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7   Functional morphology of Richardson's ground squirrel (*Spermophilus richardsonii*)

8   alarm calls: the meaning of chirps, whistles and chucks.

9

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14   Running Head: SLOAN ET AL.: GROUND SQUIRREL ALARM SIGNALS

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24   Repetitive alarm vocalizations of Richardson's ground squirrels (*Spermophilus*

25   *richardsonii*) vary in terms of the acoustic structure of their primary syllables and the

26   inclusion of brief, lower amplitude, frequency-modulated elements trailing those

27   syllables which we term "chucks". Chucks are included in calls of both males and

28   females and increase in prevalence with the proximity of the caller to the alarm-evoking

29   stimulus. Further, chuck presence is not independent of primary syllable type: chucks

30   follow primary syllables that have constant frequency and diminishing amplitude

31   producing a "whistle", but do not trail primary syllables with diminishing frequency and

32   non-descending amplitude spectra ("chirps"). Playbacks to free-living squirrels of

33   repeated alarm calls having whistle- or chirp-like primary syllables and factorially

34   combining those with chuck presence or absence revealed that chirp-like syllables

35   elicited greater vigilance from call recipients during signal propagation. The addition of

36   chucks to the end of primary syllables of either type, however, increased initial vigilance

37   duration, and both the proportion of time devoted to vigilance during and after signal

38   reception. Chucks thus promote increased and lasting vigilance on the part of call

39   recipients. Beyond enhancing vigilance, however, the inclusion of frequency-modulated

40   chucks and chirps facilitates the orientation of receivers to the signaler. Multiple

41   acoustic parameters of Richardson's ground squirrel alarm vocalizations thus interact to

42   communicate information regarding several aspects of a predator encounter. Receivers

43   utilize such information to their advantage, affording greater attention to calls that

44   would be more readily located by predators, and hence are more costly for signalers to

45   produce.

46

47   Alarm signals warn conspecific and sometimes allospecific individuals of potential

48   danger posed by predators. Considerable diversity exists, however, in the nature of the

49   information conveyed by these signals. Specific attributes of predators may be encoded,

50   such that referential information allows signal recipients to respond in a manner that

51   best suits certain predator types or characteristics (referential alarm signaling: Seyfarth

52   et al. 1980; Cheney & Seyfarth 1988; Pereira & Macedonia 1991). Information regarding

53   the situation imposed by the encounter may also supplement or take the place of

54   referential information (situationally-specific alarm signaling: Ficken 1989; Blumstein

55   1995; Blumstein & Arnold 1995) as is the case where signals convey response urgency

56   (Warkentin et al. 2001).

57   Decoding the information conveyed in a given signal affords insight into the biology

58   of the organism, and into the basic economics by which natural selection operates in

59   refining communication (Marler 1955; Klump & Shalter 1984). Such insights are

60   garnered, however, only via comprehensive consideration of both the circumstances

61   surrounding variation in signal production and documentation of the response to such

62   signals, thereby addressing the perception of the signal by potential receivers (Evans et

63   al. 1993; Macedonia & Evans 1993).

64   Davis (1984) reported productional specificity in the alarm calling system of

65   Richardson's ground squirrels, wherein squirrels produced short "chirps" in response to

66   aerial predators, and longer "whistles" that were often repeated in response to

67   terrestrial predators. Warkentin et al. (2001) noted, however, that such productional

68   specificity could result from the more imminent threat imposed by faster approaching

69   avian versus terrestrial predator types, and revealed that Richardson's ground squirrels

70   encode the extent of threat imposed by predators via variation in the rate of repetitive

71   calling. Differing spectral properties of the syllables underlying repetitive calls may thus

72   act to verify or even refine information regarding response urgency. Indeed, Macedonia

73   & Evans (1993) and Blumstein (1995, 1999) similarly concluded that referential signaling

74   is unlikely to be exhibited by ground-dwelling squirrels (but see Slobodchikoff et al.

75   1991).

76   If the spectral variability in Richardson's ground squirrel alarm calls does not provide

77   functional referentiality, why does such pronounced variation exist, and what, if any,

78   information does such variation encode? Davis's dichotomy of whistles and chirps

79   drastically under-represents the many parameters of Richardson's ground squirrel alarm

80   vocalizations that show spectral variation (Koeppl et al. 1978). Among these, we sought

81   to understand the function of the brief, relatively low amplitude, frequency-modulated

82   elements, which we termed "chucks" that often follow the offset of primary syllables in

83   repeated alarm vocalizations (see Koeppl et al. 1978 Fig. 5H).

84   While lower amplitude elements in some cases represent echoes of preceding

85   louder components, the elaboration of fine structure within a vocalization may enhance

86   signal transmission or expand information content (Owings & Hennessy 1984; Bradbury

87   & Vehrencamp 1998). Roosters (*Gallus gallus*) often incorporate a relatively brief, but

88   intense broadband pulse of sound immediately before the first syllable of a repeated

89   alarm call, which functions to alert receivers to the subsequent call (Gyger et al. 1987;

90   Bayly & Evans 2003). Similarly, male Túngara frogs (*Physalaemus pustulosus*) append

91   one or more broadband "chucks" to the end of their tonal advertisement call, which

92   increase the effectiveness of the signal in terms of attracting females (Rand & Ryan

93   1981) and act as honest indicators of male body size (Ryan 1985).

94   Both broadband and frequency-modulated sounds are more readily locatable than

95   signals that are restricted to a narrow frequency range (Bradbury & Vehrencamp 1998).

96   Temporally segregated trailing elements may also facilitate localization of the signaler.

97   In harbor seals (*Phoca vitulina*), clicks following grunt vocalizations provide discrete

98   temporal cues that allow localization of the signal source based on interaural differences

99   in their time of arrival (Terhune 1974). The inclusion of such elements in alarm

100   vocalizations may thus increase the signaler's risk of predation (Ryan et al. 1982), which

101   in turn would select for honest signaling (Bradbury & Vehrencamp 1998).

102   We used Richardson's ground squirrel alarm vocalizations recorded in the context of

103   previous research (Hare 1998) to describe the spectral properties of chucks, and

104   examine the contextual correlates of their inclusion in repeated calls. Further, we

105   conducted a factorial playback experiment to determine how natural primary syllable

106   attributes and chucks interact in affecting the alarm responses of the squirrels.

107

108   **METHODS**

109   **General Methods**

110   Research involved the characterization of signals and analysis of contextual

111   elements underlying signal production from recordings made in the context of previous

112   alarm communication studies (Hare 1998; Hare & Atkins 2001; Sloan & Hare 2004,

113   Warkentin et al. 2001; Wilson & Hare 2004). Alarm calls used in those studies were

114   elicited by presenting free-living juvenile Richardson's ground squirrels (Michener &

115   Koeppl 1985) with a model predator: a tan-coloured Biltmore hat (32.5 x 19.5 cm brim x

116   13 cm high). The use of models is common in studies of antipredator calling behaviour

117   as models allow greater contextual control than do natural predator encounters

118   (MacWhirter 1992; Hare 1998). All presentations and call recordings were made by JFH

119   while wearing the same outer clothing to minimize any confounding effects of the

120   observer (see Slobodchikoff et al. 1991). Recording methods followed those described in

121   Hare (1998). Subjects that had not previously been presented with the predator model

122   (hat) were approached to within 15 m. The hat was tossed from hip level with a flip of

123   the wrist to within 1-8 m of the intended subject at an angle of 0-30° relative to a line

124   between the observer and the subject (but never directly over the subject). In all cases,

125   calling did not begin until after the hat landed on the ground. For each recording

126   session, the time of day, position of the recording on the tape, locations of the

127   microphone, predator model, and subject at the outset of recording, and the behaviour

128   of the subject coinciding with the presentation of the model (particularly whether the

129   subject faced the model while calling) were recorded. Only sessions in which juveniles

130   faced the predator model while calling were used in subsequent analysis and playbacks,

131   thus decreasing the probability of spurious responses to the experimenter or other

132   elements in the squirrels' environment.

133   We conducted additional fieldwork from 8 April through 14 July 2004 on free-

134   living Richardson's ground squirrels occupying mowed lawns at the Assiniboine Park Zoo

135   (49° 52' N, 97° 14' W) in Winnipeg, Manitoba. Juvenile squirrels were live-trapped using

136   National or Tomahawk traps baited with peanut butter, permanently marked with metal

137   ear tags (National Band & Tag Company #1005) and given unique marks on their dorsal

138   pelage with hair dye (Clairol Hydrience™ 52, Black Pearl). Experimenters wore the same

139   outer clothing each day to habituate the squirrels to our appearance. All work involving

140   animals conformed to the guidelines for the ethical use of animals in research set forth

141   by the Canadian Council on Animal Care and those outlined under the Animal Behaviour

142   Society's guidelines for the treatment of animals in behavioural research and teaching.

143

144   **Spectral Analysis of Call Structure**

145   Preliminary examination of the spectral properties of juvenile Richardson's ground

146   squirrel alarm calls recorded by Hare at sites across southern Manitoba between 1994

147   and 1998 (see Hare 1998) revealed that in addition to primary syllable attributes, calls

148   could be categorized according to the presence or absence of a relatively low amplitude

149   acoustic element that trailed the offset of primary syllables (ca. -20 dB relative to

150   primary) within repetitive calls, after a brief (ca. 10 - 40 msec) intervening silence (Fig.

151   1). We refer to these elements as "chucks" (although they lack the overlap in time with

152   the primary syllable, increased amplitude, and abundant and powerful harmonics of

153   Túngara frog chucks; Ryan 1985) as their audible effect is to harshen the offset of each

154   syllable, interjecting a pulsatile beat into the end of each utterance.

155   Of the 34 juvenile Richardson's ground squirrel repeated calls selected for their high

156   signal-to-noise ratio and used in playback studies by Sloan &Hare (2004) and Wilson &

157   Hare (2003), 14 included at least some syllables accompanied by chucks. To avoid

158   problems associated with pseudoreplication (Machlis et al. 1985) in describing chucks, a

159   single syllable/chuck pair was sampled arbitrarily from each calling individual. We used

160   Canary™ 2.04 to parameterize the spectral properties of those chucks, measuring their

161   duration, latency and frequency at onset relative to the offset of the preceding primary

162   syllable, frequency at offset and harmonic structure (Fig. 1). All spectra were generated

163   using an FFT size of 256 points and Hamming windowing. As both males and females

164   issued chucks in some of their calls, we also compared each acoustic parameter of male-

165   versus female-produced chucks using Mann-Whitney U-tests.

166   We tested for an association between chuck presence and both the general

167   frequency and amplitude characteristics of the primary syllables contained in 32 of the

168   34 repeated calls with the highest signal-to-noise ratio. We employed Fisher's exact

169   tests on contingency tables examining the presence or absence of chucks relative to

170   primary syllable frequency type (categorized from spectra as chirps with frequency

171   descending over time or whistle-like with constant frequency) and amplitude type

172   (categorized from spectra as descending, ascending, bi-peaked, or multi-peaked, though

173   calls of the latter 3 types were relatively rare and thus pooled into a category called

174   "other" for the purpose of contrasts with descending amplitude calls). To ensure that

175   these association tests were not subject to bias introduced via the arbitrary selection of

176   a single syllable from within each call, contingency tables were formed considering both

177   the attributes of the preceding syllable relative to chuck presence, and the attributes of

178   the majority of syllables (≥ 75%) relative to chuck presence in the entire call sample.

179   Further, we used logistic regression to test for any association between the rate at

180   which syllables were produced (estimated from the time taken to produce the first 5

181   syllables in the call) and the inclusion of chucks within those calls.

182

183   **Context of Chuck Production**

184   We reviewed field notes documenting contextual elements associated with the

185   production of the 34 calls used in our studies, including the sex of the caller, distance of

186   the caller from the predator model, distance of the caller from the

187   observer/microphone, date (day within year), time of day, wind speed (an ordinal

188   ranging from 0 - calm, to 3 - very windy), and cloud cover (an ordinal ranging from

189   0- clear to 2 - total overcast). We subjected data on the sex of the caller versus chuck

190   presence or absence to contingency table analysis using a Fisher's exact test. The

191   remaining contextual data were analysed using logistic regression to determine whether

192   the environmental parameters measured affected the propensity of individuals to

193   include chucks in their repeated calls.

194

195   **Playback Trials - Call Perception**

196   To determine how alarm call recipients perceive chucks, to ascertain whether chucks

197   exert an effect on receivers independent of the primary syllables they accompany, and

198   to test for any differential effect of those two general primary syllable types, we

199   examined responses of juvenile Richardson's ground squirrels to playbacks of recorded

200   calls. Playback trials were conducted when both wind and potential public interference

201   were minimal between 0700 and 2055 hours CST from 5 through 14 July 2004 following

202   a factorial design. Each of 60 subjects received a single 5-syllable playback (3 sec

203   intersyllable latency) of one of four possible call types formed via the manipulation of

204   two syllable attributes: primary syllable type (whistle-like with constant frequency and

205   descending amplitude versus chirps with descending frequency and multi-peaked

206   amplitude within each syllable) and chuck presence (present versus absent). Because

207   the rate at which syllables are uttered in repeated calls significantly affects the vigilance

208   responses of call recipients (Warkentin et al. 2001), we held intersyllable latency

209   constant among call types. Thus calls including chucks and calls composed of whistle-like

210   primary syllables were of longer duration than those without chucks and those

211   composed of chirps. Calls were constructed on Canary™ 2.04 via the repetition of single

212   syllables derived from unique juvenile callers (within the 34 calls above) recorded at

213   sites other than the zoo. Calls having primary syllables with constant frequency,

214   diminishing amplitude and incorporating a chuck were described by Davis (1984) as

215   "whistles", while those having primary syllables with diminishing frequency, multi-

216   peaked amplitude and lacking a chuck fall within the call types Davis described as

217   "chirps". The two artificial call types in our experiment - primary syllables of constant

218   frequency and diminishing amplitude with no chuck, and primary syllables having

219   diminishing frequency but multi-peaked amplitude with a chuck - were created by

220   deleting chucks from the whistles used above and appending those chucks to the

221   aforementioned chirps respectively. Whistles serving as the source of chucks were

222   matched to chirps receiving those chucks so as to minimize the difference in the onset

223   frequencies of the primary syllables.

224   Field playbacks of alarm calls followed the general methods described in Hare &

225   Atkins (2001). Upon identification of a previously untested squirrel, we approached the

226   prospective subject to within 15 - 25 m and set up the playback apparatus, including a

227   minidisc player (Sony MZ-N707), Sony XM-2025 audio amplifier and a Genexxa Pro LX5

228   loudspeaker. The playback system collectively reproduced frequencies ranging from 85

229   Hz to 22 kHz. While the peak sound pressure level (SPL) of playback exemplars of all four

230   call types diminished with distance from the source, no significant difference in SPL

231   (measured with a Realistic™ 33-2050 sound level meter, A weighting, fast response) was

232   detected at either 15 or 25 m from the speaker during a series of SPL measurement

233   trials conducted over similar terrain at a remote site (Table 1). Videotaping (via a tripod-

234   mounted Sony DCR-TRV120 camcorder) commenced when squirrels began to forage

235   and continued from 30 sec prior to call playback (pre-playback) until 30 sec

236   post-playback. Calls were arbitrarily assigned to subjects, though the order in which calls

237   of the four possible types were presented was randomized. Playbacks of different callers

238   within a given day were performed at least 50 m apart from one another, or if within

239   the same general area, were staged at least one hour apart.

240   Vigilant Richardson's ground squirrels elevate their head above the horizontal plane.

241   Thus postural responses to alarm calls provide an assay of vigilance in call recipients

242   (Holmes 1984; Hare 1998; Hare & Atkins 2001). Using a stopwatch and the video record,

243   we quantified responsiveness to alarm calls as the initial vigilance duration of call

244 recipients (the time from the initial expression of vigilance after the first syllable of the

245 playback to any reduction in vigilance posture) and as the total proportion of time spent

246 vigilant (including any posture in which the head is elevated above the horizontal plane;

247 see Hare 1998) during the playback and post-playback periods. In addition, to assess

248 whether certain call parameters facilitate localization of the signaler, we quantified the

249 orientation of call recipients relative to the signal source. We estimated the angular

250 deviation of the subject squirrel's nose over the majority (≥75%) of the playback period

251 in 5 degree increments from the speaker, which itself was consistently positioned 9 m to

252 the right of the observers and at roughly the same distance as the observers were to the

253 call recipient. Data were coded from videotape by observers who were blind to the

254 treatment conditions for each trial, but were provided with the time code for the onset

255 and offset of the pre-playback, playback and post-playback periods. Data from three

256 trials (one constant frequency chuck present and two constant frequency chuck absent)

257 were excluded from the analysis, however, because of loud natural calling during the

258 playback period that could have affected the response of call recipients.

259   We used two-factor analysis of variance (the parametric assumptions of normality

260 and homogeneity of variance were met, all $P > 0.05$) to test for effects of primary

261 syllable type, chuck presence and their interaction on initial vigilance duration, the

262 proportion of time call recipients engaged in vigilance during the playback and post-

263 playback periods, and orientation relative to the signal source. Miscellaneous grouping

264 factors including: time of trial (0700 - 2055 hours CST), date (187th - 196th day within

265 year), wind speed (0 - 11.4 kph), temperature (13.1 - 29.6°C), relative humidity (35 -

266   86%), cloud cover (0 - 100%), the angle of the speaker relative to the recipient (0 - 45°),

267   the distance between the speaker and the call recipient (6.3 - 24.1 m), the number of

268   natural callers heard during the playback (categorized as none or one, few, or many),

269   and caller sex were balanced across chuck presence versus absence and primary syllable

270   type (all P > 0.05) and thus do not confound the interpretation of receiver responses.

271   Statistical analyses were performed on Statview™ 5.01 and differences were considered

272   significant where P £ 0.05.

273

274   **RESULTS**

275   **Spectral Properties of Chucks**

276   A spectrographic representation of a chuck along with its preceding primary syllable is

277   shown in Fig. 1. While thirty-two high-quality calls were initially examined, all 14 calls

278   recorded in 1994 and 1995 were omitted from further spectral analysis because of

279   potential biases introduced by year, variation among study populations, or the

280   microphone used to record calls in those years. Indeed, chucks were observed in only 2

281   of 14 calls (14.3%) recorded in 1994 and 1995 with the parabolic microphone (Dan

282   Gibson P-650), but were present in 12 of 18 calls (66.7%) recorded with the shotgun

283   microphone (Audio-Technica AT815B) in 1997 and 1998 despite the fact that the same

284   experimenter, wearing the same outer clothing, presented the same call-eliciting model

285   in the same way in all of those years. In the 12 chuck-containing calling bouts recorded

286   in 1997 and 1998, chucks followed 50 to 97% of the primary syllables sampled (a

287   proportion of 0.87 ± 0.04 of the syllables, mean ± SE), trailed primary syllables by a

288   latency of 10.2 to 40.7 msec (23.2 ± 2.5 msec), and had a duration of 8.7 to 37.8 msec

289   (21.0 ± 2.6 msec). The onset frequency of the chuck was 1.13 to 5.07 KHz (2.84 ± 0.34

290   KHz) below the offset frequency of the preceding syllable, and chucks themselves were

291   invariably frequency modulated from a higher frequency at their onset (range: 4.48 to

292   7.53 KHz, mean ± SE: 6.13 ± 0.23 KHz) to a lower frequency at their offset (range: 2.74 to

293   4.63 KHz, mean ± SE: 3.52 ± 0.19 KHz). Frequency within chucks thus declined anywhere

294   from 1.33 to 4.67 KHz (mean ± SE: 2.61 ± 0.27 KHz) at a rate of 0.13 ± 0.004 KHz/msec

295   (mean ± SE), and all chucks exhibited a pattern of declining amplitude over their

296   duration. No harmonics or sub-dominant carriers were detected in any of the chucks

297   recorded in 1997 or 1998.

298

299   **Contextual Correlates of Chuck Production**

300   <u>Signaler attributes</u>

301   Female and male juveniles had an equal propensity to include chucks in their

302   repeated calls (seven females produced repeated calls with chucks and four produced

303   calls without chucks whereas five males produced repeated calls with chucks and two

304   produced calls without chucks: Fisher's exact test, P = 1.0). Females and males also

305   incorporated chucks into a similar proportion of their syllables (Table 2: Z_c = 0.49, P =

306   0.62). Further, no significant differences were detected between male- and female-

307   produced chucks in terms of their maximum frequency, minimum frequency, change in

308   frequency from onset to offset, the rate of change in frequency, the difference in their

309   onset frequency relative to the offset frequency of the preceding syllable, the latency

310   from the primary syllable to chuck onset or chuck duration (all $P \geq 0.22$; see Table 2).

311   The statistical power of these contrasts is limited, however, by the small samples of

312   male- and female-produced calls.

313

314   <u>Influence of primary syllables</u>

315   The presence of chucks was significantly correlated with both the general amplitude

316   and frequency attributes of the primary syllables found within repeated calls. Chucks

317   were more likely to be present when either the preceding syllable (Fisher's exact test, P

318   = 0.01) or the majority of syllables in the call (Fisher's exact test, P = 0.00) decreased in

319   amplitude from onset to offset (Table 3). Chucks were also more likely to be present

320   when either the preceding syllable (Fisher's exact test, P = 0.00) or the majority of

321   syllables in the call (Fisher's exact test, P = 0.00) had constant as opposed to a

322   descending fundamental frequency from their onset to offset (Table 4). The rate at

323   which syllables were uttered had no effect, however, on the likelihood of chucks

324   accompanying those syllables (Logit(P) = 0.95 -1.0x, X^2_1 = 0.02, P = 0.89, R^2 = 0.001).

325

326   <u>Environmental factors</u>

327   Only the distance between the predator model and the signaler had a significant

328   influence on whether repeated calls included chucks (Table 5). The likelihood of calls

329   incorporating chucks increased as the model was positioned in closer proximity to

330   the caller.

331

332   **Playback Trials - Chuck Versus Primary Syllable Effects**

333   The inclusion of chucks in repeated calls significantly increased the initial vigilance

334   duration of call recipients and the total proportion of time devoted to vigilance during

335   and immediately after the playback (Table 6). Whereas the proportion of time devoted

336   to vigilance was significantly greater for chirp-like (decreasing frequency and multi-

337   peaked amplitude) primary syllables during the playback, and squirrels tended to

338   prolong initial vigilance in response to chirp-like syllables, primary syllable type did not

339   affect the proportion of time devoted to vigilance after the playback (Table 6). Further,

340   primary syllable type did not interact with chuck presence for any of the vigilance

341   response measures (Table 6).

342   Unlike vigilance proper, orientation of call recipients to the signal source was

343   unaffected by either chuck presence or primary syllable type, though a significant

344   interaction was apparent such that chuck presence increased orientation to the source

345   when paired with whistle-like primary syllables but not chirp-like syllables (Table 6).

346   Orientation of the head to the signal source was most pronounced for chirp-like primary

347   syllables without chucks (chirps *sensu* Davis 1984) and less so for whistle-like syllables

348   with chucks (whistles *sensu* Davis 1984), chirp-like syllables with chucks and whistle-like

349   syllables without chucks in that order (Table 6).

350

351   **DISCUSSION**

352   We investigated the function of chucks, chirps and whistles that comprise the audible

353   alarm vocalizations of Richardson's ground squirrels. The likelihood of chucks being

354   incorporated into repeated alarm calls increased with proximity to the call-eliciting

355   stimulus. Further, the broadcast of chucks increased both the initial vigilance duration

356   and exerted a tonic effect (Schleidt 1973; Owings et al. 1986), prompting squirrels to

357   devote a greater proportion of their time to vigilance once the alarm signal had ceased.

358   Thus chucks appear to heighten the perception of threat by call recipients, lending

359   credence to the message conveyed by their preceding primary syllables. In that sense,

360   calls incorporating chucks are treated as more reliable indicators of threat, and are

361   afforded greater attention by signal recipients, just as juvenile Richardson's ground

362   squirrels attend to more reliable signalers (Hare & Atkins 2001) and signals that

363   temporally convey the extent of threat with greater certainty (Sloan & Hare 2004).

364   Our playback results also reveal, however, that the two primary syllable types

365   differed in their salience to receivers over the short term. Chirp-like primary syllables

366   elicited greater vigilance responses than whistle-like primary syllables during their

367   broadcast, though that difference did not persist into the post-playback period. The

368   observed difference in response to chirps versus whistles may be explained by Davis's

369   (1984) finding that chirps tend to be produced in response to avian predators and

370   whistles in response to terrestrial predators. Avian predators typically appear suddenly,

371   stoop on prospective prey and retreat to cover. Thus they present an immediate but

372   transitory threat, which would require immediate and pronounced response.

373   Consistent with that interpretation, we found that in natural calls, chucks trailed

374   syllables with constant frequency and descending amplitude, producing whistles, but

375   not primary syllables uttered as chirps. Chucks then, may be incorporated into whistles

376   in cases where predators present an immediate threat, but omitted where the caller

377   perceives a lesser threat. In our playback experiment, receivers oriented more directly

378   to the source when chucks were left in whistles than when chucks were appended to

379   chirps. Because squirrels can enhance their safety in the face of terrestrial predators by

380   monitoring the location of the predator (Lima & Dill 1990), the inclusion of chucks in

381   calls issued to terrestrial predators may result from selection favoring localization of the

382   signaler, whose calls may serve in part as a pronouncement of vigilance, but ultimately

383   benefit the signaler by warning others of the predator's presence (Sherman 1977).

384   Indeed, by discriminating among individual callers (Hare 1998), and estimating the

385   distance of the predator from the signaler via perception of the rate of repetitive calling

386   (Warkentin et al. 2001), receivers that could locate the signaler in space could infer their

387   distance from the predator based on alarm vocalizations alone, perhaps even

388   integrating information from multiple signalers to pinpoint the position of the

389   presumptive predator within the colony. The persistence of vigilance beyond the end of

390   the repeated call where chucks are present likely reflects receiver's attempts to visually

391   locate the predator that elicited the signal.

392   Whereas the retention of chucks where primary syllables had constant frequency

393   enhanced orientation toward the signal source, the most direct orientation to the

394   source was observed for chirps which lacked chucks altogether (Table 6). It is likely that

395   the highly frequency modulated nature of the chirps, along with the high response

396   urgency such syllables convey, promote this pronounced orientation to the signal

397   source. Monitoring positional changes of a predator imposing an imminent threat may

398   not be practical, though it would prove selectively advantageous if alarm signals given in

399   that context provided information allowing receivers to orient their evasive response

400   accordingly. Diminution of the orientation response when chucks are appended to the

401   chirps, or when chucks are deleted from whistles, may reflect potentially conflicting or

402   incomplete information in those two artificial call types respectively.

403   Whereas uttering whistles containing chucks would serve squirrels encountering

404   terrestrial predators, avian predators sometimes perch, or even land on the ground,

405   within or in close proximity to a colony, resuming their attack from those positions. It is

406   not surprising then that the level of productional specificity reported by Davis (1984) is

407   not absolute: whistles are sometimes given to aerial predators and chirps to terrestrial

408   predators. Indeed both the chirps and the whistles used in our study were elicited by

409   tossing the same tan-coloured hat toward squirrels in the field (see Hare 1998).

410   Contrary to Davis then, Richardson's ground squirrels appear to use chirps and whistles

411   to communicate different information - chirps for immediate threat and whistles

412   incorporating chucks for more tonic threats that should be tracked independent of

413   taxonomic affiliation - rather than using spectrally distinct calls to represent different

414   predator classes per se. Further experimentation employing live, or at least life-like

415   models of terrestrial and avian predators is necessary, however, to address the extent

416   to which information regarding predator type, or specific predator attributes, may also

417   be communicated in Richardson's ground squirrel alarm signals.

418   Because chucks are temporally segregated from the primary syllables they

419   accompany, they provide a discrete temporal cue that may facilitate localization of the

420   signal's source (Terhune 1974). Like chirps themselves, however, the

421   frequency-modulated nature of chucks would also promote such localization (Bradbury

422   & Vehrencamp 1998). Whereas locating the signal source may prove advantageous to

423   conspecifics, individuals producing such localizable calls would incur an increased cost

424   given that eavesdropping predators could more readily locate the caller (Sherman 1977;

425   Ryan 1985). That such signaling is costly, however, provides further reason why call

426   recipients can rely on those signals and show enhanced responsiveness to repeated calls

427   containing frequency-modulated elements such as chirps and chucks. The inclusion of

428   frequency-modulated components within Richardson's ground squirrel repeated calls

429   expands their information content and communicates underlying signal veracity.

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620   Table 1. Peak sound pressure level (mean ± SE dB) of the four Richardson's ground squirrel call types (n = 15 exemplars/ call type) at

621   15 and 25 m from the loudspeaker.

622

	Call Type				
	Whistle-like		Chirp-like		ANOVA
Distance From Speaker	With Chuck	No Chuck	With Chuck	No Chuck	$F_{3,56}$, *P*
15 m	62.7 ± 1.0	61.0 ± 0.8	60.3 ± 0.5	60.4 ± 0.8	2.05, 0.12
25 m	58.5 ± 0.8	58.0 ± 0.6	57.8 ± 0.5	58.1 ± 0.6	0.22, 0.88

528   Table 2. Comparison of chuck parameters (mean ± SE) in male- versus

529   female-produced Richardson's ground squirrel alarm calls.

530

531   Sex of Caller

532 Call Attribute	Male (N = 5)	Female (N = 7)	Z_c	P
533 Proportion of syllables	0.85 ± 0.09	0.90 ± 0.02	0.49	0.62
534 with a chuck				
535 Chuck duration (msec)	22.5 ± 4.4	19.9 ± 3.5	0.65	0.52
536 Latency to chuck (msec)	20.9 ± 3.4	24.8 ± 3.6	0.73	0.46
537 Minimum frequency (KHz)	3.18 ± 0.15	3.76 ± 0.29	1.22	0.22
538 Maximum frequency (KHz)	5.86 ± 0.41	6.31 ± 0.28	0.89	0.37
539 Frequency change (KHz)	2.68 ± 0.35	2.56 ± 0.42	0.57	0.57
540 Frequency rate change	0.13 ± 0.01	0.13 ± 0.01	0.41	0.68
541 (KHz / msec)				
542 Frequency drop from primary	3.40 ± 0.64	2.45 ± 0.32	1.06	0.29
543 offset to chuck onset (KHz)				

544

545   Table 3. Amplitude type of preceding primary syllables and majority (≥ 75%) of primary

546   syllables in calls with and without an accompanying chuck (note: "other"

547   includes bi-peaked, multi-peaked and ascending) in Richardson's ground

548   squirrel alarm calls.

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		Preceding Syllable Amplitude Type		Majority Amplitude Type	
		Descending	Other	Descending	Other
Chucks	Yes	8	4	12	0
Present?	No	0	6	0	6

555 Table 4. Frequency type of preceding primary syllables and the majority (≥ 75%) of

556   syllables in calls with and without an accompanying chuck in Richardson's

557   ground squirrel alarm calls.

558

		Preceding Syllable Frequency Type		Majority Frequency Type	
559		Descending	Constant	Descending	Constant
561 Chucks	Yes	0	12	0	12
562 Present?	No	6	0	6	0

563   Table 5. Summary of contextual influences on chuck production in Richardson's ground squirrel alarm calls.

564

565   Logistic Likelihood

Variable	Logit(P) =	Chi-Square	P	Correct Predictions	Effect Size (R^2)
Date	15.07 - 0.08x	2.18	0.14	70%	0.08
Time	4.45 - 0.39x	0.75	0.38	60%	0.03
Cloud Cover	0.91 - 0.39x	0.43	0.51	65%	0.02
Wind Speed	0.67 - 0.13x	0.03	0.87	65%	0.00
Caller/Observer Distance	4.79 - 0.68x	2.95	0.09	69%	0.12
Caller/Hat Distance	5.90 - 2.50x	11.40	0.00	85%	0.44

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573 Table 6. The influence of primary syllable type, chuck presence and their interaction on the vigilance responses of Richardson's

574 ground squirrel call recipients. Results are shown as mean ± SE sec (N).

575

	Call Type				Significance Tests		
	Whistle-like		Chirp-like		1° Syllable	Chuck	Interaction
Dependent Variable	With Chuck	No Chuck	With Chuck	No Chuck	$F_{1,53}$, *P*	$F_{1,53}$, *P*	$F_{1,53}$, *P*
Initial Vigilance Duration (sec)	7.4 ± 3.1 (14)	2.2 ± 0.6 (13)	12.9 ± 3.7 (15)	6.6 ± 1.7 (15)	3.4, 0.07	4.6, 0.04	0.0, 0.83
Total Vigilance (Playback)	0.6 ± 0.1 (14)	0.4 ± 0.1 (13)	0.7 ± 0.1 (15)	0.6 ± 0.1 (15)	6.5, 0.01	7.2, 0.01	0.0, 0.89
Total Vigilance (Post-playback)	0.5 ± 0.1 (14)	0.5 ± 0.1 (13)	0.7 ± 0.1 (15)	0.4 ± 0.1 (15)	0.2, 0.68	4.5, 0.04	3.7, 0.06
Orientation to Source (*$F_{1,54}$)	74.3 ± 12.1 (15)	100.0 ± 17.3 (14)	84.3 ± 14.3 (14)	54.0 ± 9.0 (10)	1.8*, 0.18	0.0*, 0.86	4.4*, 0.04

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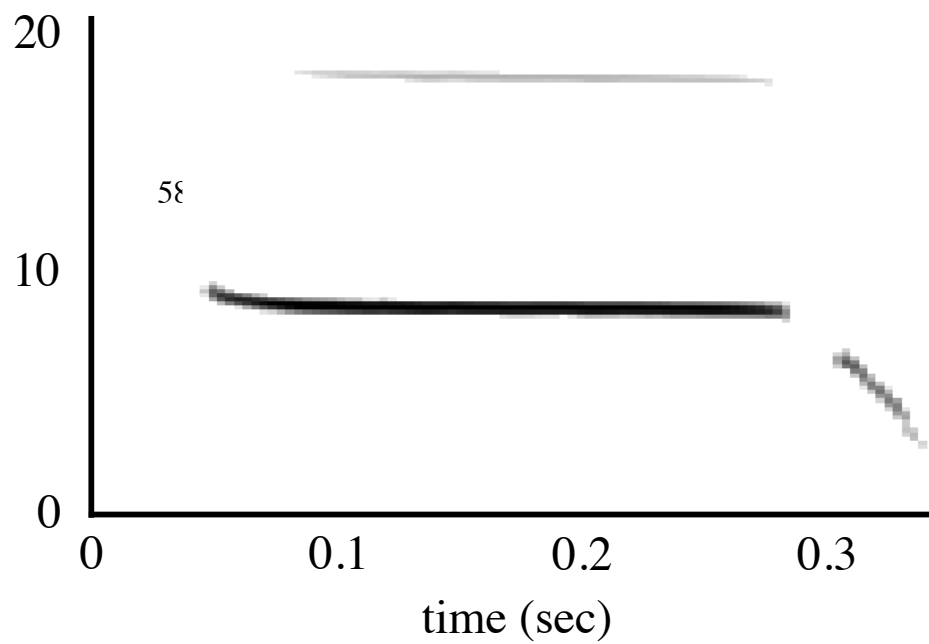
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Frequency (kHz)

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594   Figure 1. Spectrographic representation of the frequency versus time domain of a

595   Richardson's ground squirrel "whistle" (*sensu* Davis 1984) with a "chuck"

596   trailing the primary syllable.