



## When bats are boxing: aggressive behaviour and communication in male Seba's short-tailed fruit bat



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Aggressive behaviours have an important impact on the social organization of animals and on the social status of individuals, especially in gregarious species. Agonistic interactions between territory holders are essential to set and reinforce territorial borders. Additionally, agonistic displays are used to demonstrate ownership of a territorial site and may indicate social status of the signaller. Between neighbouring territory owners, dynamic borders require frequent interactions. In daily repeated aggressive encounters, ritualization can help to avoid the need for serious fights and their costly consequences. In the bat *Carollia perspicillata*, a gregarious frugivore with resource defence polygyny, males defend territories at valuable roosting sites for females. Working with a captive bat colony of about 400 individuals, housed under seminatural conditions in a tropical zoo, we found that males defended territories aggressively by the use of a succession of displays forming a ritualized structure. Simultaneously, males used three different vocalization types during aggressive displays, namely down-sweeps, warbles and aggressive trills. A statistical analysis of 58 aggressive trills from five adult males showed that they contained sufficient variation to encode an individual signature. Using a habituation–dishabituation paradigm playback experiment, we found that males could discriminate between vocalizing males based on aggressive calls alone. Such discrimination is probably useful for distinguishing between neighbouring territory owners and more unfamiliar intruders, and thus allows for an economical response.

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Individuals attempting to gain ownership over the same finite resource may meet as contestants in an aggressive encounter (Bradbury & Vehrencamp, 2011; Hardy & Briffa, 2013). Animal contests can range from gentle agonistic displays of butterflies to deadly encounters among ants. They can be costly, require energy and entail risk of injury or even death (Clutton-Brock, Albon, Gibson, & Guinness, 1979; Mercier, Lenoir, & Dejean, 1997; Neat, Taylor, & Huntingford, 1998; Piper, Walcott, Mager, & Spilker, 2008). Thus, contestants usually have an interest in resolving conflicts before escalating to costly physical fights (Bradbury & Vehrencamp, 2011). Game theory approaches seek to explain how and why intraspecific contests are resolved (Smith & Price, 1973). The motivation to fight (hawk or dove) is a key factor in these theoretical models, but can be adjusted to avoid serious injuries with the so-called 'limited war type', in which individuals in a

conflict use inefficient weapons or ritualized tactics (Smith & Price, 1973). Ritualized encounters are composed of successive stages, each offering information about the contestants' motivation or resource-holding potential (i.e. showing fighting ability, sensu Bradbury & Vehrencamp, 2011). A continuous update during an interaction allows each contestant to decide at each successive stage whether to retreat or to engage further in the conflict. The resolution of a conflict at an earlier stage can be facilitated when both contestants use signals to provide information about their willingness to engage further in the conflict (Burmeister, Ophir, Ryan, & Wilczynski, 2002; Hofman & Schildberger, 2001; Logue et al., 2010), or reveal asymmetries between contestants (Davies & Halliday, 1978; Ladich, 1998; Mager, Walcott, & Piper, 2007). In the presence of a third party, the bystander can be influenced by the outcome of the conflict and adjust its own strategy: the winner and loser effect (Earley & Dugatkin, 2002; Hsu & Wolf, 1999). As the third party may be less willing to engage in a conflict with the winner, the latter has an interest in communicating its victory (Field & Rind, 1992; Grafe & Bitz, 2004). Such behaviours can be performed visually, but are mostly acoustically displayed to reach potential rivals further away (Bower, 2005).

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Bats are successful in their nocturnal environment because they are capable of using echolocation calls for orientation. Undoubtedly, echolocation calls serve bats to orient themselves in their environment and for foraging (Balcombe & Fenton, 1988; Barclay, 1982). But echolocation calls are not only limited to these functions. Several studies have demonstrated that sonar calls have a communicative function as well and can facilitate behavioural interactions (Fenton, 2003; Knörnschild, Jung, Nagy, Metz, & Kalko, 2012; Voigt-Heucke, Taborsky, & Dechmann, 2010). Aside from echolocation calls, bats possess a repertoire of social signals for purely communicative reasons.

The social function of communication signals in general is defined as transformation of information about the current motivational state of an individual or a certain social situation, directed to the receivers (Simmons, 2003). Acoustic signals produced during social interactions can influence the behaviour of both the signaller and the receiver, and also of individuals not directly involved (e.g. Otter et al., 1999). Social vocalizations can encode vocal or acoustic characteristics allowing for discrimination between frequently interacting individuals (McComb, Moss, Sayialel, & Baker, 2000; Rendall, Rodman, & Emond, 1996) and may also provide information about colony, group and/or individual identity (Arnold & Wilkinson, 2011; Boughman, 1997; Eckenweber & Knörnschild, 2013; Holekamp et al., 1999; Janik, Sayigh, & Wells, 2006; Kastein, Winter, Kumar, Kandula, & Schmidt, 2013; Semple, 2001).

The ability to discriminate or even individually recognize individuals based on vocalizations can be a valuable social skill (e.g. Carter, Skowronski, Faure, & Fenton, 2008; Müller & Manser, 2008; Rendall et al., 1996; Semple, 2001), especially in repeated encounters (Godard, 1991). Individual signatures in aggressive vocalizations can facilitate interactions between opponents. Individuality encoded in vocalizations allows animals to discriminate between well-known individuals and strangers (Mackin, 2005), providing information that can facilitate decision making in different social circumstances, e.g. during aggressive encounters (Bradbury & Vehrencamp, 2011).

Research on aggressive behaviour, aggressive signalling and vocalizations has been conducted in a range of species, for example anurans (Reichert & Gerhardt, 2013) crickets (Hack, 1997; Hofman & Schildberger, 2001; Logue et al., 2010), chameleons (Stuart-Fox, 2006), birds (Searcy, Anderson, & Nowicki, 2006) and monkeys (Kitchen, 2004). In bats, however, studies on aggressive interactions and associated vocal signals are still scarce (i.e. Behr & von Helversen, 2004; Markus, 2002; Ortega & Arita, 2000; Porter, 1979a, 1979b).

Seba's short-tailed bat, *Carollia perspicillata*, is a highly social species occurring in the Neotropics from southern Mexico to southern Brazil (Cloutier & Thomas, 1992). The bats live in colonies, occupying hollows (e.g. trees, buildings, caves, bridges) for their day roost sites. There, they form groups composed of one male together with up to 18 females (Williams, 1986). Males occupy spots in the day roost, which they defend vigorously against other males (Porter, 1979a; Williams, 1986). Male territories are then selected by females for roosting. This selection is assumed to be based on territory quality, and the *C. perspicillata* mating system is consequently considered to be resource defence polygyny (Fleming, 1988). The harem males have privileged access to females (Porter, 1979a), but nevertheless may not father every young born in the respective harem (Porter & McCracken, 1983). Males not competitive enough to own a territory cluster together in bachelor groups during the day. To obtain mating opportunities, the bachelor males attempt to gain access to females in nearby territories. Males are thus daily involved in aggressive encounters, either between neighbouring territory holders or between sneaker males and the respective harem owner. Aside from bachelor groups and harems,

solitary bachelors and mixed-sex subadult groups can be found in day roosts (Porter, 1979a).

Here we studied aggressive interactions between resident males in a large captive colony of *C. perspicillata* to investigate the behavioural patterns during aggressive encounters between males and to document the vocalizations associated with these encounters. Because males are daily involved in agonistic encounters, we hypothesized that these encounters are ritualized. Ritualized encounters allow animals to minimize energy expenditure and costly fights. First, we predicted that during an aggressive encounter several distinct behavioural displays would be observed. Second, we predicted that the different behavioural displays would follow a sequential structure. Our second hypothesis proposed that males utter distinct aggressive vocalization types during agonistic encounters that are not produced in other contexts. Finally, we hypothesized that male aggressive vocalizations possess an individual signature to facilitate discrimination. As males are repeatedly involved in agonistic encounters they should benefit from being able to discriminate between different individuals. Following our hypothesis we predicted that males would be able to discriminate between two opponents based on aggressive vocalizations only, and that the aggressive vocalizations would encode a statistically detectable individual signature.

## METHODS

### Study Animals

The study was performed in a captive breeding colony of *C. perspicillata*, housed in a tropical zoo (Nocturama; in the Papiliorama Kerzers FR; Switzerland, [www.papiliorama.ch](http://www.papiliorama.ch)). All work was approved by the University of Bern, the veterinary office of Fribourg (permit number 2012-15E-FR), reviewed by the cantonal ethics committee and adhered to the ASAB/ABS Guidelines for the use of animals in research. In the Nocturama, a 40 m diameter dome, a colony of about 400 bats lives in a reversed light cycle tropical environment (night: 0930–2130 hours; day: 2130–0930 hours), which mimics the bats' original habitat in Central America. The bats fly freely under the dome, while their roosting sites are situated in an artificial cave. Bats are fed twice a night with a house-made fruit-based mixture. The dome is open to visitors during the day from 0900 to 1800 hours, and we thus worked outside of the visiting hours to avoid potential disturbance (between 0700 and 0900 hours and 1800–2100 hours). A unique combination of three different coloured plastic rings (AC Hughes Ltd., U.K., size XB), positioned on both forearms, allowed for visual individual identification.

Based on regular spatial and behavioural monitoring of the bats at their roosting site, and on the literature (Porter, 1979a; Williams, 1986), we differentiated between two categories of male social status (territorial versus nonterritorial). Territorial (or harem) males were individuals occupying a roosting site as their territory and defending it vigorously against any male intrusion. Moreover, during our social monitoring, these males were very faithful to their territory. Some males protected a group of females (harem males), but as we were interested in aggressive behaviours, we considered single territorial males with similar fidelity to their roosting site as having the same social status. The other status category comprised the nonterritorial bachelor males (or sneakers). These males were not territorial as they were observed in several places in the cave during the social monitoring and rarely initiated aggressive behaviours. They mainly roosted in large bachelor groups (groups composed of males only). As harem males do not sire all the young born in their harem, they share their reproductive success with bachelor males (Porter & McCracken, 1983). The costs of guarding females imposed on harem males may therefore offer the

opportunity for reproductive sneaking behaviours and drive the evolution of these alternative reproductive tactics in *C. perspicillata* (see Wolff, 2008).

#### *Analysis of Aggressive Male–Male Encounters*

In 2012 (May) and early 2013 (February), we recorded videos of 10 different harem groups and one bachelor group. Each recording session lasted for 25 min per group and was performed twice a night. Behaviours and accompanying vocalizations were recorded during the main activity periods around the roosting sites, either before bats left to forage, namely 1.5 h before dusk, or when they returned 1.5 h before dawn. Camcorders (Sony HDR-CX700VE, Sony Corporation, Weybridge, U.K.) with infrared projectors (IRLamp6 Illuminator, Bat conservation and Management, Inc. Carlisle, PA, U.S.A.) were used to monitor the behaviour of every member of the harem under observation. For the analysis of the videos, we used a VCL media player (v.2.1.0; VideoLAN Organisation, Paris, France). A behavioural repertoire was established based on the data from the first observation period (approximately 32 h of videos were analysed in total). We analysed 48 aggressive encounters between contestants to establish a precise description of aggressive behavioural displays between males, the types of encounter that occurred and the modes of ritualization. We defined the different stages that occurred during the aggressive encounters. We analysed whether the succession of the stages was consistent throughout the aggressive encounters and hence formed a ritualized sequence. A Yates' chi-square test was used to compare observed and expected first-order transition frequencies between successive stages. Statistics were performed using R v3.0.2 (The R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>).

#### *Sound Recordings for Aggressive Vocal Repertoire Description*

In 2012 and early 2013, sound recordings were made twice a night (for details see above). To analyse the likely social function of the different vocalization types, we filmed the harems at the same time. The videos allowed us to identify the sound-producing individual. Sound recordings were made with an ultrasonic recording set-up consisting of automatic bat recorders (Pettersson D500x, Pettersson Elektronik AB, Uppsala, Sweden; 500 kHz sampling rate, 16-bit depth resolution) connected to external microphones (Pettersson external microphone for D500X; flat frequency response: 5–190 kHz). Both bat recorders were installed at the same height as the camcorders. The first recording period in 2012 was used to establish the species' vocal repertoire. To account for the differences between vocalization types, different structural and temporal patterns were assessed visually from sonograms using the software Raven Pro 64, v.1.4 (Cornell Lab of Ornithology, New York, U.S.A.). Further, a combination of video analysis and direct observations was used to associate social function with the different vocalization types. By matching 37 aggressive encounters with accompanying aggressive vocalizations, we determined the vocalization types occurring in agonistic interactions. By analysing 37 excerpts (each 5 s long) of vocal encounters and performing a chi-square test we elucidated whether the aggressive vocalization types were produced in a consistent succession. Statistics were performed using R v3.0.2.

#### *Analysis of Individuality in Male Aggressive Vocalizations*

Aggressive trills of five different harem males were selected for statistical analyses. In total, 58 aggressive trills with a good signal-to-noise ratio were analysed using the software Avisoft-SASLab Pro v.5.2 (R. Specht, Berlin, Germany). Measurements were taken from spectrograms, generated with a Hamming window, a 1.042-point

fast Fourier transform and a 93.75% overlap, resulting in a frequency resolution of 488 Hz and a temporal resolution of 0.128 ms. The start and end of vocalizations were determined automatically (–20 dB relative to the peak frequency of the signal). To best depict male aggressive trills, five spectral parameters (peak frequency, minimum and maximum frequency, bandwidth, entropy), one waveform parameter (energy) and two temporal parameters (duration, distance from start to maximum amplitude) were measured over the entire aggressive call, which resulted in a total of eight acoustic parameters. Additionally, two spectral parameters (peak and minimum frequency) were measured at 11 different locations spread equally over the entire call. Thus, we obtained 22 acoustic parameters to depict the call curvature, resulting in a total of 30 acoustic parameters per call. We performed a principal component analysis with varimax rotation on the 22 parameters depicting call curvature, which resulted in five principal components (with eigenvalues >1) that explained 73.1% of the total variance. The appropriateness of our data set for a principal component analysis was ascertained by performing Bartlett's test and by calculating the Kaiser-Meyer-Olkin (KMO) index, which measures sampling adequacy. The KMO index and Bartlett's criteria (KMO index: 0.829; Bartlett:  $\chi^2_{231} = 905.443$ ,  $P < 0.0001$ ) confirmed the suitability of the principal component analysis for our data. The five principal components describing call curvature were used together with the remaining eight original parameters to conduct a stepwise discriminant function analysis (DFA). The DFA allowed us to test for an individual signature in the aggressive trills of males. The DFA used a leave-one-out-cross-validation procedure that classified each trill based on discriminant functions established with all trills except the one being classified. The significance of the classification success resulting from the DFA was evaluated using a one-tailed binomial test (following Mundry & Sommer, 2007). We used SPSS v20.0 (SPSS Inc., Chicago, IL, U.S.A.) for our statistical analysis on individual signatures in aggressive trills.

#### *Stimulus Acquisition for Playback Experiments*

For the playback experiments, we recorded aggressive calls under standardized conditions during spring 2013. In total, 18 harem males were used as stimulus donors. The stimulus acquisition followed a specific routine. For 6 consecutive days, a harem male and two to three females were housed in a large flight cage where they could fly freely (2.1 × 0.9 m × 1.4 m high). Water and food were provided ad libitum. During the first 3 days, the animals acclimatized to the new environment. Every harem clustered in the same corner of the cage, i.e. furthest away from the door. The position of the harems in the respective corners ensured that the set-up for the playback experiments was the same for every tested individual. During days 4 and 5, another harem owner was caught with a hand-net from the main colony and introduced into the flight cage to challenge the harem male. During the resulting aggressive encounters, both bats produced aggressive vocalizations. The introduced male was left in the cage for 45 min. During this period, aggressive vocalizations were recorded with the ultrasonic bat detector (Pettersson D500X) connected to an external microphone (for details see above). From outside of the flight cage we documented the fight and the vocalizing males. Additionally, two camcorders (details see above) were installed at two different positions of the cage. Typically the intruding male approached the corner occupied by the harem male and his females, and then the harem holder started to defend his corner. Thus, the recording of aggressive vocalizations during a male–male encounter occurred under standardized conditions in the absence of potential human disturbance. After 45 min, the introduced male was recaptured with a hand-net and released back into the main colony.

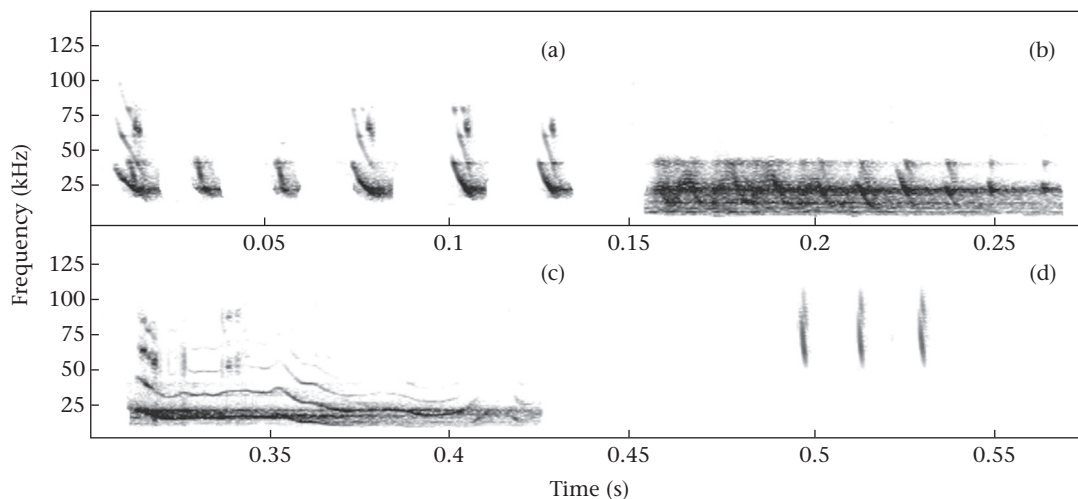
### Experimental Design

Ten focal harem males were tested in playback experiments. Every focal male was accompanied by two to three females from his original harem to mimic a natural situation. We used a habituation–dishabituation paradigm (Cheney & Seyfarth, 1988; Hauser, 1998). Calls from one stimulus donor were broadcast until the focal male habituated, i.e. no longer reacted to the stimuli. Subsequently, calls from a second stimulus donor were broadcast to test whether the focal male could discriminate between the two, i.e. showed a full response (dishabituation). The habituation stimuli consisted of at least 13 different aggressive vocalizations (Fig. 1). The calls were separated by silent intervals of 0.9–34 s, mimicking the natural intervals between calls uttered in aggressive encounters. An individual was considered habituated when the aggressive calls of the original stimulus donor no longer elicited a response, i.e. the male either started grooming or closed his eyes, while his ears and body remained motionless. When the male was habituated, the dishabituation stimuli were played back in a single sound file. These files were all 15 s long, each containing five different calls interspaced by silence. Immediately after the dishabituation, we broadcast a control stimulus (i.e. the sound of tossing keys) to monitor attentiveness, for example to test whether an animal was distracted or sleeping. The files for the habituation and dishabituation period were prepared in Cool Edit 2000 (Syntrillium, Phoenix, AZ, U.S.A.), which allowed us to reduce the background noise of the recordings. Every file was unique because no aggressive call was used twice, and the silent intervals between calls were of random length. The calls recorded from an encounter between two males were not used later for testing the same two males in the habituation–dishabituation experiment. The calls were broadcast using a loudspeaker (Pettersson L400 Ultrasound Speaker, frequency range: 10–110 kHz) connected to the ultrasonic bat recorder D1000X (Pettersson D1000X; flat frequency response: 5–235 kHz). The maximal peak amplitude was normalized for all stimuli (500 kHz sampling rate and 16-bit depth resolution) used in playback. Sound level settings of the speaker were the same for all experiments. The loudspeaker was always positioned in the opposite corner to that occupied by the harem group. This allowed the harem male to show aggressive displays, including flying towards the speaker and hovering in front of it. We documented the focal

**Table 1**  
Ritualized aggressive display of male *C. perspicillata*

Displays	Label	Description
Ear movements	A	Detection of an opponent (either by low-frequency vocalizations or by ultrasonic sounds) The distance between contestants is at least a full wing length so physical contact is not possible
Head lift/turn	B	Opponents lift and/or turn heads to face each other The distance between contestants is at least a full wing length so physical contact is not possible
Craning of neck, unfolding wings	C	Wings are unfolded partially, opponents start fast wing flapping. The body is leaned forwards in a conspicuous way while the neck is craned towards the opponent. Often opponents start to produce aggressive vocalizations The distance between contestants is at least a full wing length so physical contact is not possible
Walk and mimicked punching	D	Opponents start to walk towards each other, with partially unfolded wings. Fast wing flapping is alternated with mimicked punching (i.e. virtual boxing behaviour without physical contact). Aggressive vocalizations accompany the fast wing movements Opponents are in front of each other, approximately one wing length away
Boxing	E	Escalation of conflict, physical contact through punching with the wrist, a behaviour called boxing: Both wings are partially unfolded, the upper arm tucked firmly into the body, the forearm stretched out slightly, finger digits (i.e. digits 3, 4, 5) are spread. The chest protrudes between both wings. Then one forearm with the wrist headmost (i.e. where digit 1 with the claw is located) is used for fast repetitive boxing towards the opponent. Usually, only one forearm is used for boxing; the other is kept partially unfolded, but is flapped fast

male's behavioural response by observing from outside the flight cage. Five ranks according to increasing aggressive behavioural displays between males were established, with rank 1 being the lowest-ranking response: ear movement (rank 1), head lift and/or head turn (rank 2), body turn accompanied by opening of the wings (rank 3), flight towards the speaker (rank 4) and flight towards the speaker and hovering in front of it (rank 5). The flight towards the speaker was rated highest because the approach towards the



**Figure 1.** Male aggressive calls of *C. perspicillata*. Males produced three different types of aggressive calls during conflicts. We differentiated between aggressive (a) down-sweeps, (b) aggressive warbles and (c) male aggressive trills. For comparison, (d) echolocation calls are also shown. The spectrograms depict frequency as a function of time and were created with the program 'open office'.

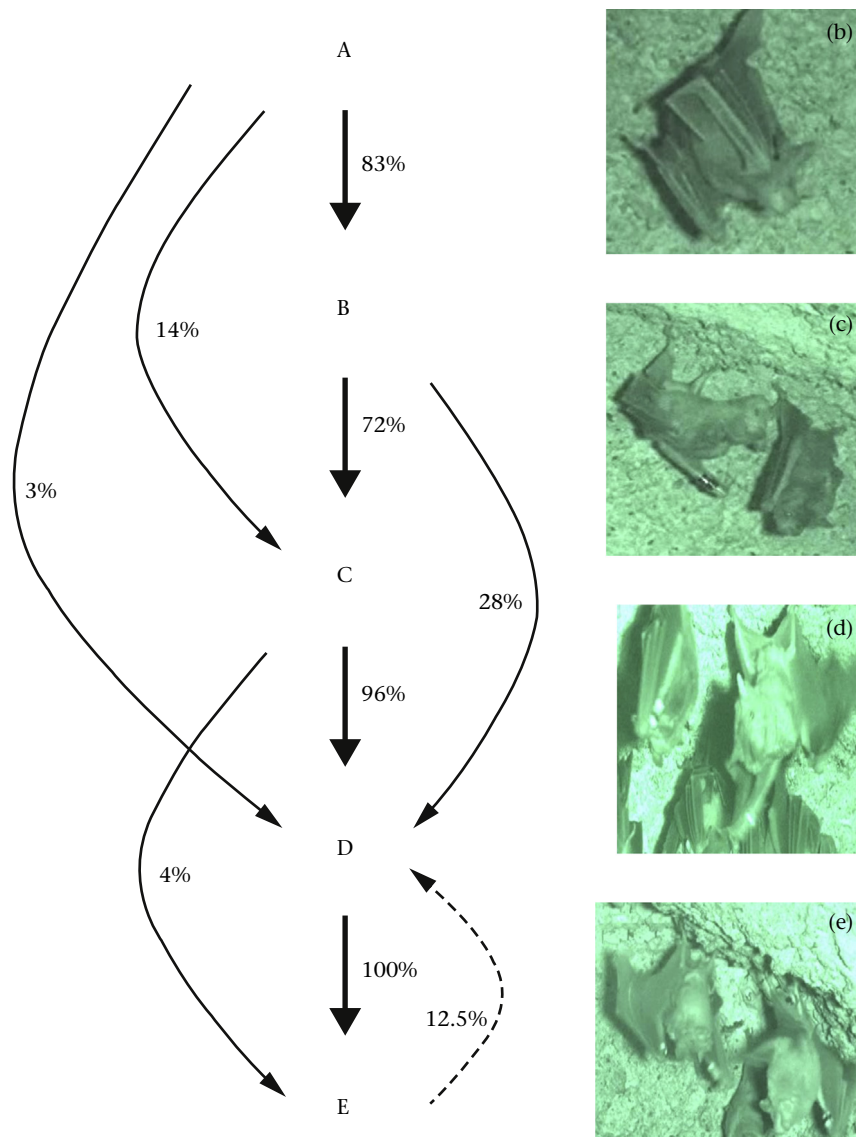
speaker corresponds to a male approaching an opponent under natural circumstances. After the completion of the playback experiment, all bats were caught and released into the main colony.

**RESULTS**

*Ritualized Aggressive Encounters Between Male Contestants*

Aggressive encounters between males followed a defined succession of behavioural stages with increasing levels of aggression (Table 1) as predicted for ritualized interactions. Ear movements (stage A) and head lifts or turns (stage B) proceeded to neck craning and unfolding of both wings (stage C) which, in turn, proceeded to walking and mimicked punching (stage D) and, ultimately, boxing (stage E). The encounters could last for more than 1 min and ended when one of the males retreated, i.e. started self-grooming, turned away or flew off. Both opponents could cease conflicts at any stage. Despite the aggressive appearance, injuries were not common. We never had to intervene in a fight to prevent it from becoming too

severe. The worst injuries of males were grazed elbows, scratches on the wrist or little holes in the wing membranes and ears, which we noted when we caught the animals for ringing. However, we could not deduce whether all of these injuries were a consequence of aggressive male–male encounters. Observed fights never caused injuries and, throughout the project, we observed a single case of bats falling on the ground during a fight. Both contestants took off immediately afterwards. Consequently, we never had to remove a bat. The succession of behavioural stages (A–B–C–D–E; Fig. 2) was highly significant in all cases; first-order transitions diverged significantly from random transitions (chi-square tests with Yates' correction; probability of A followed by B:  $\chi^2 = 36.407, P < 0.0001$ ; probability of B followed by C:  $\chi^2 = 6.25, P = 0.012$ ; probability of C followed by D:  $\chi^2 = 22.321, P < 0.001$ ; probability of D followed by E:  $\chi^2 = 18.050, P < 0.0001$ ). Sixteen of the 48 encounters lasted until the last stage (E), and six of 16 encounters went back from stage E to stage D (hatched line in Fig. 2). In seven cases, the encounter started directly with stage E, usually after a clear violation of the territorial boundary without any previous warnings.



**Figure 2.** Sequence of behavioural stages (A–E) during ritualized aggressive encounters of male *C. perspicillata*. The numbers represent the transitions between behavioural stages as percentages. The pictures depict the behavioural displays of males; the lowercase letters indicate the affiliation to the corresponding stage. Stage A has no accompanying picture.

Loud aggressive vocalizations were produced immediately by both opponents and accompanied the boxing behaviour.

### Aggressive Vocalization Types

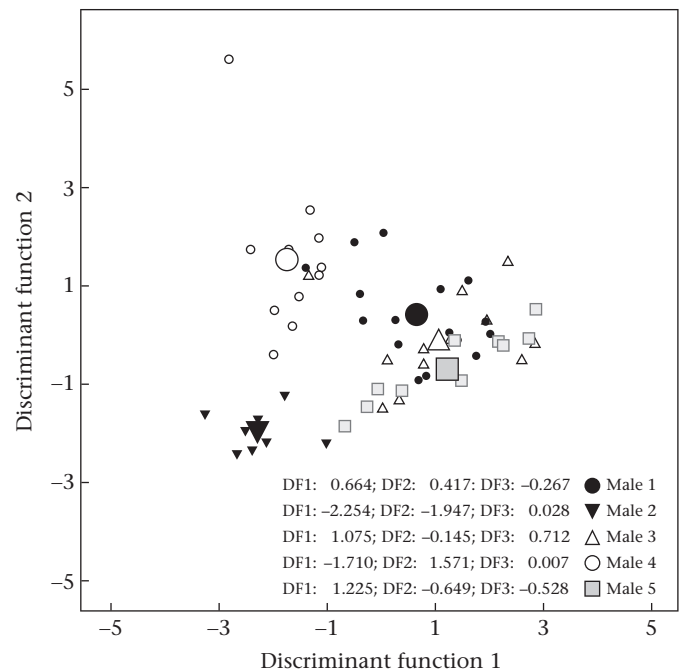
Males produced three different vocalization types during aggressive encounters: down-sweeps, warbles and trills (Fig. 1a–c). All three vocalization types were uttered by both harem and bachelor males. In 37 of the 48 encounters, the recordings were of sufficient quality to be analysed. The analysis of the call sequence showed that the three call types did not follow each other in a specific sequential order. Down-sweeps ( $\chi^2 = 54.08$ ,  $P < 0.0001$ ) and aggressive warbles ( $\chi^2 = 7.483$ ,  $P = 0.023$ ) were followed significantly more often by themselves than by other vocalization types, suggesting that repetition rather than a specific syntax conveyed meaning. Aggressive trills, however, were significantly more often followed by aggressive down-sweeps than by aggressive trills or warbles ( $\chi^2 = 31.6$ ,  $P < 0.0001$ ). During behavioural stages A and B (see Table 1), no aggressive vocalization types were recorded. In three of 37 encounters lasting at least until stage D, aggressive vocalizations started at stage C, and in 34 of 37 encounters they started at stage D. In the 16 encounters that continued to stage E, vocalizations were always produced.

### Individual Signature in Male Aggressive Trills

A stepwise DFA (Table 2) with cross-validation classified 62.1% of aggressive trills to the correct harem male (Fig. 3), which was significantly higher than the 20% expected by chance (binomial test:  $P < 0.0001$ ). Three acoustic parameters were sufficient for individual discrimination, namely energy and two principal components describing call curvature (PC1 and PC2, Table 3). PC1 and PC3 contributed mostly to discriminant functions 1 and 3, respectively, whereas energy contributed most to discriminant function 2. Thus, male aggressive trills (Fig. 4) encoded an individual signature. When we tested whether focal males could use that signature to discriminate between males in a habituation–dishabituation paradigm, all 10 males reacted to the discrimination stimuli (100% discrimination), thus discriminating aggressive calls based on the encoded individual signature. As a response to the dishabituation stimuli, one male showed display stage A only, three males proceeded to stage B, three males to stage C and three males to stage D, one of them even hovering in front of the loudspeaker.

## DISCUSSION

In agreement with our first hypothesis we found that aggressive encounters of *C. perspicillata* followed a succession of defined stages, as is typical for ritualized behaviours. Our results correspond to the description of ritualized aggressive encounters found in a variety of different taxa, such as ants (Ettershank & Ettershank, 1982), cichlids (Maan, Groothuis, & Wittenberg, 2001; Neat et al., 1998), spiders (DeCarvalho, Watson, & Field, 2004) and deer (Bartoš et al., 2007; Clutton-Brock et al., 1979). A succession of



**Figure 3.** Individual signature in male aggressive trills of *C. perspicillata*. The DFA signal space depicted here is defined by the first two discriminant functions, which were most important for individual discrimination. Different males are represented by different symbols. Small symbols depict trills; large symbols depict centroids of males. Numbers next to each centroid report values for the respective discriminant functions 1–3.

stages from mild to escalated interactions is thought to reduce the costs of conflicts for contestants since both can decide to escalate further or retreat at any stage. Contests between *C. perspicillata* males covered two to five stages. The occasional skipping of a stage (Fig. 2) may indicate that either contestants started at a too low a level or that they used additional cues that were not obvious to the observer. In our study, aggressive encounters occurred between bachelor as well as harem males. In particular, harem males were engaged daily in aggressive encounters at the territorial boundaries. These boundaries were not entirely fixed, and both acoustic and behavioural signals were used in negotiations. In seven cases, as a consequence of a territorial violation, opponents directly escalated in physical interactions. Interestingly, these immediate escalations were only observed to occur between neighbouring harem males. Bachelor males did not invade territories by immediate attacks. This may indicate that bachelor males are weaker or of lower resource-holding potential than harem males. But it may also result from a sneaker/satellite strategy that allows non-territorial males to reproduce successfully in social systems. Different mating tactics (i.e. dominant versus subordinate status) have been observed and described not only for mammals (e.g. Heckel & von Helversen, 2002) but also for fish (e.g. cichlids: Martin & Taborsky, 1997; Oliveira & Almada, 1998), mites (Sato, Sabelis, & Egas, 2014; Sato, Sabelis, Egas, & Faraji, 2013) and lizards (Zamudio

**Table 2**

Statistical evidence for an individual signature in male aggressive trills of *C. perspicillata* (58 calls from five males)

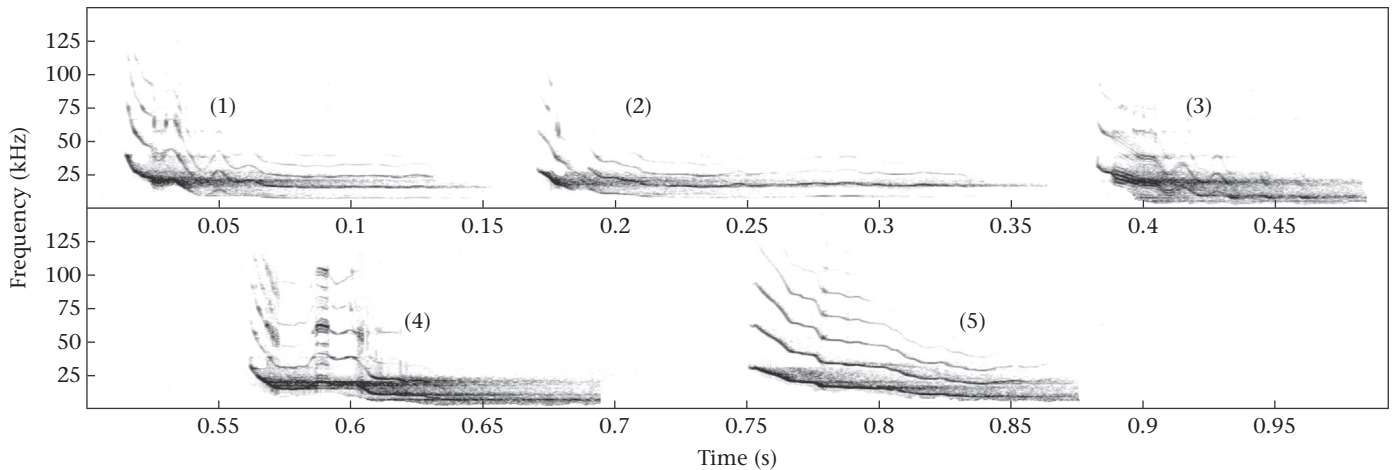
Assessment of a model fit <sup>a</sup>	DF1	DF2	DF3
Eigenvalue	2.07	1.22	0.2
Percentage of variation	59.4	35.0	5.7
Test of functions	DF1–DF3	DF2–DF3	DF3
Wilk's lambda	0.12	0.38	0.834

<sup>a</sup> Stepwise discrimination function (DF) analysis with cross-validation.

**Table 3**

Structure matrix showing the canonical loading of the discriminant functions (DF) for the three acoustic parameters included in the stepwise discriminant function analysis (sorted by their relevance for discrimination)

Acoustic parameters	DF1	DF2	DF3
Principal component 1	0.808	0.529	–0.259
Energy	–0.401	0.833	0.380
Principal component 2	0.980	0.560	0.994



**Figure 4.** Aggressive trills from five *C. perspicillata* males, visualizing their individual signatures. 1 = male 1, 2 = male 2, 3 = male 3, 4 = male 4 and 5 = male 5. The spectrograms depict frequency as a function of time.

& Sinervo, 2000). Alternatively, neighbouring harem holders may be of equal strength and know each other well enough that an assessment over several stages is redundant. Hence they may directly enter at the highest escalation level. An interesting aspect of future research would be to address the costs associated with different male strategies by estimating the relative frequencies at which aggressive interactions between males take place.

In agreement with the predictions of our second hypothesis, three different vocalization types (down-sweeps, aggressive warbles, aggressive trills; Fig. 1) were produced exclusively during aggressive contests. Since the three call types did not occur in a fixed succession it is unlikely that they contribute to the ritualized structure. In other species, costs of agonistic interactions were mitigated when accompanied by acoustic communication (Logue et al., 2010; Silk, Kaldor, & Boyd, 2000). This may be predicted if acoustic signals convey information on colony, group or individual identity (e.g. Behr & von Helversen, 2004; Eckenweber & Knörnschild, 2013). Correspondingly, male aggressive trills encoded an individual signature that allowed males to discriminate between different competitors, thus supporting our third hypothesis. In a gregarious species such as *C. perspicillata*, harem males in their roost are surrounded by several other territory owners. Thus, it is probably highly useful to be able to discriminate between neighbouring territory owners and more unfamiliar intruders, and this may provide the basis for an economical response (e.g. Godard, 1991; Godard, 1993; Myrberg & Riggio, 1985; Price, Boutin, & Ydenberg, 1990).

Although we could show that the aggressive vocalizations contained sufficient information for individual discrimination, the experimental paradigm used here does not allow us to conclude that males are capable of individual recognition in the sense that the receiver learns, associates and uses individually distinct characteristics of the signaller for recognition (Tibbets & Dale, 2007; Tibbets, Sheehan & Dale, 2008). Both individual discrimination and recognition can facilitate interactions between individuals, but recognition is thought to further reduce costs in territorial defence since response levels can be adjusted as a consequence of previous interactions with the same individual. Experience from past interactions can be integrated into future decisions (Gherardi & Tiedemann, 2004; Karavanich & Atema, 1998), and should therefore reduce costs, increase benefits and ultimately enhance fitness. Individual recognition may also allow conspecifics to eavesdrop on information about winners and losers of contests and subsequently challenge the loser rather than the winner. On the one hand, broadcasting individual information can thus establish and

strengthen hierarchies (Oliveira & McGregor, 1998), but, on the other, eavesdropping may be detrimental for the signaller when information cannot be selectively masked. Furthermore, eavesdropping on agonistic interactions influences the behaviour not only of the same sex (e.g. Naguib, Amrhein, & Kunc, 2004; Peake, Terry, McGregor, & Dablesteen, 2002) but also of the opposite sex and thus may have implications for mate choice (e.g. Otter et al., 1999).

In conclusion, our study demonstrated the capability of male *C. perspicillata* to discriminate between male conspecifics based on aggressive vocalizations. Furthermore, our study provides a detailed description of daily aggressive displays during agonistic interactions between males. Further experiments are needed to elucidate whether true individual recognition (*sensu* Tibbets & Dale, 2007) between adult males is occurring and to what extent eavesdropping influences the signalling behaviour (e.g. Naguib et al., 2004).

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