Dung beetle distress signals may be correlated with sex and male morph: a case study on Copris lunaris (Coleoptera:Scarabaeidae, Coprini)

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Dung eetle Distress Signals May Be Correlated with Sex and Male Morph: the Case Study on

Copris lunaris **(Coleoptera: Scarabaeidae, Coprini)**

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Abstract

Insect sounds are predominantly produced by stridulations, where specialized body parts contact repeatedly to induce acoustic pulse trains. We studied the stridulatory organ and sound emissions in *Copris lunaris*, by focusing separately on females, major and minor males. Results highlighted an isometric growth of *pars stridens* in response to body size, and identified a wing-pygidium locking structure that assists sound emission. Sex-specific acoustic differences of some degree were detected between major males and females, as sounds emitted by majors showed higher frequencies and shorter pulses with elevated impulse rates. This cannot be immediately explained by size differences in the components of stridulatory apparatus. Rather, divergence might be an indicator of some underlying behavioural difference in response to distressing events. In minor males, acoustic properties overlapped with both females and major males, although incomplete stridulations had a lower ratio in minor than major males. This paper provides the very first perspective of the potential role of sex and male polymorphism on sound production. However, future categorisations of sounds coupled to behavioural observations of specific interactions are needed to reveal the function of sex and morph-specific differences.

Keywords: Bioacoustics, dung beetles; male dimorphism; elytro-abdominal stridulatory organ; *pars stridens*; *plectrum*

Introduction

Acoustic communication has been studied quite extensively in insects, as they have unique adaptations for sound production that are achieved by their sclerotized and segmented bodies (Virant-Doberlet and Cokl 2004; Cocroft and Rodriguez 2005; Hedwig 2014; Greenfield 2016; Pollack et al. 2016). Stridulation is the most widespread mechanism for generating sound in insect groups (Alexander 1957; Claridge 2005), where repeated contact and rubbing activity of a mobile scraper (*plectrum*) and a fixed file-like structure (*pars stridens*) create a series of pulse trains within a certain frequency range (Alexander et al. 1963; Ewing 1989; Hall et al. 2015). Beetles have a diverse set and location of stridulatory structures that are utilized to produce sound (Alexander et al. 1963; Wessel 2005). For example, the location of *pars stridens* can be cephalic (Cane et al. 1990), thoracic (Finn et al. 1972), elytral (Grant et al. 2014), or can be found on the hind legs (Carisio et al. 2004). These basic types exhibit further variations and nuances in their structural and morphological combinations, forming up to 20 distinct stridulatory organs found to date in beetles (Wessel 2005). In dung beetles the *pars stridens* can be located on the ventral surface of the elytra, forming an elytro-abdominal structure (Palestrini et al. 1991, 1998) or near or on the hindwings, forming an abdomino-alary type (Hirschberger and Rohrseitz 1995; Kasper and Hirschberger 2005a, 2005b). In some Geotrupidae species, more than one type of pars stridens can be found on a single individual, both on thorax and on the hindlegs (Palestrini and Zunino 1987; Palestrini et al. 1988; Palestrini and Pavan 1995). Nevertheless, only the coxo-abdominal stridulatory organ has been documented to function as stridulatory files during sound production (Palestrini and Pavan 1995; Carisio et al. 2004).

The morphological diversity of the stridulatory organ can explain some of the interspecific variation in the sound production of distinct beetle groups (Kasper and Hirschberger 2005b; Carisio et al. 2004). Kasper and Hirschberger (2005) noticed that in Aphodiidae the frequencies of stridulations are likely to be directly related to the density of the ridges on plectrum, where densely packed ridges causing pulse trains with higher frequencies. In addition, they surmised that the number of

stridulatory pulses per unit time is expected to be higher for smaller individuals, since individuals with small body sizes tend to support shorter abdominal region that could be moved back and forth at higher frequencies without any difficulty. Interestingly, these results match with what was reported in Mexican pine beetles (Yturralde and Hofstetter 2015). When Carisio et al. (2004) investigated the sound production mechanism of three *Trypocopris* species, they found that an increase in the length of *pars stridens* located on the coxa was associated with longer pulses within a pulse train. Furthermore, the subpulse rate had a negative relationship with the density of crests found along the *pars stridens*.

In beetles, acoustic properties of stridulations might vary even within the same species in response to particular functional needs such as male-female interactions and courtship behaviour (Lewis and Cane 1992; Kasper and Hirschberger 2005a; Moeseneder and Cook 2014; Yturralde and Hofstetter 2015), startle display against predators or distress (Masters 1979; Lewis and Cane 1990; Palestini et al. 2003; Panneton et al. 2005), intraspecific competition (Ryker and Rudinsky 1976; West-Eberhard 1984; Fleming et al. 2013), or mother-offspring communication (Klemperer 1982a, 1982b; Palestrini et al. 1990; Halffter et al. 1996).

In cases where stridulations are given in response to physical disturbance, beetles are known to generate bursts of tooth-strike pulses with distinct but repeated subunits (Masters 1980). In Geotrupidae there is some evidence for sex-specific differences in sound production under stress (Palestrini et al. 1988; Carisio et al. 2004). Palestrini et al. (1988) found that the males of the species *Geotrupes stercorarius* (Linnaeus, 1758) exhibit higher repetition rates in pulse trains than females, while in *Trypocopris pyrenaeus* (Charpentier, 1825) and *Anoplotrupes stercorosus* (Scriba, 1791) the length of the second subunit was found to be different between sexes (longer and shorter, respectively). Carisio et al. (2004) corroborated these findings for *T. pyrenaeus* and *T. vernalis* (Linnaeus, 1758). Another difference has been observed in the courtship behaviour of Aphodiidae where males produce stridulations to advertise their qualities as potential mates (Hirschberger 2001; Kasper and Herschberger 2005a). Females only copulate with males that produce complex

courtship calls with high repetition rate, potentially reflecting the female choice for honest indicators of male quality through acoustic signals (Hirschberger 2001; Kasper and Hirschberger 2005a).

In comparison to other taxa, very few studies have explored sex-specific contrast in the production of stridulations in Coprini, as *Copris incertus* Say, 1835 (Palestrini et al. 1991) and *C. hispanus* (Linnaeus, 1764) (Palestrini et al. 2000), but neither of those studies found a marked difference between sexes in their distress stridulations.

In addition to sex-specific expression of acoustic signals, one interesting aspect that requires further attention is the potential role of male polymorphism, especially on the expression of distress calls. Male dimorphism is relatively common in insect, and it is known (among others) also in some insect taxa which exihibit complex sound communication as the Orthoptera (Kelly 2005; Kelly and Adams 2010). However, several of the best-known example of male dimorphism are those found in Scarabaeidae genera such as *Onthophagus* (Emlen et al. 2005; McCullough and Simmons 2016) and *Copris* (Sugiura et al. 2007; Akamine 2016, 2019; Kerman et al. 2018), where some males have large bodies with long and sturdy horns (i.e. majors), while others possessing smaller body sizes with short, highly diminished horn structures (i.e. minors). Horn dimorphism is usually correlated with the type of reproductive strategy implemented by males to gain access to fertile females or crucial resources such as dung pats (Moczek 2009a, 2009b). Dung beetles majors tend to defend the nest entrances against other intruding males, acting as "guards", and minors can use sneaking tactics to avoid majors guarding the tunnels leading to key reproductive resources (Moczek and Emlen 2000; Simmons et al. 2007).

Stridulations are usually observed during intraspecific contests, where females defend their nests against intruders that are experimentally introduced into their nesting tunnels or placed near their brood balls (Klemperer 1982b, 1986; Halffter et al. 1996). Nevertheless, Klemperer (1984) hints that in the Geotrupidae *Ceratophyus hoffmannseggi* (Fairmaire, 1856) males too can produce

stridulations when fighting with intruding males. This observation suggests that males can be using stridulations as much as females do during their nest guarding behaviour.

Here we focused on the effect of sex and male polymorphism on sound production, by reporting morphological measurements of the stridulatory organ and acoustic analysis of distress calls in a horn dimorphic species, *Copris lunaris* (Linnaeus, 1758). In the genus *Copris*, male dimorphism has been described in more than one species (Kerman et al. 2018), and defensive stridulations are known to occur frequently in semi-natural contexts (Klemperer 1982b, 1986). Although Klemperer (1982b, 1986) notes the presence of stridulations in *Copris* in experimentally monitored nesting chambers, detailed acoustic examinations regarding the structural properties of these calls are only available for *C. hispanus* and *C. incertus* (Palestrini et al. 1991, 2000). Moreover, these aforementioned studies did not consider the role of male dimorphism as a potential factor in the differential expression of distress signals. Initially, we measured the length of toothed rows along *pars stridens* as a correlate of the general sound production organ (Carisio et al. 2004), then compared its scaling relationship with body size across male morphs and sexes. Following that, we investigated any intraspecific differences that would occur in our selected list of acoustic parameters.

Materials and Methods

Material collection

We collected several individuals of *C. lunaris* (N = 20; 11 females and 9 males) in Northwest Italy (Torino, Piedmont) during the spring and summer of 2015. We transferred the dung beetles to the vivarium facilities of the Department of Life Sciences and Systems Biology (DBIOS) at the University of Torino. Individuals were housed solitarily in plastic terrariums (diameter 25 cm) at a constant temperature (20°C) and humidity (40%) for the entire reproductive season.

Sound recordings

For sound recordings, we used a subset of the collected individuals excluding 3 poorly reactive males (N = 17; 11 females and 6 males), and placed them inside a waiting arena in the recording room. We randomly selected individuals from the arena, and captured their distress calls one at a time. We recorded the stridulations inside an anechoic chamber at a constant temperature (20°C) using a sound level meter (Brüel & Kjær model 2235) connected directly to a computer. Individuals were held 1 cm away from the sound level meter with either pliers or by hand. The prepolarized condenser microphone was calibrated with a 1000 Hz sound produced by a Brüel & Kjær 4230 acoustic calibrator. Sampling rate of the sound recording instrument was set to 44.1kHz/16 bit, which was suitable to detect stridulations in *Copris* (Palestrini et al. 1991). In addition, we videotaped (Sony Digital HD Video Camera Recorder HDR-CX240E) some of the individuals during sound emissions for an accurate depiction of their movement patterns during stridulations (see supplemental material, movie S1).

We implemented the sound analysis software Avisoft-SAS Lab Pro v5.2.13 (2019) to identify and categorize the sound recordings. All emitted sounds used in our analysis are quantifiable as acoustic distress signals (Palestrini et al. 1991), which consist of pulse trains with high repetition rates. Each complete stridulation is composed of two subunits of opposite phases with harmonic structure, where subunit A is emitted by the scraping of pars stridens against the plectrum during the extension of the abdomen, and subunit B when the abdomen is constricted back to its original position. For each individual, we randomly selected at least six (up to 20) complete stridulations with high acoustic quality and low background noise to capture four temporal, two structural, and four spectral parameters to be used in the statistical analyses (Palestrini et al. 1991; Carisio et al. 2004, see Table 1 for abbreviations, descriptions, and units). Finally, in addition to the complete stridulations described above, we surveyed the sound emissions to detect the partial stridulations (i.e., only subunit A or B) for each individual.

Morphological data acquisition and measurements

We collected morphological data in the geometric morphometrics laboratory at DBIOS. We captured the images of head, pronotum, and stridulatory organ by the software LAS-Leica Application Suite (Leica Microsystems AG, Wetzlar, Germany), using a Leica® DMC4500 digital camera connected to a stereoscopic dissecting scope Leica® Z16APO.

We obtained the measures from head and pronotum: maximum pronotum width (*proW*), and cephalic and pronotal horn lengths (*cHL* and *pHL*, respectively). We used *proW* as a reliable approximation for body size (Knell 2009). We defined male morphs based on the switchpoint value reported in Kerman et al. (2018): males with *proW* higher than the switchpoint value were considered majors while the opposite was true for minors (Akamine 2019). Horn lengths (*cHL* and *pHL*) were used to corroborate the assignment of males to each morph. Based on that information, we separated individuals into three groups (major males, minor males, and females), and used this terminology consistently. Finally, we measured the length of the toothed rows (Carisio et al. 2004) at 20 diffenent points along the *pars stridens* (*rPS* from now on) of each individual, and used the mean values in the statistical analyses.

We applied the microCT scanning techniques to perform a detailed visual, non-invasive survey of the stridulatory organ and elytral structure in a three dimensional space (see supplemental material, movie S2). The scans were performed by the Bruker® SkyScan 1174 (Bruker microCT, Kontich, Belgium), using the Bruker SkyScan software series for data acquisition and reconstruction applying the same parameters as in Kerman et al. (2018).

Statistical analyses

We investigated the presence of *pars stridens* allometry within each sex by calculating the scaling relationship between *rPS* and *proW* for males and females separately. We applied the Akaike Information Criterion (AIC) to determine the model that best describes the scaling relationship by

using the software PAST v3.22 (Hammer et al. 2001). Details of this allometric analysis can be found in Kerman et al. (2018). We then compared *rPS* and *proW* values across groups using the Kruskal–Wallis test. Pairwise comparisons among groups were achieved by using the Dunn's multiple comparison test (Dunn 1964). We implemented the Holm-Bonferroni method to obtain adjusted p-values for pairwise comparisons (Holm 1979). We investigated the influence of *rPS* on acoustic parameters by averaging parameter values over multiple measurements from each individual, and correlating them with individual *rPS* scores using Spearman's correlation method. In order to document the potential effect of categories on acoustic parameters, we ran separate linear mixed effect models for each acoustic parameter where categories were factored as the predictor variable while individual identity was treated as the random effect. We checked the normality of our data through Shapiro normality tests and Q-Q plots (Shapiro and Wilk 1965), and the homogeneity of variance through Levene's test of homogeneity (Levene 1960). For models that showed significant violations from these assumptions, we used generalized linear mixed effect models (GLMMs) with appropriate link function for the error distribution. We used analysis of deviance with Wald test to assess the overall effect of categories in each model. In the case of a significant difference between categories, we implemented the least squares method on back-transformed data to detect pairwise differences among coefficients with post-hoc adjustments (see Table 1). P-value adjustments for post-hoc pairwise comparisons were accomplished by Tukey method.

After identifying partial stridulations, we calculated the ratio of partial stridulations to the complete ones for individual, and tested whether this ratio differed between categories using the Kruskal-Wallis test. We used the aforementioned method for post-hoc pairwise comparisons.

All statistical analyses were performed in R 3.4.3. (R Core Studio, 2017). We used the package 'nlme' for linear mixed effect models (Pinheiro et al. 2017), package 'lme4' for mixed effect models (Bates et al. 2015), and package 'lsmeans' for pairwise comparisons (Lenth 2018).

Results

Description of the stridulatory organ

The *pars stridens* (Figure 1a) was positioned on the distal third of the inner surface of both elytra, with the right part usually covered by the left one when the two elytra were joined (see supplemental material – movie S2). It consisted of a symmetrical, narrow, thickened area near the elytral margin (the juxta-sutural edge). This area was constituted by a series of distinct rows (Figure 1b-c) showing no difference in its structural feature across categories. The *plectrum* was located on the 6th tergite (Figure 2), which was covered entirely by thick rows of small teeth in the central part (Figure 2a), and many small fringes of short, thickened setae extending on both sides (Figure 2, marked by an arrow). A thickened, well-sclerotized, very narrow area was present on the proximal margin. The other tergites were smooth, except for a small sclerotized area in the central part, which was always less developed than that on the 6th tergite (Figure 3). On the proximal part of the pygidium (Figure 3b), a triangular-shaped groove was present, matching with two concave parts on the elytra (Figure 3a). The structures together constituted a sort of locking mechanism which likely contributed to the joining of the stridulatory parts. For the hindwing, we identified a large, concave area on the basal side of each elytron (Figure 4). This area housed the dorsal, sclerotized part (near the apical hinge) of the hindwings. So, the lateral position of the folded hindwing at rest freed the central portion of the tergites (Figure 4), where the *plectrum* is placed.

Scaling relationship and size of pars stridens

We identified 4 major males and 5 minor males based on the body measurements (see the supplemental material, Table S1). Similar to what was reported in Kerman et al. (2018), minor males were smaller than majors ($\chi2 = 3.22$; $p = 0.004$) and females ($\chi2 = 2.48$; $p = 0.03$), while no significant difference was observed between majors and females ($\chi2 = -1.41$; $p = 0.16$).

When examining the stridulatory organ, the scaling relationship produced by the linear model ($AIC_M = 6.83$ and $AIC_F = 6.42$) showed a better fit than the Hill's sigmoidal curve ($AIC_M = 18.48$ and $AIC_F = 15.22$), indicating the presence of an isometric growth for the *pars stridens* in response to body size in both males and females. This linear relationship was further supported by a highly significant correlation between *rPS* and *proW* (Spearman's $rho = 0.83$, $p < 0.01$).

Description of distress calls

The distress signals of *C. lunari*s share the same characters already described for other dung beetle species: the complete stridulation (*sAB*) is composed of two opposite phases with harmonic structure, where subunit A is always followed by subunit B (*sA* and *sB*, respectively; see Figure 5). Aside from the complete stridulations, we detected two types of partial stridulations that missed one of the subunits; stridulations that were composed only of *sA*, and stridulations that were composed only of *sB*. Since *sB* was either none existent or exhibited very low rates of occurrence in most individuals (see supplemental material, Table S2), we decided to exclude *sB* from the final analysis. We found a strong relationship between the frequencies of *sA* and *sAB* when all the categories combined (Spearman's $rho = -0.62$, $p = 0.01$). Furthermore, partial stridulation ratios showed a significant difference across male morphs ($\chi^2 = 6.05$; $p = 0.04$). Major males had higher ratio of partial stridulation than minor males ($z = 2.46$; $p = 0.04$). Females, on the other hand, did not reveal any significant difference from neither majors ($\chi^2 = -1.30$; $p = 0.19$) nor minors ($\chi^2 = 1.78$; $p = 0.15$).

Acoustic parameters

We used GLMMs since acoustic parameters violated the assumptions required for linear models (Tables 1 and 2). Considering the temporal parameters, we found a significant contrast between categories for the parameters *dA* ($\chi^2 = 6.29$; $p = 0.04$), *dB* ($\chi^2 = 7.56$; $p = 0.02$), and *dAB* ($\chi^2 = 7.66$; $p = 0.02$). Major males had distress calls with shorter duration than females whereas minors exhibited considerable variation, and were not significantly different from both majors and females (Tables 2

and 3). We failed to observe the same effect for the remaining temporal parameter, iAB ($\chi^2 = 0.86$; $p = 0.65$).

In terms of structural parameters, we detected a significant influence of groups on pA ($\chi^2 = 6.07$; $p = 0.04$). Major males had higher impulse rates for subunit A than females, but they did not differ from minors (Table 3). Akin to temporal parameters described above, impulse rates of minors also shown considerable overlap with those of the remaining groups (Table 3). As for pB, however, the influence of groups was insignificant ($\chi^2 = 5.22$; $p = 0.07$).

Among the spectral parameters, only the frequencies described by FF_A ($\chi^2 = 16.19$; $p = 0.0003$) and FF_B ($\chi^2 = 10.59$; $p = 0.005$) were significantly different across categories (Table 3). For FF_A, females had lower frequency values than major males while no difference was documented in other pairwise comparison (Table 3). For FF_B, majors had lower frequency values than minor males (Table 3) whereas no significant divergence was observed for the remaining pairwise comparisons. We did not observe any differences between categories in the remaining spectral parameters PF_A ($\chi^2 = 1.25$; $p = 0.54$) and PF_B ($\chi^2 = 3.18$; $p = 0.20$).

Acoustic parameters and pars stridens

We found a negative relationship between sAB and rPS (Spearman's $rho = -0.51$, $p = 0.03$) while the remaining acoustic parameters failed to show such significant trends. Furthermore, we detected a positive correlation between the ratio of partial to complete stridulations and rPS (Spearman's $rho = 0.56$, $p = 0.02$).

Discussion

In this research we studied stridulatory organs and sound emissions in the subsocial dung beetle species *Copris lunaris.* In a previous paper we evidenced a clear male dimorphism in this species,

which gives rise to major and minor males (Kerman et al. 2018). Therefore, analyses of sound emissions were separately carried out for females, major and minor males. In the following discussion we first focused on the stridulatory organ and acoustic characteristics in a comparative framework, then we concentrated on the potential behavioural implications of acoustic differences between sexes and male morphs.

Stridulatory organ and acoustic profile

The elytro-abdominal stridulatory organ of *C. lunaris* (consisting in the *pars stridens* positioned on the surface of both elytra and in the *plectrum* located on the 6th tergite) structurally resembles to those of other congeneric species (Palestrini et al. 1991, 2000). We highlighted an isometric growth for the *pars stridens* in response to body size, indicating that minor males have a smaller organ just because of their smaller body size.

We also described a wing-pygidium locking structure that assists sound emission. It consists in a large, concave area on the basal side of each elytron (as for the wing), and in the triangular-shaped groove that matches with two concave parts on the elytra (as for the pygidium).

Such structure was also observed in other *Copris* species (i.e. *C. hispanus* and *C. incertus*), although its locking function was not properly recognized (Palestrini et al. 1991, 2000). This stridulatory organ pattern is not exclusive of the genus *Copris* because it is shared with other insect species such as the rhinoceros beetle *Oryctes rhinoceros* (Linnaeus, 1758) (Mini and Prabhu 1990). In this species, the hindwings were not essential for stridulation but the sound was distinctly different without their presence, thus they could potentially act as a resonator. Even in *O. rhinoceros* there is evidence for a wing-locking mechanism to keep the elytra in the stridulatory position, in which the sound production areas do not overlap with each other (Mini and Prabhu 1990).

The acoustic profile of distress signals produced by the stridulatory organ in *C. lunaris* – in terms of its structural, spectral, and temporal characteristics – is also similar across the studied *Copris*

species (Palestrini et al. 1991; 2000). Nevertheless, the complete biphasic stridulations associated with distress calls are longer and higher-pitched in *C. lunaris* and *C. hispanus* than in *C. incertus*. Furthermore, *C. lunaris* and *C. hispanus* have similar levels of peak fundamental frequencies for complete stridulations, and these frequencies tend to be higher in the first subunit (in our terminology, subunit A). Since *C. hispanus* is somewhat larger than *C. lunaris*, while *C. lunaris* and *C. incertus* tend to be of similar dimensions, it is highly unlikely that these broad acoustic differences are a direct result of body size. However, the considerable overlap between *C. lunaris* and *C. hispanus* may depend on their close phylogenetic relationships as Palearctic species (Villalba et al. 2002; Monaghan et al. 2007). Since *C. incertus* is a Neotropical species (Palestrini e al. 1991; Darling and Génier 2018), it is likely to be more distantly related to the other two. Lastly, previous studies failed to document sex-specific differences in acoustic properties, potentially because male dimorphism was ignored in the sound analyses (Palestrini et al. 1991; 2000). It is quite plausible that dimorphic differences would emerge if the other two species were to be investigated with the same approach as we used in this study.

Sex and male morph modulation of acoustic signals

Our results suggest that there is some degree of sex-specific acoustic differences between major males and females in *C. lunaris*, as stridulations emitted by majors have higher frequencies, and are composed of shorter pulses with elevated impulse rates. This differential trend is unlikely to be explained by differences in the components of the stridulatory organ, because both major males and females exhibit similar body sizes and toothed row lengths. Since morphological differences in the stridulatory organs of major males and females are negligible, the observed acoustic divergence between them might be an indicator of some underlying behavioural difference in response to physical challenges and distressing events. Some dung beetles (Klemperer 1982b, 1984, 1986; Hirschberger and Rohrseitz 1995; Hirschberger 2001) are known to utilize stridulations under stress and/or against intruders. In his extensive study on the nesting behaviour of *C. lunaris* and *C.*

laeviceps Harold, 1869, Klemperer (1982b; 1986) reports acoustically recognizable stridulatory activity when resident females try to repel the experimentally introduced unfamiliar conspecifics away from their own nesting areas. In a previous pilot study, we observed nesting females to emit excited sound emission when their cocoons were experimentally removed. In short, female defensive behaviour in dung beetles are closely associated with stridulations.

Although this type of acoustic display has not been described in detail for male dung beetles (but see Klemperer 1984), the importance of stridulations in the context of same-sex territorial disputes and access to females have been demonstrated in several other insect groups such as bark beetles (Swedenborg et al. 1989; Lindeman and Yack 2015), passalid beetles (Schuster and Schuster 1985; Palestrini et al. 2003; Snell-Rood and Moczek 2013), burying beetles (Hall et al. 2013), pine beetles (Ryker and Rudinsky 1976), woodboring beetles (Breidbach 1986), weta (Field and Rind 1992; Kelly 2005; Kelly and Adam 2010), and field crickets (Hoffart et al. 2002). It might be hypothesised that major males of *C. lunaris* also employ a vigorous stridulation tactic to accompany their active blockage of tunnel entrances with their large bodies and well-developed horn structures (Klemperer 1983). The fact that majors tend to produce higher impulse rates, and exhibit higher call frequencies than females may suggest its role as honest signal (Hirschberger 2001; Kasper and Hirschberger 2005; Arriaga-Osnaya et al. 2017), by which competing males can reliably assess the physical quality of potential challenger before an actual physical fight. However, we also stress that in *C. lunaris* pair bond and cooperation between partners is very important for a successful reproduction (Klemperer 1982a, 1982b, 1983). Therefore, sound emission may also concurrently reflect the need of an optimal communication between males and females.

Minor males avoid direct competition with major males either by employing a sneaker tactic (Moczek and Emlen, 2000; Simmons et al. 2007; Moczek 2009a, 2009b; Buzatto et al. 2015), or by increasing their activity levels later in the breeding season when majors are less abundant and low in density (Akamine 2019). Hence, we should expect to see no functional need for minors to implement intense stridulations during intraspecific contests. This might explain why minors in *C.*

lunaris show considerable overlap both with females and majors (FF_B a part) in their acoustic properties and why major males are the ones that exhibit a significant divergence from females - potentially due to a history of directional selection on major males for stridulatory contests, as h highlighted in other taxa (Oh and Shaw 2013; Anichini et al. 2017).

Nevertheless, with our current evidence, we cannot discount the complementary effects of morphological differences between male morphs on the stridulatory output. For example, we found that the ratio of incomplete stridulations (*sA*) within a pulse train increases in parallel with body size, which explains why major males tend to have more incomplete stridulations than minor males. It is possible that longer *pars stridens* in major males causing the elytra to skip some sections of the toothed rows during the extension phase of the abdomen. Nevertheless, another acoustic property, the duration of pulses, which also exhibits strong correlation with body size as described earlier, failed to show a similar difference between male morphs. It is possible that the dimorphic contrast in the ratio of incomplete stridulations is behavioural, where major males, with their intense distress calls during an encounter with a similar-sized male, might also be producing more incomplete stridulations as they try to stridulate faster to deter intruders. Since sound production in male dung beetles was only assessed in mating and mate choice contexts, as in some Aphodiini species (Hirschberger 2001), this general impression needs to be confirmed with further acoustic studies.

In conclusion, our paper provides the very first perspective on the potential role of sex and male polymorphism in insect acoustic communication. Male dimorphism, and its link to alternative reproductive strategies, have been documented across many arthropod groups (Clark 1997; Moczek and Emlen 2000; Buzatto and Machado 2014), but to our knowledge, how acoustic communication might be associated with the expression and functional maintenance of different male morphs is yet to be explored in its detail and complexity. Future work on this and other dung beetle species, where careful categorisation of distress calls in combination with the actual behavioural

observations of conspecific interactions (e.g. nest defence and brood protection) should reveal more clues as to the main eco-ethological function of sex and morph-specific acoustic differences.

Acknowledgments

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Table 1. List of acoustic parameters obtained from complete stridulations. The abbreviation of the parameter type, description of the parameters, unit of measurement and error distribution (including the link function) used in GLMMs are reported. Parameters were derived from Palestrini et al. (1991) and Carisio et al. (2004).

Parameter	Parameter Type	Description	Unit	Error Distribution (Link Function)
dA	Temporal	Temporal length of subunit/pulse A	Millisecond	Gamma (inverse)
dB	Temporal	Temporal length of subunit/pulse B	Millisecond	Gamma (inverse)
iAB	Temporal	Temporal length of the interval between subunits/pulses A and B	Millisecond	Gamma (inverse)
dAB	Temporal	Temporal length of a complete acoustic set ($dA + dB + iAB$)	Millisecond	Gamma (inverse)
pA	Structural	Impulse rate in subunit/pulse A	Pulse/second	Poisson (log)
pB	Structural	Impulse rate in subunit/pulse B	Pulse/second	Poisson (log)
PF$_A$	Spectral	Peak frequency at the maximum parameter of subunit/pulse A	Hertz	Poisson (log)
FF$_A$	Spectral	Fundamental frequency at the maximum parameter of subunit/pulse A	Hertz	Poisson (log)
PF$_B$	Spectral	Peak frequency at the maximum parameter of subunit/pulse B	Hertz	Poisson (log)
FF$_B$	Spectral	Fundamental frequency at the maximum parameter of subunit/pulse B	Hertz	Poisson (log)

Table 2. Summary statistics. Mean and standard deviation of the acoustic parameters major males (4 individuals, Number of complete stridulations =58), minor males (2 individuals, N= 30), and females (11 individuals, N= 175).

	Major Males	Minor Males	Females
dA	92.11 ± 23.5	91.13 ± 6.19	108.12 ± 25.56
dB	102.58 ± 54.48	149.54 ± 19.84	132.92 ± 51.11
dAB	227.81 ± 71.74	262.65 ± 22.50	271.98 ± 66.45
iAB	33.21 ± 28.64	21.98 ± 11.68	30.94 ± 17.41
pA	1697.55 ± 581.38	1327.7 ± 178.51	1255.66 ± 325.47
pB	973.55 ± 238.29	839.23 ± 190.74	778.22 ± 233.16
PF$_a$	4632.76 ± 590.68	5396.67 ± 779.25	4825.14 ± 1127.27
FF$_A$	3334.48 ± 999.75	3693.33 ± 1177.35	2619.43 ± 983.70
PF$_B$	3303.45 ± 734.84	4126.67 ± 804.27	3377.14 ± 564.81
FF$_B$	$1194.83 + 884.85$	$1473.33 + 1025.51$	1426.86 ± 1049.42

Table 3. Pairwise comparison of model output for measures of acoustic parameters with significant differences among groups. Least-square method was used to calculate model estimates for each

contrast. Lower (Lower CL) and upper (Upper CL) confidence intervals were also reported. P-values were adjusted using Tukey method (* p<0.05, ** p<0.01, *** p<0.001, n.s. = not significant).

			Estimate	Lower CL	Upper CL	t	p-value
Females	Major males	dA	-3.10	-6.03	-0.16	-2.47	*
		dB	-10.59	-20.2	-0.94	-2.57	*
		dAB	-2.41	-4.54	-0.29	-2.66	*
		pA	0.74	0.55	0.99	-2.43	*
		FF$_A$	0.82	0.64	1.03	-2.01	n.s.
		FF$_B$	1.57	0.88	2.82	1.82	n.s.
Females	Minor males	dA	-1.74	-5.61	2.14	-1.05	n.s.
		dB	2.81	-11.6	17.19	0.46	n.s.
		dAB	0.21	-2.94	3.35	0.16	n.s.
		pA	0.86	0.58	1.27	-0.89	n.s.
		FF$_A$	0.61	0.45	0.83	-3.82	***
		FF$_B$	0.48	0.22	1.03	-2.27	n.s.
Major males	Minor males	dA	1.35	-2.89	5.59	0.75	n.s.
		dB	13.40	-2.28	29.09	2.00	n.s.
		dAB	2.62	-0.76	6.00	1.82	n.s.
		pA	1.17	0.76	1.82	0.84	n.s.
		FF$_A$	0.74	0.53	1.05	-2.04	n.s.
		FF$_B$	0.30	0.13	0.72	-3.24	**

Figure Legends

Figure 1. Stridulatory organ, *pars stridens*. (a) Distal third of both elytra, on ventral view. (b) Detailed view of the central portion of the elytra, with the medial rim. (c) A more detailed view evidencing the toothed rows of the pars stridens, which is placed along the elytral medial rim.

Figure 2. Stridulatory organ, *plectrum*. The 6th tergite carries thick rows on the whole surface (marked by an arrow on the right side). (a) Detailed view of the central area of the 6th tergite.

Figure 3. Stridulatory organ, locker structure between pygidium and elytra. The whole abdomen is shown in side view, after the right elytron was removed. (a) Detailed view of the terminal portion of the right elytron. (b) Detailed view of the basal part of the pygidium with the large groove.

Figure 4. Stridulatory organ, posterior view of the elytra and pygidium. (a) Rested position of the folded hindwing. (b) Ventral side of the elytron, with the concave area in which the hindwing is placed at rest marked by an asterisk.

Figure 5. Visualization of acoustic distress signals. (a) Oscillographic and (b) spectographic representation of a pulse train with complete emissions. Fast Fourier Transformation with Hamming window function was used on 256 temporal slices to avoid spectral distortions. (c) Each complete stridulation (sAB) was composed of two subunits/pulses: subunit A (sA) and subunit B (sB) with an interval between the two subunit (iAB). Each subunit/pulse is further composed of impulses as the most basic structure of a distress call.