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Responses of short-nosed fruit bat, *Cynopterus sphinx* (Vahl 1797) towards distress calls of their conspecifics from related and unrelated sites: implications for building a social relationship

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Distress calls emitted by bats signal their conspecifics either to warn them or inform them about the situations. Conspecifics may also get attracted towards distress calls as a behaviour of cooperative mobbing or just selfishly assessing the potential source of danger. The exact functions of distress calls in bats therefore vary to a great degree and are very hard to pinpoint. We conducted playback experiments to test the response of short-nosed fruit bat, *Cynopterus sphinx* towards the distress calls of their conspecifics from related and unrelated sites. Bats were attracted to their conspecifics from both related and unrelated sites and in one occasion towards fruit bat (*Rousettus leschenaulti*) of another genus within the same family. The response towards the opposite sex was significant in most of the playback trials and the reasons remain unclear. This symmetric response towards conspecifics from related and unrelated sites suggests the possibility of fruit bats building social relationships among unrelated individuals and probably between species.

Keywords: Chiroptera, *Cynopterus sphinx*, conspecifics, distress calls, social relationship.

SHORT-NOSED fruit bat (*Cynopterus sphinx*) is a non-echolocating, frugivorous bat that is very common in India and South East Asia¹. They usually roost in small groups of 3 to 6 individuals in so-called tents constructed typically by the harem males within the fronds of palm leaves or behind creeping vines². Morphologically they exhibit sexual dimorphism where males are larger than females and also characteristically orange tinted on the shoulders, sides of chest and thighs¹. *C. sphinx* emits characteristic audible distress vocalizations when they are entangled in mist nets to warn or inform their counterpart or conspecifics³. During such distress call emissions, the

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levels of neurotransmitters (serotonin, dopamine and norepinephrine) as well as the stress hormones (adrenocorticotropic hormone and corticosterone) are significantly elevated in the amygdala of call emitters⁴. Thus, distress calls in *C. sphinx* are emitted particularly in stressful situations. Although the physiology behind the emission of distress vocalizations is well explained in *C. sphinx*⁴, the exact functions of distress calls in the context of social behaviour are still not clear.

The acoustic features of distress calls of *C. sphinx* are remarkable in their spectral feature. It consists of long phrases of multi-harmonic, loud distress calls with irregular time structure³. Each unit of phrases consists of two phases, phases I and II that alternate with each other (Figure 1). Phase I may be defined as 'exhalation phase' with high sound pressure and phase II as 'inhalation phase' with low sound pressure, where the maximum energy is distributed over discrete bands of 7 kHz and concentrates at low-frequency ranges⁵. Recently, the syllable phases I and II were renamed as narrowband arched frequency modulation, nAFM and broadband arched frequency modulation, bAFM⁶. The temporal and spectral features of distress calls in *C. sphinx* differed between sexes in the parameters of peak frequency in phase I and duration of both phases⁵. These differences in the call parameters between sexes indicate that individual bats and their sex can be recognized by their distinct call features. Playback experiments in the laboratory have shown that *C. sphinx* elucidated limited response towards the 'modified distress calls' (the sequence order of phases I and II are interchanged) than the normal distress calls⁶.

Field studies clearly show that when *C. sphinx* was trapped in a mist net, they tried to escape and often produced distress calls³. These distress calls attracted other bats that were foraging in the close vicinity and proved that distress calls significantly attract a large number of bats to the capture site, than the mist netting trials without any distress sounds³. From our earlier observations we found that when males were entangled in the mist net, they produced distress calls and the first bat that was trapped after males was a female ($n = 45$). In order to validate these observations playback experiments were designed to investigate the response of bats towards the distress calls of their opposite sex. Furthermore, we also address whether playbacks of distress calls of bats from one foraging site evoke the response from bats of another foraging site. The aim of this study was: (i) to evaluate the response of fruit bat, *C. sphinx* towards the distress calls of their opposite sex; (ii) to predict as to what extent the response of fruit bats towards distress calls varied between their own conspecific (bats foraging in the same site) and unrelated conspecifics (bats from a different site).

The field study was carried out from August 2014 to March 2015 at two different sites in Madurai, one at the American College campus (lat 9.9297°N, long

78.1321°E) – site I and the other at the botanical garden of Madurai Kamaraj University campus (lat 9.9405°N, long 78.0105°E) – site II. These two sites were 15 km apart and playback experiments were done specifically during the non-breeding months of the year, September to November¹. 3♂ *C. sphinx* were captured from site II and the distress calls were recorded using a Philips SBC MD110 microphone (frequency response: 1–12 kHz \pm 3 dB) connected to Sony TCM-353V cassette recorder. The recordings from the cassette were digitized using BatSound ver. 2.0 and stored as .wav files. The intensity of distress calls was measured using the sound level meter (Model: SL-4001; Lutron, Taiwan). The natural calling intensity of distress calls recorded in the field ranges from 67.6 to 78.5 dB sound pressure level (SPL) and the mean corresponds to 73.3 \pm 4.0 dB SPL ($n = 5$ bats).

Fine sequences of distress calls were selected on the basis of the quality of sound recordings (signal to noise ratio). Playback trials in the field were carried out in site I and the response of bats was determined based on the number of bats caught in the mist net. A mist net (9 \times 2 m; Avinet-Dryden, USA) was placed near the regular flight paths of bats 30 min before sunset. A duration of 30 s of representative distress calls from a single male of site II (Figure 1 c) were played back at the natural calling intensity of *C. sphinx* using amplifier (Stranger Model C29, Stranger Audio Pvt Ltd, Kolkata, India). The speaker was placed near the mist net at a distance of about 1 m from ground level with the help of a stand to avoid atmospheric attenuation of the sounds during playback. A playback netting session comprises 5 h netting sampling with a playback of distress call stimuli between 18.30 and 23.30 h that correspond to the first peak foraging activities of *C. sphinx*⁷. A silent netting session also refers to the same, but without any sounds. In playback netting sessions, the 30 s distress calls were repeatedly played back for 1 to 3 min and repeated at the interval of 60 min, that provides 4 trials per day. The netting sessions were randomized at different locations of the site I for both playback netting sessions and silent netting sessions for 14 different days. The number of bats captured during playback netting sessions ($n = 7$ days) and silent netting sessions ($n = 7$ days) was compared. Since our assessment of bat's response towards distress calls was based on the number of bat captures in the mist net, it is necessary to do more number of frequent mist netting sampling with various kinds of distress call stimuli. It is well known that frequent netting may reduce capture effort of bats within a small study area⁸. For this reason, we restricted our playback experiment in the field with single male distress call stimulus. To address our main objectives, laboratory experiments were carried out with various distress sound stimuli.

2♂ and 3♀ *C. sphinx* were captured from the site I and the distress calls were recorded using the methods described above. Six bats (3♂ and 3♀) were captured

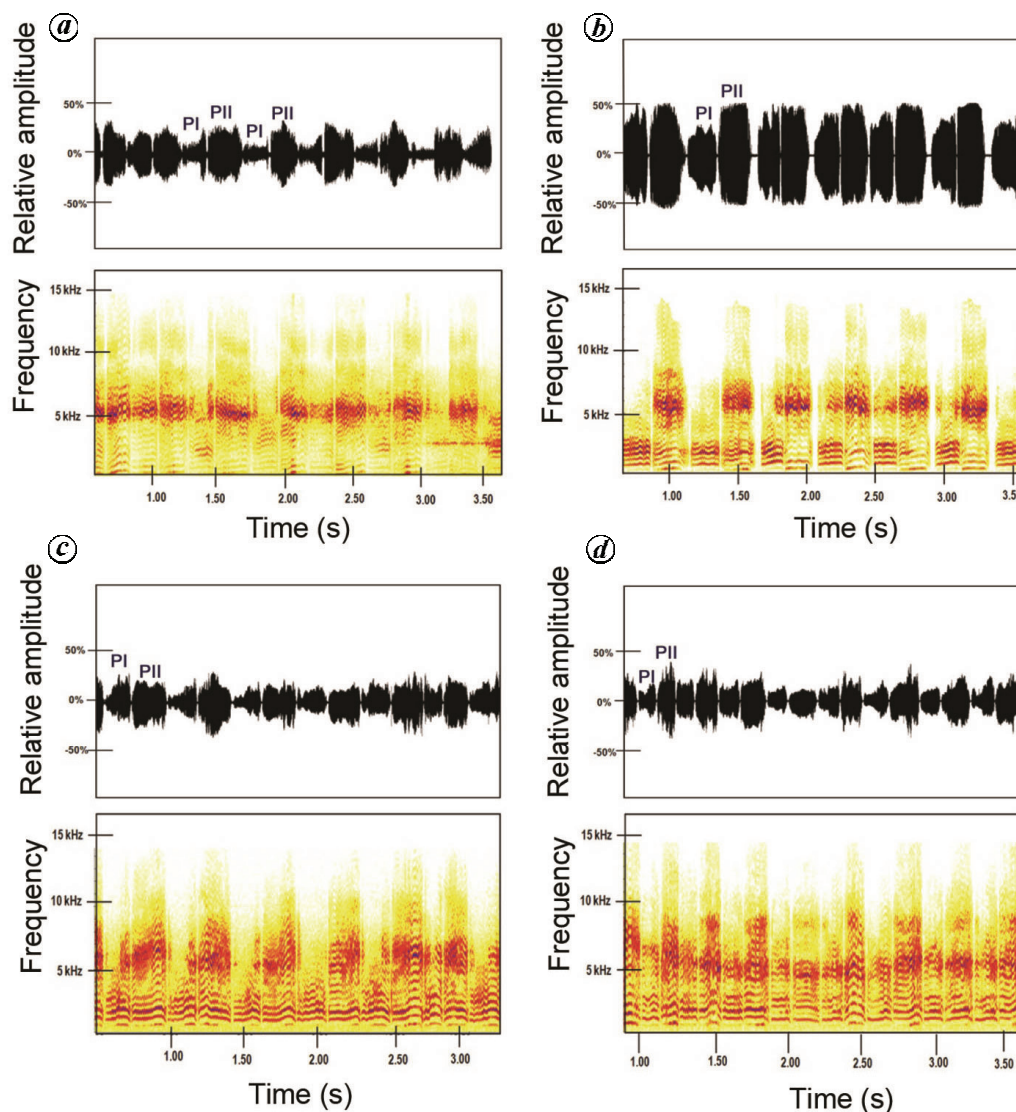


Figure 1. Representative spectrograms and relative amplitudes of *C. sphinx* distress sounds used as a playback stimulus. *a*, Site I male; *b*, Site I female; *c*, site II male and *d*, site II female. PI is the phase I and PII is phase II. Site II male (*e*) was also used as a playback stimulus in the field at site I. (Note: Only partial calls of about 3.5 sec were depicted here.)

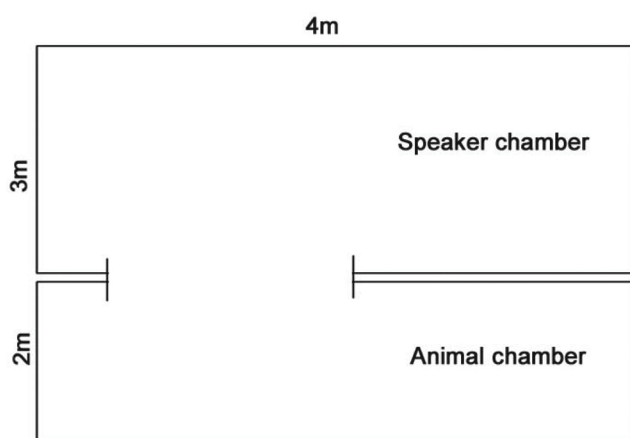


Figure 2. The flight room consisting of two chambers, one was the speaker room and the other has perch for the bats.

from site II and were kept in 1 × 1 m cages made of cotton mosquito net on iron frames. The bats were fed *ad libitum* with locally available fruit such as guava, sapota, papaya, grapes, banana, etc. They were allowed to acclimatize for two days before carrying out the laboratory playback experiments. The flight room consists of two chambers, one with a dimension of 2 × 4 m and the other of 3 × 4 m. The former was the ‘animal chamber’ where the bats were allowed to acclimatize and the latter was the ‘speaker chamber’ (Figure 2).

The playback experiment was conducted between 19.00 and 12.30 h and a single bat was tested per day. The playback stimuli included the four different distress calls of bats; site I male, site I female, site II male and site II female (Figure 1). The pulse consisted of 30 s of each stimulus that was played back continuously for

2 min using amplispeaker kept in the speaker chamber (Figure 2). In a night, any one of the above stimuli was presented for 5 times at an interval of 60 min and the bats were randomized for testing between nights. Based on the response of bats, we calculated the 'response score' as bats flying above the speaker 30 cm away (40), flying above the speaker 2 m away (30), changing the perch or crawl from its perch (20), stretching the wings/turned the ears on either side (10) and for no response, a score of 0 was given. The sum of scores for all five trials in a night represents the total response score of a playback session. Before each playback trial to bats, duration of 10 min 'silent period' was given as the control. The activities of bats were continuously observed during the silent control trials. The position of the amplispeaker was changed for each playback trial. Bats were kept in captivity not more than a week and all of them were released at the site of capture when all the experiments were done.

In natural situations, a total of 20 bats (8♂ and 12♀) were caught in playback netting sessions and 15 bats (9♂ and 6♀) in silent netting session. An average of 2 ± 1.46 ($n = 7$) and 3 ± 2.5 ($n = 7$) bats were caught per silent netting and playback netting sessions respectively. Although the total number of bats caught in playback netting sessions was more than that in silent netting sessions, the data did not show any significant variation between the sexes ($\chi^2 = 1.37$, $P > 0.05$). In one of the playback netting sessions, a single male fulvous fruit, *Rousettus leschenaulti* was caught in the mist net.

In laboratory conditions, site II bats of both sexes responded to all the stimuli of distress calls (Figure 3). When site I male and female distress calls were presented individually, the response score of site II bats was more towards the opposite sex (Figure 3a and b). For instance, the response score of site II males towards site I female stimulus was more than the site I male stimulus and this response was vice-versa for site II females. A similar response was obtained for the site II bats towards their own conspecifics (Figure 3c and d). Thus, distress calls attract opposite sex more frequently than similar sex when site II bats are presented with four different distress stimuli ($\chi^2 = 49.57$, $P < 0.05$). Bats never showed any movements and always rested in their perch during the silent control period.

The distress calls of *C. sphinx* are loud, noisy and audible for both conspecifics as well as heterospecifics (other sympatric bat species and predators such as barn owl). These characteristics are consistent with essential bioacoustic requirements for long-distance propagation of vocal signals in rainforest habitats^{9,10} and possibly in urban noisy environments. However, in general, the functions of distress calls in bats are still under debate on whether to warn other conspecifics or to seek their help^{11,12}. In *Saccopteryx bilineata*, the distress calls of the same elucidated higher response when playback was done near roost than in foraging sites. Thus, it implies that the

role of distress calls seems to help the bats in selfishly assessing the situations of predation risk near the roost¹¹. Carter *et al.*¹² compared the response of bat species of highly maneuverable *S. bilineata* with poorly maneuverable *Molassus molassus* towards their distress calls and found that both species performed individual passing inspection flights, but did not approach in groups near the broadcasting speaker. Here cooperative mobbing is ruled out. Contrastingly, cooperative mobbing is well known in sac-winged bat, *Taphazous nudivenris*¹³.

Playback experiments with distress calls of bats and the analysis of behavioural responses of conspecifics have been extensively investigated in insectivorous bats¹⁴⁻¹⁷. However, there are limited studies in frugivorous bats³⁻⁶. Ryan *et al.*¹⁸ observed that the distress call of the Phyllostomid bat *Artibeus jamaicensis* attracted bat species belonging to the same family. Ganesh *et al.*³ suggested that in Pteropodid bat *C. sphinx*, the distress calls elucidated the response only towards their conspecifics and irrespective of sex. The present study further substantiates that not only bats of both sexes, but also bats from different foraging area responded to the majority of playback trials. The symmetric response towards distress calls from related and unrelated sites suggests that distress calls presumably help build social relationships between bats of different foraging area. However, it is unclear why the response of bats was greater towards the opposite sex. Results of our field study with a larger number of females (12 out of 20) captured in the mist net after playing back the male distress calls are consistent with our earlier observations and also with the laboratory playback experiments. Therefore, there was no difference in the response of bats towards distress calls between field and laboratory conditions. The present study was restricted to non-breeding seasons of the year and a detailed study is needed to address the influence of breeding seasons, where the response of bats towards distress calls may be compared between breeding and non-breeding seasons.

The distress calls were recorded from the bats of American College (site I) and played back to the bats of another site, MKU campus (site II), located at a distance of 15 km from site I. The home ranges of male and female *C. sphinx* from MKU site are within 0.75 sq. km and 0.83 sq. km respectively¹⁹. Thus, the populations of site II and site I would be different. However, we do not know whether these bats are genetically related and it is well known that *C. sphinx* exhibit extensive dispersion in space and time. Further studies are needed in this context as to whether the distress calls elucidate the response from the bats of a different geographic area; for example populations of bats between North and South India.

In one instance of playback netting session with distress calls of site II male stimuli, a single individual *Rousettus leschenaulti* (body mass ~100 g; larger than *C. sphinx*) was trapped in the mist net. It is true that both *R. leschenaulti* and *C. sphinx* forage in the same area¹ and

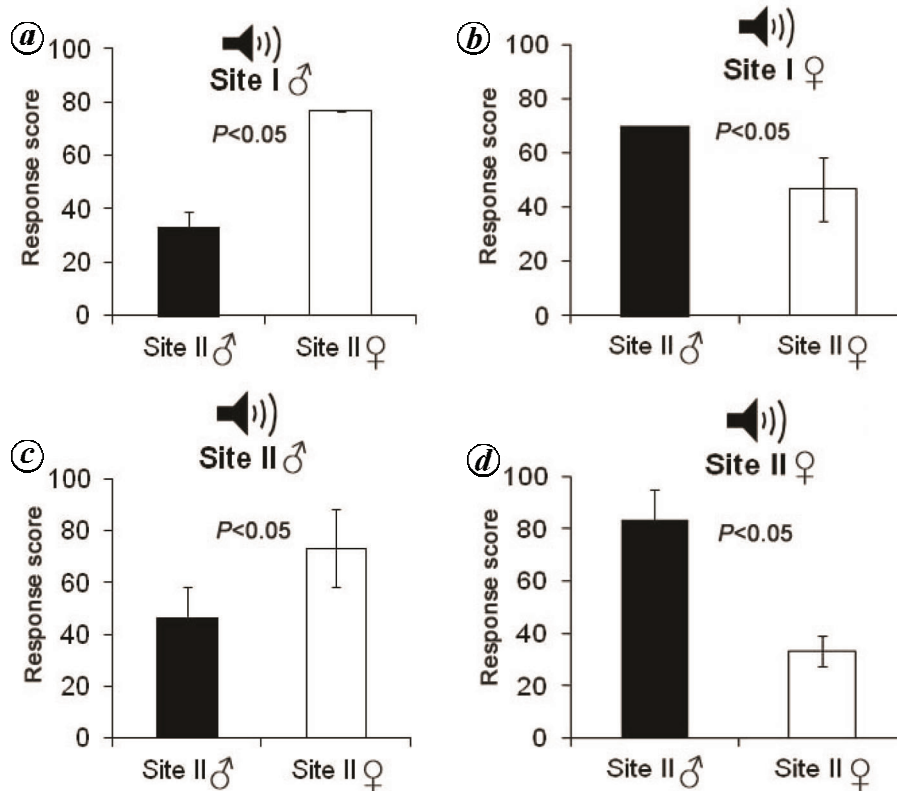


Figure 3. Response score of site II male and female *Cynopterus sphinx* towards playback sounds of distress calls of unrelated conspecifics – (a) site I male and (b) site I female and towards their own conspecifics – (c) site II male (d) and site II female. Data are presented as mean of response score \pm SD for $n = 3$ bats for both males and females.

the auditory sensitivity of *R. leschenaulti* falls within the frequency range of distress calls of *C. sphinx*²⁰. However, a single instance cannot be large enough to confirm the possibility of the response to distress calls by another related fruit bat species. Ganesh *et al.*³ in their study observed that, *R. leschenaulti*, foraging in the same area, was not attracted by the distress call of *C. sphinx*. Therefore, we substantiate that *R. leschenaulti* neither forage in the same area nor eavesdrop the distress calls of *C. sphinx* to recognize the presence of a food resource. It is likely that most of the food plants of *R. leschenaulti* overlap with food plants of *C. sphinx*^{21,22}. In New World bats, interspecific responses to the distress calls were well known in Phyllostomid bat species^{18,23}. In these bats, distress calls of smaller species attracted larger species and thus distress calls serve as a purpose in scaring a potential predator away from a foraging site²³.

The playback experiments in the field were restricted only to site I with a single male stimulus because our study area is small and therefore constrains an extensive playback sampling. It is true that frequent mist-netting with playback sounds in a smaller foraging area neither reduces the sampling effort nor forces the bats to habituate to playback sounds⁸. A similar situation prevails in site II and due to time constraints we were unable to find

another suitable site (alternate sites) for our playback experiments within Madurai. However, playback experiments in the site I substantiated the conceptual framework of our study that when males are caught in mist-net, the females were the first to be attracted in most trials. This study may be extended over a large foraging area such as the rainforests in the Western Ghats to provide a detailed confirmatory result. In conclusion, the distress calls serve for certain important functions that differ in contexts. The study on the response of harem males and non-harem males towards the distress calls of conspecifics and other unrelated bats will further throw light on the functions of distress calls in the social behaviour of bats.

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Response of methane diffusion in varying degrees of deformed coals to different solvent treatments

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In the communication, we analysed four semi-anthracite coals with different degrees of deformation from the Huoerxinhe colliery in China that were extracted by tetrahydrofuran (THF) and carbon disulphide (CS₂), and treated with hydrochloric acid (HCl) solution. Low-temperature nitrogen adsorption, water contact angle measurement and methane diffusion of untreated coals and their residues were carried out. As well, mineral composition of untreated coals and their residues treated with HCl are performed. Overall, compared with untreated coals, specific surface area has increased tendency after THF and CS₂ extraction due to the removal of the soluble organic components in coal, depends jointly on mineral types and their respective content after HCl treatment. Regardless of coal un- or treated by solvents, diffusion coefficient grows with increasing coal deformation. Further study shows that the diffusion coefficient of

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