



The first cut is the deepest: primary syllables of Richardson's ground squirrel, *Spermophilus richardsonii*, repeated calls alert receivers

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Richardson's ground squirrels produce alarm calls that warn conspecifics of potential predatory threat. Variation in the acoustic structure of individual syllables within a call carries information regarding the extent of threat. Given this variation, along with the production of multiple syllables within a repeated call, the syntactical structure of the call could also encode meaningful information. To test for evidence of syntax in Richardson's ground squirrel alarm communication, we presented adult and juvenile subjects with repeated alarm calls with unaltered syllable order and those same calls with syllable order randomized. There was limited evidence that syllable order affected receiver response, but squirrels were more responsive to primary syllables of repeated calls even when these syllables were embedded within randomized calls. This suggests that primary syllables serve a general alerting function, possibly priming receivers for information that follows. Distinctive structural elements of primary syllables, including reduced syllable duration and reduced harmonic durations relative to the fundamental frequency, were correlated with the heightened vigilance responses that these syllables evoked.

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Ground-dwelling squirrels (tribe Marmotini) alert conspecifics of potential predatory threat by producing alarm vocalizations (Macedonia & Evans 1993); however, situationally specific information regarding the nature of threat (Owings & Leger 1980; Robinson 1981; Blumstein 1995a, b; Blumstein & Armitage 1997; Warkentin et al. 2001; Kiriazis & Slobodchikoff 2006) or the status of the signaller (Nesterova 1996; Hare 1998; Hare & Atkins 2001; Blumstein & Daniel 2004; Blumstein et al. 2004; Blumstein & Munos 2005) can vary greatly among calls. Alarm calls exist as discrete acoustic elements (syllables) temporally separated from other sounds or as repeated vocalizations where individual syllables are separated by varying periods of silence. Receivers extract information communicated within alarm calls by attending to variation in the acoustic structure of individual syllables (Waring 1970; Robinson 1981; Slobodchikoff et al. 1991; Sloan

et al. 2005), the number of callers (Blumstein & Daniel 2004; Sloan & Hare 2008) or the overall temporal pattern of a repeated call (Waring 1970; Blumstein & Armitage 1997; Warkentin et al. 2001).

Where multiple utterances are juxtaposed temporally, variation among those utterances could also, theoretically, encode information on the nature of a particular predatory threat or signaller status. Syntax is critical in human vocal communication (Chomsky 1972) and probably enhances the information content of vocalizations in other species (Marler 1977; Mitani & Marler 1989; Blumstein 1999). Subtleties in the urgency associated with predatory threat are expressed via rate changes in Richardson's ground squirrel alarm calls (Warkentin et al. 2001; Sloan & Hare 2004). Furthermore, Sloan et al. (2005) showed that variation in the acoustic parameters of individual syllables within repeated alarm calls carries information regarding the extent of threat and the location of the signaller. Given this variation, where multiple syllables are produced in a repeated alarm call, lexical recombination could drastically alter the message expressed by the overall call, thereby providing evidence that repeated alarm calls

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have syntactical structure. To explore this possibility, we presented juvenile and adult Richardson's ground squirrels with two call exemplars derived from the same calling individual in which the order of syllables produced by the original caller was retained (control) or randomized (experimental). The mechanisms by which Richardson's ground squirrels perceive call urgency change over the life of a squirrel (Sloan & Hare 2006; Swan & Hare, *in press*), so we accounted for both signaller and receiver age in our study. We compared vigilance responses of call recipients to control and experimental treatments created from both juvenile and adult signallers to address whether (1) repeated calls have an element of syntax, (2) whether such syntax is differentially produced by signalers from different age cohorts and (3) whether juveniles and adults manifest differential abilities to perceive syntactical content of repeated calls.

Beyond syllable-order effects on alarm call perception, this design also permitted an examination of potential disparities among individual syllables that could affect the vigilance response of receivers. Where behavioural differences appear in response to individual syllables within calls, they cannot be attributed to syntax proper. That said, the initial syllable of a repeated alarm call could convey unique information that serves a general warning function, which would have been retained in calls presented in normal order, but communicated only in the later syllables of randomized calls. Soha & Marler (2000) showed that introductory notes in white-crowned sparrows, *Zonotrichia leucophrys oriantha*, are critical in song learning and species recognition. In the context of alarm vocalizations, such unique elements may serve as an initial alerting function, priming receivers for information that follows (Hauber *et al.* 2001). We tested this hypothesis by comparing the vigilance response to the transposed original first syllables within randomized calls relative to the syllables in a normally ordered call with the same temporal position. To determine whether primary syllables elicit dissimilar responses, it is important to examine not only perceptual differences between syllables but also production differences (Macedonia & Evans 1993; Blumstein 1995a). Therefore, in addition to examining behavioural responses to individual syllables, we contrasted various acoustic elements of primary syllables (first acoustic element uttered in a calling bout) with those of subsequent syllables.

METHODS

General Methods

Research was conducted on a population of free-living Richardson's ground squirrels occupying mowed lawns at the Assiniboine Park Zoo (49°52'N, 97°14'W) in Winnipeg, Manitoba, Canada during 5 May–25 July 2005 and 7 April–7 July 2006. The Zoo offered easily observable subjects that were previously habituated to humans. Habituation to humans does not, however, denote general habituation to nonhuman predators, because natural predation is common within the Zoo and squirrels within our study population readily utter and react to alarm signals (Sloan & Hare 2006).

Adult (2 years and older) and juvenile (first year above ground) squirrels were live-trapped using National or Tomahawk traps baited with peanut butter following emergence from hibernation. Subjects were permanently marked with metal eartags (National Band and Tag Company, Monel no. 1, Newport, KY, U.S.A.) and given a unique design on their dorsal pelage with hair dye (Clairol Hydrience, no. 52 Pearl Black, Stamford, CT, U.S.A.) to distinguish individuals from afar. All work with animals conformed to the guidelines set forth by the Canadian Council on Animal Care and by the ASAB/ABS (Protocol no. F03-042).

Treatment construction

Our study followed a randomized block design where 42 adult and 59 juvenile subjects were presented with four treatments in a matched-pairs fashion. All treatments were constructed from repeated alarm calls, manipulated on Canary (Cornell Laboratory of Ornithology, Ithaca, NY, U.S.A.) to have a uniform 4 s latency between syllables, roughly equivalent to the average intersyllable latency in naturally produced calls (Sloan & Hare 2004, 2006). In all cases, the first six syllables produced by callers were used for treatment construction. Treatment 1 consisted of an adult alarm call with syllable order equivalent to that of the original recording. Treatment 2 was derived from the same adult call as in treatment 1 but with the syllable order randomized. Treatment 3 was a juvenile alarm call with syllables in their original order. Treatment 4 was constructed using the same call as in treatment 3 but with syllable order randomized. The randomization of syllable order in treatments 2 and 4 was subject to the restriction that no single syllable held the same temporal position as the call from which it was derived. Because Richardson's ground squirrels can identify individual callers (Hare 1998) and alter their vigilance response according to the past reliability of callers (Hare & Atkins 2001), we used multiple call exemplars for each treatment. Although it would have been ideal to use calls from distinct callers for each call recipient, the number of high quality recordings from unique adult callers was limited. Therefore, to keep variability of calls similar between age classes, we used 10 different adult calls to create treatments 1 and 2, and 10 distinct juvenile calls to create treatments 3 and 4. All subjects were randomly assigned treatments, but each individual received treatments 1 and 2 from the same adult signaller and treatments 3 and 4 from the same juvenile signaller.

To construct the treatment playbacks, J.F.H. recorded alarm calls of female squirrels in southern Manitoba using either a Sony TCD-D7 digital audiotape (DAT) recorder (Sony Corporation, Oradell, NJ, U.S.A.) and a Dan Gibson EPM P-650 parabolic microphone (R.D. Systems of Canada, Toronto, Ontario; 1994–1996), or a Sony TCD-D8 DAT recorder and an Audio-Technica AT-815b condenser microphone (Audio-Technica Inc., OH, U.S.A.; 1997–1998) (Hare 1998; Warkentin *et al.* 2001). To keep contextual information about the nature of threat conveyed within calls as similar as possible, a tan Biltmore hat (32.5 × 19.5 brim × 13.0 cm high) was consistently used

as a call-eliciting stimulus (see Hare 1998). Calls were elicited from squirrels at sites in southern Manitoba at least 85 km from the Zoo to avoid familiarity between signallers and receivers that could bias responses according to past reliability of known signallers (Hare & Atkins 2001). Recorded calls were transferred from DAT using the Sony TCD-D8 recorder onto a Macintosh computer and manipulated into the four treatments using the program Canary. Tracks were then transferred to MiniDisc (using a Sony MZ-N707 MiniDisc Recorder) at a sampling rate of 44.1 kHz for playback in the field.

Playback trials

The same outer clothing was worn during all trials to minimize confounding effects of researcher presence on the squirrels' reactions. We approached each of the 101 subjects to within 8.3–27.1 m and then erected the playback apparatus. A Sony DCR-TRV110 camcorder was placed atop a Manfrotto 055NAT tripod and a Genexxa Pro LX5 (InterTan Ltd, Barrie, Ontario, Canada) loud-speaker was positioned 8 m to our left and oriented towards the subject. The distance from the speaker to the subject ranged from 5.8 to 31 m (mean \pm SE = 19.01 \pm 0.306 m). A treatment track was then broadcast from the speaker using a Sony XM-2025 amplifier and a Sony MZ-N707 MiniDisc Recorder. Playbacks were broadcast at 90 dB SPL (measured at 1 m from the speaker using a Techcessories 33-2050 sound level meter, weighting C, response fast; InterTan Ltd), which is within the natural range of Richardson's ground squirrel alarm call amplitude (Hare 1998). Treatments were presented to subjects in a random order and no subject received more than one playback within an hour, but most (81%) intertreatment times were at least a day. An assistant videotaped subjects for 30 s before playback (Pre-PB), throughout the playback (PB) and 30 s after the playback had concluded (Post-PB). For a trial to be used in analyses, the subject had to remain nonvigilant (as defined by Hare 1998: with all feet on the ground and head down) for at least 50% of the Pre-PB period. Furthermore, trials were terminated if the subject was out of view for more than 30% of the Pre-PB, PB or Post-PB period. A trial also ceased if a subject descended into a burrow and remained there for more than one syllable of the playback.

Environmental and miscellaneous factors that could influence the subjects' responses, including maximum and average wind speeds (km/h over 2 min using a Kestrel 3000 Pocket Weather Meter; Nielsen Kellerman, Chester, PA, U.S.A.), temperature ($^{\circ}$ C using the Kestrel 3000), cloud cover (1 = clear; 4 = overcast), date, time, distance (m) and angle (degrees) of speaker to the subject were recorded following each trial. These factors were balanced across treatments (Mann–Whitney *U* tests: all $P > 0.05$) and thus do not confound the evaluation of dependent variable effects.

Data coding and analyses

For each trial, we coded subject behavioural response for each of the three time periods (Pre-PB, PB, Post-PB) from the videotape records using a Sony Trinitron KV 20FV10

television and a Sony DCR-TRV110 camcorder. The time codes (within a 30th of a second) for each postural change were recorded along with broadcast time (within 1 s) of each syllable. While coding data, we were unaware of which treatment was being presented until after the trial had concluded, thus avoiding any experimenter bias. As per Hare (1998), subject vigilance response was categorized in order of increasing vigilance as follows: S4hd (four feet on substrate with head below the horizontal; considered nonvigilant behaviour); S4hu (four feet on substrate with head raised above the horizontal; considered the least vigilant posture); Slouch (resting on hindlegs with back arched forward); Alert (standing on hindlegs with back erect); and Run (considered escape behaviour). Vigilant behaviour was defined as any posture in which the head was raised above the horizontal plane (S4hu, Slouch, Alert).

To evaluate the effect of syntax on subjects' vigilance responses, we determined the proportion of time spent vigilant in the Pre-PB, PB and Post-PB periods and calculated the difference in the proportion of time spent vigilant between the PB and the Pre-PB periods and between the Post-PB and Pre-PB periods to adjust for differences in responsiveness of subjects before playback. Where dependent variables reflect behavioural responses to playbacks as a whole, the comparison of treatments 1 and 2 examined whether the syntax of an adult's alarm call affected vigilance responses of adult or juvenile receivers, suggestive of semantic information encoded via syllable order. Similarly, comparing dependent variables between treatments 3 and 4 tested whether the syntax of juvenile-produced alarm calls affected the vigilance response of receivers from either age cohort, suggesting that semantic information was encoded in the syllable order of those calls. We found no significant differences (Mann–Whitney *U* tests: all $P > 0.10$) between male and female subjects of either age cohort in any treatment, so we pooled the data into strictly adult and juvenile categories.

To examine the effect of individual syllable variation on subjects' vigilance responses, we coded and analysed two response variables following each syllable within a call: relative postural change (where S4hd = 1, S4hu = 2, Slouch = 3, Alert = 4 and Run = 5) and, where a positive postural change occurred, the duration of vigilance posture. Because treatments 1 and 2 and treatments 3 and 4 were derived from the same adult and juvenile calls, respectively, it was possible to compare reactions to individual syllables within each of these treatment couplets. We defined six syllable types for each treatment couplet as follows (see Fig. 1): NN (the first syllables broadcast within the naturally ordered treatment; primary syllables); RR (the first syllables broadcast within the randomized treatment); RN (the same primary syllables as NN but embedded within the randomized treatment); NR (the same syllable as RR but within the naturally ordered treatment); NC (the syllable within the naturally ordered treatment that holds the same temporal position as RN); and RC (the syllable within the randomized treatment that holds the same temporal position as NR). There were thus three relevant comparisons that could be made between these

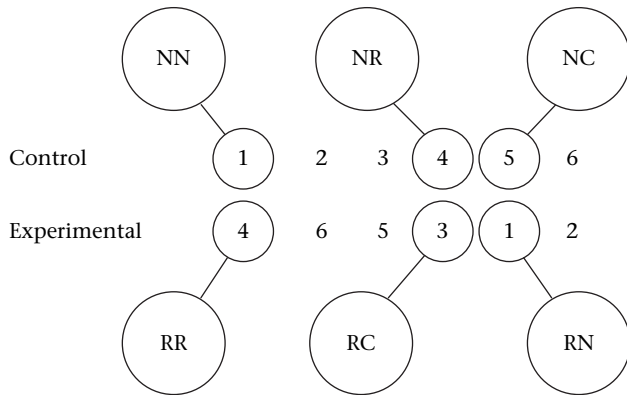


Figure 1. Schematic representation of syllable types in control and experimental treatment couplets derived from alarm calls of adult and juvenile Richardson's ground squirrels. NN: first syllable broadcast within naturally ordered treatments (primary syllable); RR: first syllable broadcast within the randomized treatments; RN: primary syllable embedded within randomized treatment; NR: the same syllable as RR but in its natural temporal position; NC: syllable within the naturally ordered treatment that holds the same temporal position as RN; RC: the syllable within the randomized treatment that holds the same temporal position as NR.

syllables for each of the adult- and juvenile-produced couplets. (1) We compared relative postural change and vigilance duration following NN versus RR to test for reaction differences caused by the primary syllables of each treatment while they were the first syllable broadcast. (2) We compared reactions to RN versus NC to test for reaction differences following primary syllables embedded within a randomized call and syllables that held the same temporal position. (3) We compared reactions following NR versus RC to test whether squirrels responded differently to the first syllables broadcast in randomized calls in their natural temporal positions and to syllables that held the same temporal position.

To test for acoustic differences within the naturally occurring first syllable that may account for behavioural differences, we compared the acoustic structure of NN syllables to that of RR syllables (Wilcoxon signed-ranks tests) for each of the spectral parameters outlined below (obtained using Canary). Because adult and juvenile calls do not differ in any of these parameters (Swan & Hare, *in press*), we pooled adult and juvenile data. Dependent variables included frequency of maximum amplitude (kHz), syllable duration (ms), maximum and minimum fundamental frequency (kHz), fundamental frequency range (maximum–minimum; kHz), first harmonic (where present) duration (ms), fundamental frequency at harmonic onset and offset (kHz), latency between syllable and harmonic onset (ms), latency between syllable and harmonic offset (ms) and the ratio of harmonic duration to syllable duration derived from those measurements.

All statistical analyses were done using Statview 5.01 (SAS Institute, Cary, NC, U.S.A.). We used nonparametric tests throughout because our data were not drawn from a normally distributed population of differences (Kolmogorov–Smirnov tests: all $P < 0.05$) and error deviations were not homogeneous across treatments (Bartlett's tests: all

$P < 0.05$). In accordance with the dependent-groups nature of this study, where each call recipient received all possible playback types, we used Wilcoxon signed-ranks tests when evaluating effects of the main independent variables (syllable order, syllable type). Differences were considered significant where $P \leq 0.05$. For all pairwise comparisons, we calculated Cohen's d as a measure of effect size, where $d = 0.2$, $d = 0.5$, and $d = 0.8$ correspond to small, medium and large effect sizes, respectively (Cohen 1988).

RESULTS

There were no significant differences in the proportion of time spent vigilant during the PB or Post-PB periods (after adjusting for differences in subject behaviour before playback) between control and experimental treatments derived from adult- or juvenile-produced calls. While not significant, adult (Wilcoxon signed-ranks test: $Z = -1.926$, $N = 34$, $P = 0.054$, $d = 0.390$; Table 1) and juvenile ($Z = -1.764$, $N = 46$, $P = 0.077$, $d = 0.219$; Table 1) receivers spent more time vigilant in the Post-PB period after adult-produced calls with unaltered syllable order (treatment 1) than after randomized adult calls (treatment 2).

The primary syllables of adult- and juvenile-produced repeated alarm calls induced a markedly greater vigilance response in both adult and juvenile call recipients than did subsequent syllables. Neither adult nor juvenile receivers, however, showed differential reaction to those first syllables presented in experimental treatments when in their natural temporal positions (NR) relative to nonprimary syllables that held the same temporal position (RC).

Adult receivers showed significantly longer vigilance duration ($Z = -1.995$, $N = 39$, $P = 0.046$, $d = 0.415$; Table 2) and significantly greater postural change ($Z = -2.785$, $N = 39$, $P = 0.005$, $d = 0.701$; Table 2) after playback of primary syllables of naturally ordered adult calls (NN) than after playback of the first syllables of randomized adult calls (RR). Even when primary syllables were embedded within randomized calls (RN), they induced greater postural change ($Z = -2.744$, $N = 39$, $P = 0.006$, $d = 0.763$; Table 2) and longer vigilance duration ($Z = -3.332$, $N = 39$, $P = 0.001$, $d = 0.524$; Table 2) than syllables that held the same temporal position in the control treatment (NC). Although adult receivers did not show differential response to NN (primary) versus RR (first broadcast in experimental treatment) syllables of juvenile-produced calls, they did show a greater postural change ($Z = -3.501$, $N = 40$, $P = 0.001$, $d = 0.927$; Table 2) and longer vigilance duration ($Z = -2.840$, $N = 40$, $P = 0.005$, $d = 0.538$; Table 2) after playback of RN syllables (primary syllables embedded within randomized calls) than after playback of NC syllables (same temporal position as RN) of juvenile-produced calls.

Juvenile receivers increased their vigilance posture to a greater degree after playback of adult-produced NN syllables than after playback of RR syllables ($Z = -2.163$, $N = 46$, $P = 0.031$, $d = 0.452$; Table 2), but showed no differential reaction to adult-produced RN versus NC syllables. They also manifested no differential responses to NN

Table 1. Proportion of time that juvenile and adult Richardson's ground squirrels spent vigilant during (PB) and after (Post-PB) playback of naturally ordered (control) and randomized (experimental) alarm call exemplars

| Dependent variable | Recipient age | Caller age | Control | Experimental | Z | P | N | d |
|--------------------|---------------|------------|-------------|--------------|--------|-------|----|-------|
| | | | (mean±SE) | (mean±SE) | | | | |
| PB vigilance | Adult | Adult | 0.458±0.037 | 0.460±0.037 | -0.377 | 0.706 | 39 | 0.009 |
| | | Juvenile | 0.504±0.038 | 0.434±0.048 | -1.001 | 0.317 | 40 | 0.256 |
| | Juvenile | Adult | 0.427±0.041 | 0.405±0.044 | -0.672 | 0.502 | 46 | 0.076 |
| | | Juvenile | 0.496±0.040 | 0.411±0.038 | -1.475 | 0.140 | 52 | 0.302 |
| Post-PB vigilance | Adult | Adult | 0.404±0.047 | 0.301±0.037 | -1.926 | 0.054 | 39 | 0.390 |
| | | Juvenile | 0.331±0.039 | 0.232±0.051 | -1.640 | 0.101 | 40 | 0.345 |
| | Juvenile | Adult | 0.325±0.042 | 0.258±0.048 | -1.764 | 0.077 | 46 | 0.219 |
| | | Juvenile | 0.345±0.048 | 0.246±0.036 | -1.668 | 0.095 | 52 | 0.324 |

versus RR juvenile-produced syllables but did show longer vigilance duration ($Z = -2.661$, $N = 51$, $P = 0.008$, $d = 0.430$; Table 2) and greater postural response ($Z = -3.467$, $N = 51$, $P = 0.001$, $d = 0.857$; Table 2) to RN versus NC juvenile-produced syllables.

The acoustic structure of primary syllables (NN) is contrasted with the first syllables broadcast in randomized calls (RR) in Table 3. NN syllables had shorter duration ($Z = -2.09$, $N = 20$, $P = 0.037$, $d = 0.202$), smaller differences between maximum and minimum frequency

Table 2. Vigilance duration (VD) and postural response (PR) of juvenile and adult Richardson's ground squirrels following playback of alarm calls containing different syllable types (NN versus RR; RN versus NC; NR versus RC)

| Receiver age | Caller age | Dependent variable | Response to syllable types | | Z | P | N | d |
|-----------------|------------|--------------------|----------------------------|--------------|--------|-------|----|-------|
| | | | (mean±SE) | (mean±SE) | | | | |
| NN vs RR | | | | | | | | |
| Adult | Adult | VD | 12.468±2.464 | 6.812±1.859 | -1.995 | 0.046 | 39 | 0.415 |
| | | PR | 1.564±0.179 | 0.821±0.160 | -2.785 | 0.005 | 39 | 0.701 |
| | Juvenile | VD | 11.252±2.518 | 8.657±2.124 | -0.270 | 0.787 | 40 | 0.176 |
| | | PR | 1.200±0.169 | 0.975±0.121 | -1.043 | 0.297 | 40 | 0.242 |
| Juvenile | Adult | VD | 8.187±1.923 | 7.310±1.976 | -0.428 | 0.669 | 46 | 0.066 |
| | | PR | 1.304±0.152 | 0.870±0.130 | -2.163 | 0.031 | 46 | 0.452 |
| | Juvenile | VD | 8.419±1.778 | 8.520±1.875 | -0.014 | 0.988 | 51 | 0.008 |
| | | PR | 1.608±0.184 | 1.275±0.159 | -1.425 | 0.154 | 51 | 0.271 |
| RN vs NC | | | | | | | | |
| Adult | Adult | VD | 3.689±1.317 | 0.536±0.351 | -3.332 | 0.001 | 39 | 0.524 |
| | | PR | 0.462±0.089 | 0.026±0.094 | -2.744 | 0.006 | 39 | 0.763 |
| | Juvenile | VD | 2.738±0.999 | 0.283±0.212 | -2.840 | 0.005 | 40 | 0.538 |
| | | PR | 0.575±0.129 | 0.000±0.051 | -3.501 | 0.001 | 40 | 0.927 |
| Juvenile | Adult | VD | 0.575±0.205 | 0.929±0.518 | -0.402 | 0.687 | 46 | 0.132 |
| | | PR | 0.304±0.081 | 0.043±0.107 | -1.753 | 0.080 | 46 | 0.406 |
| | Juvenile | VD | 1.393±0.442 | 0.356±0.180 | -2.661 | 0.008 | 51 | 0.430 |
| | | PR | 0.451±0.076 | -0.059±0.090 | -3.467 | 0.001 | 51 | 0.857 |
| NR vs RC | | | | | | | | |
| Adult | Adult | VD | 1.795±1.113 | 0.741±0.482 | -0.357 | 0.721 | 39 | 0.197 |
| | | PR | 0.028±0.101 | 0.128±0.041 | -0.785 | 0.433 | 39 | 0.208 |
| | Juvenile | VD | 0.999±0.877 | 0.223±0.091 | -0.267 | 0.790 | 40 | 0.197 |
| | | PR | 0.025±0.091 | 0.075±0.083 | -0.245 | 0.807 | 40 | 0.091 |
| Juvenile | Adult | VD | 0.729±0.471 | 0.232±0.118 | -0.866 | 0.386 | 46 | 0.213 |
| | | PR | 0.109±0.133 | -0.022±0.073 | -0.563 | 0.573 | 46 | 0.180 |
| | Juvenile | VD | 0.288±0.112 | 0.629±0.335 | -0.170 | 0.865 | 51 | 0.191 |
| | | PR | -0.196±0.131 | -0.020±0.099 | -0.915 | 0.360 | 51 | 0.212 |

Syllable types are defined in Fig. 1.

Table 3. Results from Wilcoxon signed-ranks tests comparing the acoustic structure of the first syllable of Richardson's ground squirrel alarm calls broadcast in the naturally ordered treatments (NN; treatments 1 and 3) and in the randomized treatments (RR; treatments 2 and 4)

| Dependent variable | NN | RR | Z | P | N | d |
|--|--------------|--------------|-------|------|----|--------|
| | (mean±SE) | (mean±SE) | | | | |
| Frequency max. amplitude (kHz) | 7.55±0.43 | 7.55±0.41 | -0.26 | 0.80 | 20 | 0.000 |
| Syllable duration (ms) | 161.51±15.05 | 175.41±15.67 | -2.09 | 0.04 | 20 | -0.202 |
| Syllable max. freq. (kHz) | 8.77±0.26 | 8.87±0.28 | -0.59 | 0.55 | 20 | -0.083 |
| Syllable min. freq. (kHz) | 6.45±0.68 | 6.03±0.73 | -1.55 | 0.12 | 20 | 0.133 |
| Syllable freq. max.-min. (kHz) | 2.08±0.50 | 2.58±0.56 | -2.37 | 0.02 | 20 | -0.211 |
| First harmonic duration (ms) | 71.14±14.24 | 94.06±15.53 | -2.50 | 0.01 | 10 | -0.486 |
| Harmonic duration: syllable duration | 0.59±0.07 | 0.73±0.07 | -2.29 | 0.02 | 10 | -0.632 |
| Fundamental freq. at harmonic onset (kHz) | 6.86±0.56 | 7.39±0.43 | -1.35 | 0.18 | 8 | -0.375 |
| Fundamental freq. at harmonic offset (kHz) | 4.36±0.95 | 4.15±0.96 | -0.91 | 0.36 | 8 | 0.078 |
| Latency to harmonic onset (ms) | 33.08±6.97 | 24.67±7.66 | -1.27 | 0.20 | 10 | 0.363 |
| Latency from harmonic offset (ms) | 18.97±11.38 | 16.18±11.64 | -1.99 | 0.05 | 10 | 0.077 |

See text for details.

($Z = -2.37$, $N = 20$, $P = 0.018$, $d = 0.211$), shorter harmonic duration ($Z = -2.50$, $N = 10$, $P = 0.013$, $d = 0.487$) and longer latency between harmonic offset and syllable offset ($Z = -1.99$, $N = 10$, $P = 0.047$, $d = 0.077$) than did RR syllables. The ratio of harmonic duration to syllable duration was also greater in RR than in NN syllables ($Z = -2.29$, $N = 10$, $P = 0.022$, $d = 0.430$).

DISCUSSION

Our results provide limited evidence that the syllable order of Richardson's ground squirrel alarm calls carries meaningful information to call receivers. Adult and juvenile receivers maintained a greater degree of vigilance following control playbacks derived from adult-produced calls than they did following experimental playbacks, although those differences were not significant. Therefore, while we cannot conclude with certainty that syntax contributes to alarm communication in Richardson's ground squirrels, it remains a compelling possibility. The order of acoustic elements encodes information in the vocal communication of other social vertebrates (Robinson 1979, 1984; Marler & Pickert 1984; Hailman et al. 1985). The arrangement of notes in male gibbon, *Hylobates agilis*, songs qualitatively affects behavioural responses of conspecific receivers (Mitani & Marler 1989). Furthermore, Vancouver Island marmots, *Marmota vancouverensis*, show different levels of alarm response when call order in multinote calls is manipulated experimentally (Blumstein 1999).

What appears to be semantic information encoded within syllable order could be an artefact of the unique information encoded within the natural first syllable of repeated alarm calls of Richardson's ground squirrels that promotes increased vigilance response in call recipients. The initial syllable of a repeated call retains its more salient alerting function even after losing temporal primacy. Indeed, the primary syllable almost consistently elicited longer vigilance duration and increased postural change in adult and juvenile subjects than did other syllables. Unique functionality of the initial elements of animal vocalizations is not unusual (Marshall 1964;

Herzog & Hopf 1984; Soha & Marler 2000; Templeton et al. 2005; Mennill & Rogers 2006). Brown-headed cowbirds, *Molothrus ater*, respond differently to playbacks of partial flight whistles and complete calls (Dufty & Pugh 1994). Moreover, Hailman et al. (1987) suggested that semantic information is encoded via the ratio of certain notes included in the 'chick-a-dee' calls of black-capped chickadees, *Poecile atricapillus*. In Richardson's ground squirrels, it seems that the first acoustic element uttered in a repeated alarm call is perceived as a more urgent warning than subsequent syllables.

The notion of an alerting function preparing receivers for information to follow has considerable merit (Hauber et al. 2001; Peters & Evans 2003). Where predatory attacks depend on an element of surprise, alarm components produced when the threat is first detected would be vital in readying colony members. Furthermore, in that the rate of syllable production in Richardson's ground squirrels repeated calls is correlated with proximity to predators (Warkentin et al. 2001) and that variation in the pattern of subsequent syllables communicates signaller certainty (Sloan & Hare 2004), an initial alerting signal could direct conspecific attention to the temporal pattern of subsequent syllables, in effect priming receivers to assimilate and integrate information that follows.

With differential response we expect some structural difference between primary and subsequent syllables. Primary syllables were significantly shorter than trailing syllables. Intuitively, where threat is most urgent (i.e. when predators are first noticed), time available to vocalize is limited. One could expect, then, that initial syllable duration should be minimized (Owings & Hennesy 1984; Macedonia & Evans 1993). Shortening primary syllables may also allow alarm signallers to warn conspecifics while reducing their own conspicuousness (Bayly & Evans 2003). Davis (1984) asserted that Richardson's ground squirrels utter short chirps to avian predators and longer whistles to terrestrial threats; however, this differential production probably reflects degree of threat and not referential signalling (Warkentin et al. 2001). Indeed, an airborne attack may not allow enough time for

production of a whistle, let alone a polysyllabic call. Within a repeated call, it is plausible that brief primary syllables also communicate elevated threat. The smaller frequency range and shorter harmonic duration in the primary syllables may also enhance alerting potential, but could simply be a product of the shorter fundamental duration. Reducing the frequency range of a call would also decrease a predator's ability to locate the signaller (Terhune 1974; Bradbury & Vehrencamp 1998). Interestingly, primary syllables had a smaller ratio of harmonic duration to syllable duration and a greater latency between fundamental and harmonic offset. Such variation in the harmonic elements may convey information regarding signaller status/identity or predatory threat (Slobodchikoff et al. 1991; Fischer et al. 2002; Templeton et al. 2005; Mennill & Rogers 2006). In the alarm calls of black-capped chickadees, the bandwidth of notes is directly related to predator size (Templeton et al. 2005). There is also evidence that Gunnison's prairie dogs, *Cynomys gunnisoni*, encode information about specific predator attributes into the harmonics of their alarm calls (Slobodchikoff et al. 1991) and that such referential signaling is communicated to conspecifics (Kiriazis & Slobodchikoff 2006).

The structural differences unique to primary syllables in repeated calls of Richardson's ground squirrels provide readily testable hypotheses that can be investigated in future studies. Regardless of what the results of such tests reveal, it is apparent that what we perceive as simple chirps are actually complex vocalizations, replete with information.

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