



Social context mediates testosterone's effect on snort acoustics in male hyrax songs

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ABSTRACT

Testosterone affects physical and motivational states, both of which may strongly influence vocalization structure and acoustics. The loud complex calls (i.e., songs) of male rock hyraxes (*Procavia capensis*) are used as honest signals for advertising physical and social states. The snort, a low frequency, noisy element of the song, encodes information on the singer's age and social rank via harshness, as measured by jitter (i.e., acoustic frequency stability) and duration; suggesting that the snort concomitantly advertises both vocal stability and aggression. Our past findings revealed that testosterone levels are related to both vocal elements and social status of male hyraxes, suggesting that hormonal mechanisms mediate the motivation for aggressive and courtship behaviors. Here we examined whether long-term androgen levels are related to snort acoustics and song structure by comparing levels of testosterone in hair with acoustic and structural parameters. We found that songs performed by individuals with higher testosterone levels include more singing bouts and longer, smoother snorts, but only in those songs induced by external triggers. It is possible that hyraxes with higher levels of testosterone possess the ability to perform higher-quality singing, but only invest in situations of high social arousal and potential benefit. Surprisingly, in spontaneous songs, hyraxes with high testosterone were found to snort more harshly than low-testosterone males. The context dependent effects of high testosterone on snort acoustics suggest that the aggressive emotional arousal associated with testosterone is naturally reflected in the jittery hyrax snort, but that it can be masked by high-quality performance.

1. Introduction

Androgens are crucial for the development of reproduction-related behaviors (reviewed by Wingfield et al., 1990). In many vertebrate taxa, male testosterone levels increase during the mating season (e.g., Girard-Buttoz et al., 2015; Johnston et al., 1994; Minter and DeLiberto, 2008), mediating sexual and territorial behavior and inducing aggressive, as well as courtship displays (Ketterson and Nolan, 1992). Changes in testosterone mediate the synchronization of vocalization in displays of aggression and courtship. Thus, variation in testosterone can be associated with acoustic and structural qualities. This behavioral-motivational pathway is especially well-documented in association with bird song (e.g., Alward et al., 2017b; Beani et al., 1995; Johnsen, 1998;

reviewed by: Harding, 2004; Ketterson and Nolan, 1992; Schlinger and Brenowitz, 2017).

Testosterone also affects vocalization via an anatomical-physiological pathway, in line with the source-filter theory (Fant, 1960; Taylor and Reby, 2010; Titze, 2000). This theory suggests that acoustics are shaped by the physical properties of both the vocal source (i.e., vocal fold vibration frequencies) and filter (i.e., vocal tract morphology selectively amplifying specific frequencies by resonance). The anatomical and physiological effects of testosterone can be either direct or indirect. Androgens affect vocalization directly by stimulating laryngeal development (Beckford et al., 1985), through an increase in androgen receptor concentrations in the larynx (Saez and Sakai, 1976). Furthermore, it appears that testosterone targets brain regions that influence

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the laryngeal muscles controlling the fundamental frequency (i.e., the lowest frequency of the emitted sound wave; Beani et al., 1995). Testosterone may also affect vocalization indirectly, by increasing body size. Body size poses a major constraint on acoustic fundamental frequencies (Charlton and Reby, 2016), and as testosterone stimulates male body growth (Cox et al., 2009) the voice is indirectly affected. Regardless of size, in multiple systems testosterone is associated with voice pitch either inversely (e.g., human males; Dabbs and Mallinger, 1999) or directly (e.g., white-handed gibbons, *Hylobates lar*; Barelli et al., 2013). Giant panda (*Ailuropoda melanoleuca*) bleats sounded by males with higher testosterone are longer and have higher fundamental frequency modulation rates (Charlton et al., 2011b). High modulation may be due to stiffer vocal-fold ligaments, caused by a high collagen-to-elastin ratio that is mediated by testosterone (Fischer and Swain, 1980). Thus, in animal communication, calls can potentially be used as honest indicators of circulating testosterone concentrations. Information on male testosterone levels is significant for both male and female conspecifics (Charlton et al., 2011a; Demartsev et al., 2016a), as high testosterone is related to competitive achievement (Zielinski and Vandenbergh, 1993) and sperm count (Johnston et al., 1994; Kishk, 2008).

Rock hyraxes (*Procavia capensis*) are one of a few mammal species that use singing to advertise male quality. Before and during the mating season, males produce a loud complex call (i.e., a song) composed of three elements, arranged in singing bouts (Koren and Geffen, 2009a). Our past findings suggest that males that sing more have higher testosterone levels (Koren, 2006), and that testosterone levels are related to social status (Koren et al., 2006). As multiple structural and acoustic cues contain information on age and social status (Koren and Geffen, 2009a; Weissman et al., 2019), hyrax songs may serve as an advertisement for singer quality (Demartsev et al., 2014; Ilany et al., 2013). Age, social status, and residency may all reflect on individual quality in the hyrax. Longevity holds survival skills and experience, and resident males have access to the more experienced mothers in the group (Bar-Ziv et al., 2016). The snorts, which are low frequency, noisy elements within complex hyrax songs (Fig. 1), encode information on the singer's physical and social state via harshness, as measured by jitter (i.e., waveform frequency stability) and length (Weissman et al., 2019). Manipulating these parameters in playback experiments influenced conspecific responses. We theorized that while low harshness may indicate a longer vocal tract and therefore a larger individual, elevated acoustic harshness may signal aggression.

We have found that hyrax snort harshness constitutes a unique channel for information transfer (Weissman et al., 2019). No previous studies have found a significant association between vocal harshness and either dominance and age of adult animals (e.g., Briefer et al., 2010; Stoeger et al., 2014) or testosterone levels (Charlton et al., 2011b). We have previously found that testosterone is connected to singing behavior and acoustics in hyraxes (Koren and Geffen, 2009a). In the present study, we assess the hormonal effects that mediate morphology, motivation for aggressive, and courting behavior, along with the social context of these behaviors, as expressed in the acoustics and structure of male hyrax songs.

To understand these associations, we examined the relationship between long-term integrated hair testosterone levels and hyrax snort acoustics (i.e., harshness and length). We predicted that these acoustic parameters would provide information on the singer's testosterone levels to listening conspecifics, who may associate acoustic factors with the cumulative effects of testosterone on morphology and behavior. Our predictions stemmed from the findings that testosterone stimulates body growth (Cox et al., 2009), specifically in the larynx (Beckford et al., 1985), and that vocal harshness has been shown to decrease with body size in both hyraxes (Weissman et al., 2019) and developing harbor seal (*Phoca vitulina richardii*) pups (Khan et al., 2006). Additionally, testosterone is known to positively affect element length and overall singing time in birds (e.g., canaries, *Serinus canaria*; Alward

et al., 2017a, 2017b), and mammals (e.g., giant pandas; Charlton et al., 2011b). Therefore, we expected that as testosterone level increases, snorts will become longer and smoother and that whole songs will be extended.

Finally, we examined how the snort elements varied according to song context. Hyrax singing events may be induced by external occurrences such as a singing conspecific, an approaching predator, or pup alarm calls; but the majority of songs (~75% of recorded events) are performed spontaneously with no evident outer trigger. Both bachelor and resident males sing either type of song (i.e., induced and spontaneous). As induced songs tend to be longer and more complex (Demartsev et al., 2014), hyraxes may be taking advantage of arousing situations, in which conspecifics are alert and listening (Ilany et al., 2011). Thus, we expected that differences in singing quality linked to testosterone levels would be more accentuated in induced songs, as hyraxes would make a stronger effort to perform at their best in these occasions.

2. Material and methods

2.1. Field procedures

We have been monitoring free-living groups of rock hyraxes as part of an ongoing study since 1999 in the Ein Gedi nature reserve, a desert oasis located on the western shores of the Dead Sea, Israel (31° 27' N, 35° 23' E). Rock hyrax groups comprise several females and a single mature (resident) male (Rubsamen et al., 1982). Other males (bachelors), live in the vicinity of groups; and although not socially bound to them, interact with the group members (Barocas et al., 2011). We trapped hyraxes annually using live box traps (Tomahawk Live Trap Co, Tomahawk, WI, USA), weighed and measured them, and acquired hair samples for steroid measurements, using scissors or electric beard trimmers. Animals were individually marked for external identification using collars (5 g; only for adults) and earrings. One of six focal groups was daily randomly selected for observation, four or five days a week, throughout the field season (March–August). We used a telescope (×50 and ×75 magnifications; Fieldscope ED82, Nikon, Japan) and binoculars (10 × 42; Monarch, Nikon, Japan) to observe the presence and behavior of focal individuals (Ilany et al., 2011; Koren and Geffen, 2011, 2009a).

2.2. Vocal recording and acoustic analysis

During observations, male songs were recorded using a Sennheiser ME 67 shotgun microphone (frequency response 50–20,000 Hz), powered by a Sennheiser K6 module and covered with a Sennheiser MZW70-1 blimp widescreen (Sennheiser Electronic GmbH & Co. K. G., Wedemark, Germany). The microphone was mounted on a tripod or handheld using a MZS20-1 shock-mount with a pistol grip. The songs were recorded in mono (Marantz PMD-222 cassette recorder, Marantz, Japan or Tascam HD-P2 digital audio recorder Tascam Corporation, USA) with a sampling frequency of 48 kHz and a sampling width of 24 bits (Demartsev et al., 2014; Ilany et al., 2011).

Our data relate to males for which we had both hair samples and audio recordings including snorts in the same year. From this data-set, we randomly selected songs that contained snorts for each male and sought to analyze a minimum of five snorts from a minimum of two songs per male and year, though not all hair samples had more than one matching song. When data from multiple songs were analyzed, songs from different months were included in order to present a wide time frame along the season. If more than two snorts occurred in a single bout, we analyzed the first and last snorts to represent the bout.

We used the same songs previously used by Demartsev et al. (2014) and by Weissman et al. (2019). We manually detected snorts using the Avisoft SAS Lab Pro software (version 5.2.07; Avisoft Bioacoustics, Berlin, Germany). Snorts are composed of a series of consecutive pulses

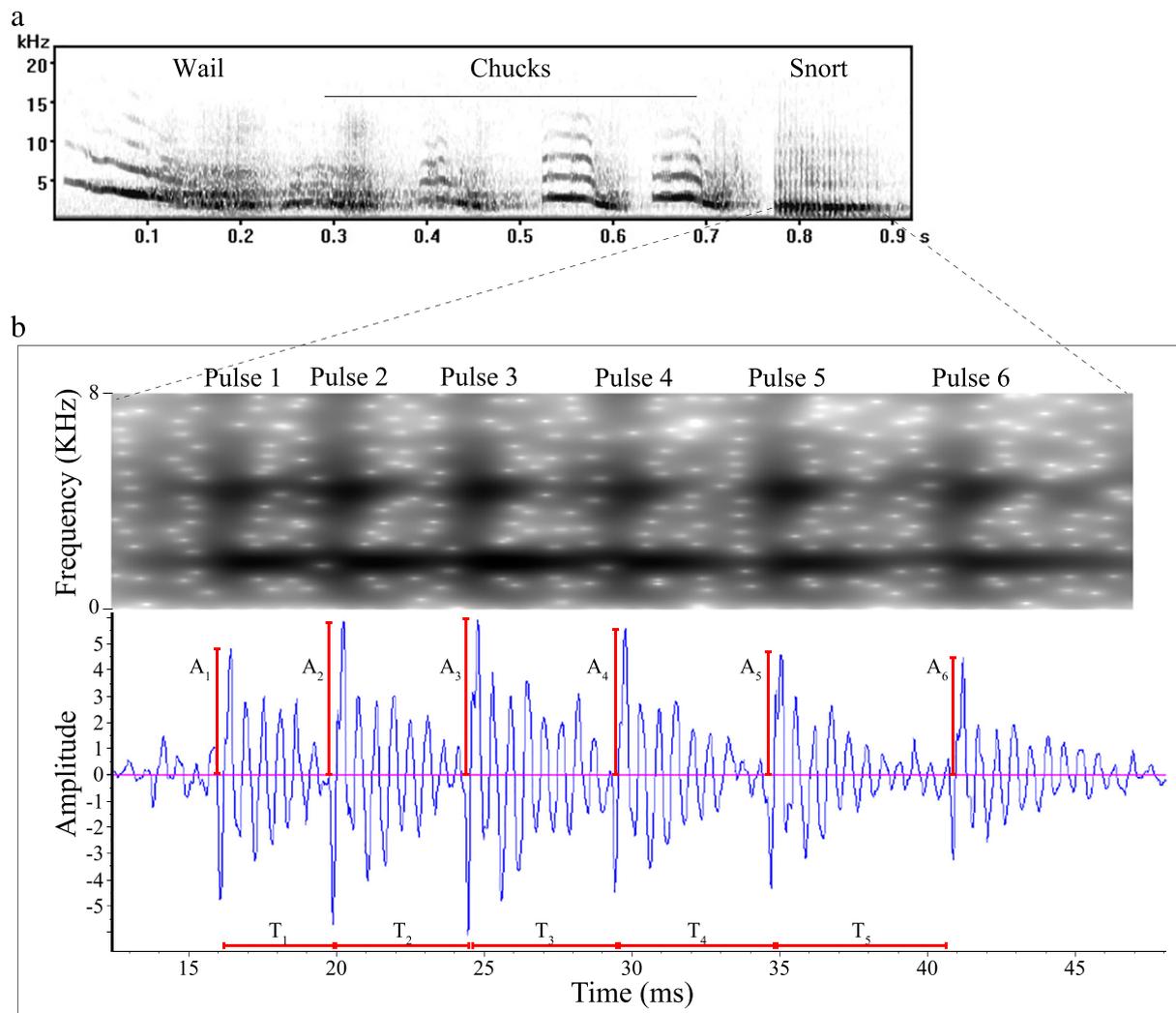


Fig. 1. A typical bout from a male rock hyrax song includes wails and chucks, and occasionally one or more snorts (sonogram; a). The snort is formed by a series of consecutive pulses (sonogram and waveform; b). Pulse frequency (A_i) and pulse maximal amplitude (T_i) vary. Amplitude units are schematic. Figure was adapted from Weissman et al. (2019).

(Fig. 1), that are not evenly spaced in time and may differ in amplitude. These variations are measured by jitter (variation of pulse duration; Eq. (1)) and shimmer (variation of maximal pulse amplitude; Eq. (2)) respectively (Farrús and Hernando, 2009). Pulses were detected using Avisoft's Pulse Train Analysis tool when possible, or manually. For each snort, we calculated the following seven variables: number of bouts in the song, snort length (seconds), number of pulses, mean interval length between pulses (seconds), mean amplitude (standardized separately within each snort relative to the maximum amplitude), jitter, and shimmer (Eqs. (1) and (2)). The latter two were used separately as they are not necessarily correlated, and each captures a different aspect of vocal instability. We excluded from the analysis snorts with < 4 pulses because such short snorts would not permit a reliable estimation of jitter and shimmer parameters.

$$Jitter = \frac{1}{N-1} \sum_{i=1}^{N-1} |T_i - T_{i+1}| \quad (1)$$

$$Shimmer = \frac{1}{N-1} \sum_{i=1}^{N-1} \left| 20 \cdot \log \left(\frac{A_{i+1}}{A_i} \right) \right| \quad (2)$$

A is the peak amplitude of pulse i , T is the time interval between the peak amplitude of pulse i and the peak amplitude of pulse $i + 1$, and N is the number of pulses in each given snort (Fig. 1). Higher values

indicate greater variation in peak amplitudes and intervals between pulses and are perceived as sounding harsher by the human ear. Lower values indicate more even peak amplitudes and intervals between pulses, and are perceived as smoother sounds. Both jitter and shimmer were calculated using MATLAB (version R2015a Natick, Massachusetts: The MathWorks Inc., 2010).

2.3. Steroid measurements

We quantified testosterone in hyrax hair. Hair-testing has several advantages over blood or fecal sampling. First, hair is collected with scissors or shaved, making the procedure relatively quick and non-invasive compared to blood sampling, which may necessitate anesthesia. Second, hair can be kept for many years and does not require freezing (Webb et al., 2010; Wilson et al., 2013). Finally and most importantly, as free hormones from the bloodstream are embedded in the hair as it grows, samples provide a long-term profile of integrated steroid levels, unaffected by the momentary stress of capture or diurnal changes (Schell et al., 2017). To avoid differences in quality and growth properties that may affect steroid levels in hair, hair samples were collected from the same body region on all animals, the back of the upper thigh. For this study, we used hair samples collected annually from 20 individual males between 2002 and 2012. We analyzed a total of 30 hair samples from males whose songs included snorts in the specific year.

Table 1

The effect (model estimate, $F_{(1,df)}$, P) of male residency status (M_{RS} ; resident or bachelor male), singing context (M_{SC} ; induced or spontaneous songs), long-term testosterone levels (T , standardized by year), and body weight (BW) on the number of bouts in the male song, snort length, and number of pulses in the snort. Effect size is presented as total effect.

Model	Estimate	F	df	P	Total effect
Number of bouts/song					
M_{RS}	-0.735	0.0	22	0.881	0.343
M_{SC}	0.038	0.0	260	0.969	0.547
T	-0.775	0.0	27	0.873	0.871
M_{RS}^*T	4.267	1.1	23	0.297	
M_{SC}^*T	5.194	22.2	259	< 0.001	
BW	7.688	1.1	240	0.282	0.109
Snort length					
M_{RS}	-0.011	1.4	22	0.185	0.287
M_{SC}	0.019	15.7	159	< 0.001	0.427
T	-0.010	1.2	35	0.246	0.387
M_{RS}^*T	-0.011	2.0	34	0.127	
M_{SC}^*T	0.009	4.6	202	0.033	
BW	0.037	2.7	48	0.096	0.158
Number of pulses/snort					
M_{RS}	-0.190	0.0	22	0.835	0.289
M_{SC}	2.106	14.0	148	< 0.001	0.531
T	-1.028	1.0	35	0.297	0.592
M_{RS}^*T	-1.649	3.3	35	0.059	
M_{SC}^*T	1.321	7.0	196	0.009	
BW	-2.311	0.8	42	0.348	0.055

Statistically significant parameters are marked in bold.

Hair testosterone was extracted and quantified using protocols previously described in [Koren and Geffen, \(2009b\)](#). Briefly, prior to testosterone extraction, hair samples were washed in isopropyl alcohol (Sigma-Aldrich Israel LTD, Rehovot, Israel) while gently shaking (twice for 3 min; 100 RPM). Hair was dried and either ground (25 Hz, 200MM, Retsch, Germany) or left whole, depending on the year. Testosterone was extracted by sonicating 20 mg hair samples (weighed to the nearest 0.01 mg by electronic balance; BJ610c, Precisa, Switzerland) with methanol (1 ml, 30 min; Sigma-Aldrich Israel LTD, Rehovot, Israel) followed by incubation with shaking (50 °C, 130 RPM, 20 h). Methanol was then decanted and evaporated with nitrogen at 45 °C. Extracts were reconstituted with the zero standard that was provided with the commercial ELISA kit (EIA-1559, DRG International Inc.). Samples were run in duplicates following the kit protocol, which had been previously validated for hyrax hair ([Koren et al., 2008, 2006; Koren and Geffen, 2009a, 2009b](#)). The intra-assay variability for six duplicates of the hyrax hair pool run on a single plate was 4.09%. Duplicates of the pool quantified on different days gave an inter-assay coefficient of variation of 7.08%. Serial dilutions of the pool showed parallelism with the provided kit standards (univariate analysis of variance in SPSS; $P = 0.356$), and linearity was demonstrated between 5 and 40 mg hair. Recovery was calculated to be 101.9% through the addition of a known amount of testosterone to the pool.

2.4. Statistical methods

To evaluate whether long-term testosterone has a significant effect on each of the above seven snort variables, we used linear mixed models. We set each of the snort parameters as the dependent variable in the model, male residency status (i.e., resident or bachelor); and the singing context (i.e., induced or spontaneous) as the fixed effects (these have been shown to affect acoustics and structure of hyrax song, as well as reply probability ([Demartsev et al., 2017, 2016b; Ilany et al., 2011; Weissman et al., 2019](#))); hair testosterone level (standardized by year) and body weight (tight proxy for male age) as covariates; and male identity as a random effect. Although snort length is correlated with the number of pulses, because the variation in the number of pulses

between snorts could affect estimation of the interval length between pulses, the mean amplitude, jitter, and shimmer, and thus be used to assess snort quality, we also added the number of pulses as an additional covariate in these models. All models were fitted by the REML approach using JMP (version 13.1; SAS Inc.), and the exact probability of each effect was calculated by randomizations in the R environment using the package “pgirmess” (<http://perso.orange.fr/giraudoux>). Effect size was expressed as total effect, an index showing the relative contribution of each factor both alone and in combination with other factors. The total effect is calculated using a Monte Carlo sampling, a procedure that estimates the importance of factors in a model independently of the model type or fitting method ([Saltelli, 2002](#)).

2.5. Ethics

Permits for capturing and handling the hyraxes at the Ein Gedi Nature Reserve were obtained from the Israeli Nature and Parks Authority (2000/8871, 2001/8871, 2002/14674, 2003/14674, 2004/17687, 2005/17687, 2007/27210, 2008/31138, 2009/32871, 2010/37520, 2011/38061, 2012/38400). No observable long-term stress or other interference effects were detected in individual animals or in the overall study population. The population in the research area remained stable for the duration of our long-term study ([Barocas et al., 2011](#)).

3. Results

Altogether, we analyzed 267 snorts taken from 46 songs that were performed by 20 different male hyraxes during 2002–2012. Of these, 28 songs, including 181 snorts, were induced, and 18 songs, including 86 snorts, were spontaneous. On average (\pm SD; range), we analyzed 5.8 (\pm 7.1; 1–46) snorts from 2.3 (\pm 1.2; 1–5) songs from each male. All snorts had associated data on the singer's hair testosterone and body weight, collected during the same year in which the song was recorded.

First, we examined whether male residency status, singing context, hair testosterone levels, and the two-way interactions between the fixed effects and hair testosterone levels, affected the number of bouts in the song, snort length, and the number of pulses in the snort. In all three models, we detected a significant interaction between singing context (i.e., induced or spontaneous) and testosterone level ([Table 1, Fig. 2](#)). Number of bouts in a song (coefficient estimate = 14.6, $P < 0.001$), snort length (estimate = 1.5, $P = 0.004$), and number of pulses in a snort (estimate = 0.01, $P = 0.021$) increased in induced songs with the increase in hair testosterone level ([Fig. 2](#)). In contrast, the number of bouts in a song (estimate = -3.0, $P = 0.262$), snort length (estimate = -0.5, $P = 0.484$), and number of pulses in a snort (estimate = -0.005, $P = 0.310$) in spontaneous songs did not change by the increase in hair testosterone level. Male residency status and body weight showed no significant effect on the above dependent variables ([Table 1](#)). However, the number of pulses tended to increase in residents (estimate = 1.1, $P = 0.027$), but not to change in bachelors (estimate = -0.9, $P = 0.360$), with the increase in testosterone levels ($P = 0.059$; [Table 1](#)).

The number of pulses in a snort negatively correlated with the values of shimmer, jitter, mean snort amplitude, and mean pulse interval ([Table 2](#)). In addition, we detected a significant interaction between singing context and testosterone levels only in jitter ($P = 0.001$; [Table 2, Fig. 3](#)). In the induced songs, the jitter decreased with the rise in testosterone level (estimate = -0.0002, $P = 0.020$), whereas in the spontaneous songs jitter increased with the rise in testosterone level (estimate = 0.0004, $P = 0.005$). Hair testosterone level did not correlate with shimmer, mean snort amplitude, or mean pulse interval ([Table 2](#)). Jitter tended to be higher in residents than in bachelors ($P = 0.054$; [Table 2](#)). Finally, body weight only correlated with the mean interval duration, which significantly increased with the increase in body weight ($P = 0.002$; [Table 2](#)).

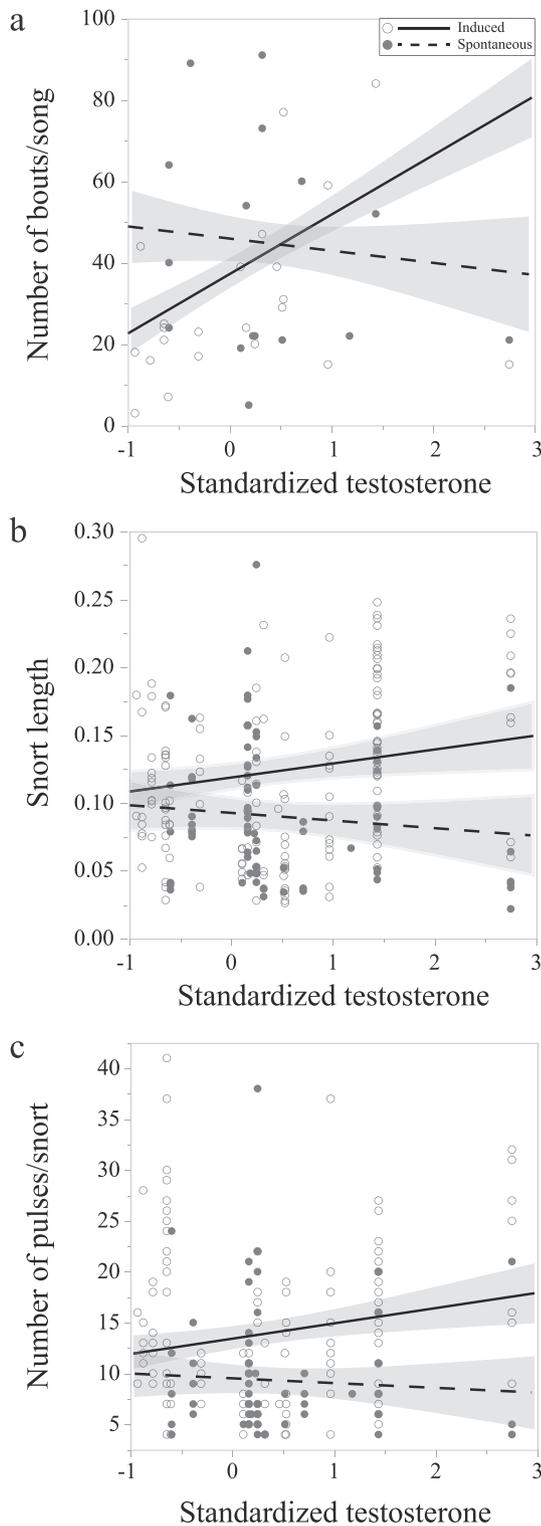


Fig. 2. The interaction between song context (induced vs. spontaneous songs) and testosterone levels in number of bouts per song (a: $P_{induced} < 0.001$, $P_{spontaneous} = 0.262$), snort length (seconds; b: $P_{induced} = 0.004$, $P_{spontaneous} = 0.484$) and number of pulses per snort (c: $P_{induced} = 0.021$, $P_{spontaneous} = 0.310$). Gray areas denote 95% confidence intervals. Levels of testosterone were standardized by year.

Table 2

The effect (β , $F_{(1,df)}$, P) of male residency status (M_{RS}), singing context (M_{SC}), number of pulses per snort (N_{Pulses}), body weight (BW), and long-term testosterone levels (T; standardized by year) on snort shimmer, snort jitter, mean snort amplitude, and mean interval duration between pulses. Effect size is presented as total effect.

Model	Estimate	F	df	P	Total effect
Shimmer					
M_{RS}	0.046	0.1	15	0.769	0.005
M_{SC}	0.122	2.2	138	0.129	0.039
T	0.179	1.5	24	0.191	0.141
$M_{RS} * T$	-0.007	0.0	24	0.956	
$M_{SC} * T$	-0.049	0.5	173	0.469	
N_{Pulses}	-0.052	35.2	259	< 0.001	0.762
BW	-0.412	1.2	30	0.217	0.061
Jitter					
M_{RS}	-2.9e-4	3.5	17	0.054	0.292
M_{SC}	6.4e-5	0.5	132	0.499	0.271
T	-6.2e-5	0.2	27	0.687	0.396
$M_{RS} * T$	-1.9e-4	1.8	28	0.172	
$M_{SC} * T$	-3.1e-4	14.5	171	0.001	
N_{Pulses}	-4.6e-5	19.7	259	< 0.001	0.418
BW	1.8e-4	0.2	28	0.649	0.009
Mean amplitude					
M_{RS}	-1.254	2.8	22	0.103	0.047
M_{SC}	-2.176	10.2	18	0.002	0.138
T	-0.385	0.2	38	0.705	0.012
$M_{RS} * T$	0.188	0.1	21	0.661	
$M_{SC} * T$	0.233	0.2	169	0.770	
N_{Pulses}	-0.483	30.4	40	< 0.001	0.791
BW	-2.112	1.2	2	0.508	0.021
Mean interval duration					
M_{RS}	-9.1e-4	2.7	22	0.076	0.168
M_{SC}	3.3e-4	1.6	205	0.183	0.050
T	-2.5e-4	0.2	33	0.623	0.057
$M_{RS} * T$	1.7e-4	0.1	30	0.709	
$M_{SC} * T$	-2.8e-4	1.5	223	0.212	
N_{Pulses}	-1.1e-4	18.1	255	< 0.001	0.302
BW	3.8e-3	8.6	71	0.002	0.474

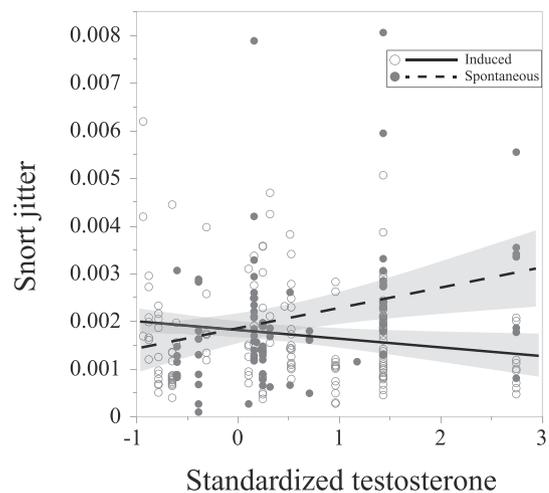


Fig. 3. The interaction between song context (i.e. of induced and spontaneous songs) and testosterone levels in snort jitter ($P_{induced} = 0.020$, $P_{spontaneous} = 0.005$). Gray areas denote 95% confidence intervals. Values of testosterone are standardized by year.

4. Discussion

4.1. Physiological effects

The combination of acoustic analysis with behavioral observations and long-term testosterone measurements, indicated that hyraxes with

higher testosterone levels might be more skilled singers. When songs were induced by events that alerted conspecifics, high testosterone singers demonstrated a superior performance: structurally, by increasing the number of singing bouts in each song; and acoustically, by increasing the duration of snorts. Moreover, longer snort duration is not achieved by performing slower snorts (i.e., exhaling less air) but, rather, by maintaining the typical pulse rate for an extended period of time. This enhanced performance may require larger lungs, or the ability to increase the inhaled air volume while controlling vocal stability. Thus, the ability to sing more bouts and to elongate snorts may be due to the physical attributes that are influenced by testosterone such as body size (Cox et al., 2009), rib cage size, or the direct effect of testosterone on the larynx itself (Beckford et al., 1985), through its androgen receptors (Saez and Sakai, 1976). The enhanced performance seen in induced songs may be due to an increased effort made by these males to advertise themselves in situations in which conspecifics are more alert and will pay more attention to a message that might otherwise have gone unnoticed (Demartsev et al., 2014; Ilany et al., 2011). Hence, it seems that even for hyraxes possessing the physical capabilities to sing higher-quality songs, doing so is costly and perhaps not wasted on spontaneous songs.

4.2. Behavioral-motivational effects

An alternative but not mutually exclusive explanation may be that the performance of longer elements and songs is due to the higher motivation for courtship and aggressive behavior associated with testosterone (Charlton et al., 2011b), as high arousal was shown to induce the production of longer and more numerous vocal elements (Rendall, 2003). Furthermore, we detected these effects solely in induced songs that were emitted in situations of high excitement. In these situations, the behavioral-motivational effects of testosterone may be more strongly expressed due to higher arousal. Thus, the enhanced performance displayed in induced songs may not be due to an advertisement effort but, rather, by a situation of higher behavioral motivation, whether the context of singing is that of conspecific aggression or female courtship behavior. Interestingly, an opposite pattern was found in the green tree frog (*Hyla cinerea*, Burmeister and Wilczynski, 2001), in which higher testosterone levels stimulated males to call spontaneously, whereas no effect of testosterone was seen in induced songs. Perhaps all frogs have the capability to produce higher-quality calls, but only individuals with high testosterone have the stamina to make the effort in spontaneous calls. These findings are supported by the effects of androgens observed in males of various vertebrates, which affect length of elements and singing duration, rate of singing, and the acoustic frequency of calls (e.g., gray partridge, *Perdix perdix*, Beani et al., 1995; red-winged blackbird, *Agelaius phoeniceus*, Johnsen, 1998; canary, Alward et al., 2017a, 2017b; Alston's singing mouse, *Scotinomys teguina*, Pasch et al., 2011; chimpanzee, *Pan troglodytes*, Fedurek et al., 2016; giant panda, Charlton et al., 2011b).

Regarding acoustic harshness, we found that jitter increased with elevated testosterone in spontaneous songs but decreased in induced songs. Acoustic harshness in vocalizations can range from nonlinear phenomena, in which there is no detectible frequency or amplitude trend in the sound wave, to slight harshness or roughness due to frequency or amplitude instability in a linear wave form (Titze, 2000). Given that hyraxes with elevated testosterone attempt, have the ability, or are strongly motivated to perform higher-quality songs in induced singing, it is not surprising to find that individuals with higher testosterone levels demonstrated higher vocal control in these songs. However, such an explanation does not fit our findings for spontaneous songs in which higher testosterone seemingly caused vocal instability. These apparently contradicting results lead us to speculate regarding alternative explanations that may uncover the underlying mechanism behind these phenomena.

4.3. Emotional effects

We previously noted two effects of testosterone on call acoustics: the physiological and the motivational. Here we suggest a third type of effect, the emotional-arousal aspect. We theorize that the higher aggressive arousal of hyraxes with higher testosterone levels may be reflected in snorts as high jitter, as the emotional state of a vocal performer may affect the acoustics of its vocalization. Morton (1977) contended that across taxa, calls in appeasing circumstances tend to be high and tonal, whereas typically aggressive calls are low and harsh. Although widely debated and criticized, these rules present a trend found in acoustic repertoires of many mammals (August and Anderson, 1987) and birds (e.g., the African penguin, *Spheniscus demersus*; Favaro et al., 2014). Moreover, vocal harshness increases in intensity and rate with arousal. For example, the fraction of non-linear phenomena in meerkat (*Suricata suricatta*) alarm calls rises with the level of urgency (Manser, 2001). Levels of jitter in African elephants (*Loxodonta africana*), rhesus macaques (*Macaca mulatta*), and human vocalizations are good predictors of the intensity of emotional arousal (Fuller et al., 1992; Li et al., 2007; Soltis et al., 2005). As vocalizations are controlled by neural mechanisms linked to the autonomous nervous system, situations of high arousal result in a reduction in vocal control, resulting in harsh-sounding calls (the polyvagal theory; Porges, 2001). Thus, the acoustic qualities of a call can serve as a reliable signal of the emotional state of the caller.

Given that the snort is a low and harsh sound, while other song elements are tonal and high, in accordance with Morton's motivational-structural rules, it is likely that the snort in itself expresses aggression and that this aggression is reflected acoustically as high jitter in snorts. Therefore, hyraxes with higher testosterone, who are in a higher aggressive-arousal state, use harsher snorts in spontaneous songs. The correlation between snort harshness and testosterone in spontaneous songs also fits our previous findings that the snort element is related to social status (Koren and Geffen, 2009a; Weissman et al., 2019) and that the snorts of higher ranked males have higher jitter (Weissman et al., 2019). We have also shown previously that higher ranked male hyraxes have higher testosterone levels (Koren et al., 2002; Koren and Geffen, 2009b). Since hierarchy is often determined by agonistic interactions (as in our system, using David's score (Gammell et al., 2003)), higher-ranked males are expected to have higher testosterone levels (Mazur and Booth, 1998). The triangular connection between testosterone, aggressive behavior, and social status is well established (reviewed by Eisenegger et al., 2011), as documented in fish (e.g. *Astatotilapia burtoni*, Maruska and Fernald, 2018), birds (e.g. potted antbirds *Hylophylax naevioides*, Hau et al., 2000; reviewed by Soma, 2006), and mammals (e.g., rats, Bernhardt, 1997; African wild dogs, *Lycaon pictus*, Creel et al., 1997; olive baboons *Papio anubis*, Sapolsky, 1982).

In line with our results on snort length and the number of bouts, we have explained that jitter decreases as testosterone levels increase in induced songs, as part of an overall elevated performance, advertising vocal control ability. It is also possible, however, that the source of the major vocal instability in induced songs is fear rather than aggression. Vocal harshness is not limited to aggressive calls but is found in a range of calls sounded in situations of high inner arousal (e.g., anxiety, fear, anger, happiness) in animals (Blumstein et al., 2008; Owren and Rendall, 2001) and humans (Whiteside, 1998). Answering a conspecific's song means engaging in a vocal confrontation, with the potential to escalate into a physical combat. High levels of testosterone are related to risk-taking (Carney et al., 2010; Ronay and Von Hippel, 2010; Stanton et al., 2011) and competitive behavior (Carré and McCormick, 2008; Zielinski and Vandenberg, 1993). Therefore, individuals with elevated testosterone levels may be less fearful of a confrontation. The high levels of jitter found in the induced snorts of low testosterone males may reflect their state of fear or excitement when they confront a conspecific. Morgan et al. (2004) suggested that hormones may affect behavior differently depending on context of action or fear, thus

optimizing the animal's response to given situations. Our suggested mechanism for context dependent effect of testosterone on snort harshness, may provide a plausible explanation for such a process.

Our results indicate that calls sounded in situations of high arousal share a similar acoustic structure, whether they are reflecting states of aggression or fear. Therefore, in order to effectively assess the acoustic data, listening conspecifics must also rely on external cues for contextual information. Recent findings show a similar phenomenon in humans vocalizations, where calls of intense fear are non-distinguishable from calls of intense happiness, when external contextual data is not available (Atias et al., 2018). Comparably, our results show that testosterone levels affect snort acoustics differently between residents and bachelors, and therefore listeners would have to consider the signaler's status when decoding the acoustic information. Hyraxes likely compile multiple signals, to form a cognitive impression of the singing that they hear (Koren and Geffen, 2009a). Since individual identity is also communicated through hyrax songs (Koren and Geffen, 2011), we cannot rule out that the social status and other singer attributes are known to most of the listeners. This is especially true for a relatively isolated population like that of Ein Gedi.

Previously, non-human mammalian androgen levels were associated with physiological and motivational aspects of vocalizations, reflected in vocal pitch, call length, and call frequency (e.g., Charlton et al., 2011b; Fedurek et al., 2016). However, androgen levels have not been linked to emotional aspects, or detected in vocal harshness. To the best of our knowledge, our results are the first to show that information regarding an individual's androgen levels is encoded via the acoustic harshness of its vocalizations. Hyraxes react to snort length and harshness in playback experiments (Weissman et al., 2019). It is possible that, listening conspecifics, both potential rival males and mates, can associate acoustic factors with the collective effects of testosterone on physiology, behavior, and emotional arousal, including reproductive quality, body size, fighting motivation, and fear.

Charlton et al. (2011b) found that in male pandas with higher testosterone levels, bleats had higher frequency modulation rates. This has been hypothesized to be caused by high collagen\elastin ratios that are mediated by testosterone (Fischer and Swain, 1980), resulting in stiffened vocal-fold ligaments, and causing vocal instability. Despite the possible explanation of high hyrax snort jitter in spontaneous songs being caused by high collagen\elastin ratios, this hypothesis is contradicted by the stabilizing effect of high testosterone that we observed in induced songs. In line with our hypothesis, an alternative explanation for Charlton's results could be that vocal instability in panda bleats is caused by high testosterone via an emotional pathway.

4.4. Synthesis

Testosterone has a wide array of influences that can be divided into three categories: **physiological aspects** such as laryngeal and body growth; **motivational aspects** such as courtship and risk-taking behaviors; and **emotional aspects** such as fearful or aggressive arousal. In this study, we found that these three aspects may all be expressed in hyrax songs. **Physiological effects** increase acoustic abilities, allowing hyraxes to maintain high quality snort structures (i.e., pulse density) for a longer period of time. **Behavioral-motivational effects** induce hyraxes to express their ability to produce high-quality snorts, and to add more bouts to each song. **Emotional effects**, expressed as acoustic harshness, are high in both aggressive high-testosterone individuals in spontaneous songs, and in fearful low-testosterone individuals in induced songs. Our findings demonstrate the multifaceted effects of testosterone, all expressed in the complex acoustics of a single element in the rock hyrax song, transmitting important information on androgen levels to conspecifics via multiple parallel pathways.

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Declaration of Competing Interest

The authors declare no competing interests.

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