84	3.40	9.05	3.36	105.40	– 65.16
10.33	PIC	1.01	1.03	1.36	1.29	1.25	1.07	1.29	1.01	1.02
	Individual *F*	1.29	1.48	3.88	9.83*	7.98*	2.64	7.60*	0.99	4.66

TABLE III. Means, standard deviations, between-bird coefficients of vatiation (CV_b), within-bird coefficients of variation (CV_w), and PIC for each feature measured in *tseet* calls. ∑PIC is the sum of the PIC values for each of the three rearing environments. The Individual *F* value is the value from the Univariate ANOVAs that test for differences in each acoustic feature between individuals. Significant values (Bonferroni corrected for multiple comparisons) are starred. The ANOVA by rearing group *F* value is from the univariate ANOVA testing for differences in each acoustic feature between the three rearing environments. Bonferroni corrected probabilities associate with each between group comparison are presented the comparison *p* values rows. MO re is a comparison with the birds reared with mountain chickadees and ISO is a comparison with birds reared in isolation from adults.

Black-cap Reared		TD	AD	DD	SF	HF	EF	PF	FMasc	FMdesc
n = 10	X	60.65	28.98	13.22	4973.64	6202.57	5072.39	5843.91	48.69	– 76.98
	SD	25.99	25.49	20.16	1607.37	1412.11	1431.67	1308.42	39.14	108.67
	CVb	42.86	87.96	152.54	32.32	22.77	28.22	22.39	80.38	– 141.16
∑ PIC	CVw	25.68	58.48	93.30	8.06	5.02	8.35	7.59	56.29	– 89.64
11.05	PIC	1.67	1.50	1.64	4.01	4.53	3.38	2.95	1.43	1.57
	Individual *F*	6.72*	10.16*	3.24*	87.68*	143.17*	90.86*	48.73*	15.49*	3.93*
ANOVA by rearing group *F*		0.28	17.59	5.59	47.97	26.88	4.84	25.31	4.65	3.60
Comparison *p* value										
	MO re	—	0.14	1.00	0.04*	1.00	1.00	1.00	0.09	0.009*
	ISO	—	< 0.001*	0.01*	< 0.001*	< 0.001*	0.02*	< 0.001*	1.00	0.06

Mountain Reared		TD	AD	DD	SF	HF	EF	PF	FMasc	FMdesc
n = 10	X	58.91	36.06	12.50	4506.98	6263.16	5038.78	5817.62	75.79	– 109.38
	SD	19.74	28.99	21.44	1220.10	733.20	923.17	727.22	88.17	106.80
	CVb	33.51	80.39	171.53	27.07	11.71	18.32	12.50	116.32	– 97.64
∑ PIC	CVw	18.52	64.76	78.76	18.01	7.98	12.43	8.08	66.08	– 68.29
14.41	PIC	1.81	1.24	2.18	1.50	1.47	1.47	1.55	1.76	1.43
	Individual *F*	7.61*	5.24*	1.83	11.47*	4.26*	8.40*	2.16	4.68*	6.66*
Comparison *p* value										
	ISO	—	< 0.001*	0.005*	< 0.001*	< 0.001*	0.01*	< 0.001*	1.00	0.20

Isolate		TD	AD	DD	SF	HF	EF	PF	FMasc	FMdesc
n = 5	X	61.37	10.52	23.74	6729.13	7468.03	5613.68	6961.36	55.32	– 119.67
	SD	11.13	11.08	19.86	811.00	789.85	808.06	753.74	46.08	96.84
	CVb	18.13	105.36	83.65	12.05	10.58	14.39	10.83	83.29	– 80.93
∑ PIC	CVw	13.57	93.15	71.05	7.97	7.87	13.12	7.53	85.15	– 77.72
11.05	PIC	1.34	1.13	1.18	1.51	1.34	1.10	1.44	0.98	1.04
	Individual *F*	4.03	4.10	0.92	11.16*	3.93	2.19	8.36*	1.81	1.06

significantly lower PIC values that males reared with adult chickadees (conspecifics, $p < 0.001$; hereospecifics, $p = 0.01$). A parallel Kruskal-Wallis test was conducted on the females, and was also significant $H(2, \mathrm{N} = 27) = 14.0$, $p < 0.001$. Mann-Whitney U tests revealed that females isolates had lower PIC scores than females reared with adult chickadees (conspecifics, $p < 0.001$; hereospecifics, $p = 0.01$). Mann-Whitney U tests conducted to test for differences between PIC values between males and females in each rearing condition were all non-significant (black-capped reared, $U(\mathrm{N} = 9) = 33$, $p = 0.53$; mountain reared, $U(\mathrm{N} = 9) = 19$, $p = 0.06$, reared in isolation, $U(\mathrm{N} = 4) = 19$, $p = 0.06$). Complimentary MANOVAs were conducted to test for statistical differences in the nine acoustic features between individuals in the different rearing groups. First, all data were standardized within each acoustic features, across all subjects. This allowed spectral (i.e., Hz) and temporal (i.e., ms) features to be compared on the same scale. The MANOVAs were significant for all groups (black-capped reared, $F_{81,539} = 16.6$, $p < 0.001$; male black-capped reared, $F_{36,140} = 16.73$, $p < 0.001$, female black-capped reared, $F_{36,140} = 30.92$, $p < 0.001$; mountain chickadee reared, $F_{81,539} = 6.10$, $p < 0.001$; male mountain reared, $F_{36,140} = 4.5$, $p < 0.001$, female mountain reared, $F_{36,140} = 9.77$, $p < 0.001$; isolate, $F_{36,140} = 3.23$, $p < 0.001$; male isolate, $F_{9,10} = 8.33$, $p = 0.001$, female isolate, $F_{18,38} = 2.77$, $p = 0.004$). Therefore, univariate ANOVAs were conducted on each acoustic feature. The results of the univariate ANOVAs are presented in Tables II and III. The Bonferroni correction (nine comparisons) was used to control for Family-wise error. Table IV is a summary of the LDAs conducted to classify *tseet* calls by the individual that produced the call.

B. Classification of *tseet* calls into groups

We performed another LDA to classify *tseet* calls into three groups. The purpose of this LDA was to classify each call in terms of the rearing environment (black-capped reared, mountain reared, or isolate) from which the bird that produced the call came from, using the nine acoustic measures. Two discriminant functions (Function 1 eigenvalue = 0.664, $R_c^2 = 0.632$, Function 2 eigenvalue = 0.047, $R_c^2 = 0.212$) correctly classified *tseet* calls in terms of rearing environment in 54.4% of cases. A *z*-test to compare the difference between two proportions shows that the predictions yielded by the LDA are significantly better than predictions expected by chance for isolate chickadees ($z = -9.462$, $p < 0.001$, correct classification 88%, as black-capped reared 8%, as mountain

TABLE IV. The original (Orig) and cross-validated (Cross) percent of cases correctly classified by individual identity by stepwise linear discriminant analysis for all groups of birds, M = male, F = female, BC re = reared with black-capped chickadees, MO re = reared with mountain chickadees, Isolate = reared in isolation from adults. The eigenvalue (Eigen) and canonical correlation (Rc^2), acoustic feature with the highest standardized canonical function coefficient (Std coeff) and highest structure coefficient (Str coeff) for the first discriminant function. PIC represents the acoustic feature which yielded the highest PIC value. TD = total duration, SF = start frequency, EF = end frequency, HF = high frequency, DD = descending duration.

Group	Orig	Cross	Eigen	Rc^2	Std coeff	Str coeff	PIC
BC re M	90	84	45.898	0.989	TD	SF	SF
BC re F	100	98	49.384	0.99	EF	EF	HF
MO re M	62	58	1.941	0.812	TD	TD	DD
MO re F	78	72	6.113	0.927	SF	EF	DD
BC re	78	73	30.785	0.984	HF	HF	HF
MO re	69	58	5.146	0.915	HF	SF	DD
Isolate	64	58	1.002	0.707	SF	SF	SF

reared 4%) and birds reared with mountain chickadees ($z = -2.046$, $p = 0.02$, correct classification 52%, as black-capped reared 42%, as reared in isolation 6%), but not chickadees reared with adult black-capped chickadees ($z = 0$, $p = 0.05$, correct classification 40%, as mountain reared 35%, as reared in isolation 25%; Betz 1987; Glass and Stanley, 1970). Four acoustic measures contributed to the discriminant functions: TD, AD, SF and HF (see Table V).

Examination of group centroid loadings on the discriminant functions reveals that Function 1 separates chickadees reared in isolation from chickadees reared with adults. The acoustic measure with the largest standardized coefficient for Function 1 is AD. The second discriminant function separates the birds reared with adult black-capped chickadees from the birds reared in isolation and the birds reared with adult mountain chickadees. The feature with the largest standardized coefficient for Function 2 is HF. Both functions combined yielded a Wilks' $\lambda = 0.574$, distributed as a $\chi^2(8,\ N = 250) = 136.370$, $p < 0.001$, indicating that the group centroids differed significantly from each other, and that the model successfully discriminated among the groups.

TABLE V. Group centroids, discriminant structure matrix, and the standardized canonical discriminant function coefficients for discriminant analysis of the acoustic measures used to classify *tseet* calls in terms of the group of birds producing each call. BC re = reared with black-capped chickadees, MO re = reared with mountain chickadees, ISO = reared in isolation from adults, SF = start frequency, AD = ascending duration, HF = high frequency and TD = total duration.

	Discriminant Function	
	1	2
Group	Group centroids	
BC re	−0.141	−0.262
MO re	−0.638	0.203
ISO	1.558	0.118
Discriminant structure matrix		
SF	0.765	0.061
AD	−0.462	0.118
HF	0.541	0.701
TD	0.048	−0.124
Standardized coefficients		
SF	0.605	−1.086
AD	−0.830	−0.005
HF	0.218	1.527
TD	0.741	0.030

Because the LDA was able to classify *tseet* calls by the rearing environment, we conducted a one-way MANOVA to test for significant differences in the nine acoustic features between the three rearing environments. The overall MANOVA was significant, $F_{18,478} = 9.57$, $p < 0.001$, therefore nine univariate ANOVAs were conducted. We applied the Bonferroni correction for multiple comparisons (nine comparisons) to control for family-wise error. The univariate *F* and probability values for the between group comparisons associated with each acoustic feature are presented in Table III. The birds reared in isolation from adults were significantly different from birds reared with either conspecifics or heterospecifics for most of the acoustic features. Birds reared with adults conspecifics significantly differed from birds reared with adult heterospecifics for only two acoustic features.

The final LDA classified *tseet* calls into six groups, namely, by classifying the birds by sex and by rearing environment (i.e., calls produced by male chickadees reared with adult black-capped chickadees, female chickadees reared with adult black-capped chickadees, male chickadees reared with adult mountain chickadees, female chickadees reared with adult mountain chickadees, male chickadees reared in isolation, or females chickadees reared in isolation) also based on the nine acoustic measures obtained from our sample of *tseet* calls. Two discriminant functions correctly classified *tseet* calls in terms of sex and rearing environment in 58.8% of all cases (see Table VI). Both functions combined yielded a Wilks's $\lambda = 0.384$, distributed as a χ^2 (10, $N = 250) = 234.705$, $p < 0.001$. Only two acoustic measures, SF and HF contributed to the discriminant functions (see Table VII). A *z*-test for the differences between two proportions show that the predictions yielded by the LDA are significantly better ($p < 0.001$) than predictions expected by chance for all groups (female chickadees reared in isolation,

TABLE VI. Matrix of classification by group membership and sex of the actual groups of chickadee *tseet* calls and the Linear Discriminant Analysis (LDA) predicted group classification based on nine measured acoustic features. Correct LDA classifications are presented (in percentages) along the diagonal in bold. Misclassifications are presented (in percentages) in corresponding rows and columns. BC re = reared with black-capped chickadees, MO re = reared with mountain chickadees, Isolate = reared in isolation from adults. Overall, 58.8% of original and cross-validated cases are classified correctly.

	LDA PREDICTED GROUP CLASSIFICATION					
ACTUAL GROUP	Male BC re	Female BC re	Male MO re	Female MO re	Male Isolate	Female Isolate
Male BC re	74	16	0	6	0	4
Female BC re	18	66	0	4	0	12
Male MO re	18	26	50	6	0	0
Female MO re	4	16	22	58	0	0
Male Isolate	10	60	15	0	0	15
Female Isolate	0	23	0	0	0	77

TABLE VII. Group centroids, discriminant structure matrix, and the standardized canonical discriminant function coefficients for discriminant analysis of the acoustic measures used to classify *tseet* calls in terms of the group of birds producing each call. BC re = reared with black-capped chickadees, MO re = reared with mountain chickadees, Isolate = reared in isolation from adults, SF = start frequency, HF = high frequency.

	Discriminant Function	
	1	2
Group	Group centroids	
Male BC re	− 0.596	− 0.925
Female BC re	0.439	0.292
Male MO re	0.035	− 0.559
Female MO re	− 1.201	0.910
Male Isolate	0.814	0.101
Female Isolate	1.660	0.402
Discriminant structure matrix		
SF	0.980	0.199
HF	0.630	0.777
Standardized coefficients		
SF	1.222	− 0.990
HF	− 0.314	1.541

$z = -8.574$; male chickadees reared with black-capped chickadees, $z = -9.541$; females chickadees reared with black-capped chickadees, $z = -8.259$; male chickadees reared with mountain chickadees, $z = 5.612$, and females chickadees reared with mountain chickadees, $z = 9.952$), except male chickadees reared in isolation ($z = -1.315$, $p = 0.094$).

IV. DISCUSSION

Bioacoustic analysis can provide insight into the particular features of vocal signals that may potentially convey species, sex, geographic, and individual identity. In a previous study (Guillette *et al.*, 2010) we used bioacoustic analysis to characterize temporal and spectral regularities found in chickadees *tseet* calls produced by different species (black-capped and mountain chickadees), sexes, and individuals from different geographic regions (allopatric black-capped chickadees, sympatric black-capped and mountain chickadees). In the current study, we used the same acoustic features as previously used; however, we analyzed the *tseet* calls produced by allopatric chickadees that were reared in one of three different acoustic environments, with adult black-capped chickadees, with adult mountain chickadees, or in acoustic isolation from adults.

A. Classification by individuals

The results suggest that male and female black-capped chickadees need to hear adult vocalizations to develop *tseet* calls with acoustic features that are highly individualized. Although there were no statistical differences found between the PIC values of birds reared by conspecifics or heterospecifics, classification by the LDA show that *tseet* calls produced by black-capped chickadees that were reared with conspecifics were correctly classified by individual in 73% of all cases. The *tseet* calls produced by birds that were reared in the absence of conspecifics were only correctly classified by individual in 58% of all cases.

B. Classification of *tseet* calls into groups

The performance of the LDA suggests that the rearing environment affects the vocal development of *tseet* calls in a group of allopatric black-capped chickadees. The results of the ANOVAs show us that the starting frequency of the *tseet* call was different between the birds reared in each of the three different environments. In fact, starting frequency and descending frequency modulation were the two features that differed significantly between birds reared with either conspecifics or heterospecifics. Several studies show that pitch is an important factor for song (e.g., Brémond, 1986; Lohr *et al.*, 1994, Weisman *et al.*, 1998) and call recognition (Charrier *et al.*, 2005) in songbirds. Charrier *et al.* (2005) shifted the different note-types from the *chick-a-dee* call of the black-capped chickadee either up or down in starting frequency and found this feature controlled the chickadees' perception of the note-type. In sum, pitch, especially starting frequency, has been shown to be important for vocal perception in songbirds. On the production end, in the current study, we find that this same feature is differentially affected by the rearing environment of the birds.

C. General discussion

The current findings demonstrate that rearing environment affects the vocal production of the *tseet* contact call in allopatric black-capped chickadees. This is consistent with a previous study that examined the role of experience in the development of the *chick-a-dee* call in black-capped chickadees (Hughes *et al.*, 1998). Hughes *et al.* reared black-capped chickadees in environments with varying degrees of exposure to adult black-capped chickadee vocalizations (ranging from none to being housed in separate cages in a colony room with adult birds). Hughes *et al.* found that the production of normal B and C notes (from the introductory *chick-a* portion of the *chick-a-dee* call) was more dependent on experience, compared to the production of normal A notes. That is, regardless of rearing condition, the A notes produced by all birds fell into the normal range of A notes produced by wild-caught adults.

The results of Hughes *et al.* (1998) are interesting because they suggest that learning plays a differential role for the development of different note-types within the same call. Compared to the *chick-a-dee* call, the *tseet* call is much less complex; the *tseet* call consists of a single note whereas the *chick-a-dee* call has at least four note types. Although the *tseet* call is lower in amplitude than the *chick-a-dee* call, the structure of the *tseet* call is very similar to that of the A and B notes from the *chick-a-dee* call. Our results here suggest that in addition to the structural similarity between *tseet* calls and note types from the *chick-a-dee* call (A and B notes), *tseet* calls also demonstrate the influence of learning on some of their acoustic features. Why this would be the case is unclear. Clemmons and Howitz (1990) suggested that A notes are derived from 'simple peeps,' a begging call produced by hatchling black-capped chickadees and therefore were less dependent on acoustic exposure during development. However, while Hughes *et al.* (1998) shows that A notes of normal phonology are produced in absence of experience with adult calls, they suggest that development serves to fine tune these notes. While this fine tuning of *tseet* call may occur on some acoustic features, but not others, our results suggest that the starting frequency and descending frequency modulation may be the components of the *tseet* contact call that are learned during development.

In sum, by demonstrating differences in acoustic features between members of the same species that were reared in different acoustic environments, we suggest that even acoustically-simple bird calls are not innate, as was once thought. Rather, we find that rearing young, wild-caught black-capped chickadees with adults of the same species, adults of a different, but closely-related species or with no adults present result in markedly different *tseet* calls. This evidence is in line with a previous study that demonstrated early acoustic experience resulted in acoustic differences in a more acoustically complex vocalization, namely, the *chick-a-dee* call (Hughes *et al.*, 1998). Because calls are produced by both sexes, throughout the annual cycle, and in a variety of different contexts, the authors urge researchers to further examine the role of experience in the development of bird calls.

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