Univerzita Palackého v Olomouci

Přírodovědecká fakulta

Katedra zoologie a Ornitologická laboratoř

Behaviorální studie nosorožce tuponosého a Cottonova v zoologických zahradách a ve volné přírodě, se zaměřením na vokální komunikaci

Behavioural study of the captive and free-ranging southern and northern white rhinos with focus on their vocal communication

Doktorská dizertační práce
Ivana Cinková

Vedoucí práce: Prof. RNDr. Vítězslav Bičík, CSc.
Konzultant: Mgr. Richard Policht, PhD.

Olomouc 2015

"If all the beasts were gone, man would die from great loneliness of spirit. For whatever happens to the beast, also happens to the man. All things are connected."

Chief Seattle

"The greatest danger to our future is apathy."

Jane Goodall

Prohlašuji, že jsem tuto práci vypracovala samostatně s použitím uvedené literatury. Výjimku tvoří přiložené články, na kterých se podílel spoluautor. Žádnou část této práce jsem nepředložila k získání jiného akademického titulu.

V Olomouci dne 22. 4. 2015

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Bibliografická identifikace:

Jméno a příjmení autora: Ivana Cinková

Název práce: Behaviorální studie nosorožce tuponosého a Cottonova v zoologických zahradách a ve volné přírodě, se zaměřením na vokální komunikaci

Typ práce: Dizertační práce

Pracoviště: Katedra zoologie a Ornitologická laboratoř, Univerzita Palackého

Vedoucí práce: Prof. RNDr. Vítězslav Bičík, CSc.

Konzultant: Mgr. Richard Policht, PhD.

Rok obhajoby práce: 2015

Abstrakt

Dobře vyvinutý komunikační systém může být zvláště důležitý u sociálně žijících druhů živočichů a umožňovat vytvoření větší sítě sociálních vazeb. Savci často používají akustické a olfaktorické signály k získávání informací o ostatních příslušnících svého druhu. Vokalizační signály jsou většinou namířeny ke konkrétnímu jedinci/jedincům a mohou být použity na delší vzdálenost, kdežto olfaktorické signály se šíří pomalu, ale mohou přetrvávat v prostředí po delší dobu. Nosorožci mají výborný sluch a čich, ale pouze slabší zrak. Pro jejich komunikaci jsou tedy velmi důležité hlasové a olfaktorické signály, ale komunikace nosorožců byla donedávna studována jen velmi málo a experimentální studie z volné přírody úplně chyběly.

Tato studie jako první popisuje informace zakódované v hlasech nosorožců a schopnost volně žijících nosorožců rozlišovat informace z hlasových a olfaktorických signálů. Nosorožci tuponosí (*Ceratotherium simum*) a kriticky ohrožení nosorožci Cottonovi (*C. cottoni*) mají nejvíce vyvinutý sociální systém ze všech druhů nosorožců, používají kontaktní hlasy a vyprazdňují se na společných hnojištích. Oba druhy se v zajetí velmi špatně rozmnožují a znalost jejich komunikace může najít významné využití v jejich ochraně a managementu.

Prvním cílem této práce bylo analyzovat kontaktní hlasy nosorožců tuponosých a Cottonových a zjistit, jaké obsahují informace. Analýzy ukázaly, že hlasy umožňují určit identitu vokalizujícího jedince a že na základě výpočtu informační kapacity obsahují u obou druhů dostatek informací pro rozlišení přibližně devíti jedinců. Kontaktní hlasy je také možné s vysokou pravděpodobností přiřadit ke správnému druhu. Nosorožci Cottonovi mají delší hlasy a liší se od nosorožců tuponosých také v některých frekvenčních parametrech. Hlasy nosorožců tuponosých se také liší v závislosti na pohlaví, věku zvířete a sociální situaci.

Dále byly provedeny playbackové a olfaktorické experimenty, abychom zjistili, jaké informace dokáží nosorožci ze svých hlasů a z trusu rozpoznat. Volně žijící teritoriální samci

nosorožce tuponosého rozlišovali mezi kontaktními hlasy dospělých samců a samic svého druhu a reagovali výrazně intenzivněji na hlasy samic, což naznačuje, že kontaktní hlasy jsou významnější pro komunikaci mezi samcem a samicí než mezi dvěma teritoriálními samci. Samci také rozlišovali mezi hlasy obou pohlaví nosorožců Cottonových a reagovali intenzivněji na hlasy samců nosorožce Cottonova než tuponosého, což mohlo být způsobeno efektem novosti, jelikož jsou oba druhy allopatrické a rozlišování mezi nimi tedy nemůže být způsobeno učením. Experimenty s trusem ukázaly, že volně žijící nosorožci tuponosí dokáží rozpoznat informace o familiaritě a pohlaví ostatních příslušníků svého druhu pouze na základě pachu jejich trusu. Nosorožci čichali více než dvakrát déle k trusu neznámých než familiárních jedinců. Ostražitost v reakci na trus samců nastávala rychleji při čichání k trusu familiárních než neznámých samců a ostražitost v reakci na trus samic nastávala naopak rychleji při čichání k trusu neznámých než familiárních samic.

Výsledky této studie ukazují, že kontaktní hlasy nosorožců tuponosých a Cottonových obsahují mnoho informací o vokalizujícím jedinci a že nosorožci tuponosí používají kontaktní hlasy a trus k zjišťování informací o ostatních. Tyto signály tedy hrají významnou roli v sociálním chování nosorožců. Intenzivní reakce zvířat na playbacky kontaktních hlasů a vzorky trusu naznačují, že tyto signály stimulují jejich sociální a teritoriální chování. Aplikace vokálních a olfaktorických podnětů by tedy mohla najít významné využití v ochraně a managementu nosorožců a zvýšit šanci na jejich reprodukci v zajetí.

Klíčová slova: nosorožec tuponosý, nosorožec Cottonův, *Ceratotherium simum*, *Ceratotherium cottoni*, sociální chování, vokální komunikace, kontaktní hlas, olfaktorická komunikace

Počet stran: 95
Počet příloh: –
Jazyk: český, anglický

Bibliographical identification

Author's first name and surname: Ivana Cinková

Title: Behavioural study of the captive and free-ranging southern and northern white rhinos with focus on their vocal communication

Type of thesis: PhD. thesis

Department: Department of Zoology and Laboratory of Ornithology, Palacký University

Supervisor: Prof. RNDr. Vítězslav Bičík, CSc.

Consultant: Mgr. Richard Policht, PhD.

The year of presentation: 2015

Abstract

Well-developed communication system can be particularly important in socially living species and allow for an establishment of a large network of social relationships. Mammals often use vocal and olfactory signals to obtain information about conspecifics. Vocalizations are mostly directed to concrete individual/s and can be used for communication over a long distance while olfactory signals are transmitted slowly, but can remain in the environment for a long time. Rhinos have an excellent sense of hearing and smelling, but their eyesight is poor. Vocal and olfactory signals are therefore very important in their social behaviour, but communication in rhinos has only been studied to a limited extent until recently and experimental studies from the wild have been missing.

This study describes for the first time the information encoded in the calls of rhinos and the ability of wild rhinos to recognize information from the vocal and olfactory signals. The southern (*Ceratotherium simum*) and critically endangered northern (*C. cottoni*) white rhinos have the most developed social system out of all the rhinoceros species, use contact pant calls and defecate at common dungheaps. Both species reproduce poorly in captivity and better knowledge of their communication could be very useful in their management and conservation.

The first aim of this study was to analyse contact calls of the southern and northern white rhinos to determine what information they encode. Discriminant function analyses revealed that contact calls allow for the identification of individual identity and the calculation of information capacity in calls showed that they contain sufficient information for the recognition of nine individuals in both species. Pant calls can also be assigned with a high probability to a correct species. The northern white rhinos have longer calls and also differ from the southern species in several frequency parameters of their calls. The calls of the southern white rhinos also vary according to the sex, age-class and social situation of the caller.

Playback experiments and manipulations with dung were conducted to determine if rhinos are able to discriminate the information about other animals from their contact pant calls and dung. Wild territorial southern white rhinoceros bulls discriminated between the calls of adult male and female conspecifics and reacted significantly more intensively to the calls of females, which suggests that contact calls are more important for the communication between a male and a female than between two territorial males. Bulls also discriminated between the calls of both sexes of northern white rhinos and reacted more intensively to the calls of northern than southern males. This could be caused by a novelty effect as both species are allopatric and discrimination between them therefore cannot result from learning. Experiments with dung showed that wild southern white rhinos are able to discriminate familiarity and sex of conspecifics based only on the smell of their dung. The rhinos sniffed the dung of unfamiliar animals more than twice as long as the dung of familiar rhinos. The latency of vigilance posture was shorter in reaction to the dung of familiar than unfamiliar males and longer in reaction to the dung of familiar than unfamiliar females.

This study shows that the contact calls of southern and northern white rhinos encode a lot of information about a vocalizing animal and that the southern white rhinos use the information encoded in contact calls and dung to assess other individuals. These signals therefore play an important role in the social behaviour of rhinos. The intensive reactions of the animals to the playbacks and to the dung samples show that they stimulate their social and territorial behaviour. Manipulation of vocal and olfactory signals could therefore be used in the management and conservation of rhinos and improve the chance for their reproduction in captivity.

Keywords: southern white rhinoceros, northern white rhinoceros, Nile rhinoceros, *Ceratotherium simum*, *Ceratotherium cottoni*, social behaviour, vocal communication, contact call, olfactory communication

Number of pages: 95
Number of appendices: –
Language: Czech, English

Poděkování

Na tomto místě bych ráda poděkovala mnoha lidem, díky jejichž pomoci a podpoře se mi podařilo tento projekt uskutečnit, pracovat s mými milovanými nosorožci a i zažít spoustu dobrodružství během terénní práce v Jihoafrické republice. Ačkoliv mnoho dat z mého terénního výzkumu teprve čeká na zpracování, základní výsledky studie se mi, myslím, podařilo dát do této dizertační práce.

Na prvním místě bych chtěla vyjádřit velké poděkování svému školiteli Prof. Vítězslavu Bičíkovi za veškerou jeho podporu, důvěru a pomoc během celého mého studia, nejen doktorského, ale i magisterského a bakalářského. Dr. Richardu Polichtovi bych ráda poděkovala za to, že navzdory mému přesvědčení, že bych chtěla po magisterském studiu pracovat s nosorožci přímo v praxi a že na doktorát nepůjdu, tak že mě přemluvil, abych šla. Dále moc děkuji za zasvěcení do výzkumu akustické komunikace a za rady a konzultace v průběhu mého studia. Jsem také moc vděčná Prof. Stanislavu Burešovi za podporu mého projektu.

Velké poděkování patří managementu a majitelům rezervací a zoologických zahrad, že mi umožnili studovat jejich zvířata a za veškerou ochotu a vstřícnost během mé terénní práce. Jelikož při práci v divočině se stává, že vznikají nějaké zádrhely a komplikace, jsem moc vděčná, že se mi ve všech rezervacích, kde jsem pracovala, dostalo v případě potřeby vždy pomocné ruky. Díky: Jonathan Swart, Gerhardt Lorist, André Burger, Shaun McCartney (Welgevonden Game Reserve), Anton Walker, Anthony Roberts, Hermann Müller, Elias Mangwane, Lin Insel (Lapalala Wilderness), Lucky Phiri, Louis Loock, Annelize Steyn, Johan Eksteen (Mthethomusha Nature Reserve), Thomas Sikhwivhilu, Thompson Phakalane, Khanyisile Mbatha (Lichtenburg biodiversity conservation centre), Jochen Lengger (zoo Salzburg), Jiří Hrubý (zoo Dvůr Králové), Ivo Klika, Markéta Horská (zoo Zlín), Anton Freivolt, Martin Krug (zoo Bratislava). Rezervacím a zoo Dvůr Králové děkuji za poskytnutí ubytování.

Velký dík patří mému kamarádovi Gordonu Whyatt-Johnovi za poskytnutí zázemí v Johannesburgu a za veškeré rady a pomoc při zařizovaní se a orientaci v Jihoafrické republice, od koupení terénního auta pro výzkum po různé běhání a vyřizování po úřadech. Velké poděkování patří i Dr. Pavlu Krchňákovi a Martinu Deutschlovi (katedra Biofyziky, Univerzita Palackého) za sestrojení reproduktoru, který jsem používala k playbackovým experimentům a za jejich veškerou vstřícnost.

Acknowledgements

I would like to express my thanks and gratitude to a lot of people thanks to whose help and support I was able to realize this project, work with my beloved rhinos and also experience a lot of adventures during my field work in South Africa. Although I still need to analyse a lot of data from my field work, I believe that I was able to include the essential results into this dissertation.

Firstly, I would like to thank very much my supervisor Prof. Vítězslav Bičík for all his support, trust and help during my studies, not only doctoral, but also during my master's and bachelor's studies. I am thankful to my consultant Dr. Richard Policht that although I was confident that after my master's studies, I would like to find a job and work with the rhinos and I didn't want to go to study for a PhD., he persuaded me otherwise. I am also very grateful to him for showing me the basics of the research on acoustic communication and for the suggestions and consultations during the course of my studies. I would also like to thank very much Prof. Stanislav Bureš for his support of this project.

I am very grateful to the management and owners of all the wildlife reserves and zoological gardens for allowing me to work with their rhinos and for all their willingness and helpfulness during my field work. Since various complications and problems can sometimes arise during the field work in the wild, I am very thankful that whenever it was necessary I received an assistance. Thank you: Jonathan Swart, Gerhardt Lorist, André Burger, Shaun McCartney (Welgevonden Game Reserve), Anton Walker, Anthony Roberts, Hermann Müller, Elias Mangwane, Lin Insel (Lapalala Wilderness), Lucky Phiri, Louis Loock, Annelize Steyn, Johan Eksteen (Mthethomusha Nature Reserve), Thomas Sikhwivhilu, Thompson Phakalane, Khanyisile Mbatha (Lichtenburg biodiversity conservation centre), Jochen Lengger (Salzburg zoo), Jiří Hrubý (Dvůr Králové zoo), Ivo Klika, Markéta Horská (Zlín zoo), Anton Freivolt, Martin Krug (Bratislava zoo). Thanks to the wildlife reserves and the zoo Dvůr Králové for personal accommodation.

A big thank you goes to my friend Gordon Whyatt-John for giving me a base in Johannesburg and for the logistic help from buying a vehicle for my field work to various errands at local authorities. I am also very grateful to the late Dr. Pavel Krchňák and to Martin Deutschl (Department of Biophysics, Palacký University) for the construction of speaker, which I was using for the playback experiments and for all their helpfulness.

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(submitted)

Úvod

Všech šest druhů nosorožců je do nějaké míry ohroženo působením člověka kvůli pytláctví, ztrátě habitatu, lovu nebo civilním nepokojům (Hutchins a Kreger 2006). Čtyři z těchto druhů jsou v současné době klasifikovány jako kriticky ohrožené a dva z poddruhů, západní poddruh nosorožce dvourohého (*Diceros bicornis longipes*) a vietnamský poddruh nosorožce jávského (*Rhinoceros sondaicus annamiticus*), byly v tomto desetiletí vyhubeny (The IUCN Red List of Threatened Species 2014). Nosorožec tuponosý (*Ceratotherium simum*) a Cottonův (*Ceratotherium cottoni*) jsou blízce příbuzné sesterské druhy, které se oddělily před asi jedním milionem let (Groves et al. 2010). Nosorožec Cottonův byl dříve označován jako severní poddruh nosorožce tuponosého (Robovský et al. 2010), nicméně nedávno publikovaná revize tohoto taxonu doporučila jeho povýšení na samostatný druh na základě morfologických a genetických rozdílů mezi oběma formami (Groves et al. 2010).

Přirozený areál výskytu nosorožce tuponosého je jižní Afrika na jih od řeky Zambezi (Rookmaaker a Antoine 2012). Na přelomu 19. a 20. století byl ale tento druh na pokraji vyhubení kvůli střílení lovci, kdy přežívalo pouze kolem 20–50 zvířat v Jihoafrické republice (Emslie a Brooks 2002) v oblasti kolem řeky Umfolozi, která byla velmi špatně dostupná a zamořená mouchami tse-tse. V roce 1897 byla tato oblast vyhlášena přírodní rezervací a nosorožce tuponosého se podařilo zachránit. V roce 1953 už bylo v Umfolozi Game Reserve při leteckém sčítání napočítáno 437 zvířat a jejich počty dále stoupaly. Následně byl vyvinut program „Operace nosorožec" a mezi lety 1961–1972 bylo převezeno za využití sedace více než 1 100 nosorožců do původních míst výskytu a do zoologických zahrad (Player 1972). V roce 2001 už jejich počty v Africe překročily hranici 11 000 jedinců (Emslie 2008) a nyní dosahují 20 424 zvířat, z nichž 93% žije v Jihoafrické republice (Knight 2013). Dnes je nosorožec tuponosý nejpočetnějším druhem nosorožce, ale jeho populace je v poslední době ohrožována pytláctvím, které se soustředí hlavně do Jihoafrické republiky. Hlavními důvody pro pytláctví jsou použití nosorožčího rohu v tradiční čínské medicíně k léčbě nemocí spojených s vysokými horečkami, k léčbě otrav, epilepsie nebo jako posilující tonikum (Nowell et al. 1992). Hlavně dříve se také používal v zemích Středního Východu k výrobě tradičních dýk zvaných jambiya (Emslie a Brooks 1999). Nosorožčí roh se skládá z velkého množství těsně spojených filament a jeho pevná struktura je dána orientací buněk korneocytů, které vznikají podobným procesem keratinizace jako při vývoji a růstu lidských nehtů (Boy et al. 2015). Pytláctví začalo od roku 2008 silně stoupat a jen za poslední tři roky v letech 2012–2014

bylo v Africe zabito více jak 3 000 nosorožců (Knight 2014; Department of Environmental Affairs 2015). Obchod s nosorožčími rohy se v poslední době soustředí především do Vietnamu, kde bývá roh používán jako přípravek k léčbě rakoviny a zároveň i jako ukázka statusu bohatství a významného postavení (Milliken a Shaw 2012).

Situace nosorožců Cottonových je velmi odlišná. Na přelomu 19. a 20. století, kdy byli nosorožci tuponosí na pokraji vyhubení (Emslie a Brooks 2002), se nosorožci Cottonovi vyskytovali ještě v poměrně velkém počtu ve střední Africe v Demokratické republice Kongo, Ugandě, Súdánu, Čadu a Středoafrické republice (Smith a Smith 1993). Ačkoliv jejich počty dosahovaly v roce 1964 kolem 2 800 zvířat (Schomber 1966), velká míra pytláctví způsobila, že v roce 1984 se už ve volné přírodě vyskytovali pouze v Národním Parku Garamba v Demokratické republice Kongo v počtu 15 zvířat. V tomto roce byl spuštěn „Projekt Garamba" na záchranu nosorožců a do roku 2006 se v parku narodilo 50 mláďat (Hillman-Smith 2006). Přestože válka v jižním Súdánu od roku 1991 výrazně ovlivnila Garambu kvůli nájezdům ozbrojených běženců, mezi lety 1993–2003 se stále dařilo udržet tamější populaci nosorožců na počtu kolem 30 zvířat. Od roku 2003 ale došlo k výraznému nárůstu pytláctví (Hillman-Smith a Ndey 2005), poslední čtyři nosorožci byli pozorováni v roce 2006 a od roku 2007 už po nich nebyly nalezeny žádné stopy. Druh byl tedy ve volné přírodě s největší pravděpodobností vyhuben, nicméně stále ještě přežívá velmi malá populace v zajetí (Emslie 2011).

Pouze 26 nosorožců Cottonových bylo dosud chováno v zoologických zahradách, z toho jich 22 pocházelo z odchytu z volné přírody v letech 1950–1975. Nejvýznamnější chovné stádo bylo vytvořeno v zoo Dvůr Králové a jeho základ tvořili dva samci (Saút a Súdán) a čtyři samice (Nesárí, Núrí, Nádí a Nola) odchycení v roce 1975 Ing. Josefem Vágnerem v Súdánu a v roce 1977 k nim byla připojena samice Nasima ze zoo Prescot, která pocházela z odchytu v Ugandě (Vágner 1980; Mercado 2004). Zoo Dvůr Králové se jako jediné na světě podařilo nosorožce Cottonovi rozmnožit a v letech 1980, 1983, 1989 a 2000 se tu narodila čtyři mláďata (Obr. 1). Další samička, která se tu narodila v roce 1977, byla jediným kdy známým křížencem nosorožce tuponosého a Cottonova (Mercado 2004). Od roku 2000 se ale reprodukce nosorožců Cottonových nedařila a v roce 2009 byla tehdy už poslední čtyři zvířata na světě potenciálně schopná přirozené reprodukce přesunuta z Dvora Králové do rezervace Ol Pejeta v Keni, kde se jejich rozmnožení bohužel dosud nepodařilo. V současné době už žijí pouze dvě samice a jeden samec v rezervaci Ol Pejeta a dvě samice v zoo Dvůr Králové a v safari parku v San Diegu. Žádné z těchto zvířat již ale není schopno přirozené reprodukce a šance na záchranu

nosorožce Cottonova tedy leží v technikách umělého oplodnění, embryotransferu a metodách genetického inženýrství (Zoo Dvůr Králové 2015).

Obr. 1: Nosorožci Cottonovi v Zoo Dvůr Králové v roce 2009. Samec Suni (vzadu; *1980, †2014) a samičky Nesárí (*1972, †2011) a Nájin (vepředu; *1989, dnes v Ol Pejeta Conservancy v Keni). (Všechny fotky: autor: Ivana Cinková)

Nosorožci tuponosí i Cottonovi se v zajetí velmi špatně rozmnožují (Hermes et al. 2005, 2006; Swaisgood et al. 2006) a důvody stále nejsou známy. Populace nosorožců tuponosých v evropských zoologických zahradách v letech 2001–2004 čítala 240 zvířat a z nich se pouze 26% jedinců v reprodukčním věku za svůj život alespoň jednou rozmnožilo (Reid et al. 2012). Pokud se tento problém brzy nevyřeší, bude k udržení jejich populace v zajetí nutný import dalších zvířat z volné přírody, což by bylo z hlediska jejich ochrany kontraproduktivní. U zvířat ze zakladatelské F0 populace byla zjištěna úspěšnější reprodukce než u F1 generace narozené v zajetí (Swaisgood et al. 2006). Samice nosorožců tuponosých i Cottonových často trpí reprodukčními patologiemi způsobenými dlouhým obdobím bez březosti (Hermes et al. 2006) a také samci často trpí sníženou fertilitou (Hermes et al. 2005). Jako jedna z možných příčin nízké reprodukce byl uveden vyšší obsah fytoestrogenů v potravě v zajetí, které by mohly

aktivovat estrogenové receptory samic. Hlavně dlouhodobé vystavení fytoestrogenům během všech fází života, ale především během vývoje plodu by tak mohlo způsobit částečnou až trvalou neplodnost. Nicméně pro potvrzení nebo vyvrácení této hypotézy a pro pochopení vlivu potravy s vyšším obsahem fytoestrogenů na reprodukční zdraví samic je zapotřebí dalšího výzkumu (Tubbs et al. 2012).

Významný vliv na reprodukci nosorožců tuponosých a Cottonových může mít změna jejich sociálního chování v zajetí a nedostatek sociálních podnětů (Lindemann 1982; Meister 1997; Kuneš a Bičík 2001-2002; Swaisgood et al. 2006; Metrione et al. 2007; Cinková a Bičík 2013). Oba druhy mají nejvíce rozvinutý sociální systém ze všech nosorožců (Goddard 1967; Owen-Smith 1973, 1975; Laurie 1982; van Gyseghem 1984; Penny 1987). Dospělí samci jsou teritoriální a samice, mláďata a mladá zvířata žijí ve skupinkách v překrývajících se domovských okrscích (Owen-Smith 1973, 1975; van Gyseghem 1984). Nejčastější jsou skupinky po 2–3 zvířatech, nicméně i společenství až šesti zvířat nejsou nijak vzácná (Obr. 2). Domovský okrsek dospělé samice může zahrnovat až 6–7 teritorií samců, většina samic však tráví v období dešťů více než polovinu času v jednom nebo dvou sousedních teritoriích (Owen-Smith 1973, 1975). Dříve byli nosorožci v zoologických zahradách chováni pouze v párech, ale taková zvířata se nerozmnožovala skoro vůbec a větší úspěch v rozmnožování měli instituce chovající více než jednoho samce a více než jednu samici (Lindemann 1982). Samice narozené v F1 generaci se také rozmnožují častěji, pokud je ve stádě přítomna starší samice původem z volné přírody (Swaisgood et al. 2006) a složení skupiny samic v závislosti na jejich věku a původu také může výrazně ovlivnit sociální chování zvířat (Cinková and Bičík 2013). V zoologických zahradách také dochází mezi zvířaty zvláště během krmení k výrazně častějším projevům agonistického chování a následně ke zvýšené sekreci stresových hormonů, což by mohlo mít vliv i na jejich reprodukci (Meister 1997; Schmidt a Sachser 1997). Také bylo zjištěno, že samice, které spolu vyrůstaly od mala, mají nižní hodnoty stresových hormonů (Metrione a Harder 2011).

Obr. 2: Setkání dvou skupin nosorožců tuponosých.

Behaviorální výzkum a znalost komunikace jsou velmi významné v ochraně a managementu živočichů a mohou pomoci nalézt a vyřešit problémy s jejich rozmnožováním v zajetí (Lindburg a Fitch-Snyder 1994; Fisher et al. 2003; Swaisgood et al. 2003; Campbell-Palmer a Rosell 2011). Behaviorální výzkum s důrazem na studium komunikace například významně napomohl v záchranném programu na rozmnožování pandy velké (Swaisgood et al. 2000; Swaisgood et al. 2003). Znalost chování nosorožců je proto klíčovým komponentem pro vývoj optimálního managementu jejich chovu v zoologických zahradách i přírodních rezervacích (Hutchins a Kreger 2006). Nosorožci mají výborný sluch a čich, ale jejich zrak je slabší a vokální a olfaktorické signály jsou proto velmi důležité pro jejich komunikaci (Penny 1987). Jelikož mají nosorožci tuponosí a Cottonovi nejvíce rozvinuté sociální chování ze všech druhů nosorožců (Goddard 1967; Owen-Smith 1973, 1975; Laurie 1982; van Gyseghem 1984; Penny 1987), dobře vyvinutý komunikační systém může být pro ně zvláště důležitý a schopnost rozpoznávání informací ze signálů ostatních jedinců může umožnit vytvoření větší sítě sociálních vztahů.

Nosorožci tuponosí a Cottonovi jako jediní z nosorožců používají kontaktní hlas sípání (angl. pant), který se skládá ze série nádechů a výdechů a zvířata ho používají nejčastěji, když dojde k setkání jedinců z různých skupin, kteří se zdraví, když jedinec přistupuje blíže ke své skupině nebo když se zvířata z jedné skupinky navzájem ztratí a poté se volají (Owen-Smith 1973; Policht et al. 2008). Oba druhy se také vyprazdňují na společných hnojištích a samci si značí své teritorium močí a trusem (Owen-Smith 1973; van Gyseghem 1984). Například výměna trusu nosorožců mezi zoologickými zahradami byla už dříve navržena jako možnost stimulace jejich teritoriálního a reprodukčního chování (Fouraker a Wagener 1996). Komunikace nosorožců byla ale donedávna probádána jen velmi málo, experimentální studie chyběly a nevědělo se, jaké informace si zvířata prostřednictvím vokálních a olfaktorických signálů předávají.

Cíle dizertační práce

Zjistit, jestli kontaktní hlasy nosorožců tuponosých a Cottonových umožňují identifikovat vokalizujícího jedince a zda se liší mezi oběma druhy, mezi pohlavími, věkovými třídami a kontextem

Hlasy mnoha savců obsahují informace o identitě, morfologii, fyziologii nebo chování vokalizujícího jedince (např. Fichtel et al. 2001; Darden et al. 2003; Rendall et al. 2004; Charlton et al. 2007, 2009a, 2009b, 2010a; Proops et al. 2009; Schneiderová a Policht 2010; Tallet et al. 2013). U žádného z šesti druhů nosorožců nebylo ale dosud zjištěno, jaké informace si jedinci prostřednictvím vokalizace mohou sdělovat. Kontaktní hlasy živočichů bývají používány hlavně k zachování soudržnosti skupiny a k oznámení identity jedince (Kondo a Watanabe 2009). Je tedy pravděpodobné, že i kontaktní hlasy nosorožců tuponosých a Cottonových by mohly obsahovat informace o vokalizujícím jedinci. Jelikož se oba druhy oddělily před asi jedním miliónem let (Groves et al. 2010) a přirozeně se vyskytují v odlišných typech habitatu (Hillman-Smith 1982, 1987; Pienaar et al. 1992, 1993), je možné, že se charakteristiky hlasů budou mezi oběma druhy lišit.

Testovat, jestli volně žijící nosorožci tuponosí reagují rozdílně na playbacky kontaktních hlasů obou druhů, zda rozpoznají pohlaví vokalizujícího jedince a jestli jsou jejich reakce rozdílné v závislosti na sociálním kontextu během playbacku

Studiu schopnosti rozlišování různých informací z hlasů jedinců stejného druhu byla dosud u volně žijících kopytníků věnována jen velmi malá pozornost. Pokud se kontaktní hlasy nosorožců tuponosých a Cottonových liší mezi pohlavími a druhy, je třeba podniknout experimentální studii a zjistit, zda nosorožci dokáží tyto informace v hlasech opravdu rozpoznat a jestli jsou tyto hlasy důležité v jejich sociálním chování. Jelikož přirozená oblast výskytu nosorožce tuponosého zahrnuje jižní Afriku a nosorožce Cottonova střední Afriku, jejich areály výskytu se nepřekrývají a jsou od sebe vzdáleny více jak 2 000 km^2 (Hillman-Smith et al. 1986). Bylo by tedy velmi zajímavé zjistit, jestli nosorožci dokáží případné rozdíly v hlasech obou druhů rozpoznat a zda preferují signály vlastního druhu.

Zjistit, zda volně žijící nosorožci tuponosí rozpoznají signály o familiaritě a pohlaví jedinců pouze na základě pachu trusu

Pro nosorožce je kromě vokální komunikace velmi důležitá i ta olfaktorická (Goddard 1967; Owen-Smith 1973; Laurie 1982; Penny 1987). Nedávná experimentální studie nosorožců dvourohých dočasně držených v zajetí ukázala, že dokáží rozpoznat signály o věku, pohlaví a identitě jedince z jeho trusu (Linklater et al. 2013). Studie olfaktorické komunikace nosorožců tuponosých byly ale dosud pouze popisné (Owen-Smith 1973) a u žádného druhu nosorožce nebyla olfaktorická komunikace experimentálně zkoumána ve volné přírodě. Proto bylo mým cílem podniknout experimentální studii a zjistit, zda volně žijící nosorožci tuponosí rozpoznají na základě pachu trusu familiaritu a pohlaví jedince. Lepší znalost vokální a olfaktorické komunikace nosorožců by mohla najít významné využití v managementu a ochraně nosorožců v zoologických zahradách i v afrických rezervacích.

Materiál a metodika

Podrobný popis metodiky je uveden v jednotlivých příspěvcích, tato kapitola představuje pouze souhrn a obecný popis použité metodiky.

Vokalizaci nosorožců tuponosých a Cottonových jsem nahrávala v zoologických zahradách ve Dvoře Králové, Salzburgu, Zlíně a Bratislavě v letech 2009–2014. Hlasy nosorožců tuponosých jsem dále nahrávala a experimenty s playbacky prováděla v rezervacích v Jihoafrické republice: Welgevonden Game Reserve, Lapalala Wilderness (obě provincie Limpopo), Mthethomusha Nature Reserve (provincie Mpumalanga) a Lichtenburg biodiversity conservation centre (provincie North-West) v letech 2011–2012. Olfaktorickou komunikaci nosorožců tuponosých jsem studovala v roce 2012 v rezervacích Welgevonden Game Reserve a Lapalala Wilderness.

Hlasy nosorožců byly nahrávány na směrový mikrofon Sennheiser (ME 67 s napájecím modulem K6, frekvenční odpověď: 40–20 000 Hz ± 2.5 db) vybavený větrnou ochranou Rycote Softie (Obr. 3) a na digitální rekordéry Yamaha Pocketrak C24 a Marantz PMD 671. Hlasy byly analyzovány pomocí programů Avisoft SAS Lab Pro 5.2.07 (Avisoft Bioacoustics, R. Specht, Berlín) a LMA 2008 (laskavě poskytnutý Kurtem Hammerschmidtem). Playbackové experimenty s kontaktními hlasy jsem prováděla pomocí digitálního přehrávače Barrel a na

míru sestaveného reproduktoru (výkon: 50W, frekvenční odpověď: 48–20 000 Hz ± 3 db) připojeného k zesilovači (laskavě sestavené Dr. Pavlem Krchňákem a Martinem Deutschlem, Katedra biofyziky, Univerzita Palackého). Playbacky byly pouštěny s maximální amplitudou na úrovni akustického tlaku 85–97 dB (měřeno 1 m před reproduktorem) v závislosti na vzdálenosti zvířat od reproduktoru (15–40 m) a jejich hlasitost odpovídala přirozeně vokalizujícímu nosorožci. Reakce nosorožců na playbackové i olfaktorické experimenty jsem nahrávala na videokameru (Canon Legria HF M31) a později analyzovala pomocí programu Activities 2.1 (Vrba a Donát 1993).

Kontaktní hlasy nosorožců byly analyzovány s využitím klasické a permutované diskriminační analýzy, generalizovaných lineárních modelů, Mann-Whitneyho U testu a výpočtu informační kapacity hlasu v bitech (angl. signature information capacity) (**Příspěvek I., III.**). Reakce nosorožců na experimenty s playbacky byly analyzovány s využitím Friedmanovy ANOVY, Wilcoxonova párového testu a analýzy hlavních komponent (**Příspěvek III.**). K statistickým analýzám olfaktorických experimentů byly použity lineární mixované modely (**Příspěvek II.**).

Obr. 3: Nahrávání hlasů nosorožců tuponosých.

Seznam přiložených příspěvků

Příspěvek I.

Cinková I., Policht R. (2014) Contact Calls of the Northern and Southern White Rhinoceros Allow for Individual and Species Identification. PLoS ONE 9:e98475. (doi:10.1371/journal.pone.0098475) (IF 3.534, Multidisciplinary Sciences 8/55)

Příspěvek II.

Cinková I., Policht R. (2015) Discrimination of familiarity and sex from chemical cues in the dung by wild southern white rhinoceros. Animal Cognition 18: 385–392. (doi:10.1007/s10071-014-0810-8) (IF 2.631, Zoology 14/153, Behavioral Sciences 26/49)

Příspěvek III.

Cinková I., Policht R. (submitted) Sex and species recognition by wild male southern white rhinoceros using contact pant calls.

Prohlášení o autorském podílu na následujících publikacích:

Cinková I., Policht R. (2014) Contact Calls of the Northern and Southern White Rhinoceros Allow for Individual and Species Identification. PLoS ONE 9:e98475. (doi:10.1371/journal.pone.0098475) (IF 3.534, Multidisciplinary Sciences 8/55)

Podíl autorů: Richard Policht navrhl design studie a základního zpracování dat. Ivana Cinková navrhla další analýzy dat, data nasbírala, zpracovala je, provedla statistické analýzy a napsala článek. Richard Policht přispěl doplněním dat, poskytl cenné rady a konzultace během zpracovávání a analýz dat a poskytl komentář k textu článku.

Cinková I., Policht R. (2015) Discrimination of familiarity and sex from chemical cues in the dung by wild southern white rhinoceros. Animal Cognition 18: 385–392. (doi:10.1007/s10071-014-0810-8) (IF 2.631, Zoology 14/153, Behavioral Sciences 26/49)

Podíl autorů: Ivana Cinková navrhla design studie a analýz dat, data nasbírala, zpracovala je, provedla statistické analýzy a napsala článek. Richard Policht poskytoval rady a konzultace v průběhu celého výzkumu.

Cinková I., Policht R. (submitted) Sex and species recognition by wild male southern white rhinoceros using contact pant calls.

Podíl autorů: Richard Policht navrhl základní design experimentů s playbacky. Ivana Cinková doplnila a přizpůsobila design studie pro terénní práci, nasbírala data, navrhla jejich analýzy, data zpracovala, provedla statistické analýzy a napsala článek. Richard Policht poskytoval rady a konzultace v průběhu celého výzkumu.

14.4.2015
_____
Datum

Mgr. Ivana Cinková   Mgr. Richard Policht, Ph.D.

Komunikační signály

Všechny důležité projevy sociálního chování jsou zprostředkovávány komunikačními signály (McGregor a Peake 2000). Design komunikačních signálů živočichů je tvořen dvěma složkami, strategickým designem a efektivitou. Strategický design signálu přestavuje jeho formování přírodním výběrem, aby poskytl dostatek informací nezbytných pro reakci příjemce, která zvyšuje nebo zachovává biologickou zdatnost signalizujícího jedince. Efektivita se týká přenosu signálu a přírodní výběr favorizuje takové signály, které jsou jednoduše přenášeny, detekovány, rozlišeny od ostatních signálů a ty, co jsou snadno zapamatovatelné (Guilford a Dawkins 1991). Rozpoznávání je založeno na porovnávání analyzovaného signálu se specifickou předlohou, která představuje vnitřní zobrazení signálu. Pokud existují mezi zvířaty dlouhodobé asociace, rozlišování jedinců vyžaduje, aby byly předlohy pro rozpoznávání pravidelně aktualizovány (Sherman et al. 1997). Smyslové systémy, signály, chování během vysílání signálu a výběr habitatu jsou evolučně propojeny a toto spojení bývá označováno jako smyslový tah (angl. sensory drive; Endler 1992). Signalizace může probíhat v různých komunikačních kanálech a různí signalizující jedinci mohou být také vnímáni odlišným smyslem (Endler 1993).

Komunikace na větší vzdálenost může probíhat skrz zrak, vokalizaci nebo čichové podněty. Vizuální i vokální komunikace umožňují velmi rychlý přenos velkého množství informací. Pro vizuální komunikaci je nicméně nezbytný dostatek světla a také to, aby zvířata byla na dohled (Endler 1993). Vokální komunikace je výhodná pro přenos informací na delší vzdálenost než může být dosaženo olfaktorickými nebo vizuálními signály (Kondo a Watanabe 2009). Olfaktorická komunikace je pomalá a velmi specifická a olfaktorické signály velmi úzce souvisí s fyziologií a potravou jedinců, takže mohou být dobrými indikátory biologické zdatnosti (Endler 1993). Na rozdíl od olfaktorických signálů (Eisenberg a Kleiman 1972; Endler 1993) je mnoho vokálních signálů přímo namířeno ke konkrétnímu příjemci, ačkoli může být vokalizace dostupná mnoha dalším jedincům díky odposlouchávání (McGregor a Peake 2000). Olfaktorické signály mají zase velkou výhodu v tom, že v prostředí přetrvávají po delší dobu i v absenci signalizujícího jedince (Eisenberg a Kleiman 1972).

Vokální komunikace nosorožců

Vokální signály různých druhů savců obsahují informace o identitě vokalizujícího jedince (Darden et al. 2003; Frommolt et al. 2003; Charlton et al. 2009b, 2011a; Proops et al. 2009; Schneiderová a Policht 2010), jeho pohlaví (Rendall et al. 2004; Charlton et al. 2009a; Lemasson et al. 2009), věku (Charlton et al. 2009a), druhu (Schneiderová a Policht 2012), velikosti (Charlton et al. 2007, 2009a; Lemasson et al. 2009), emočním stavu (Fichtel et al. 2001), fázi reprodukčního cyklu u samic (Charlton et al. 2010a), úrovni hladiny androgenů u samců (Charlton et al. 2011b) nebo o sociální situaci, ve které se vokalizující zvíře nachází (Tallet et al. 2013). Příjemci tak mohou tyto informace v signálech rozlišovat a rozhodovat se na základě chování, fyziologie nebo morfologie ostatních jedinců (Endler 1993). Mnoho akustických signálů se také vyvinulo a je používáno v kontextu reprodukce a tyto signály často vedou k modifikaci neuroendokrinní fyziologie příjemce (Ball a Dufty 1998).

U kopytníků je schopnost rozlišovat různé informace z hlasů jedinců stejného druhu dobře probádána u některých domácích druhů nebo druhů chovaných na farmách (kůň domácí: Lemasson et al. 2009, Proops et al. 2009, Basile et al. 2009; prase domácí: Illman et al. 2002, Illman et al. 2008; koza domácí: Briefer a McElligott 2011, Briefer et al. 2012; jelen evropský: McComb 1991, Reby et al. 2001, Charlton et al. 2007; daněk evropský: Torriani et al. 2006, Pitcher et al. 2014), ale výzkumu rozpoznávání informací z hlasů jedinců stejného druhu u volně žijících kopytníků byla dosud věnována jen malá pozornost. Výzkum vokalizace jelenů evropských chovaných na farmách významně napomohl pochopení jejich reprodukční biologie. Laně preferují samce, kteří vokalizují rychleji (McComb 1991) a větší samce, které rozpoznají podle troubení, jež obsahuje nižší hodnoty rezonančních frekvencí (= formantů) v hlase a menší rozestupy mezi jednotlivými formanty (Charlton et al. 2007). Laně také dokáží rozpoznat troubení dominantního jelena ze svého stáda od troubení sousedních dominantních samců (Reby et al. 2001). Rozdíly v rychlosti troubení jelenů korelují s jejich schopností boje a rychlost troubení jedince také záleží na rychlosti troubení jeho oponenta (Clutton-Brock a Albon 1979). Takové znalosti o vokální komunikaci by byly velmi cenné v managementu a ochraně nosorožců.

Vokální projevy nosorožců byly donedávna popsány pouze ohledně kontextu, ve kterém jsou používány (Owen-Smith 1973; Laurie 1978; Goddard 1967; Budde a Klump 2003; von Muggenthaler et al. 2003; Policht et al. 2008), ale jejich funkce nebyla známá. Vokální repertoár nosorožců dvourohých (Budde a Klump 2003), sumaterských (von Muggenthaler et al. 2003)

a Cottonových (Policht et al. 2008) byl studován bioakusticky v zoologických zahradách a vokální repertoár volně žijících nosorožců tuponosých (Owen-Smith 1973) a indických (Laurie 1978) byl popsán pouze verbálně. Někteří autoři dříve uvažovali, jestli komunikace nosorožců neprobíhá na dlouhou vzdálenost podobně jako u slonů (Baskin 1991; von Muggenthaler et al. 1993), kteří reagují na hlasy ostatních jedinců až na vzdálenost několika kilometrů (Langbauer et al. 2001). Používání infrazvuku bylo zaznamenáno u všech druhů nosorožců, které jsou v současnosti drženy v zajetí (sumaterský, indický, dvourohý, tuponosý, Cottonův) (Baskin 1991; von Muggenthaler et al. 1993; Policht et al. 2008), nicméně se zdá, že nízké frekvence obsažené v některých hlasech by mohly být spíše vedlejším produktem velkého těla nosorožců než prostředkem pro komunikaci na velkou vzdálenost (Policht et al. 2008).

Vokální signály také mohou sloužit jako prezygotický reprodukčně izolační mechanismus a omezit nebo zabránit křížení příbuzných druhů (Ryan a Kime 2003). Nosorožci tuponosí a Cottonovi jsou blízce příbuzné sesterské druhy (Groves et al. 2010) a mají široký vokální repertoár, který pravděpodobně souvisí s jejich rozvinutým sociálním chováním (Owen-Smith 1973; Policht et al. 2008). U volně žijících nosorožců tuponosých bylo popsáno 10 typů hlasových projevů používaných v sociálním kontextu (Owen-Smith 1973) a u nosorožců Cottonových v zoologické zahradě Dvůr Králové bylo zaznamenáno 11 typů hlasových projevů (Policht et al. 2008). Vokální repertoár je mezi oběma druhy značně podobný (Owen-Smith 1973; Policht et al. 2008) (Tabulka 1), nicméně hlas chraptění (angl. hoarse) byl zaznamenán pouze u samců nosorožce Cottonova (**Příspěvek I.**). Tento nápadný hlas je používán samci téměř vždy během krmení, je opakován v sériích, které mají průměrnou délku 26 sec a byl popsán u všech třech samců nosorožce Cottonova chovaných v zoo Dvůr Králové (Policht et al. 2008; **Příspěvek I.)**, ale u nosorožců tuponosých zaznamenán nebyl (Owen-Smith 1973; **Příspěvek I.**). Nosorožci tuponosí a Cottonovi se také liší v některých parametrech svých kontaktních hlasů, které je možné s 91%ní pravděpodobností přiřadit ke správnému druhu. Tyto rozdíly mohly vzniknout přizpůsobením se konkrétním podmínkám habitatu, ve kterém se oba druhy přirozeně vyskytují (**Příspěvek I.**).

Tabulka 1: Vokální repertoár nosorožců Cottonových a tuponosých (Owen-Smith 1973; Policht et al. 2008; **Příspěvek I., III.**).

Typ hlasu a kontext		Použití*
Sociopozitivní projevy		
Sípání (angl. pant)	Kontaktní hlas, používán během přístupu, následování nebo zdravení jiného nosorožce nebo během izolace od zbytku stáda. Je tvořen sérií sípavých nádechů a výdechů.	Všichni
Chraplavé sípání (hic)	Součástí hlasu sípání, který se liší v závislosti na kontextu hlasu. Od klasického sípání je odlišováno intenzivnějšími chraplavými výdechy a nádechy. Používáno dospělým samcem během přístupu k samici, nejčastěji během říje.	♂
Zapištění (squeak)	Krátký, vysoký hlas. Používán jen málo, především při vizuální izolaci mláďat od matky, subadultů od ostatních členů skupiny nebo používán subadulty během utíkání před teritoriálním samcem.	Ml., Sub.
Kňučení (whine)	Nejčastěji používáno mláďaty před kojením. Bývá opakováno v sériích.	Ml.
Kňourání (squeal)	Připomíná kňučení mláďat. Používáno teritoriálním samcem, který se snaží zabránit samici, která přichází do říje, v opuštění jeho teritoria.	♂
Agonistické projevy		
Hrozba, funění ** (threat)	Hrozba zafuněním. Tvoří ji prudký výdech nebo nádech a bývá vnímán jako první varování k přistupujícímu zvířeti (Obr. 4).	Všichni
Funění (snort)**	Behaviorální kontext tohoto hlasu je variabilní, je také používán v agonistickém kontextu.	Všichni
Mručení (grunt)	Nízkofrekvenční vokalizace, většinou následuje po hrozbě zafuněním, pokud oponent neustoupil.	♀, Sub., Subdom.♂
Bručení (snarl)	Uši jsou namířené dozadu, hlava natažená dopředu a ústa jsou otevřená. Nejčastěji bývá směřován od dospělé samice, ale i subadultních zvířat nebo subdominantního samce k teritoriálnímu samci. Bývá používáno ve více extrémních situacích k zastavení přístupu oponenta.	♀, Sub., Subdom. ♂
Troubení (shriek)	Připomíná troubení slona. Používán subdominantními, subadultními nebo teritoriálními samci za hranicemi jejich teritoria, kteří jsou konfrontováni nebo pronásledováni místním teritoriálním samcem. Slouží pravděpodobně ke ztlumení agresivity teritoriálního samce.	Sub. ♂, Subdom. ♂
Vrčení (gruff-squeal)	Opakováno v sériích. Používáno teritoriálním samcem, když pronásleduje ostatní nosorožce, kteří před ním utíkají. Slouží pravděpodobně k zdůraznění přítomnosti a hrozby samce.	♂
Hlasy vydávané v dalších kontextech		
Zamručení (grouch)	Používáno během krmení a dalších aktivit v blízkosti ostatních členů stáda. Zaznamenáno především ve vokálním projevu dvou samic nosorožce Cottonova.	♀
Chraptění (hoarse)	Repetitivní hlas, který může trvat až několik minut. Byl zaznamenán pouze u dospělých samců nosorožce Cottonova. Bývá vydáván téměř vždy během krmení.	♂ *C. cottoni*
Frknutí (puff) **	Behaviorální kontext tohoto hlasu je variabilní, je používán např. během krmení, odpočinku nebo v kohezivních interakcích.	Všichni

* Nejčastější použití různými věkovými třídami nosorožců: Ml. = mláďata, Sub. = subadulti, Subdom. = subdominantní, ♀ = samice, ♂ = samec

** Owen-Smith (1973) popsal funění u nosorožců tuponosých, které používají v agonistickém kontextu jako „snort", nicméně Policht et al. (2008) rozlišili v bioakustické studii nosorožců Cottonových tři typy funění (angl. puff, snort, threat), z nichž pouze hlas popsaný jako „threat" byl používán výhradně v agonistickém kontextu.

Obr. 4. Samice nosorožce tuponosého během hrozby zafuněním.

Budde a Klump (2003) analyzovali harmonické, žadonící hlasy (angl. begging calls) nosorožců dvourohých v zoologických zahradách kvůli možnosti individuální identifikace zvířat a 61% hlasů byli schopni přiřadit ke správnému jedinci. Ačkoliv toto číslo bylo výrazně vyšší než by umožňovala náhoda, autoři došli k závěru, že pravděpodobnost přiřazení ke správnému zvířeti není dostatečně vysoká pro praktické určování jedinců podle hlasu. Kontaktní hlas sípání nosorožců tuponosých a Cottonových se skládá ze série nádechů a výdechů (Owen-Smith 1973; Policht et al. 2008) a opakování krátkých signálů může být v otevřeném habitatu, ve kterém se vyskytují (Owen-Smith 1973; Hillman-Smith 1982, 1987; Estes 1991), favorizováno kvůli snadnější detekci mezi nárazy větru na otevřených pláních (Wiley a Richards 1978). Podtypem kontaktního hlasu sípání je hlas chraplavé sípání (angl. hic), který používají dospělí samci během přístupu k samici, nejčastěji v období říje (Owen-Smith 1973; Policht et al. 2008; **Příspěvek III**.) (Obr. 5). V době, kdy samice přichází do říje, se intenzita přístupu samce k samici a frekvence jeho volání tímto hlasem neustále zvyšuje. Chraplavé sípání se liší od klasického sípání intenzivnějšími chraplavými nádechy a výdechy. V určitých sociálních situacích lze také slyšet přechodné hlasy mezi sípáním a chraplavým sípáním, které se těžko kategorizují (Owen-Smith 1973; vlastní pozorování).

Obr. 5: Páření nosorožců tuponosých. Když samice přichází do říje, samec intenzivně vokalizuje kontaktním hlasem chraplavé sípání během neustálých přístupů k samici a pokusů o páření.

Kontaktní hlasy nosorožců tuponosých a Cottonových lze s vysokou pravděpodobností přiřadit ke konkrétnímu jedinci. Jedinci se mezi sebou liší především v časových parametrech hlasu a jeho jednotlivých elementů (nádechů a výdechů) a hlasy dospělých samců také mohou obsahovat informace o dominantním nebo submisivním postavení (**Příspěvek I.**). Kontaktní hlasy nosorožců tuponosých se také liší mezi pohlavími, věkovými třídami (mladá a dospělá zvířata) a sociální situací vokalizujícího jedince (jestli je jedinec izolován nebo je ve vizuálním kontaktu s ostatními nosorožci; Obr. 6, 7). Například délka hlasu a počet elementů v hlase je u dospělých samic a mladých samců na rozdíl od dospělých samců větší v izolaci než ve vizuálním kontaktu (**Příspěvek III.**). Délka hlasu a počet hlasů v sekvenci bývají delší v situacích spojených se stavem většího rozrušení (Rendall 2003; Charlton et al. 2010a) a opakování elementů v hlase také napomáhá lokalizovat ztraceného jedince (Aubin a Jouventin 2002). Samice a mladí samci jsou tedy pravděpodobně v izolaci od vlastní skupinky ve stavu většího rozrušení a snaží se s ní znovu spojit (**Příspěvek III.**). Dospělí samci jsou samotářští (Owen-Smith 1973) a větší naléhavost situace pravděpodobně zažívají, když jsou v kontaktu se

samicí, zvláště v období říje, kdy je hlas chraplavé sípání používán nejčastěji (**Příspěvek III.**). Například samci pandy velké s vyšší hladinou androgenů mají delší hlasy (Charlton et al. 2011b) a hlasy samic jsou delší v plodné dny (Charlton et al. 2010a). Zajímavé je, že u mladých samic nosorožců tuponosých se délka hlasu v závislosti na sociální situaci neliší a počet elementů v hlase je velmi variabilní (**Příspěvek III.**).

Nosorožci tuponosí dokáží rozpoznat informace z hlasů jedinců stejného druhu i z hlasů blízce příbuzných nosorožců Cottonových. Volně žijící teritoriální samci jsou schopni rozlišit kontaktní hlasy samců a samic nosorožců tuponosých a v izolaci od ostatních nosorožců reagují výrazně intenzivněji na hlasy samic než samců, z čehož vyplývá, že kontaktní hlasy jsou důležitější pro komunikaci samce se samicí než pro komunikaci mezi teritoriálními samci (**Příspěvek III.**). Samci si značí své teritorium močí a trusem (Owen-Smith 1973, 1975) a olfaktorické signály jsou tedy pravděpodobně pro komunikaci teritoriality významnější. Samci nosorožců tuponosých také dokáží rozpoznat pohlaví z hlasů nosorožců Cottonových, ale reagují na samce i samice stejně intenzivně. Samci jsou také po playbaccích kontaktních hlasů aktivnější, stráví více času chůzí a běháním, značkují své teritorium nebo vokalizují kontaktním hlasem (**Příspěvěk III.**).

Způsob, jakým zvířata reagují na signály vlastního, a blízce příbuzného druhu může být komplexní. Jedinci na ně mohou reagovat stejně a důvodem nemusí být neschopnost mezi nimi rozlišovat, ale to, že signály příbuzného druhu jsou podobné signálům druhu vlastního a zároveň nové, pokud se jedná o allopatrické druhy (Braune et al. 2008; Ord a Stamps 2009). Reakce na signály příbuzného druhu mohou být ale i intenzivnější než na druh vlastní (Ord a Stamps 2009). Samci nosorožce tuponosého reagují intenzivněji na hlasy teritoriálního samce nosorožce Cottonova než samce vlastního druhu (**Příspěvek III.**). Oba druhy jsou allopatrické (Hillman-Smith et al. 1986) a rozlišování mezi nimi tedy nemůže být způsobeno učením a mohlo by být vyvoláno efektem novosti (angl. novelty effect; Tulving a Kroll 1995). Neznámé charakteristiky v hlasech samce nosorožce Cottonova mohou tedy vyvolat intenzivnější reakci (**Příspěvek III.**).

Se zjištěním, že kontaktní hlasy nosorožců tuponosých a Cottonových hrají významnou roli v jejich sociálním chování, vyvstává otázka, jestli i ostatní druhy nosorožců používají nějaké typy vokalizace k zjišťování informací o ostatních jedincích a jakou funkci jejich hlasy mají. Také další typy hlasů nosorožců Cottonových a tuponosých včetně chraplavého sípání, by mohly umožňovat kódování informací o vokalizujícím jedinci. Zejména znalosti o vokální komunikaci asijských druhů nosorožců jsou jen velmi malé (Laurie 1982; von Muggenthaler et al. 2003). Ačkoliv ostatní druhy nosorožců nemají tak rozvinuté sociální chování jako nosorožci

tuponosí a Cottonovi a nepoužívají stejný typ kontaktního hlasu (Goddard 1967; Owen-Smith 1973, 1975; Laurie 1982; van Gyseghem 1984; Penny 1987; Policht et al. 2008), rozpoznávání informací z hlasů ostatních jedinců pro ně může být velmi důležité stejně jako pro jiné, i solitárně žijící druhy savců (např. panda velká: Charlton et al. 2010a, 2010b; koala medvídkovitá: Charlton et al. 2011a, 2012a, 2012b).
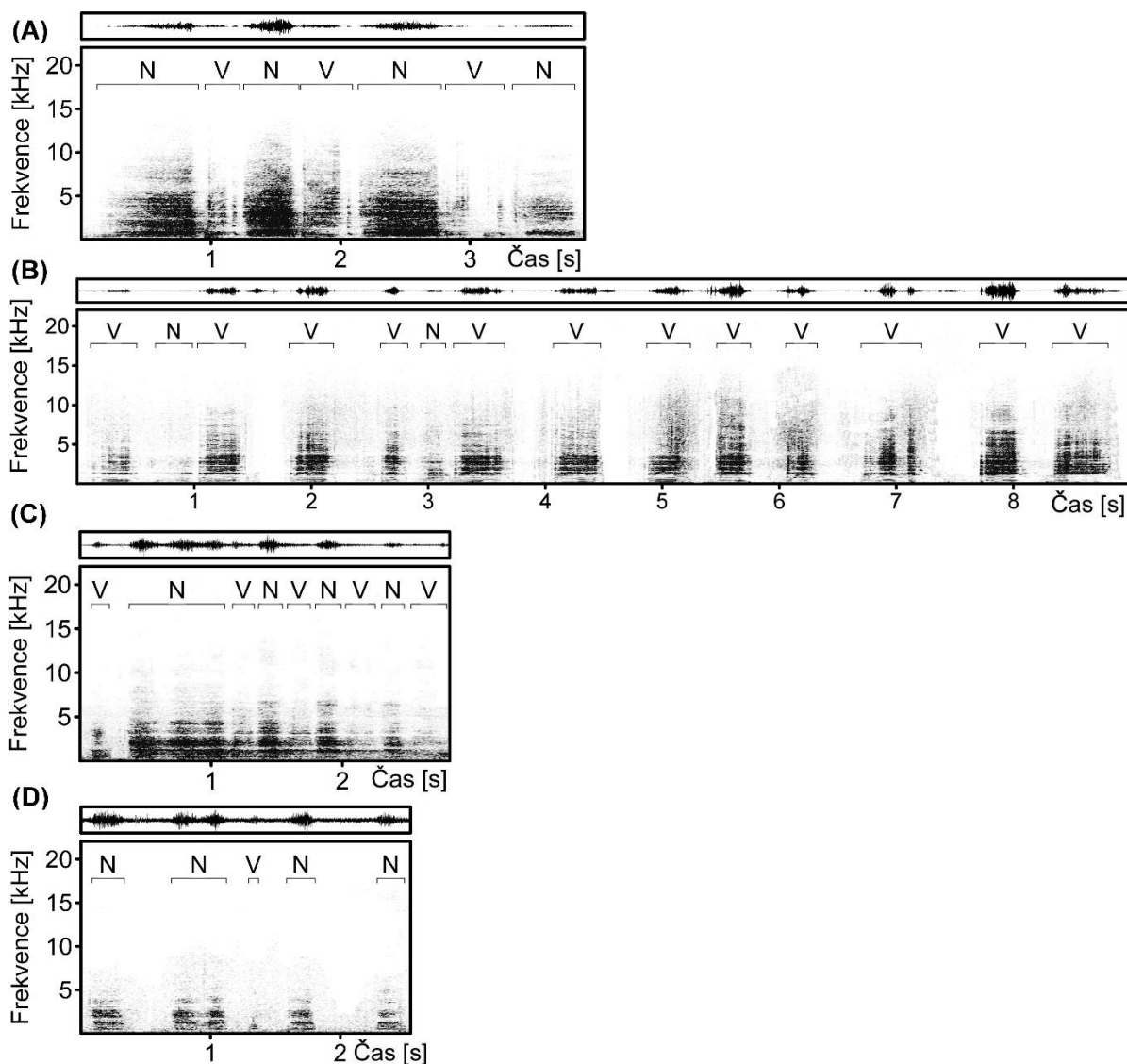
Obr. 6: Oscilogramy (nahoře) a spektrogramy (dole) kontaktních hlasů samců nosorožce tuponosého. Dospělí samci ve vizuální izolaci (A) a ve vizuálním kontaktu se samicí (během říje) (B). Subadultní samci ve vizuální izolaci (C) a ve vizuálním kontaktu s ostatními členy své skupiny (D). Jednotlivé elementy hlasu jsou označeny jako nádechy (N) a výdechy (V). Na spektrogramech jsou také nápadné individuální rozdíly v hlasech mezi zvířaty. Parametry spektrogramů: FFT length 1024, frame size 100%, overlap 87.5%, Hamming window.
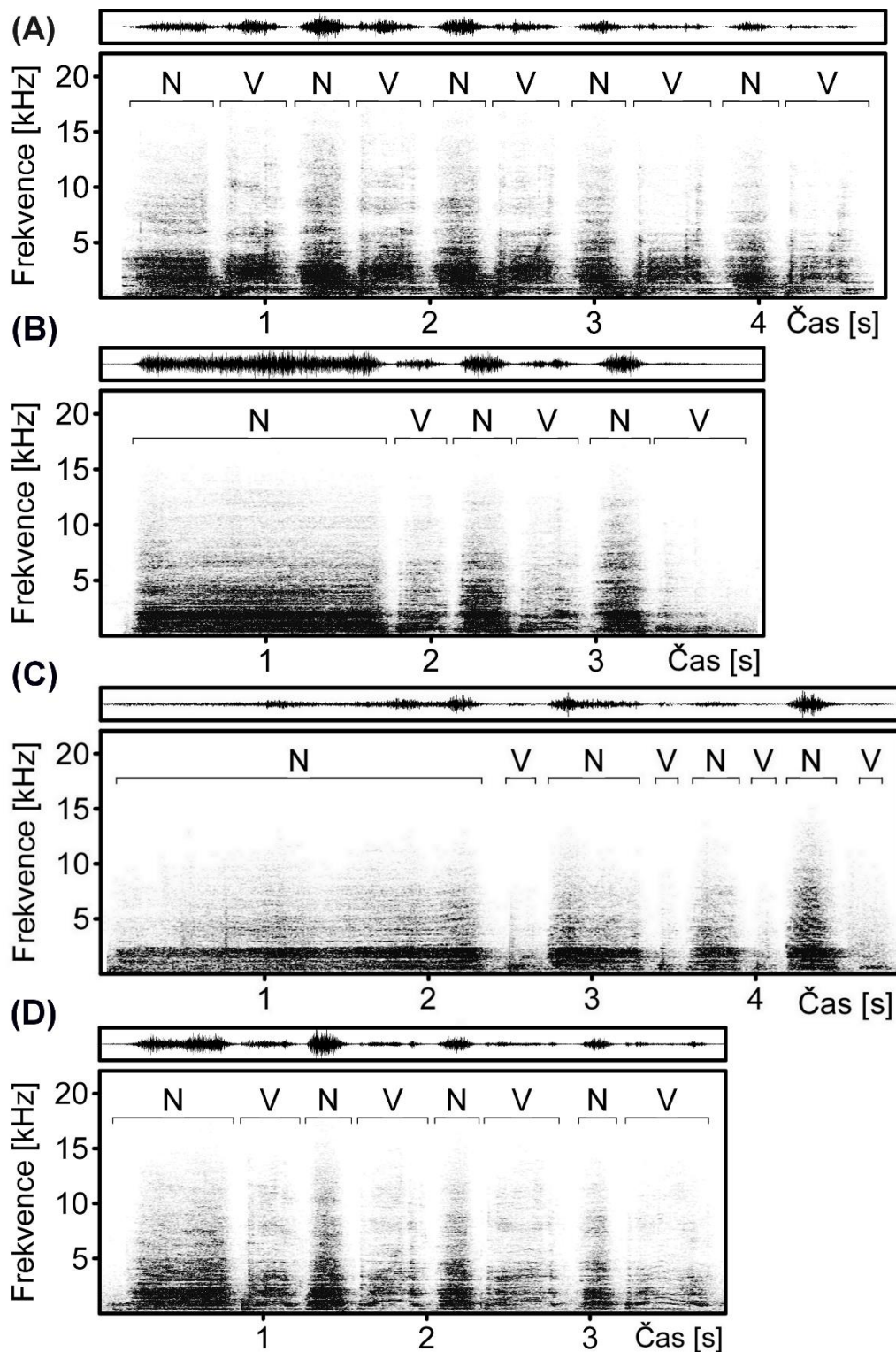
Obr. 7: Oscilogramy (nahoře) a spektrogramy (dole) kontaktních hlasů samic nosorožce tuponosého. Dospělé samice ve vizuální izolaci (A) a ve vizuálním kontaktu s ostatními členy své skupiny (B). Subadultní samice ve vizuální izolaci (C) a ve vizuálním kontaktu s ostatními členy své skupiny (D). Jednotlivé elementy hlasu jsou označeny jako nádechy (N) a výdechy (V). Na spektrogramech jsou také nápadné individuální rozdíly v hlasech mezi zvířaty. Parametry spektrogramů: FFT length 1024, frame size 100%, overlap 87.5%, Hamming window.

Olfaktorická komunikace nosorožců

Pachové signály hrají u většiny savců významnou roli v reprodukčním a sociálním chování i v jejich prostorové organizaci (Eisenberg a Kleiman 1972). Suchozemští obratlovci vnímají pachové signály hlavním čichovým systémem, který zpracovává především těkavé signály a vomeronazálním orgánem, který zpracovává hlavně netěkavé feromony (Dulac a Torello 2003). U savců rozlišujeme dva typy pachových signálů, které slouží ke komunikaci: identifikační a emotivní. Identifikační pachové signály jsou produkovány při pravidelných metabolických procesech zvířete a emotivní signály jsou produkovány pouze za zvláštních podmínek, například jako výsledek přechodného emočního stavu nebo vnějšího stimulu (Brown 1979). Pachové značky poskytují informace o kompetičních schopnostech a kvalitě signalizujícího jedince (Gosling a Roberts 2001) a signály v moči nebo trusu savců umožňují rozpoznat příbuzné (Bates et al. 2008), pohlaví a fázi reprodukčního cyklu jedince (Swaisgood et al. 2000; Charlton 2014), jeho identitu (Linklater et al. 2013) a tyto signály mohou hrát roli i při výběru partnera (Fisher et al. 2003; Johansson a Jones 2007) a umožnit rozpoznání jeho zdravotního stavu (Kavaliers a Colwell 1995). Chemické složení pachových značek, ale i jejich umístění, hustota výskytu v dané oblasti nebo čerstvost přispívají k posouzení signalizujícího jedince příjemcem ještě předtím, než se potkají (Gosling a Roberts 2001). Jelikož pachové značky bývají příjemcem nalezeny často bez přítomnosti signalizujícího jedince, může být pro potvrzení jeho identity potřeba porovnat pachové značky s jeho vlastním pachem (angl. scent-matching; Gosling 1982).

Olfaktorická komunikace je velmi důležitá v sociálním chování kopytníků, kteří mají také dobře vyvinutý vomeronazální orgán (Eisenberg a Kleiman 1972), trus a moč používají k značení svých teritorií a vyprazdňování a močení u nich může být doprovázeno ritualizovanými projevy (Grau 1976). Mnoho sudokopytníků má také specializované pachové žlázy, jejichž výměšky si značí své domovské okrsky nebo teritoria (Grau 1976; Estes 1991). U nosorožců až na několik výjimek žádné specializované pachové žlázy popsány nebyly [(nosorožci tuponosí: předkožkové žlázy (Cave 1966); nosorožci indičtí a jávští: pedální žlázy (Cave 1962)] a k olfaktorické komunikaci jim slouží především signály v moči a trusu a obecný pach těla (Goddard 1967; Owen-Smith 1973, 1975; Laurie 1982; van Gyseghem 1984; Penny 1987).

Nosorožci dvourozí signalizují pachovými značkami a chováním mnoho informací o své identitě a fyziologii. Samci i samice dělají po vyprázdnění zadníma nohama do země rýhy

a rozkopávají tak svůj trus po hnojišti. Samci dělají tyto rýhy častěji než samice, délka rýh vzrůstá u obou pohlaví s věkem a u samic je také ovlivněna fází reprodukčního cyklu (Freeman et al. 2014). Nosorožci dvourozí, kteří byli dočasně drženi v zajetí, dokáží rozpoznat signály v trusu o pohlaví, věku a identitě jedince. Zájem zvířat o trus ostatních jedinců se nesnižuje s jeho stářím až po dobu 32 dní, což naznačuje, že takto starý trus stále hraje důležitou roli v komunikaci nosorožců (Linklater et al. 2013). Také se ukázalo, že při vypouštění nosorožců dvourohých do nové oblasti může aplikování vzorků moči a trusu v okolí místa vypuštění, ovlivnit pohyb a distribuci zvířat, nicméně je zapotřebí další výzkum pro lepší pochopení všech faktorů, které ovlivňují chování nosorožců v této situaci (Linklater et al. 2006).

Nosorožci tuponosí a Cottonovi se vyprazdňují na společných hnojištích, a když prochází kolem nějakého hnojiště, většinou se zastaví a kupky trusu očichávají (Obr. 8). Samci si značí své teritorium tím, že před a po vyprázdnění zakopávají zadníma nohama, čímž rozptýlí trus po hnojišti a zadníma nohama dělají také do země rýhy, na které sprejovitě močí (Owen-Smith 1973, 1975; van Gyseghem 1984). Samci nosorožců tuponosých si značí teritorium močí intenzivněji blízko hranic svého teritoria na rozdíl od trusu, který je distribuován rovnoměrněji po celém teritoriu. Zdá se tedy, že trus by mohl poskytovat více informací o pohybu a distribuci samců (Kretzschmar et al. 2001). Samci v období sucha také mohou chodit do sousedních nebo více vzdálených teritorií k vodnímu zdroji a pro samce tedy není potkání jiného dominantního samce nebo jeho pachových značek ve svém teritoriu úplně výjimečné (Owen-Smith 1973).

Stejně jako u nosorožců dvourohých (viz nahoře), také u nosorožců tuponosých slouží trus k signalizování informací o jedinci. Volně žijící nosorožci tuponosí jsou schopni rozpoznat familiaritu a pohlaví jedince pouze na základě pachu jeho trusu. Zvířata čichají déle k trusu neznámých než familiárních jedinců a doba, za kterou nastane od začátku čichání k trusu ostražitost, se liší v závislosti na pohlaví i familiaritě zvířete, od kterého trus pochází. Na trus neznámých samic nastává ostražitost dříve než na trus familiárních samic a u trusu samců to je naopak. Trus tedy pravděpodobně hraje důležitou roli v sociálním chování a prostorové organizaci nosorožců tuponosých (**Příspěvek II.**).

Dospělí volně žijící teritoriální samci nosorožce tuponosého mají vyšší hladinu testosteronu než submisivní samci (Rachlow et al. 1998) a teritoriální status samce také ovlivňuje kvalitu jeho spermatu. Pokud jsou v zoologických zahradách drženi dva dospělí samci ve stejném zařízení se skupinou samic, pouze jeden ze samců má vysokou kvalitu spermatu, druhý samec má buď střední, nebo nízkou kvalitu (Hermes et al. 2005). Pro samice by tedy mohlo být nezbytné rozpoznat status samce z jeho olfaktorických signálů. V recentní studii se podařilo izolovat volatilní složky z trusu volně žijících nosorožců tuponosých, které jsou

asociovány s pohlavím, věkem, teritoriálním statusem samců a reprodukční fází samic (Marneweck 2013). Nosorožci by tedy mohli být schopni rozpoznat z trusu ostatních jedinců kromě familiarity a pohlaví (**Příspěvek II.**) mnoho dalších informací a ke stejnému účelu může sloužit i jejich moč. K objasnění, jaké další informace nosorožci z olfaktorických signálů zjišťují, jsou tedy nutné další studie.

Obr. 8: Samice nosorožce tuponosého se svým mládětem (samečkem) čichá k trusu na nosorožčím hnojišti, které je umístěno na cestě.

Závěr a hodnocení výsledků dizertační práce

Výsledky této dizertační práce představují první doklad o tom, že hlasy nosorožců obsahují informace o vokalizujícím jedinci a že zvířata jsou schopna tyto informace rozpoznat. Kontaktní hlasy nosorožců tuponosých a Cottonových hrají významnou roli v jejich sociálním chování, umožňují identifikaci konkrétního jedince, jeho druhu, pohlaví, věkové třídy a sociální situace (**Příspěvek I., III.**) a zvířata využívají informace obsažené v těchto hlasech k určení pohlaví nebo druhové příslušnosti jedinců (**Příspěvek III.**). Tato dizertační práce také přináší první informace o schopnosti volně žijících nosorožců rozlišovat informace o ostatních jedincích svého druhu pouze na základě pachu trusu. Nosorožci tuponosí jsou schopni z trusu rozlišit informace o familiaritě a pohlaví (**Příspěvek II.**).

Kontaktní hlas sípání nosorožců tuponosých a Cottonových je možné s vysokou pravděpodobností přiřadit ke správnému druhu (**Příspěvek I.**) a samci nosorožců tuponosých jsou schopni některé rozdíly v hlasech mezi oběma druhy rozpoznat (**Příspěvek III.**). Tyto výsledky přináší další významné informace o rozdílech mezi oběma druhy, které byly odlišeny teprve nedávno (Groves et al. 2010; Groves a Robovský 2011). K zjištění existence prezygotické reprodukční izolace mezi druhy založené na vokalizaci jsou ale relevantnější reakce samic na hlasy samců (Searcy 1992). Jelikož jsou nosorožci Cottonovi a tuponosí allopatričtí (Hillman-Smith et al. 1986), bylo by velmi zajímavé zjistit, zda rozdíly ve vokalizaci mezi nimi mohou způsobit preferenci samic pro hlasy samců vlastního druhu a zda existuje jakýkoliv náznak reprodukční izolace mezi oběma druhy, která by byla založená na vokalizaci.

Lepší znalost komunikace nosorožců nabízí možnost pro využití těchto poznatků v jejich managementu a ochraně. Mnoho samic nosorožce tuponosého a Cottonova má v zajetí nepravidelnou nebo absentující luteální aktivitu (Schwarzenberger et al. 1998; Hermes et al. 2006) a mnoho samců má pouze střední nebo nízkou kvalitu spermatu (Hermes et al. 2005). Hladina testosteronu u samců v zajetí stoupá se vzrůstajícím počtem samic, ke kterým mají přístup (Christensen et al. 2009) a dospělí volně žijící teritoriální samci mají vyšší hodnoty testosteronu než samci submisivní (Rachlow et al. 1998). Endokrinní fyziologie zvířat se často mění v odpovědi na akustickou stimulaci (Wingfield et al. 1994) a pachové signály (Eisenberg a Kleiman 1972; Campbell-Palmer a Rosell 2011). Intenzivní reakce samců na playbacky kontaktních hlasů (**Příspěvek III.**) a jejich intenzivní reakce na vzorky trusu (**Příspěvek II.**) ukazují, že tyto signály stimulují jejich sociální a teritoriální chování. Kontaktní hlasy také mohou významně ovlivňovat fyziologické procesy samic. Hlas chraplavé sípání používaný

samci nosorožců tuponosých a Cottonových je přímo asociovaný s reprodukcí (Owen-Smith 1973; **Příspěvek III.**) a mohl by stimulovat samice v říji. Trus, který samci používají k značení teritoria (Owen-Smith 1973; van Gyseghem 1984) by také mohl mít vliv na hormonální aktivitu samic, jelikož pachové značky často nejsou pouze způsobem obrany teritoria, ale slouží i k předávání informací a ke koordinaci sociálního a reprodukčního chování (Campbell-Palmer a Rosell 2011).

Playbacky kontaktních hlasů a vzorky trusu by tedy mohly ovlivňovat endokrinní fyziologii nosorožců, zlepšit luteální aktivitu samic a ovlivnit hladinu testosteronu u samců, stejně jako bylo ukázáno u jiných druhů zvířat (Knight a Lynch 1980; McComb 1987; Perret 1995; Ball a Dufty 1998; Rekwot et al. 2001; Tauck a Berardinelli 2007). Je tedy potřeba podniknout další studie zkoumající komunikaci nosorožců včetně zjištění vlivu manipulace s vokálními a olfaktorickými signály na hladinu hormonů u zvířat. Manipulace signálů volně žijících savců může mít také významné využití např. při translokaci zvířat k ovlivnění jejich pohybu po vypuštění nebo k vytvoření „virtuálních teritorií" před vypuštěním, což by mohlo snížit riziko konfliktu s rezidentními zvířaty (Swaisgood 2007; Campbell-Palmer a Rosell 2011).

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Příspěvek I.

Cinková I., Policht R. (2014) Contact Calls of the Northern and Southern White Rhinoceros Allow for Individual and Species Identification. PLoS ONE 9:e98475.
(doi:10.1371/journal.pone.0098475) (IF 3.534)

PLOS ONE

Contact Calls of the Northern and Southern White Rhinoceros Allow for Individual and Species Identification

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Abstract

Inter-individual relationships particularly in socially living mammals often require a well-developed communication system. Vocal and olfactory signals are the most important for the communication of rhinos, however, their vocal communication has been investigated to a very limited extent so far. White rhinos have the most developed social system out of all the rhinoceros species and vocal signals might therefore play an important role in their social interactions. We recorded repetitive contact pant calls from six captive northern white rhinos (*Ceratotherium cottoni*) and 14 captive and free-ranging southern white rhinos (*Ceratotherium simum*) and examined if they transmit information about individual identity, species, social context and age class. Discriminant analyses revealed that a high percentage of the pant calls of both species could be classified to a correct individual. We calculated signature information capacity of pant calls recorded from adult animals in isolation at 3.19 bits for the northern white rhinos and at 3.15 bits for the southern white rhinos, which can potentially allow for a vocal discrimination of nine individuals of both species. We found that pant calls varied by species. Northern white rhinos had longer calls and also differed from the southern white rhinos in several frequency parameters of their calls. We also analysed the pant calls of southern white rhinos for the differences between the age classes and between social contexts in which they were recorded. Our results show that pant calls carry information about individual, species, age class and context. The ability to recognize this information would allow rhinos, in addition to olfactory cues, to communicate with highly increased accuracy. A better understanding of communication of white rhinos has potential practical use in their management and conservation particularly because of the low breeding success of white rhinos in captivity.

Introduction

Certain aspects of rhinoceros acoustic communication have been compared to the communication of elephants intensively studied over recent decades (e.g. [1–3]). It has been hypothesised that rhinos might be using infrasound for long-distance communication which would be similar to the communication of elephants [4,5]. To the best of our knowledge, however, only basic descriptions of the rhinoceros vocal repertoire are known so far and no studies have reported any detailed information encoded in particular rhinoceros calls [6–8].

White rhinos have the widest vocal repertoire out of all the rhinoceros species whose vocalizations have been studied bioacoustically (see [6–8]) and also have the most developed social system (see [9–13]). Adult white rhinoceros males are territorial while females, subadults and juveniles live in groups in overlapping home-ranges [10,11,14]. The most frequently observed long-term associations of southern white rhinos include 2–3 individuals, although long-lasting groupings of up to six animals have also been

recorded [10,15]. Advanced acoustic communication might therefore be particularly useful for the white rhinoceros. Vocal recognition of offspring [16,17], mother [18], sex or age class [19,20], group membership [21,22], individual identity [23,24] and the dominant or subordinate status of males [25] have been previously described in many socially living mammals.

Acoustic signals may also serve as a premating isolating mechanism and restrict reproduction between different species [26]. Two subspecies of the white rhinoceros have recently been elevated to the species level, the northern (*Ceratotherium cottoni*) and southern white rhinoceros (*Ceratotherium simum*) [27]. More detailed research on their possible vocal distinction might contribute valuable data to this reassessment. Rookmaaker [28,29] has suggested that due to taxonomic revision, the name northern white rhinoceros is no longer appropriate and suggests following Heller [30] and calling it the Nile rhinoceros.

Rhinos are known to utter calls belonging to several categories including puffing, growling and harmonic calls (see [6–8,10,12]). The vocal repertoire of black [6], Sumatran [7] and northern

Table 1. Characteristics of the animals included in the study.

Individual (studbook no.)	Sex	Age (years)[1]	Population	Zoo/Reserve	No. of calls analysed in each context[2]				Notes
					Total	I	PI	VC	
Northern white rhinos:									
Fatu (#1305)	F	S, A (5,6,9)	Zoo	Dvůr Králové	35	26	5	4	Parents Nájin x Saút
Nabiré (#0789)	F	A (25,26)	Zoo	Dvůr Králové	53	24	18	11	Parents Nasima x Súdán
Nájin (#0943)	F	A (17,20)	Zoo	Dvůr Králové	34	24	10	–	Parents Nasima x Súdán
Nesári (#0377)	F	A (33,37,38)	Zoo	Dvůr Králové	15	6	5	4	Wild-born
Súdán (#0372)	M	A (36)	Zoo	Dvůr Králové	18	18	–	–	Wild-born
Suni (#0630)	M	A (29)	Zoo	Dvůr Králové	8	8	–	–	Parents Nasima x Saút
Southern white rhinos:									
Ada (#1154)	F	A (25)	Zoo	Bratislava	12	12	–	–	Wild-born
Kathi (#362)	F	A (37,38)	Zoo	Salzburg	23	16	–	7	Wild-born
Kifaru (#773)	F	A (27)	Zoo	Salzburg	21	21	–	–	Wild-born
Munyani	F	A (15)	Free-ranging	Lapalala	35	35	–	–	Wild-born
Yeti (#936)	F	A (11)	Zoo	Salzburg	9	–	–	9	Wild-born, mother of Malia
Malia (#938)	F	S (3)	Zoo	Salzburg	7	–	–	7	Wild-born, daughter of Yeti
Tamu (#937)	F	S (4)	Zoo	Salzburg	19	–	–	19	Wild-born
Naja (#T18)	F	S (5)	Zoo	Zlín	17	13	–	4	Wild-born
Zanta (#T17)	F	S (6)	Zoo	Zlín	26	24	–	2	Wild-born
Natal (#371)	M	A (39)	Zoo	Dvůr Králové	25	25	–	–	Wild-born
Medupi	M	S (3)	Free-ranging	Lapalala	10	10	–	–	Wild-born, stepbrother of Lekoto
Lekoto	M	S (2)	Free-ranging	Lapalala	5	–	–	5	Wild-born, stepbrother of Medupi
M06	M	S (4)	Free-ranging	Welgevonden	8	–	–	8	Wild-born
Bert	M	S (3)	Free-ranging	Lichtenburg	5	–	1	4	Wild-born

[1] Age at time of call recordings. A = adult, S = subadult. The animals were classified by age class following Owen-Smith [10,11]; females were regarded as adults at 6.5–7 years of age and males between 10 and 12 years of age.
[2] I = visual isolation from other rhinos, PI = partial isolation: the animal was visually isolated from the rest of its group, but was in the company of other rhinos, VC = in visual contact with group member(s).
doi:10.1371/journal.pone.0098475.t001

white rhinos [8] has been studied bioacoustically while the repertoire of Indian [12] and southern white rhinos [10] has only been described verbally. Northern and southern white rhinos share a similar vocal repertoire [8,10] and a detailed comparison of the vocal repertoires of all rhinoceros species revealed that white rhinos use a unique category of repetitive calls [8].

Policht et al. [8] suggested that the repetitive contact call pant of white rhinos, which is not known in other rhinoceros species, could be used for long-distance communication. White rhinos live in open habitats [10,31] and the repetition of short signals would therefore be favoured for long-distance communication in this environment due to an easier detection between bursts of wind [32]. Pant is a sequence of inhalations and exhalations and is used by all sex-age classes when greeting or approaching another rhinoceros, as a response to previous calls or during separation from a group [8,10]. Pant is usually apparently directed to a particular individual [8] and in such vocalizations, individuality in calls could be expected. The differences in vocalizations between males can also indicate the quality (red deer: [33]) or social status (horse: [22]) of the male and therefore influence female mate choice. Vocal individuality also has the potential to be used in conservation; individual discrimination and identification of animals have their implications from census tasks to monitoring the animals over time [34].

Northern white rhinos are currently on the brink of extinction with only seven surviving individuals and although the numbers of southern white rhinos have recently reached over 20,000 individuals in Africa, their population is in danger due to escalating poaching [35]. The reproduction of both species in captivity is extremely low (e.g. [36,37]). Although the reasons behind this are poorly understood, several studies have suggested that social interactions between captive rhinos might be one of the possible reasons [38–41]. Research on white rhinoceros communication might be extremely valuable for an improved understanding of their social behaviour.

We investigated whether the contact pant calls of white rhinos contain sufficient information for recognition of individuals, species, age classes and contexts. In addition, we also calculated the signature information capacity H_S present in the pant calls of northern and southern white rhinos following Beecher [42].

Materials and Methods

Ethics Statement

Research for this project including the recording of calls and playbacks of pant calls for white rhinos was approved by the Ethics and Scientific Committee of the National Zoological Gardens of South Africa (Project P11/03). The research was conducted in accordance with the guidelines of the Animal Behaviour Society for the ethical use of animals in research.

Animals and Data Collection

The pant calls of six northern white rhinos were recorded in the zoological garden Dvůr Králové and the calls of 14 southern white rhinos in zoological gardens Salzburg, Zlín, Bratislava, Dvůr Králové and in the South African wildlife reserves Lapalala Wilderness, Welgevonden Game Reserve and Lichtenburg Biodiversity Conservation Centre in 2005–2006 and 2009–2012 (Table 1). We recorded the calls with a Sennheiser directional microphone (ME 67 with K6 powering module, frequency response: 40–20,000 Hz±2.5 db) fitted with a Rycote Softie windshield and digital recorders Marantz PMD 671 or Yamaha Pocketrak C24 with a 44.1 kHz sampling rate and 16 bits resolution. The vocalizations were recorded both outside and in

the stables at distances from 0.5 to 30 m, over a minimum of two different days for each animal (mean 5±2.5 days, range 2–12 days) and with the time between the first and last recording varying from three days to five years for each subject.

The pant calls were recorded in the following context: (1) visual isolation from other white rhinos (68.1% of the calls), (2) partial isolation: the animal was visually isolated from the rest of its group, but was in the company of other white rhinos (10.1%), (3) in visual contact with group member(s) (21.8%). The animals vocalized either naturally or replied to our playback of a pant call. The rhinos became isolated when they either naturally separated themselves and lost visual contact or when they were separated in the enclosure or in the stables by the keepers. All the pant calls of adult males were recorded in visual isolation from other white rhinos.

Acoustic Analysis

We only included complete calls consisting of a series of elements in the analysis; single inhalations or exhalations, which rhinos occasionally produce in excited situations, were not analysed. We only selected calls which were recorded in good quality with low background noise. The call elements were manually marked in Avisoft SAS Lab Pro 5.2.07 (Avisoft Bioacoustics, R. Specht, Berlin, Germany) with the help of an envelope curve and spectrogram. The temporal parameters were then computed automatically using the following spectrogram parameters: FFT length 256, frame size 100%, overlap 50%, FlatTop window. These were duration, interval between particular elements, the distance from the start to maximum amplitude and start/end time. As the duration of the particular elements and the number of elements of a certain duration within the call were highly variable between individuals, we calculated various parameters in order to extract the most important temporal characteristics of the calls. We calculated the number of elements in each call, the call duration, the duration of the longest and shortest inhalation and exhalation in the call, the order of the longest inhalation and exhalation in the call, the number of inhalations and exhalations in the call in the duration from 0.0–0.4 sec (= in categories 1 and 2, see below) and the percentage of inhalations and exhalations in a duration 0.0–0.4 sec from all the inhalations and exhalations in the call. Spectral parameters were measured using the following spectrogram parameters: FFT length 1,024, frame size 100%, overlap 87.5%, Hamming window.

As pant calls are repetitive signals, we only selected certain elements of the calls to measure the spectral parameters. The calls were divided into inhalations and exhalations and several very weak elements (2% out of the total number of elements) were excluded from the analysis to avoid fluctuations in breath intensity. The inhalations and exhalations were then divided according to their duration into categories: (1) 0.0–0.2 sec (28.6% of all elements), (2) 0.21–0.4 sec (50.1%), (3) 0.41–0.8 sec (18.6%), (4) >0.81 sec (2.7%) (Figure 1). Only one element from the most numerous group of inhalations and exhalations was chosen for the analysis (in 2.3% of inhalations and 3.6% of exhalations, we used an element from the second most numerous group as there was no good quality recorded element in the first group). Within the group, the first well-recorded intensive element from the beginning of each call was chosen. The spectrograms (spectrogram parameters: FFT length 1024, frame size 100%, overlap 87.5%, Hamming window, time resolution 2.9 ms) of these elements were then analysed in the acoustic programme LMA 2008 (kindly provided by Kurt Hammerschmidt) and we computed 117 parameters for each selected element.
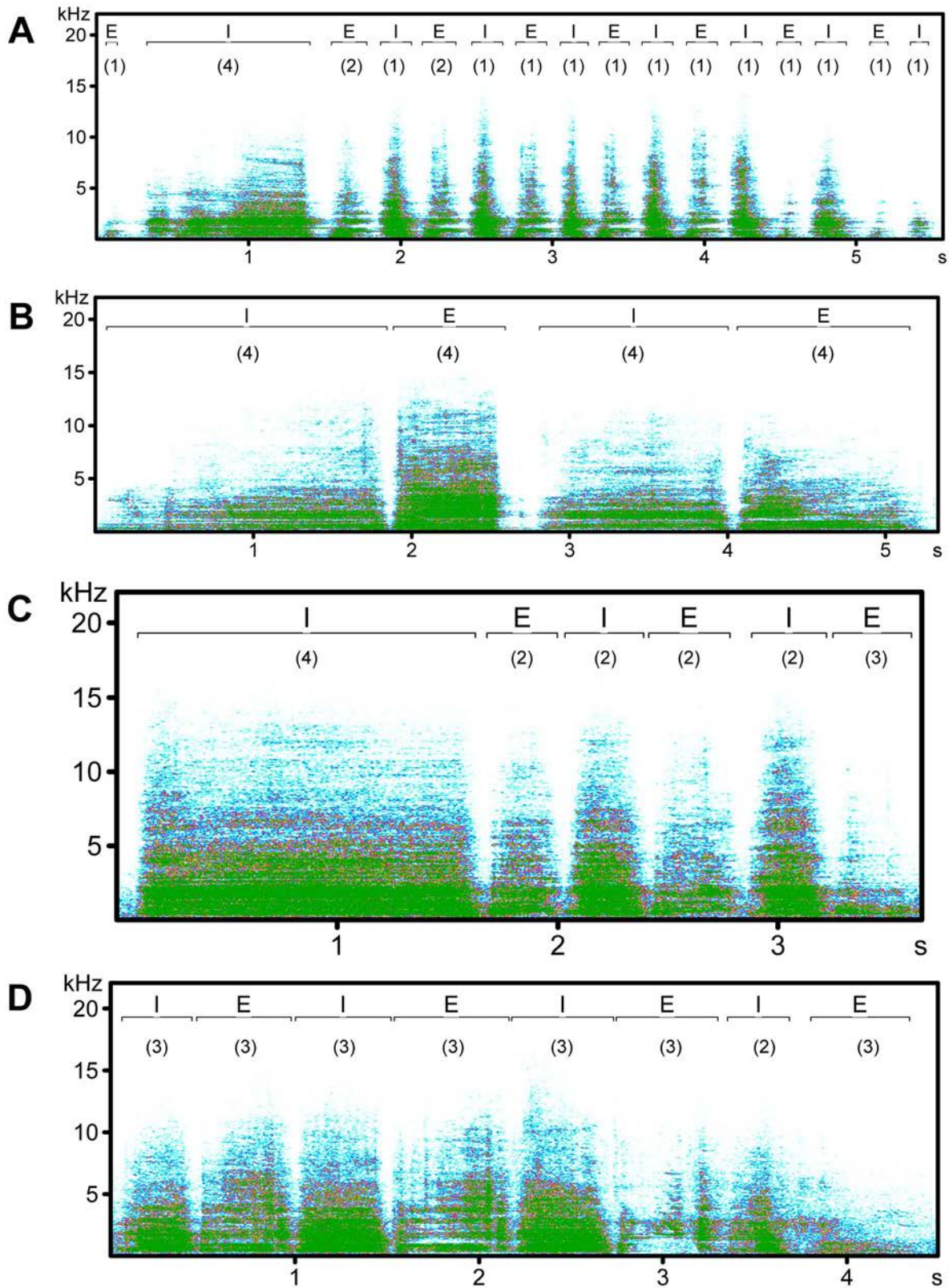
Figure 1. Spectrograms of pant calls of adult northern and southern white rhinos. Northern white rhinos: female Nabiré (A) and male Suni (B). Southern white rhinos: female Yeti (C) and male Natal (D). Inhalations (I), exhalations (E) and their affiliation to the categories based on their duration are shown: category (1): 0.0–0.2 sec, (2): 0.21–0.4 sec, (3): 0.41–0.8 sec, (4): 0.81 sec. (Spectrogram parameters: FFT length 1024, frame size 100%, overlap 87.5%, Hamming window).
doi:10.1371/journal.pone.0098475.g001

General Statistical Analyses

All the variables were Box-Cox transformed to improve the normality of their distribution. The dataset contained a few zero values; they were therefore shifted to the smallest possible value during the Box-Cox transformation. Statistical analyses were performed with software IBM SPSS Statistics 20.0 (IBM Corp., Armonk, USA) (for conventional discriminant function analyses and information calculation), R 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) (for permuted discriminant function analyses) and Statistica 12.0 (StatSoft Inc., Tulsa, USA) (for Box-Cox transformations and Mann-Whitney U tests).

Discriminant Function Analyses

We performed conventional discriminant function analyses (DFAs) and permuted discriminant function analyses (pDFAs) to test our ability to correctly assign calls to individual, species, context and age class. We checked for pair-wise correlations between variables and only one from a highly correlated pair with $r > 0.8$ has been retained in the analyses.

We conducted conventional forward stepwise DFAs to classify the calls of both species according to the individuals. The variables were added and removed based on the changes in Wilks' lambda. The resulting variables which contributed to the greatest extent to the recognition between individuals were used as a source for the final DFAs. We applied a cross-validation (leave-one-out procedure) to validate the results of the DFAs as in this case each call in the analysis was classified by the functions derived from all the calls other than that call. We studied the possibility of classifying the calls of northern white rhinos recorded in various social contexts to a correct individual (= DFA 1). To avoid overestimation or underestimation of our results due to the fact that the animals were recorded in various contexts, we re-ran the analysis using only calls recorded in isolation (= DFA 2). Similarly, we performed DFA 3 to study the possibility of correctly classifying the calls of southern white rhinos recorded in various contexts as well as calls recorded only in isolation (= DFA 4). The DFAs 2 and 4 were only conducted using the calls of the adult animals to control for any possible influence of age class on our results.

We then performed pDFAs for nested designs, which is a randomization procedure used for non-independent two-factorial data sets when one factor is nested in another. The detailed procedure is described in Mundry and Sommer [43]. The pDFAs were conducted using a script written in software R (kindly provided by Roger Mundry) using 100 random selections and 1,000 permutations. The script is based on the function Ida of the R package MASS [44]. The pDFA calculates the percentage of correctly classified objects for the original (i.e. unpermuted) data, based on the calls used to derive discriminant functions and the percentage of correctly classified calls for the cross-validated (i.e. permuted) data, which were not used to derive discriminant functions [43].

We conducted pDFAs to test our ability to correctly assign calls to context (= pDFA 1) and age class (adults and subadults; = DFA 2) in southern white rhinos and to assign calls of adult northern and southern white rhinos recorded in isolation to correct species (= pDFA 3) while controlling for individual variation. For pDFAs, we used variables which discriminated best between contexts and age classes in southern white rhinos and between species. In pDFA 1, we included calls from animals recorded in isolation (Ada, Kathi, Kifaru, Munyani, Naja, Zanta, Natal, Medupi) and in visual contact with group member(s) (Yeti, Malia, Tamu, Lekoto, M06, Bert) in order to meet the requirements of the nested design of pDFA as the calls of each individual can only be included in one context. The same analysis was not performed for the northern

white rhinos as we were limited by the number of individuals and the number of recorded calls in each context.

We conducted seven DFAs and pDFAs in total. A sequential Bonferroni correction was therefore applied to correct the p-values. A detailed description of all the variables used in the DFAs is provided in Table S1 and their descriptive statistics in Table S2. Two-tailed Mann-Whitney U tests were performed to test for differences in the call parameters between species and between contexts and age classes in southern white rhinos. We applied the sequential Bonferroni correction as the data were used for multiple comparisons.

Information Calculation

We described the variability in calls of northern and southern white rhinos following Beecher [42], Arnold and Wilkinson [45] and Carter et al. [46]. We extracted principal components (PCs) with varimax rotation from call data and ran a parallel analysis [47] to determine how many PCs to extract from our data. We saved the PC scores using the Bartlett method and then used the restricted maximum likelihood to obtain the variance component estimate (VCE) of the random factors (individual, sex, context, age class, population) for each retained PC. We weighted the VCE for each factor by the percentage variance explained by its corresponding PC to estimate the percentage of variance contributed by the random factors. As the southern white rhinos were recorded both in zoological gardens and in wildlife reserves, we divided them into two groups according to the population (captive and free-ranging) and included population as a random factor in the VCE analysis.

The signature information capacity in contact calls was calculated following Beecher [42], Arnold and Wilkinson [45] and Carter et al. [46]. We favoured this approach as it allows for a comparison of signature information content in calls across different species or sample sizes [42] and it is a standard method used in many recent papers (e.g. [45,46,48–51]). The information capacity H_S in a particular vocalization is calculated in bits [42]. The value 2^{HS} provides an estimate of the number of individuals, which can potentially be discriminated on the basis of the call parameters considered [52]. To avoid any influence of call context or age class on our results, we calculated H_S from the calls of adult northern and southern white rhinos recorded in isolation. The total signature information capacity was calculated from VCEs (obtained by the procedure described above) for individual and sex differences (S_B^2) and within-individual differences (= unexplained variation in calls) (S_W^2). The total variance (S_T^2) is the sum of S_B^2 and S_W^2. The information in each PC was then summed ($H_i = \log_2 (S_T/S_W)$) to calculate the total information capacity in the call ($H_S = \sum H_i$) and the repeatability of each PC ($S_B^2/(S_B^2+S_W^2)$) [42,45,46].

Results

Description of the Pant Calls of Both Species

We recorded 163 calls of six northern white rhinos, which consisted on average of 14 ± 4.7 elements with a call duration 6 ± 1.8 sec. The mean frequency range was 4794 ± 1609.6 Hz in inhalation and 4948 ± 2119.1 Hz in exhalation. The minimum frequency of the first and maximum frequency of the third distribution of the frequency amplitude was 175 ± 139.8 Hz and 9170 ± 3870.6 Hz, respectively in inhalation and 106 ± 102.1 Hz and 8351 ± 3825.6 Hz, respectively in exhalation. We recorded 222 calls of 14 southern white rhinos, which consisted on average of 9 ± 2.6 elements with a call duration 4 ± 0.9 sec. The mean frequency range was 4504 ± 1362.6 Hz in inhalation and

5753±1678.2 Hz in exhalation. The minimum frequency of the first and maximum frequency of the third distribution of the frequency amplitude was 530±260.2 Hz and 12003±4269.8 Hz, respectively in inhalation and 422±206.2 Hz and 14768±2757.1 Hz, respectively in exhalation (see Figure 1).

Information Encoded in the Calls of Northern White Rhinos

The pant calls of northern white rhinos were individually distinct. A discriminant function analysis assigned 81% of calls (74% cross-validated) to the correct individual (DFA 1: N = 6 animals, n = 163 calls, Wilks' lambda = 0.064, p<0.001) (Table 2, Figure 2). We included nine acoustic variables into this model (Table 3). Three extracted principal components from this model accounted for 57% of the total variance in calls. Out of this variation, 32.4% was explained by individual (14.5%), sex (10.8%), context (1.5%), interaction between the individual and context (4.7%) and between the sex and context (0.9%).

The ability to assign calls to individuals slightly increased when we only included calls recorded from the adult animals in isolation and 87% of calls (79% cross-validated) were correctly classified (DFA 2: N = 6 animals, n = 104 calls, Wilks' lambda = 0.023, p< 0.001) (Table 2). We used the same nine acoustic variables as in DFA 1. The first three discriminant functions with Eigenvalue>1 explained 94% of the variability. Based on this model, we calculated the signature information in the pant calls, which was 3.19 bits with a mean repeatability of PCs 0.52.

Information Encoded in the Calls of Southern White Rhinos

The pant calls of southern white rhinos were classified to the correct individual with 93% success (83% cross-validated) (DFA 3: N = 14 animals, n = 222 calls, Wilks' lambda = 0.0004, p<0.001)

(Table 2) by using 17 acoustic variables (Table 3, Figure 3). Six extracted principal components from this model accounted for 73% of the total variance in calls. Out of this variation, 38.8% was explained by individual (9.3%), age class (7.1%), sex (5.0%), population (3.4%), context (0.6%), interaction between the individual and context (6.0%), sex and age class (2.8%), context and age class (1.9%) and further 2.7% of the variation was explained by other interactions between these factors.

As the calls of some animals were primarily recorded in isolation and the calls of others primarily in visual contact with group member(s) (see Table 1), we conducted pDFA to test whether the calls vary by these contexts while controlling for individual variation. By using six variables (Table 4), we could correctly assign 92% of calls (89% cross-validated) (pDFA 1: N = 14 animals, n = 208 calls, p = 0.01). Pant calls of the southern white rhinos also varied by age class. We classified 88% of calls (86% cross-validated) to the correct age class when controlling for individual variation (pDFA 2: N = 14 animals, n = 222 calls, p = 0.028). We included five variables in this model (Table 4).

We ran another conventional DFA with the calls of adult southern white rhinos recorded in isolation. This analysis revealed similar results as the DFA 3 and 90% of calls (84% cross-validated) were classified to the correct individual (DFA 4: N = 5 animals, n = 109 calls, Wilks' lambda = 0.028, p<0.001) (Table 2). We included eight variables in this model (these variables were also included in DFA 3) and the first two discriminant functions with Eigenvalue>1 explained 84% of the variability. Based on this model, we calculated signature information in the pant calls, which was 3.15 bits with a mean repeatability of PCs 0.71.

Species Differences in the Pant Calls

We conducted pDFA to assess differences in pant calls between the northern and southern white rhinos while controlling for
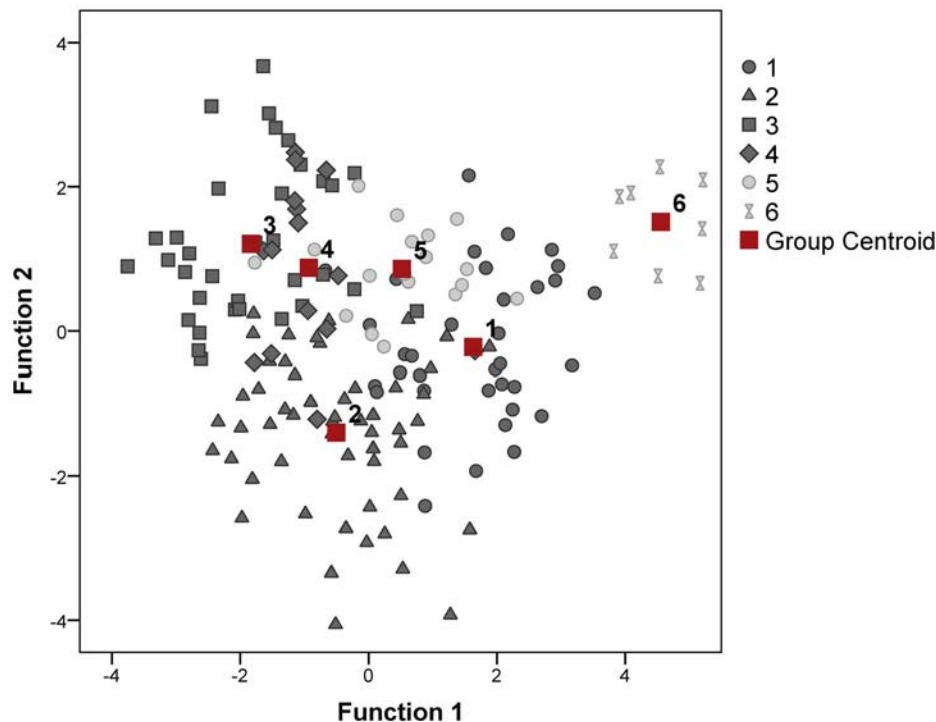
Figure 2. Vocal individuality in pant calls of northern white rhinos recorded in various social contexts. The plot shows the first two canonical discriminant functions with the centroid values of pant calls for each animal. 1 = Fatu, 2 = Nabiré, 3 = Nájin, 4 = Nesárí, 5 = Súdán, 6 = Suni.
doi:10.1371/journal.pone.0098475.g002

Table 2. Classification success of pant calls of northern and southern white rhinos in conventional discriminant function analyses conducted with calls recorded from all the animals in various contexts (DFAs 1, 3) and with calls recorded only from the adult animals in isolation (DFAs 2, 4).

Individual	Sex	Calls recorded in various contexts		Calls recorded in isolation	
		% correctly classified	% correctly classified cross-validated	% correctly classified	% correctly classified cross-validated
Northern white rhinos:					
		DFA 1	DFA 1	DFA 2	DFA 2
Fatu	F	85.7	68.6	83.3	79.2
Nabiré	F	75.5	75.5	75.0	70.8
Nájin	F	79.4	76.5	87.5	75.0
Nesárí	F	86.7	66.7	100	83.3
Súdán	M	77.8	72.2	94.4	83.3
Suni	M	100	100	100	100
Southern white rhinos:					
		DFA 3	DFA 3	DFA 4	DFA 4
Ada	F	100	91.7	100	83.3
Kathi	F	95.7	95.7	93.8	81.3
Kifaru	F	85.7	71.4	85.7	81.0
Munyani	F	97.1	88.6	80.0	80.0
Yeti	F	88.9	55.6	–	–
Malia	F	100	71.4	–	–
Tamu	F	68.4	63.2	–	–
Naja	F	94.1	88.2	–	–
Zanta	F	96.2	84.6	–	–
Natal	M	100	96.0	100	92.0
Medupi	M	100	90.0	–	–
Lekoto	M	60.0	60.0	–	–
M06	M	100	100	–	–
Bert	M	100	60.0	–	–

doi:10.1371/journal.pone.0098475.t002

individual variation. To avoid any possible influence of age class or social context on our results, we only included calls from the adult animals recorded in isolation. Five variables were included in this model (Figure 4) and 91% of calls (90% cross-validated) were assigned to the correct species (pDFA 3: N = 11 animals, n = 213 calls, p = 0.01).

Discussion

The Function of Pant Call and Factors Influencing its Structure

Despite the growing number of papers devoted to the information encoded in animal vocalizations, such studies have been completely lacking for the entire family *Rhinocerotidae*. Policht et al. [8] suggested that contact call pant of the northern and southern white rhinos might serve for long-distance communication. Since we found that pant carries the individual characteristics of the caller and contains context-, age class- and species-specific acoustic features, we agree that it could serve as a signal advertising the identity and state of the caller at longer distances than can be achieved by visual or olfactory cues, particularly since the eyesight of rhinos is weak (e.g. [31]). Certain parameters of mammalian vocalizations can be highly correlated between relatives [53], however, relatedness does not necessarily affect

the overall acoustic similarity between the animals [53,54]. Although most northern white rhinos in our study were closely related, it seems unlikely that it would affect our results because the calls of unrelated individuals (SÚDÁN and NESÁRÍ) clustered in DFAs 1 and 2 together and also along with other northern white rhinos. Only two pairs of the southern white rhinos were related (see Table 1).

We found that pant calls of the southern white rhinos varied by age class and social context in which they were recorded. Although the differences were not statistically significant, subadults produced calls with shorter total duration and with shorter duration of the longest and shortest exhalation than adults. This is in accordance with our expectations since the smaller animals have smaller lungs and less air volume available for calling. They should therefore emit shorter calls than the larger animals [55]. The five parameters, which we used to classify the calls according to the context also included a number of elements in the calls. The calls emitted in isolation contained more elements than the calls emitted in visual contact with group member(s). More frequent repetition of elements in the pant call could serve to better locate an isolated individual by the group member(s). In king penguins, more frequent repetition of syllables helps the chicks to better locate their parents because of the background noise of the colony [56]. However, when we studied the differences between the age

Table 3. DFA structure matrices for northern and southern white rhinos showing pooled within group correlations between discriminating variables and standardized canonical discriminant functions with Eigenvalues>1.

Acoustic variable	Short description	Northern white rhinos DFA 1: Discriminant functions		Southern white rhinos DFA 3: Discriminant functions				
		1.	2.	1.	2.	3.	4.	5.
Call duration	Duration of call (sec)			−0.41			0.41	
No. elements	Number of elements in call			−0.41			0.47	
I: max element length	Duration of the longest inhalation in call (sec)	0.49						
I: min element length	Duration of the shortest inhalation in call (sec)	0.46	0.38			−0.49		
Order longest inhalation	Order of the longest inhalation in call							0.40
I: no. in cat. 1,2–percentage	Percentage of exhalations in call, which are in duration from 0.0–0.4 sec					0.55		
E: max element length	Duration of the longest exhalation in call (sec)	0.50			0.55			
E: duration	Duration of exhalation (sec)				0.61			
E: no. in cat.1,2	No. of exhalations in call in duration from 0.0–0.4 sec (= in categories 1, 2)	−0.45						−0.38
I: peak freq (max)	Frequency of the maximum amplitude of spectrum [Hz]		−0.38					
I: ampratio3	Amplitude ratio between 2nd and 3rd dominant frequency band [Hz]		0.36					
I: df3mean	Mean frequency of 3rd dominant frequency band [Hz]		0.35					
Eigenvalue		2.58	1.26	4.01	3.3	2.3	1.6	1.1
% of variance		55.8	27.3	27.4	22.1	15.7	11.0	7.4
Cumulative %		55.8	83.1	27.4	49.6	65.3	76.3	83.7

Only correlations≥0.35 are shown. Eigenvalue and percentage of variance explained by each discriminant function are also shown. The DFA 1 included calls of all the northern white rhinos recorded in various contexts, DFA 3 included calls of all the southern white rhinos recorded in various contexts. I = variable measured in inhalation, E = variable measured in exhalation.

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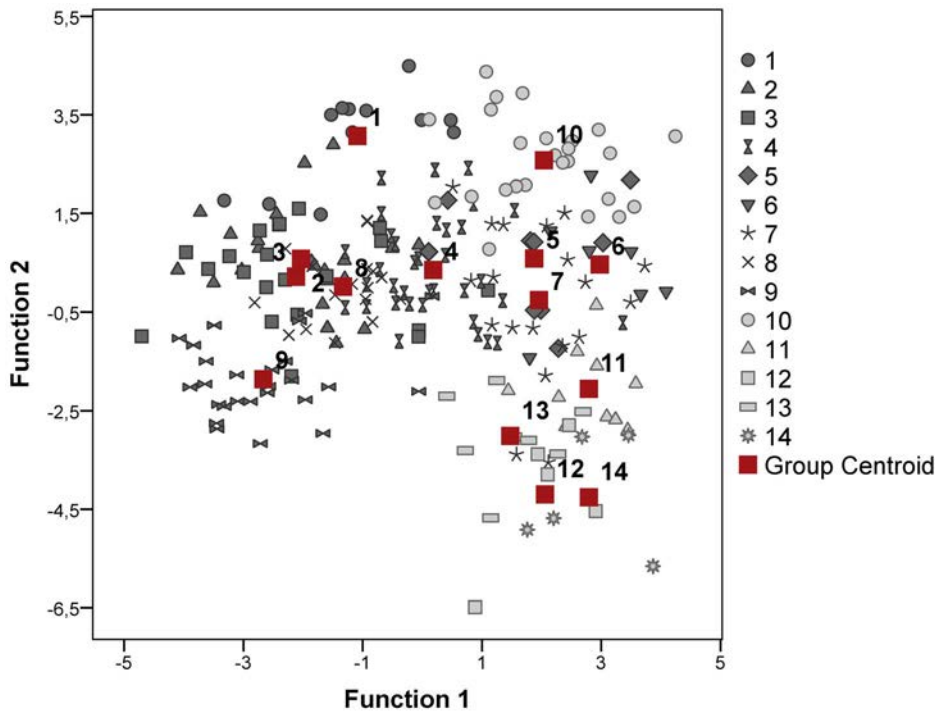
Figure 3. Vocal individuality in pant calls of southern white rhinos recorded in various social contexts. The plot shows the first two canonical discriminant functions with the centroid values of pant calls for each animal. 1 = Ada, 2 = Kathi, 3 = Kifaru, 4 = Munyani, 5 = Yeti, 6 = Malia, 7 = Tamu, 8 = Naja, 9 = Zanta, 10 = Natal, 11 = Medupi, 12 = Lekoto, 13 = M06, 14 = Bert.
doi:10.1371/journal.pone.0098475.g003

classes and social contexts in the southern white rhinos, both age class categories (adults and subadults) included calls recoded in various social contexts. Similarly, the tested categories of the social context included calls recorded from both the adult and subadult animals (see Table 1). Consequently, we cannot exclude that the differences between the social contexts were not influenced by the

differences between the age classes and the other way around. Further studies are therefore necessary to confirm our results.

Pant calls of all four subadult southern white rhinoceros males clustered together in DFA 3 and the calls of adult northern white rhinoceros male SUNI were clearly separated in DFAs 1 and 2 from the calls of other rhinos. This could indicate the influence of

Table 4. Differences in the acoustic parameters of pant calls of the southern white rhinos between the social contexts (pDFA 1) and age classes (pDFA 2).

Acoustic variable[1]	Short description	Mean±sd[2]		p-value[3]
pDFA1		**In isolation**	**In visual contact**	
No. of elements	Number of elements in call	10.6±2.58	7.6±2.76	0.07
I: fp1amax	Maximum amplitude of 1st global frequency peak (relative amplitude)	637.2±403.48	276.3±218.61	0.20
I: peak freq (max)	Frequency of maximum amplitude of spectrum [Hz]	1107.6±369.36	1713.5±482.16	0.10
I: q3med	Median frequency of 3rd distribution of frequency amplitude [Hz]	3188.7±894.02	4785.6±1384.89	0.14
E: q3med	Median frequency of 3rd distribution of frequency amplitude [Hz]	3852.5±1075.60	7463.2±1719.25	0.049
E: df1end	End frequency of 1st dominant frequency band [Hz]	771.93±472.60	583.2±500.38	0.27
pDFA2		**Adults**	**Subadults**	
Call duration	Duration of the call [sec]	4.6±0.90	3.4±0.98	0.23
E: max element length	Duration of the longest exhalation in call [sec]	0.6±0.14	0.4±0.11	0.08
E: min element length	Duration of the shortest exhalation in call [sec]	0.3±0.10	0.2±0.06	0.23
E: fp1amean	Mean amplitude of the 1st global frequency peak	135.4±110.06	34.1±37.71	0.04
I: ampratio1	Amplitude ratio between 1st and 2nd dominant frequency band	1.3±0.55	0.8±0.25	0.01

[1]I = variable measured in inhalation, E = variable measured in exhalation.
[2]The data were calculated as averages of mean values/individual.
[3]Significance of Mann-Whitney U tests after sequential Bonferroni correction.
doi:10.1371/journal.pone.0098475.t004
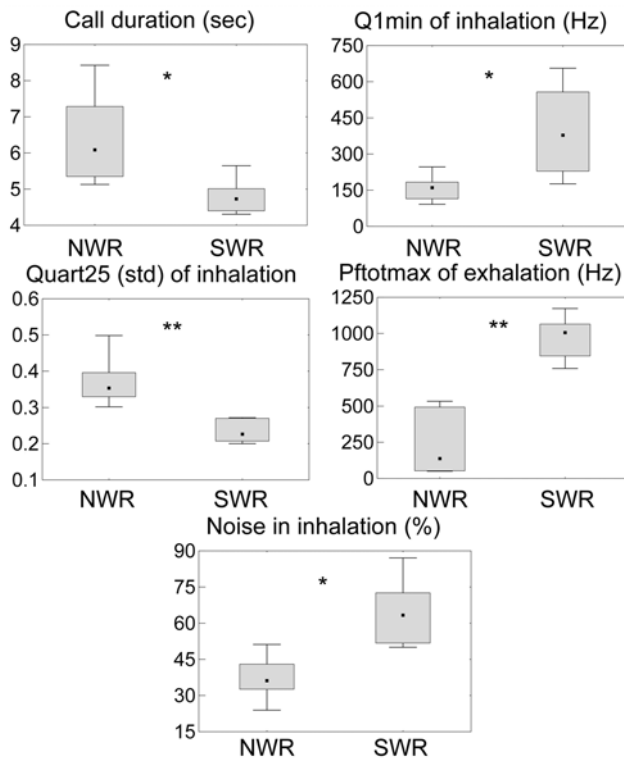
Figure 4. Differences in acoustic variables between the northern (NWR) and southern (SWR) white rhinos. The analysis only included calls of adult animals recorded in isolation. Median, box: 25–75% quartile±maximum, minimum value. Q1min = minimum frequency of the 1st distribution of the frequency amplitude, Pftotmax = frequency of the total maximum amplitude, Quart25 (std) = relative standard deviation of 25% quartile measured from all spectra between the start and the end of the element, Noise = percentage of noisy time segments. Results of Mann-Whitney U tests after sequential Bonferroni correction: *p = 0.07, **p = 0.04.
doi:10.1371/journal.pone.0098475.g004

age class and social status on the call structure of males. The bull SUNI was kept in the stables with females and with an adult male SÚDÁN, whose calls clustered together with the calls of the females. We think that SUNI might have had a subordinate status as he vocalized with the pant call and answered the playbacks of pant only in the enclosure, where he and not SÚDÁN had access. Unlike SÚDÁN, SUNI was not observed to vocalize with pant call in the stables or in the enclosure, where both bulls had access in turns and where they used olfactory territorial markings. The coding of male social status has also been recorded in squeals [57] and contact whinny calls [22] of horses.

Species Differences in White Rhinoceros Vocalizations

Signals evolve in correlation with sensory systems, signalling behaviour and micro-habitat choice [58]. Polícht et al. [59] found the link between the acoustic parameters of long-range calls and social system in equids. The northern and southern white rhinos have a similar social system [10,14,38,41,60], however, the northern males produce a unique repetitive call hoarse. All three adult northern white rhinoceros males studied by Polícht et al. [8] (two of them were also included in our study) produced hoarse calls primarily during non-social activities such as feeding with its mean duration being 26 sec. Both northern males in our study also vocalized with a hoarse call during almost all feeding bouts. We did not record a hoarse call, however, in any of the three studied

captive adult southern white rhinoceros males (two of them were not included in the results due to the low number of recorded pant calls from them) or in the adult males (n = 4) observed during feeding in several zoos, which we visited. This corresponds with the observations concerning the free-ranging southern white rhinos; no hoarse call was recorded during the studies of 11 adult males by Cinková et al. [61] and Cinková and Polícht (unpublished data) or during the long-term behavioural study by Owen-Smith [10]. A hoarse call could therefore be considered an apomorphy of the northern white rhinoceros, although, its function remains unclear. This question could be tested by playbacks of this call to both species, but unfortunately only three northern males are currently known to survive.

Species distinction in white rhinoceros puffing and growling sounds was not found by Polícht et al. [8], however, their study only included three southern white rhinos, which prevented them from performing a more detailed comparison. We found that the pant calls of northern and southern white rhinos could be classified to a correct species with a high accuracy. This may be the result of the repetitive character and more complex structure of pant calls in contrast to the puffing or growling sounds.

The sensory drive hypothesis for divergence in sexual signalling between closely related species emphasises the adaptation of communication systems to local environments [62]. The southern white rhinos in Kruger National Park in South Africa, where their largest population is found [63], prefer a habitat with good quality short grasses and an open to moderate low shrub stratum in woodland or tree and bush savannah not far from a water source [64,65]. The northern white rhinos are most probably extinct in the wild [66], however, they formerly lived in a wetter habitat with numerous watercourses and marshes in the open long grass savannah in Garamba National Park in the Democratic Republic of Congo [67] or in the Shambe area in the Sudan in seasonally flooded grasslands, wooded savannah and swamps [68]. As the genetic divergence indicates a separation time over a million years between the northern and southern white rhinos [27], ecological differences could have affected certain parameters of their vocalizations. Lower frequencies of pure tones attenuate (lose the signal intensity) more rapidly in grassland than in marsh habitat due to the ground effect [69]. A longer call duration, a lower minimum frequency of the first distribution of frequency amplitude and less noise in an inhalation of northern white rhinoceros pant calls might possibly serve to increase the chance of signal detection in a wetter habitat and therefore be favoured in selection.

Individual Signatures in Pant Calls

We showed that pant calls of both species can be classified to correct individuals with a high success, however, pant calls were also highly variable within individuals. The total information capacity of the pant calls of adult northern and southern white rhinos recorded in isolation was modest (3.19 and 3.15 bits, respectively) and would allow for a discrimination of approximately nine individuals of both species based on the call parameters used [52]. Budde and Klump [6] tested the harmonic begging calls of captive black rhinos for individual differences, but found only low inter-individual variation. White rhinos are the most social of all the rhinoceros species [9–14] and in evolution, their sociability might select for an increased need to discriminate individuals. In sciurid rodents, species living in larger social groups have more signature information in their alarm calls than species living in smaller groups, which indicates a strong evolutionary link between the social group size and the vocal individuality [49]. The most common group size of females, subadults and juveniles of

southern white rhinos is 2–3 individuals although long-term associations of up to six animals have also been observed [10,15]. The signature information of the pant calls would therefore be sufficient for vocal recognition between the members of the groups, between territorial and subordinate males or neighbouring territorial males. A similar information capacity, which we found in pant, was described in marmot alarm calls (3.37 bits) [70] and the playback experiments revealed that the marmots were indeed able to extract this information as they discriminated between the alarm calls from different individuals [19].

Conclusions

The variation between individuals, species, contexts and age classes which we found in the contact pant call of northern and southern white rhinos represents the first information reported concerning any rhinoceros call. Playback studies are now needed to investigate if the rhinos are able to extract this information. Understanding the communication of white rhinos is critical as the lack of social relationships with conspecifics and arising communication problems amongst captive rhinos might be one of the contributing factors to their low reproduction. This is crucial particularly for the northern white rhinoceros, which ranks among the most endangered mammals in the world. A knowledge of chemical communication and manipulation of chemosignals has been very successfully implemented, for instance, in the captive breeding programme of giant pandas [71]. We emphasize the need for further research on rhinoceros communication and believe that manipulated communication signals might potentially be used in rhinoceros conservation and management and might help to encourage breeding in captive rhinos.

Supporting Information

Table S1 Description of acoustic variables entered into the discriminant function analyses.
(DOCX)

Table S2 Descriptive statistics of acoustic variables entered into the discriminant function analyses.
(DOCX)

Author Contributions

Conceived and designed the experiments: RP IC. Performed the experiments: IC RP. Analyzed the data: IC RP. Contributed reagents/materials/analysis tools: IC RP. Wrote the paper: IC RP.

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Supporting information

Table S1. Description of acoustic variables entered into the discriminant function analyses.

Acoustic variable[1]	Description	Software	Inclusion of parameter into DFAs[2]		
			DFA 1,2	DFA 3	DFA 4
Call duration	Duration of the call [sec]	Avisoft		*	
No. of elements	Number of elements in call	Avisoft		*	
I: max element length	Duration of the longest inhalation in call [sec]	Avisoft	*		
E: max element length	Duration of the longest exhalation in call [sec]	Avisoft	*	*	*
I: min element length	Duration of the shortest inhalation in call [sec]	Avisoft	*	*	
I: no. in cat. 1,2 – percentage	Percentage of inhalations in duration from 0.0–0.4 sec (= in categories 1,2) from the total number of inhalations in call	Avisoft		*	*
E: no. in cat.1,2	No. of exhalations in call in duration from 0.0–0.4 sec (= in categories 1,2)	Avisoft	*		
E: duration	Duration of exhalation [sec]	Avisoft		*	*
Order longest inhalation	Order of the longest inhalation in call	Avisoft		*	*
I: peak frequency (max)	Frequency of maximum amplitude of the spectrum [Hz]	Avisoft	*	*	
I: entropy (mean)	Ratio of geometric mean to the arithmetic mean of spectrum; allows to quantify the randomness of the sounds	Avisoft	*		
I: entropy (std)	Ratio of geometric mean to the arithmetic mean of spectrum; allows to quantify the randomness of the sounds	Avisoft		*	
I: hnr (std)	Ratio of harmonic to nonharmonic energy	Avisoft		*	*
I: ampratio2	Amplitude ratio between 1st and 3rd dominant frequency band	LMA		*	
I: ampratio3	Amplitude ratio between 2nd and 3rd dominant frequency band	LMA	*		
I: q3min	Minimum frequency of the 3rd distribution of frequency amplitude [Hz]	LMA		*	
I: ranmean	Mean frequency range [Hz]	LMA		*	*
I: fp1amax	Maximum amplitude of the 1st global frequency peak [relative amplitude]	LMA		*	
I: df3mean	Mean frequency of the 3rd dominant frequency band [Hz]	LMA	*		
I: pfmin	Minimum peak frequency [Hz]	LMA		*	*
E: fp1amean	Mean amplitude of the 1st global frequency peak [relative amplitude]	LMA		*	*
E: pftotmax	Frequency of the total maximum amplitude [Hz]	LMA	*		
E: pftrmean	Mean deviation between peak frequency and linear trend [Hz]	LMA		*	

[1]I = parameter measured in inhalation; E = parameter measured in exhalation; (max) = parameter measured at the location of maximum amplitude; (mean) = parameter derived from the mean spectrum of entire element; (std) parameter is computed for all spectra between the start and the end of each element and the relative standard deviation of these parameters is calculated, this parameter can be used for quantifying frequency or amplitude modulation.

Table S2. Descriptive statistics of acoustic variables entered into the discriminant function analyses.

Acoustic variable	Northern white rhinos		Southern white rhinos	
	Mean	sd	Mean	sd
Call duration [sec]	6.1	1.76	3.9	0.95
No. of elements in call	14.1	4.67	9.2	2.64
I: max element length [sec]	1.0	0.25	0.6	0.23
E: max element length [sec]	0.4	0.17	0.4	0.13
I: min element length [sec]	0.3	0.11	0.2	0.07
I: no. in cat. 1,2 – percentage	0.6	0.19	0.7	0.2
E: no. in cat. 1,2	6.2	2.49	3.3	1.65
E: duration [sec]	0.3	0.09	0.3	0.08
Order longest inhalation	3.6	3.73	3.5	2.51
I: peak frequency (max) [Hz]	967.3	695.79	1345.5	439.14
I: entropy (mean)	0.3	0.06	0.4	0.05
I: entropy (std)	0.1	0.05	0.1	0.04
I: hnr (std)	0.1	0.04	0.1	0.03
I: ampratio2	1.5	0.72	1.4	0.67
I: ampratio3	1.4	0.41	1.3	0.31
I: q3min [Hz]	1542.0	658.91	2346.7	753.57
I: ranmean [Hz]	4793.6	1609.58	4504.6	1362.65
I: fp1amax	305.9	293.22	496.5	333.56
I: df3mean [Hz]	2578.1	853.86	2532.5	736.07
I: pfmin [Hz]	84.5	96.64	222.9	194.17
E: fp1amean	92.8	123.32	77.5	68.72
E: pftotmax [Hz]	326.6	449.38	930.9	675.68
E: pftrmean [Hz]	895.1	620.55	1438.6	624.27

Descriptive statistics were calculated as averages of mean values/individual and included pant calls recorded from all the animals in various social contexts. I = parameter measured in inhalation, E = parameter measured in exhalation.

Příspěvek II.

Cinková I., Policht R. (2015) Discrimination of familiarity and sex from chemical cues in the dung by wild southern white rhinoceros. Animal Cognition 18: 385–392.
(doi:10.1007/s10071-014-0810-8) (IF 2.631)

ORIGINAL PAPER

Discrimination of familiarity and sex from chemical cues in the dung by wild southern white rhinoceros

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Abstract Communication in rhinos is primarily mediated by the vocal and olfactory signals as they have relatively poor eyesight. White rhinos are the most social of all the rhinoceros species, they defecate at common dungheaps and the adult bulls use dung and urine to mark their territory. Chemical communication may therefore be particularly important in the social interactions of white rhinos, and its knowledge could be very helpful in their management and conservation. However, no studies have investigated up until now the olfactory discrimination in any rhinoceros species in the wild. We have experimentally studied the reactions of the wild southern white rhinos (*Ceratotherium simum*) to the dung of familiar and unfamiliar adult females and adult territorial males. We registered the number of sniffing events, the duration of sniffing and the latency of the vigilance posture from the onset of sniffing. The dung of unfamiliar rhinos was sniffed longer than that of familiar rhinos. The rhinos showed a shorter latency of vigilance posture to the familiar dung of males than that of females. For unfamiliar dung, they displayed a shorter latency of vigilance posture to female than male dung. Our results indicate that the rhinos are able to discriminate the familiarity and sex of conspecifics from the smell of their dung. Olfactory cues could therefore play an important role in the social relationships and spatial organization of the southern white rhinoceros.

Keywords Southern white rhinoceros · *Ceratotherium simum* · Olfactory discrimination · Communication · Dung · Familiarity

Introduction

Olfactory signals have an advantage in that they persist for some period of time in the absence of the depositor and allow the receivers to specifically assess certain parameters of their social environment (Eisenberg and Kleiman 1972). Terrestrial vertebrates perceive olfactory signals via the main olfactory system, which is receptive to volatile signals and through the vomeronasal system, which primarily processes non-volatile pheromones (reviewed by Dulac and Torello 2003). Olfactory signals often play an important role in the communication of ungulates (Grau 1976), which have a well-developed vomeronasal organ (Eisenberg and Kleiman 1972). Vocal and olfactory signals are extremely important for communication in rhinos as they have an excellent sense of hearing and smelling, but relatively poor eyesight (Penny 1987). Cinková and Policht (2014) have recently determined that the contact calls of white rhinos contain information on the individual identity, age class, context and species of the caller (northern versus southern white rhinoceros) and similar information about the signaller may be present in their olfactory cues. Scent-marking behaviours provide honest signals of competitive ability and quality of the signaller to conspecifics in mammals. The receivers assess the signallers by use of

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intrinsic information (for instance, the density of markings or the concentration of hormones), by using memorized information about past opponents and their odours or by scent matching (Gosling and Roberts 2001). According to the scent matching hypothesis, the receiver identifies that an individual is a resource holder and a high-status animal only after meeting it and matching its odour to the receiver's memory of the individual's scent marks (Gosling 1982).

Olfactory cues allowing for the discrimination of individual identity, sex or familiarity in mammals can be contained, for instance, in faeces (horse: Rubenstein and Hack 1992; Krueger and Flauger 2011; domestic cat: Nakabayashi et al. 2012; black rhinoceros: Linklater et al. 2013), urine (giant panda: White et al. 2004; African elephant: Bates et al. 2008; koala: Charlton 2014), body odour (horse: Péron et al. 2014), anogenital gland secretion (brown bear: Jojola et al. 2012) or in a combination of odours from anogenital scent marks, urine, faeces and the pelage (giant panda: Swaisgood et al. 2000). Urine marks can also allow the discrimination of one's own urine from the urine of other familiar conspecifics (domestic dog: Bekoff 2001).

Apart from the pedal glands that have been reported in Indian and Javan rhinos (Cave 1962) and the preputial glands in the white rhinoceros (Cave 1966), rhinos do not have any specialized scent glands. Dung, urine and general body odour thus seem to be the only carriers of scent cues in the white rhinoceros (Owen-Smith 1973). White rhinos defecate at common dungheaps and, when encountering a dungheap, often pause to sniff at dung accumulation for some time (Owen-Smith 1973). The adult females, subadults and juveniles of southern white rhinos live in overlapping home ranges in groups consisting most frequently of two to three individuals (Owen-Smith 1973, 1975; Shrader and Owen-Smith 2002). The adult males are solitary and defend territories, which are exclusive of the territories of other males. They mark their territory, including its boundaries, by kicking movements before and after the defecation to scatter their dung and by spray urination, which is preceded by scrape marking (Owen-Smith 1971, 1973, 1975; Rachlow et al. 1999).

Marking of the territory boundaries is reported more often than marking towards the centre of the territory, which is, however, more usual in very large territories (reviewed by Gosling and Roberts 2001). Kretzschmar et al. (2001) observed that the spray marks of adult southern white rhinoceros males were located closer to the territory border than their dung. Dung might therefore provide more information about the movements of an animal around its territory. In contrast to southern white rhinoceros females, who only rarely scrape their dung after defecation (Owen-Smith 1973), black rhinoceros females scrape mark more often and the length of the scrapings carries the information about their reproductive state. The length of the scrapings increases in both male and female black rhinos with age (Freeman et al. 2014).

Groves et al. (2010) have recently suggested that two distinct forms of the white rhinoceros should be elevated from the subspecies to the species level, the southern white rhinoceros (*Ceratotherium simum*) and the northern white rhinoceros (*Ceratotherium cottoni*), which has also lately been referred to as the Nile rhinoceros (e.g. Rookmaaker 2011, Rookmaaker and Antoine 2012) following Heller (1913). The population of the northern white rhinoceros presently consists of only seven animals, which makes it one of the most endangered mammals in the world. The numbers of the southern white rhinos are estimated to be 20,424, but poaching for their horn is currently on a rapid increase (Knight 2013).

White rhinos have very low reproductive success in captivity, and both females and males often suffer from reproductive pathologies (Hermes et al. 2005, 2006). Social interactions between the captive rhinos might be one of the possible reasons for the low reproduction (Meister 1997; Swaisgood et al. 2006; Metrione et al. 2007; Cinková and Bičík 2013; Cinková and Policht 2014). White rhinos are the most social out of all the rhinoceros species (see Goddard 1967; Owen-Smith 1973, 1975; Laurie 1982; Penny 1987) and develop a network of social relationships in the wild with their conspecifics inhabiting the same area (Owen-Smith 1973, 1975). This is not available, however, to the rhinos living in the limited environments of zoological gardens. A better understanding of chemical communication in another species with low reproductive success in captivity, the giant panda, has substantially helped improve their breeding programme (Swaisgood et al. 2000, 2003). The use of rhinoceros dung has been suggested as a management tool to help stimulate the reproductive and territorial behaviour of captive rhinos (Fouraker and Wagener 1996) and to assist the management of wild rhinos (Linklater et al. 2006). Linklater et al. (2013) have recently experimentally studied olfactory discrimination in temporarily captive black rhinos. Studies on the olfactory communication of white rhinos, however, remain mainly descriptive and anecdotal.

The aim of this study was to test whether the wild southern white rhinos are able to discriminate familiarity and sex of their conspecifics from the smell of their dung. To our knowledge, no such study has ever been conducted on any rhinoceros species in the wild. Dung is more easily collected than urine, and we tested the reactions of the rhinos to the fresh dung as we were unsure how long possible olfactory cues allowing the discrimination of familiarity and sex could persist in the dung.

Table 1 Characteristics of the test subjects and their inclusion in particular treatments

Subject	Age class	Sex	Donor's familiarity	Donor's sex	Donor's name	Sample number
Shrek	Adult	M	Unfamiliar	F	Labata	1
Sophie	Adult	F	Unfamiliar	F	Mohlaki	2
Susan	Adult	F	Unfamiliar	F	Kedibone	3
Dikgeto[a]	Subadult	M	Unfamiliar	F	Rosy	4
M06[a]	Subadult	M	Unfamiliar	F	Kedibone	5
Henrie[a]	Subadult	M	Unfamiliar	F	Mohlaki	6
Paris	Subadult	F	Unfamiliar	F	Mohlaki	6
Sam	Juvenile	M	Unfamiliar	F	Kedibone	3
Christy	Juvenile	F	Unfamiliar	F	Mohlaki	2
Victor	Adult	M	Familiar	F	Mamasita	7
Rosy	Adult	F	Familiar	F	Sophie	8
Lucy	Adult	F	Familiar	F	Gill	9
Mamasita[a]	Adult	F	Familiar	F	Rosy	10
Alf	Subadult	M	Familiar	F	Nandi	11
Ted	Juvenile	M	Familiar	F	Nandi	11
Rosebud	Juvenile	F	Familiar	F	Mamasita	12
Babs[a]	Juvenile	F	Familiar	F	Rosy	10
Motopi	Adult	M	Unfamiliar	M	Sidney	13
Betty	Adult	F	Unfamiliar	M	Motopi	14
Kedibone	Adult	F	Unfamiliar	M	Victor	15
Dikgeto	Subadult	M	Unfamiliar	M	Victor	16
Nnete	Juvenile	F	Unfamiliar	M	Victor	15
Sidney	Adult	M	Familiar	M	Victor	17
Mamasita	Adult	F	Familiar	M	Victor	18
Rubee	Adult	F	Familiar	M	Shrek	19
M06	Subadult	M	Familiar	M	Victor	20
Henrie	Subadult	M	Familiar	M	Victor	20
Murphy	Juvenile	M	Familiar	M	Shrek	19
Babs	Juvenile	F	Familiar	M	Victor	18

[a] The five animals who were used as a test subject for the second time

Methods

Study area, animals and sample collection

Olfactory communication in the wild southern white rhinos was studied from May–December 2012 in Welgevonden Game Reserve (375 km²) and in Lapalala Wilderness (360 km²) situated in the Waterberg Biosphere Reserve in the Limpopo Province, South Africa.

Both reserves are fenced and are situated approximately 50 km apart. The vegetation of both reserves belongs to the savanna biome and is characterized as Waterberg moist mountain bushveld. The annual rainfall in this area varies from 650 to 900 mm, and the altitude ranges from 1,200 to 1,500 m (van Rooyen and Bredenkamp 1996).

We studied the reactions of 24 individually recognizable rhinos to the dung of familiar and unfamiliar adult females and adult territorial males. We included in the study animals of all sex–age classes except for calves younger than 6 months (Table 1). Females were regarded as adults at 6.5–7 years of age when their first calf is usually born and males between 10 and 12 years of age when they achieve socio-sexual maturity and become solitary. The subadult period starts at 2–3 years of age when the juveniles are driven away by their mothers after the birth of a subsequent calf (Owen-Smith 1973, 1975).

The dung used in the experiments was only collected when an adult female or an adult territorial male was observed to defecate, and we were certain about their identity. A 2 kg dung sample was placed into air-tight plastic containers, deep frozen at −20 °C the same day and used in an experiment up to a maximum of 4 months later (four samples were stored at only −5 °C for 5–14 days before being used). Before an experiment, the samples were left for 24 h to unfreeze at room temperature and were used within 12 h after defrosting. We used a total of 20 dung samples from eight adult females and four adult territorial males in the experiments (see Table 1).

The dung used as unfamiliar in Welgevonden Game Reserve was imported from Lapalala Wilderness and vice

versa in order to be certain that the test subject had never come in contact with the donor. Familiar dung was collected from the animals which inhabited the same home range as the test subject. We never used dung from a member of a subject's group as familiar dung. Companions from the same group only rarely move further apart than 25 metres and remain within five metres distance the majority of the time (Owen-Smith 1973). Finding the fresh dung of a companion at a place where the group has just arrived could therefore alter the reactions of the subjects.

Experimental design

The rhinos were followed by car or on foot, and when the animals seemed to be walking in the direction of a dung-heap where no fresh dung was present, an experimenter placed a 2 kg dung sample there. The trials were only conducted on dry days to prevent rain from washing away the scent from the samples. The dung was placed more than one rhino body length from the nearest dung pile, and the experimenter then moved away. The distance moved away from the sample differed between the experiments, depended on the structure of the habitat (dense or open), and always allowed a clear view of the study place. The experimenter paid special attention that the rhinos would not see her placing the dung near the dungheap and that the behaviour of the rhinos would not be disturbed in any way. The reactions of the rhinos were video-recorded for 5 min using a Canon Legria HF M31 digital camcorder. Reactions were only counted if the subject was up to a distance of one rhino body length from the sample. All the subjects finished investigating the dung and left the place before the end of the observation period. After an experiment finished, the dung was removed.

In all the successful trials, the dung was approached by the rhinos within 80 min. If no animal approached the sample in a maximum of 90 min, but sometimes sooner, it was removed as the dung beetles usually began removing the dung by this time (the activity of dung beetles and the intensity of dung removal increase with increasing air temperature, see Davis 1996) and we tried to maintain the same amount of samples in all the trials. The trial began when a subject started sniffing the dung as this was the only way we could be sure that the subject was aware of it.

An attempt was made to balance the number of animals of each sex–age class in the treatments. Due to difficulties with data collection, however, it was not possible to include the same number of animals of each sex–age class in each treatment. The inclusion of certain animals in particular treatments was often random as it depended on the availability of unfrozen experimental dung on the particular day. We also did not know which animals we would be able to locate that day. A number of trials were not successful; the rhinos often did not approach the experimental dung as it was very difficult to predict the exact direction where they were heading so that we could place the dung sample there. Since the adult territorial bulls seemed to react to the dung samples the most intensively (including walking or running around and trying to find the depositor), we included the same number of bulls in each treatment. Adult bulls are territorial (Owen-Smith 1971, 1973, 1975; Rachlow et al. 1999), and there are usually more adult females, subadults and juveniles in the reserves than there are adult bulls. Due to the limited number of bulls at our study sites, we could either include only one bull in each treatment or none. In order to increase the sample size, we included one bull in each treatment.

We only registered the reactions of a maximum of the first two rhinos from the same group who came to sniff the same sample (as long as no rhinoceros urinated or defecated near the sample, which might alter the reactions of others) in order to keep the repetition of the same stimulus to a minimum. Most often, only one or two rhinos came to sniff the sample although in two cases, more animals from the same group arrived (once five and once three animals). Five rhinos were used as a test subject twice (always in two different treatments), and we allowed a mean time of 63 days between the consecutive trials on each of these animals (ranging from 3 days to 4 months) (see Table 1). This approach was the only practical solution to our problem with sample size given the difficulty of collecting these data on wild animals (Wiley 2003). We were careful to assign animal identity and sample number as random factors in statistical analyses following Webster et al. (2012), who were also dealing with a similar problem in a rare and difficult-to-study species when they investigated behavioural responses of African wild dogs to the playback experiments.

Analyses

The video recordings were analysed using the Activities 2.1 computer programme package (Vrba and Donát 1993). We registered the number of sniffing events, the duration of the sniffing and the latency of the vigilance posture from the onset of sniffing which occurred up to a distance of one rhino body length from the sample. Such methods are commonly applied in analyses of olfactory discrimination in other mammals including ungulates (Rubenstein and Hack 1992; White et al. 2004; Krueger and Flauger 2011; Jojola et al. 2012; Nakabayashi et al. 2012; Linklater et al. 2013; Chamaillé-Jammes et al. 2014; Charlton 2014). All the experiments in the field were conducted, and all the video recordings were analysed, by one experimenter (IC) to avoid any inter-observer variation. Sniffing was defined as standing with the head down, above or next to the

sample and with the nose within approximately 20 cm from the sample. Sniffing was often confirmed by a clearly visible movement of the nostrils. The vigilance posture was defined as standing and raising the head, looking and listening; the animal was either scanning the surroundings or had its gaze and ears fixed in a certain direction. If a subject did not assume the vigilance posture, a latency of 5 min was assigned.

All the analyses were performed with IBM SPSS Statistics 20.0 (IBM Corp., Armonk, USA), and we regarded all results with P value <0.05 as statistically significant. We analysed the reactions of the rhinos to the dung samples using linear mixed models with a scaled identity covariance structure and a restricted maximum likelihood estimation (West et al. 2007). This allowed us to test for the influence of the fixed effects of donor familiarity, donor sex and their interaction while accounting for the random effects of animal identity and sample number. To meet the assumptions of the linear mixed model, the variables were Box-Cox transformed (Sokal and Rohlf 2012). The final Hessian matrix of the linear mixed model with the dependent variable latency of the vigilance posture was not positive definite. In such cases, simplification of the model is recommended by removing the random effects which may not be necessary (West et al. 2007). We therefore removed five animals, which were sampled twice (in two different treatments) from the model, and kept only their first reaction to dung sample in the analyses (see Table 1). This allowed us to remove the random effect of animal identity from the model, and the linear mixed model was

then fully completed. The residuals of all the dependent variables (the number of sniffing events, the duration of sniffing and the latency of the vigilance posture) from the linear mixed models were normally distributed (confirmed with Shapiro–Wilk tests), the homogeneity of variances was confirmed by Levene's tests and the distribution of residuals was also checked visually (q–q plots, histograms, boxplots).

Neither the sample number nor the animal identity had any effect on the reactions of the rhinos in the results of the linear mixed models. The reactions of five rhinos, which were included in the study twice in two different treatments, and the reactions of a maximum of two animals, which came to sniff the same sample, could therefore be considered as independent (McGregor et al. 1992), and their inclusion in the model thus proved justified.

Results

The rhinos sniffed the dung of unfamiliar animals longer than that of familiar animals (Fig. 1; LMM: $F_{1,11.66} = 5.228$, $P = 0.042$). The duration of the sniffing did not differ between the female and male dung ($F_{1,5.94} = 0.006$, $P = 0.943$), and there was no influence of the interaction between the donor familiarity and sex on the duration of sniffing ($F_{1,7.73} = 1.200$, $P = 0.306$). The number of sniffing events was similar between the dung of familiar and unfamiliar animals (LMM: $F_{1,15.229} = 1.309$, $P = 0.270$) and between the female and male dung

Fig. 1 Investigation of the dung of unfamiliar and familiar, female and male donors. Data represent mean ± SEM. *$P < 0.05$
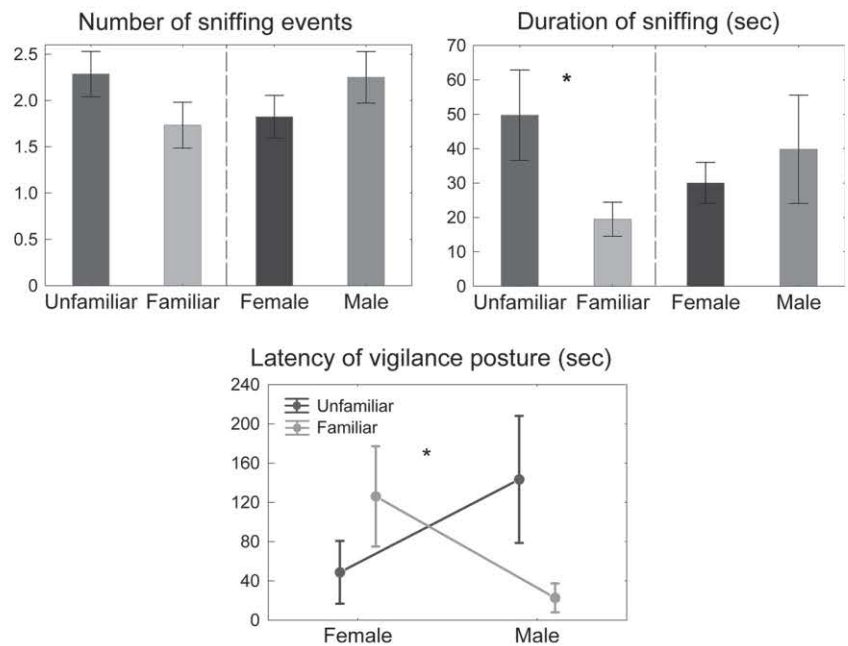
($F_{1,12.879} = 1.202$, $P = 0.293$). There was no influence of the interaction of donor familiarity and sex on the number of sniffing events ($F_{1,14.18} = 1.088$, $P = 0.314$). The latency of the vigilance posture did not differ between the familiar and unfamiliar (LMM: $F_{1,14.75} = 2.023$, $P = 0.176$) and between the female and male dung ($F_{1,14.75} = 0.016$, $P = 0.900$). However, the rhinos displayed a shorter latency of the vigilance posture to the familiar dung of males than that of females. For unfamiliar dung, they showed a shorter latency of the vigilance posture to the female than male dung (interaction donor familiarity*donor sex: $F_{1,14.75} = 5.049$, $P = 0.040$) (see Fig. 1).

Discussion

Our experiments indicate that southern white rhinos are able to discriminate the familiarity and sex of conspecifics from the smell of their dung, and to the best of our knowledge, this is the first such study on any rhinoceros species in the wild. Olfactory signals can persist in the environment for some time, and since the southern white rhinos defecate at common dungheaps (Owen-Smith 1973, 1975), such places could be important sources of information for them. We do not know how long the scent cues allowing for the discrimination of familiarity and sex can persist in white rhinoceros dung. The ability, however, to discriminate such signals in the fresh dung as we present here, could provide the animals with important information about the movements of other rhinos inside their territory or home range. Linklater et al. (2013) studied olfactory communication in temporarily captive wild-caught black rhinos and found that their interest in the dung of conspecifics did not decline with the age of the dung for up to 32 days, with the dung being kept protected from rainfall and direct sunlight. We could therefore predict that the dung of southern white rhinos might retain important chemical information for longer than 1 day, a claim which was supported by our observations of southern white rhinos being interested in older and dry dung.

In our study, the dung of unfamiliar rhinos was sniffed on average more than twice as long as the familiar dung and the differences in the reactions of rhinos to the dung of females and males were shown in the latency of the vigilance posture, which was at the same time dependent on the familiarity of the donor. A shorter vigilance posture to the dung of familiar males compared with the dung of unfamiliar males might be explained by the fact that the rhinos quickly tried to seek out a familiar resident territorial bull as soon as identity could be established, whereas with unfamiliar dung, the rhinos spent time sniffing and attempting to recognize the depositor.

The scent marks are often positioned at places where they can be more easily detected, such as along trails and pathways. In addition, the receiver often benefits from finding scent marks, and in that case, it should actively seek them out (reviewed by Gosling and Roberts 2001). The ability to discriminate the familiarity and sex based on the dung odours can be very important for the social behaviour and spatial organization of the southern white rhinoceros. The territorial bulls often move, for example, in dry seasons to adjacent or more distant territories to seek water (Owen-Smith 1973), and in areas with higher densities of bulls, each territory can be co-inhabited by adult submissive males (Owen-Smith 1971, 1973, 1975; Rachlow et al. 1999). It can therefore be essential for the adult bulls to pay special attention to the scent signals and to distinguish between the dung of familiar submissive males and territorial neighbours and the dung of unknown intruders as well as to recognize the dung of an unknown female walking in their territory. Females and subadults can similarly benefit from discriminating the familiarity and sex from odour cues in dung and register the movements of other groups and of adult bulls inside their home range. Adult territorial bulls can sometimes attack the subadults (Owen-Smith 1973, 1975). After finding the fresh dung of a resident territorial bull, the subadults might avoid confrontation with him by leaving the location as; in such a case, it is likely that the bull is nearby.

Chemical compounds that allow for the discrimination of familiarity or possible individual signature in the dung of southern white rhinos remain to be identified. Research on the vegetation differences between the two study sites was beyond the scope of our study. We believe that the higher interest of rhinos in unfamiliar dung was not triggered by the interest in unknown vegetation since the vegetation of both reserves belongs to the same vegetation type (Waterberg moist mountain bushveld) (van Rooyen and Bredenkamp 1996) and the reserves are only situated 50 km from each other (see "Methods").

Black rhinos can use chemical signals in the dung for identifying individuals and sex–age classes. Although the adult females and males investigate the dung of both sexes similarly overall, there are differences in their reactions towards the dung of subadult males (Linklater et al. 2013). In our study, we pooled the subjects from all sex–age classes because we did not manage to include a sufficient number of animals to be able to test them separately. Nevertheless, it seemed from our experiments that the adult territorial bulls were interested in the experimental dung the most as they also reacted by marking their territory with scrapes and spray urination or by walking or running around and attempting to locate the depositor. Further research should therefore focus on possible differences in

the reactions of females and males to the scent cues of various sex–age classes of rhinos and also on the practical use of olfactory signals in the management and conservation of the white rhinoceros.

Linklater et al. (2006) found that black rhinoceros chemosignals could affect the post-release movements of the animals and they might therefore have a promising use in wildlife management. A knowledge of olfactory communication can also be important for encouraging breeding in mammals, which reproduce poorly in captivity (reviewed by Campbell-Palmer and Rosell 2011). Since the reproductive success of white rhinos is very low in captivity (e.g. Hermes et al. 2005, 2006; Swaisgood et al. 2006), it might be helpful to provide them with additional olfactory stimuli to help simulate the presence of other rhinos and therefore stimulate their social and reproductive behaviour. A knowledge of olfactory communication in southern white rhinos might also help us better understand the communication system of their close relatives, critically endangered northern white rhinos, and increase the chance of their reproduction.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The project was approved by the Ethical Commission of the Faculty of Science, Palacký University, and adhered to the "Guidelines for the treatment of animals in behavioural research and teaching" as published by the ASAB (2012). The project complies with the current laws of South Africa and the Czech Republic.

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Příspěvek III.

Cinková I., Policht R. (submitted): Sex and species recognition by wild male southern white rhinoceros using contact calls.

Sex and species recognition by wild male southern white rhinoceros using contact pant calls

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Recognizing information from acoustic signals is crucial in many animals and individuals are under strong selection pressure to discriminate between the signals of conspecifics and closely related species. The role of communication signals in species recognition may be complicated. Here, we first report that rhinos use information encoded in their calls to assess conspecifics and individuals of closely related species. The southern (*Ceratotherium simum*) and critically endangered northern (*C. cottoni*) white rhinos are the most social out of all the rhinoceros species and use a unique contact call pant. We studied the structure of pant calls in southern white rhinos and found that certain temporal and frequency parameters carry information about sex, age-class and social situation of the caller. To test whether the rhinos attend to the sex and species differences in pant calls, we conducted playback experiments on wild territorial southern white rhinoceros males. Males recognized sex from the calls of conspecifics and responded more strongly to the calls of females than territorial males, which suggests that pant calls are more important in male-female than male-male communication. We also found that southern males discriminated between the female and male calls of northern species and reacted more intensively to the calls of northern than southern males, which could have been caused by a novelty effect as both species naturally live in allopatry and discrimination between them therefore cannot result from learning. Better understanding of vocal communication in white rhinos has potential management and conservation implications particularly because of their low reproduction in captivity.

Keywords Southern white rhinoceros, Northern white rhinoceros, Vocal communication, Vocalization, Sex recognition, Species recognition, Playback experiment

INTRODUCTION

Communication is a central mediator of all important social behaviours (McGregor and Peake 2000). In contrast to the mammalian olfactory signals (Eisenberg and Kleiman 1972), many vocal signals are apparently directed to concrete individual/s although they can be available to many other potential receivers through eavesdropping (McGregor and Peake 2000). Acoustic signals of mammals have been shown to encode information about individual identity, sex, species, dominant or subordinate status, fertile phase of females, kinship, signal context and motivation of the caller (McComb et al. 2000; Lemasson et al. 2009; Charlton et al. 2010; Mathevon et al. 2010; Schneiderová and Policht 2012; Tallet et al. 2013; Cinková and Policht 2014; Pfefferle et al. 2014; Pitcher et al. 2014). Receivers can therefore make decisions based upon the behaviour, physiology or morphology of others through the use of their signals (Endler 1993).

Signalling via vocalizations is very fast and can have high information transfer rate while olfactory signals are slow with poor directionality (Endler 1993). Vocal signals mediate agonistic and affiliative interactions between animals and play a crucial role in determining the outcome of intra- and intersexual competition (Owings and Morton 1998). Different signallers can also be perceived in different communication channels (Endler 1993). For instance, vocal signals regulate intra-group cohesion and coordination in golden brown mouse lemurs and olfactory signals control distribution of various groups (Braune et al. 2005). All rhinoceros species have an excellent sense of hearing and smelling, but their eyesight is weak (Penny 1987). Rhinos defecate at common dungheaps and use vocalizations in intra-specific interactions (Goddard 1967; Owen-Smith 1973, 1975; Laurie 1982; Penny 1987; von Muggenthaler et al. 2003; Policht et al. 2008; Linklater et al. 2013). Recent research has shown that the southern white rhinos (*Ceratotherium simum*) use olfactory signals in dung to assess familiarity and sex of conspecifics (Cinková and Policht 2015) and that contact pant calls of southern and northern (*Ceratotherium cottoni*) white rhinos contain sufficient information for the identification of caller's identity and its species (Cinková and Policht 2014). However, it has not yet been experimentally studied in any rhinoceros species if the animals indeed attend to the cues encoded in their calls and to what degree vocalizations are preferred as a communication channel from different signallers.

Sensory systems and signals coevolve with species' breeding behaviour and microhabitat choice (Endler 1992). There is a strong selection pressure to discriminate between the vocal signals of conspecifics and closely related species (reviewed by Irwin and

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Price 1999; de Kort and ten Cate 2001; Braune et al. 2008; Schuchmann and Siemers 2010; Seddon and Tobias 2010) and divergence among the signals of different species may be favoured especially when they occur in sympatry (Ryan and Kime 2003). The reactions of the animals to heterospecific calls can, however, be taxon specific and in some species, the responses are greater in sympatry than allopatry, in other species the opposite is true (reviewed by Irwin and Price 1999; Braune et al. 2008). The northern and southern white rhinos live in allopatry and have only lately been recognized as distinct species, which have been separated for over a million years (Groves et al. 2010). A natural range of the southern species is southern Africa and of the northern species central Africa (Hillman-Smith 1986a). Their contact pant calls differ in several call characteristics and allow for a species identification, which could be caused by adaption to different microhabitats in which they live (Cinková and Policht 2014). Until now, however, it is not known whether the rhinos discriminate between the calls of both species.

White rhinos are the most social out of all the rhinoceros species (Goddard 1967; Owen-Smith 1973, 1975; Laurie 1982; van Gyseghem 1984; Penny 1987) and adult females, calves and subadults live in groups in overlapping home-ranges. Adult white rhinoceros bulls are territorial and mark their territories with urine and dung (Owen-Smith 1973, 1975; van Gyseghem 1984). The northern and southern white rhinos have a large vocal repertoire (Owen-Smith 1973; Policht et al. 2008) and use a unique contact call pant, which consists of a series of inhalations and exhalations. Pant call is used by the animals when approaching or greeting another rhinoceros or during separation from a group (Owen-Smith 1973; Policht et al. 2008; Cinková and Policht 2014). Adult bulls use during the approach of a female, particularly during courtship a conspicuous type of a pant call called hic (Owen-Smith 1973; Policht et al. 2008), which can be distinguished from a typical pant call by the erratic and choking pattern of breathing (Owen-Smith 1973).

Although the population of southern white rhinoceros (*Ceratotherium simum*) currently numbers 20,424 individuals, a high number of animals are killed every year by the poachers (Knight 2013). The northern white rhinoceros (which is also being referred to as the Nile rhinoceros) is currently on the brink of extinction with only five surviving animals. Both species reproduce poorly in captivity (e.g., Hermes et al. 2005, 2006; Swaisgood et al. 2006) and the changes in their social behaviour, a lack of social relationships with conspecifics and arising communication problems could be one of the possible reasons (Swaisgood et al. 2006; Metrione et al. 2007; Cinková and Bičík 2013; Cinková and Policht 2014, 2015). Behavioural research can significantly aid conservation and help identify and solve problems with

breeding of captive populations (Lindburg and Fitch-Snyder 1994; Fisher et al. 2003; Swaisgood et al. 2003). Better knowledge of vocal communication in white rhinos might allow us to manipulate their vocal environment through the use of acoustic stimulation and modify their endocrine physiology as has been shown in other animals (McComb 1987; Ball and Dufty 1998) to improve their chance for reproduction.

The aim of this study was to examine if pant calls of the southern white rhinos encode information on the caller's sex, age-class and context of the call and to conduct playback experiments with wild territorial southern white rhinoceros males. We studied if males are able to discriminate sex from the pant calls of conspecifics and if pant calls are an important communication channel for male-female and male-male communication. We describe in detail the reactions to the playbacks with regards to their possible use for stimulation of social and reproductive behaviour of rhinos. We also studied discrimination of males between the male and female pant calls of northern white rhinos to determine if they perceive the differences between the sexes in both white rhinoceros species in the same way and if they react to them with the same intensity. The way how animals respond to conspecific versus heterospecific signals can be more complex and although they generally respond more strongly to the signals of conspecifics than allopatric closely related species, they can also react to them equally intensively or show a greater reaction to a heterospecific signal (Braune et al. 2008; reviewed by Ord and Stamps 2009).

METHODS

Study sites and animals
Playback experiments on the adult territorial southern white rhinoceros males were conducted between June–November 2011 and May–December 2012 in South African wildlife reserves Welgevonden Game Reserve (375 km^2), Lapalala Wilderness (360 km^2) and Mthethomusha Nature Reserve (72 km^2). The calls of the southern white rhinos were recorded in the reserves mentioned above, in Lichtenburg biodiversity conservation centre (South Africa) and in zoological gardens in Salzburg, Zlín, Dvůr Králové and Bratislava from 2009–2014. The calls of the northern white rhinos were recorded in zoological garden Dvůr Králové from 2009–2012.

This study including the playback experiments and recording of calls was approved by the Ethics and Scientific Committee of the National Zoological Gardens of South Africa (Project P11/03) and adhered to the "Guidelines for the treatment of animals in behavioural research and teaching" as published by the ASAB (2012).

Recording procedure and acoustic analysis

The calls were recorded using a Sennheiser directional microphone (ME 67 with K6 powering module, frequency response: 40–20,000 Hz ±2.5 db) fitted with a Rycote Softie windshield and digital recorders Yamaha Pocketrak C24 or Marantz PMD 671 with a 44.1 kHz sampling rate and 16 bits resolution. The calls were recorded at distances from 1–30 m from a calling individual. We analysed pant calls (Fig. 1) of 33 southern white rhinos and tested if they differ between sexes, age-classes (subadults versus adults) and contexts. Since we could not record calls from a sufficient number of northern white rhinos due to their critical conservation status, their calls were only used in playback experiments. Calls were recorded in the following contexts: (1) in visual isolation from other rhinos and (2) in visual contact with members of a subject's group (for females and subadult males) or an adult female (for adult males). Subadult period starts at 2–3 years of age when a juvenile is chased away by its mother after the birth of a subsequent calf. Females become adult around seven years of age at first parturition and males between about 10–12 years of age when they attain socio-sexual maturity and become solitary (Owen-Smith 1973, 1975).
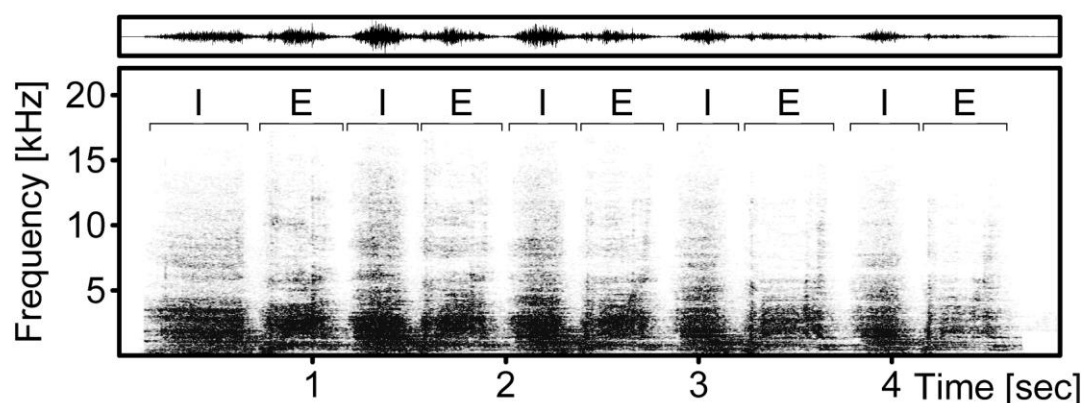
Fig. 1 Representative pant call of adult female southern white rhinoceros recorded in visual isolation from other rhinos. Waveform (above) and spectrogram (below; spectrogram parameters: FFT length 1024, frame size 100%, overlap 87.5%, Hamming window) are shown. I = inhalation, E = exhalation

We analysed one call from each animal to avoid pseudo-replication (McGregor et al. 1992) and included into the analysis only calls, which were recorded in a good quality with low background noise. If more calls were recorded from the same individual, we pseudo-randomly selected one of them as calls from both contexts were available for several animals and we wanted to achieve approximately similar sample sizes in both contexts. We analysed the calls following Cinková and Policht (2014) using Avisoft SAS Lab Pro 5.2.07 (Avisoft Bioacoustics, R Specht, Berlin, Germany) and computed their temporal parameters (using the following spectrogram parameters: FFT length 256, frame size 100%, overlap 50%, FlatTop window). Particular call elements were classified as inhalations and exhalations and categorized according to their duration: (1) 0.0–0.2 sec, (2) 0.21–0.4 sec, (3) 0.41–0.8 sec, (4) >0.81 sec. One representative inhalation and exhalation from the most numerous category were selected for the measurement of spectral parameters. The spectrograms (FFT length 1024, frame size 100%, overlap 87.5%, Hamming window, time resolution 2.9 ms) of these elements were also analysed using LMA 2008 (developed by Kurt Hammerschmidt), which allows for the extraction of different sets of call parameters (Schrader and Hammerschmidt 1997). Based on the results of Cinková and Policht (2014), we selected and calculated 12 temporal and spectral parameters of calls.

Using Avisoft SAS Lab Pro 5.2.07, we calculated call duration (sec), number of elements in the call, duration of representative exhalation (sec), duration of the longest exhalation and shortest inhalation and exhalation in the call (sec), the order of the longest exhalation in call, peak frequency (max) in an inhalation (= frequency of maximum amplitude of the spectrum; measured at the location of maximum amplitude; Hz) and entropy (std) in an inhalation (= allows to quantify the randomness of the sound; the parameter was computed for all spectra between the start and the end of element and then the relative standard deviation was calculated). Using LMA 2008, we calculated ampratio 1 in an inhalation (= amplitude ratio between 1st and 2nd dominant frequency band), fp1 amax in an inhalation (= maximum amplitude of the 1st global frequency peak; relative amplitude) and pftrmean in an exhalation (= mean deviation between peak frequency and linear trend; Hz).

Playback experiments
We tested the ability of nine adult bulls to discriminate between the pant calls of unfamiliar female and male southern and northern white rhinos. We used for playbacks the calls of seven subadult and adult female (aged ≥ 4 years) and three adult territorial male southern white rhinos and the calls of three adult female and one adult territorial male northern white rhinos.

The calls of southern males and northern male and females were recorded in isolation and calls of southern females were recorded both in isolation and in visual contact with group members. Each call was only used once to avoid pseudo-replication (McGregor et al. 1992). Playback stimuli were equalized in terms of root mean square amplitude using Avisoft SAS Lab Pro 5.2.07.

We conducted three sets of playback experiments and each of them consisted of a playback of control sound, female and male pant call. In experiment 1, we tested if males (N = 9) in visual isolation from other rhinos discriminate between the calls of female and male southern white rhinos. In experiment 2, we tested if males (N = 7) in visual isolation discriminate between the calls of female and male northern white rhinos. In experiment 3, we studied if males (N = 6) in visual contact with a female (in distance from 2 to 7 rhinoceros body lengths from her) react differently to the calls of female and male southern white rhinos. The calls were only played when a female's group consisted of a maximum of three rhinos since a higher number of animals could increase a probability that their behaviour would affect the reaction of a subject. We used calls of widespread South African birds as a control: African grey hornbill (*Tockus nasutus*), Swainson's francolin (*Pternistes swainsonii*), pied crow *(Corvus albus)*, hadeda ibis *(Bostrychia hagedash)*, blacksmith plover (*Vanellus armatus)* and white-faced duck (*Dendrocygna viduata*).

The stimuli were broadcast using a Barrel digital player and a custom-built loudspeaker (power output: 50W, frequency response: 48–20,000 Hz ± 3 db) connected to an amplifier (kindly constructed by P Krchňák and M Deutschl, Department of Biophysics, Palacký University). The loudspeaker was placed in the vehicle and the calls were broadcast at a distance of about 15–40 m from the subjects. The rhinos in the study areas were habituated to the presence of the vehicles and the stimuli were played when a subject was in a relaxed state (i.e. grazing, standing or lying) and was not looking in the direction of speaker. There was a mean interval of 11 days between consecutive experiments on one animal and bulls were exposed to particular experiments in random order.

Pant calls within an experiment were played in random order, were separated by at least six minutes and the second call was only played when a subject was in a relaxed state again. Pant calls were broadcast with peak amplitudes at sound pressure level of 85–97 dB (measured at 1 m from the speaker) depending on the distance of rhinos from the speaker (85 dB for 15 m and 97 dB for 40 m) and their volume was equivalent to a naturally panting rhinoceros. The subject's behaviour was video-recorded using digital camcorder Canon Legria HF M31 one minute after the onset of control sound and five minutes after the onset of

a pant call. We chose shorter recording time after the control sound to minimize the possibility that a subject would leave a study site before pant call was played and we considered it as sufficient since the animals either reacted to it with a low intensity or did not react at all (see Fig. 3). Since locating the animals for the study and waiting for the right time to play a call is much more difficult in the wild than e.g. in controlled settings of zoological gardens, we preferred a higher chance to obtain the data over the equal recording times after the playbacks.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, USA). A significance level of $p < 0.05$ was accepted and all tests were two-tailed.

We tested if the acoustic parameters of pant calls of the southern white rhinos differ between sexes, age-classes and contexts using univariate general linear models (GLM). The call parameters were BoxCox transformed (Sokal and Rohlf 2012) prior to the analyses to normalize their distribution (confirmed with Shapiro-Wilk tests) and achieve homogeneity of their variances (confirmed by Levene's tests). We only report results that were statistically significant or closely approached significance (for other results see Table S1 in the Supplementary material).

The video recordings with the reactions of males to the playbacks were analysed using the computer program package Activities 2.1 (Vrba and Donát 1993) and we used for statistical analyses exact non-parametric tests due to the small sample size (Mundry and Fischer 1998). The reactions to the control sound, female and male pant calls in each experiment were scored according to their increasing intensity (Table 1) and tested with exact Friedman ANOVA. Multiple comparisons were conducted using exact Wilcoxon matched-pairs tests followed by a sequential Bonferroni correction. Potentially confounding factors speaker-subject distance, volume of playback and order of a call in an experiment had no effect on the intensity of reaction of males to the pant calls (see Table S2 in the Supplementary material). The activity of the subjects after the playbacks (proportion of time, which they devoted to grazing, walking / running, standing and lying) was tested using exact Friedman ANOVA. If the results were significant, we tested particular categories with exact Wilcoxon matched-pairs tests and applied sequential Bonferroni correction. As the dataset contained zero values, we replaced them with the value 0.001.

We further analysed only the reactions to the pant calls and registered the latency and duration of vigilance, looking and turning body towards the speaker and the latency of

approaching, calling with contact call and marking the territory with dung or urine (see Table 1 for description of these behaviours) from the onset of playback. If certain behaviour did not occur, latency of 300 sec was assigned. We conducted principal component (PC) analysis (for rationale see McGregor 1992) for all three experiments using varimax rotation and saved PC scores using the Bartlett method. We included in each PC analysis only such behavioural measures, which fulfilled our criteria. The latency of a particular behaviour was only included when the behaviour was registered in at least > 25% of reactions in a particular experiment. If a certain behaviour was recorded in all the reactions in an experiment, we used its duration instead of latency since we believe that it provides more information. In experiment 2, the latency of approaching fulfilled our criteria, however, it was only little correlated with other behavioural measures and the Kaiser-Meyer-Olkin and Bartlett's criteria for the use of PC analysis were not fulfilled. We therefore removed the latency of approaching from the analysis. Similarly, we removed the latency of calling with contact call from the PC analysis in experiment 3. All the three PC analyses then fulfilled Kaiser-Meyer-Olkin and Bartlett's criteria so their use was justified (McGregor 1992). The scores of the first PC in each experiment were compared using exact Wilcoxon matched-pairs test to determine whether the responses to the playback stimuli differed. In addition, particular reactions were also tested separately using exact Wilcoxon matched-pairs tests to help us identify concrete behaviours, which differed between both types of playback.

Table 1 Reactions of the rhinos to the playback experiments ordered according to their increasing intensity

No.	Reaction
(0)	No reaction
(1)	Vigilance: standing or lying with raised head, gaze fixed or scanning
(2)	Looking: head is raised facing the speaker in an angle equal to or less than 30°
(3)	Turning body towards the speaker in an angle equal to or less than 30°
(4)	Turning body towards the speaker and marking the territory with dung or urine
(5)	Walking or running away from the speaker*
(6)	Approaching the speaker or a female
(7)	Approaching the speaker or a female and marking the territory with dung or urine or calling with pant
(8)	Approaching the speaker or a female, marking and calling
(9)	Running towards the speaker or a female
(10)	Running towards the speaker or a female and marking or calling
(11)	Running towards the speaker or a female, marking and calling
(12)	Running towards the speaker, then approaching a female and marking or calling
(13)	Running towards the speaker, then approaching a female and marking and calling

* This activity was only registered if it was not affected by other stimulation as e.g. walking away from the speaker because the animal started walking towards the waterhole, which was nearby in that direction.

RESULTS

Sex, age and context differences in pant calls

Males had smaller fp1 amax in an inhalation (GLM: $F_{1,25}$=6.497, P=0.017), shorter duration of exhalation ($F_{1,25}$ =6.012, P=0.022) and shorter duration of the shortest inhalation in call ($F_{1,25}$=5.316, P=0.03) than females (Fig. 2). Subadults had shorter call duration ($F_{1,25}$=9.175, P=0.006), lower ampratio 1 in an inhalation ($F_{1,25}$=8.432, P=0.008), less call elements ($F_{1,25}$=5.408, P=0.028) and shorter duration of the longest exhalation in the call ($F_{1,25}$=4.377, P=0.047) than adults. The animals in visual isolation tended to have lower entropy (std) in an inhalation than animals in visual contact with others ($F_{1,25}$=4.205, P=0.051). There was an interaction between the sex and age-class in the duration of shortest inhalation in call ($F_{1,25}$=5.448, P=0.028). We found an interaction between the age-class and context in the duration of exhalation ($F_{1,25}$=10.884, P=0.003), the duration of shortest exhalation ($F_{1,25}$=8.691, P=0.007), pftrmean in an exhalation ($F_{1,25}$=5.749, P=0.024), the order of the

longest exhalation in call ($F_{1,25}$=4.283, P=0.049) and entropy (std) in an inhalation ($F_{1,25}$=4.272, P=0.049). Statistical interactions also indicated differences between the sexes, age-classes and contexts in the number of elements in call ($F_{1,25}$=13.903, P=0.001), call duration ($F_{1,25}$=7.977, P=0.009), order of the longest exhalation in call ($F_{1,25}$=7.887, P=0.01) and duration of the shortest inhalation in call ($F_{1,25}$=4.406, P=0.046).

Discrimination of sex from the calls of southern and northern white rhinos

The males in isolation reacted to the control sound, female and male southern white rhinoceros calls in experiment 1 with significantly different intensity (exact Friedman ANOVA: N=9, χ^2_2=14.824, P<0.001) and their reaction was more intensive towards the calls of females than males (exact Wilcoxon test: N=9, Z=-2.673, Bonferroni corrected P=0.012) (Fig. 3). The time, which the males devoted to walking and running after the playback of control sound, male and female pant call was significantly different (exact Friedman ANOVA: N=9, χ^2_2=8.267, P=0.012) (Fig. 4). We included five behavioural measures in PC analysis (Fig. 5) and the first principal component (PC1) explained 56% of the total variance (see Table S3 in the Supplementary material). By comparing PC1 scores, males' reactions to the male and female calls were found to be significantly different (exact Wilcoxon test: N=9, Z=-2.547, P=0.008).
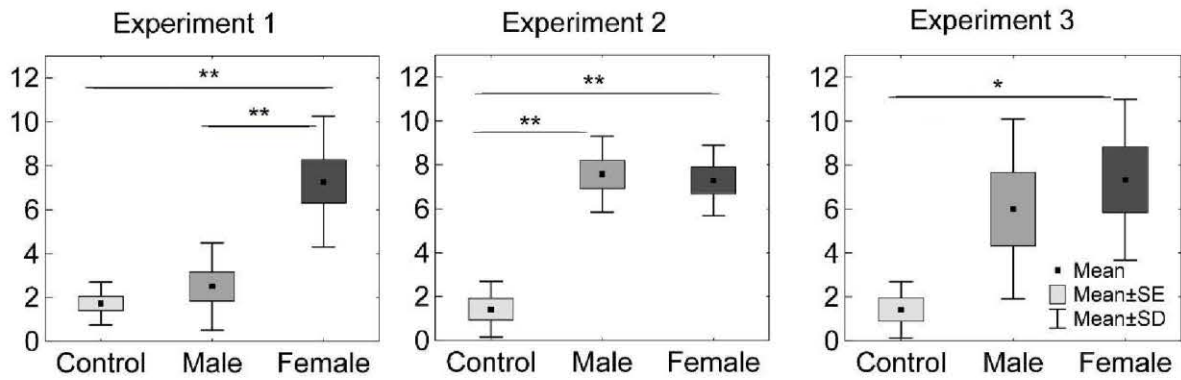
The intensity of reaction of the males in isolation to the control sound, female and male northern white rhinoceros calls in experiment 2 significantly differed (exact Friedman ANOVA: N=7, χ^2_2=11.385, P=0.001), but males reacted to the female and male calls with the same intensity (exact Wilcoxon test: N=7, ties=2, Z=-0.135, Bonferroni corrected P=1) (Fig. 3). Males devoted significantly different time to walking and running after the playback of control sound, male and female pant call (exact Friedman ANOVA: N=7, χ^2_2=8.000, P=0.016) (Fig. 4). We included five behavioural measures in PC analysis (Fig. 5) and the PC1 explained 47% of the total variance (see Table S3 in the Supplementary material). By comparing PC1 scores, we found that the reactions to the male and female calls significantly differed (exact Wilcoxon test: N=7, Z=-2.197, P=0.031).

The differences in the intensity of reaction of males in visual contact with a female to the control sound, female and male southern white rhinoceros calls in experiment 3 closely approached significance (exact Friedman ANOVA: N=6, χ^2_2=6.333, P=0.052). The intensity of reaction to the calls of females and males did not differ (exact Wilcoxon test: N=6, Z=-0.632, Bonferroni corrected P=1) (Fig. 3) as well as the activity of males after the playbacks (Fig. 4). Five behavioural measures were included in PC analysis (Fig. 5) and the PC1

explained 45% of the total variance (see Table S3 in the Supplementary material). We compared PC1 scores and found that there were no differences in the reactions to the male and female calls (exact Wilcoxon test: N=6, Z=-0.524, P=0.688).
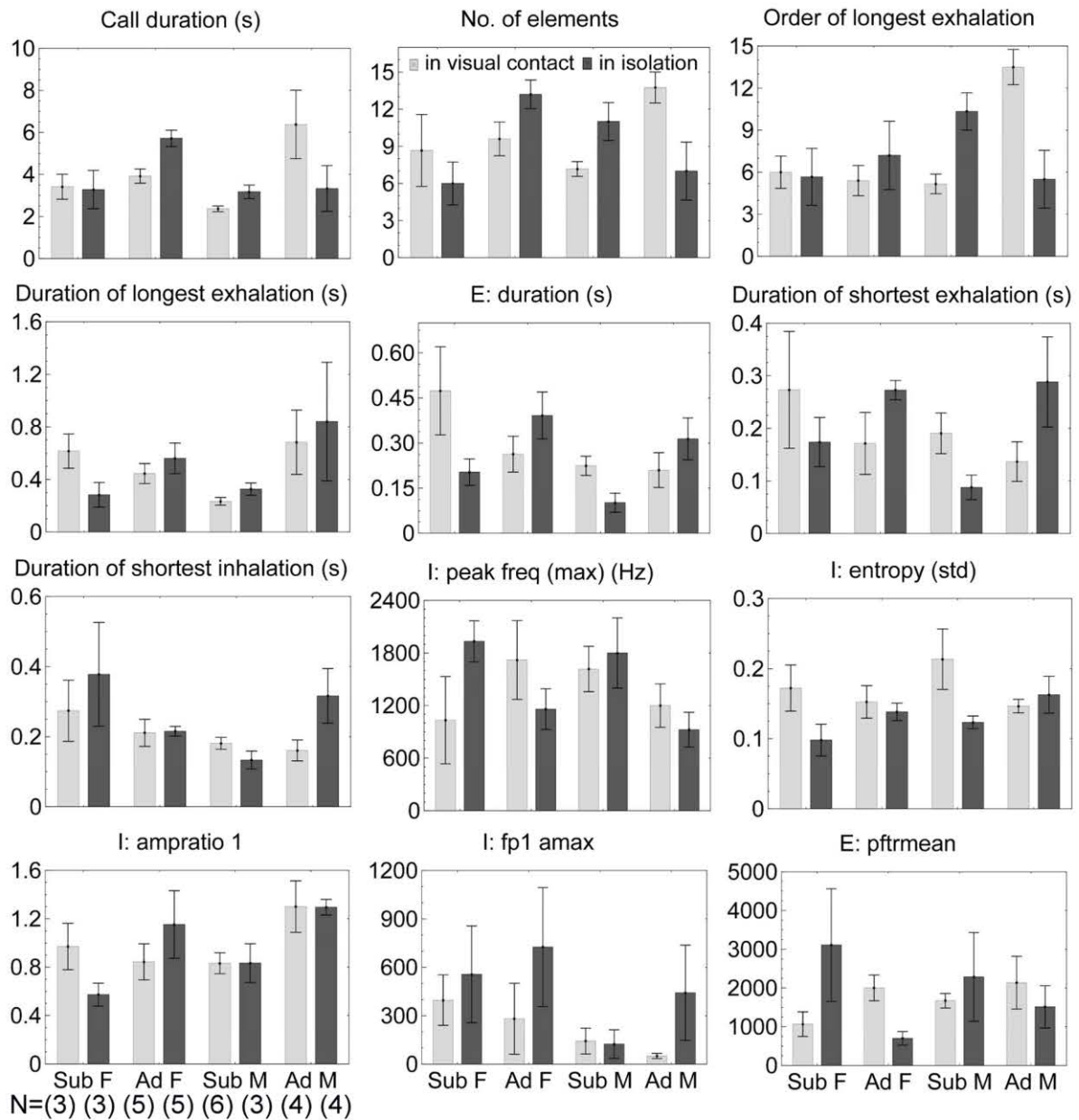
Fig. 2 Acoustic parameters (mean ± SE) of southern white rhinoceros pant calls according to sex, age-class and context. E = exhalation, I = inhalation, Sub = subadult, Ad = adult, F = female, M = male. See methods for explanation of abbreviations of acoustic parameters

Fig. 3 Intensity of reaction of males to the playbacks in particular experiments. The intensity increases on scale from 0–13 (see Table 1). ** $P < 0.05$, * $P < 0.1$
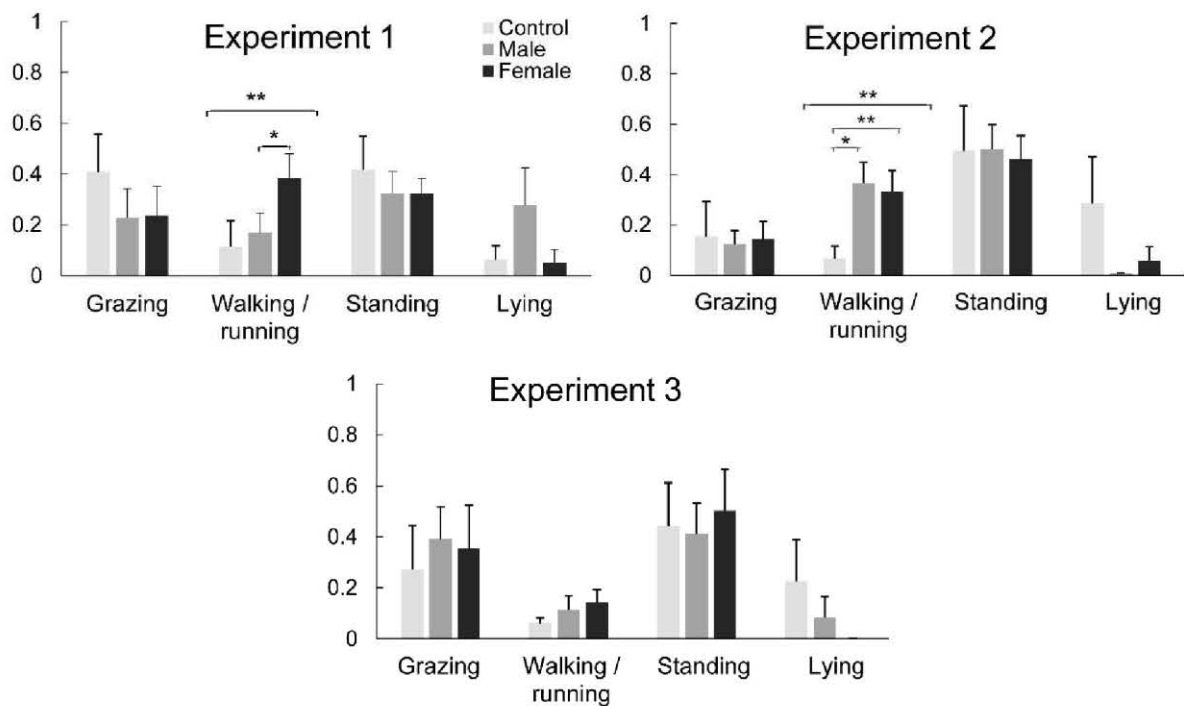
Fig. 4 Proportion of time devoted by the males to grazing, walking/running, standing and lying after the playbacks in particular experiments (mean ± SE). ** $P < 0.05$, * $P < 0.1$
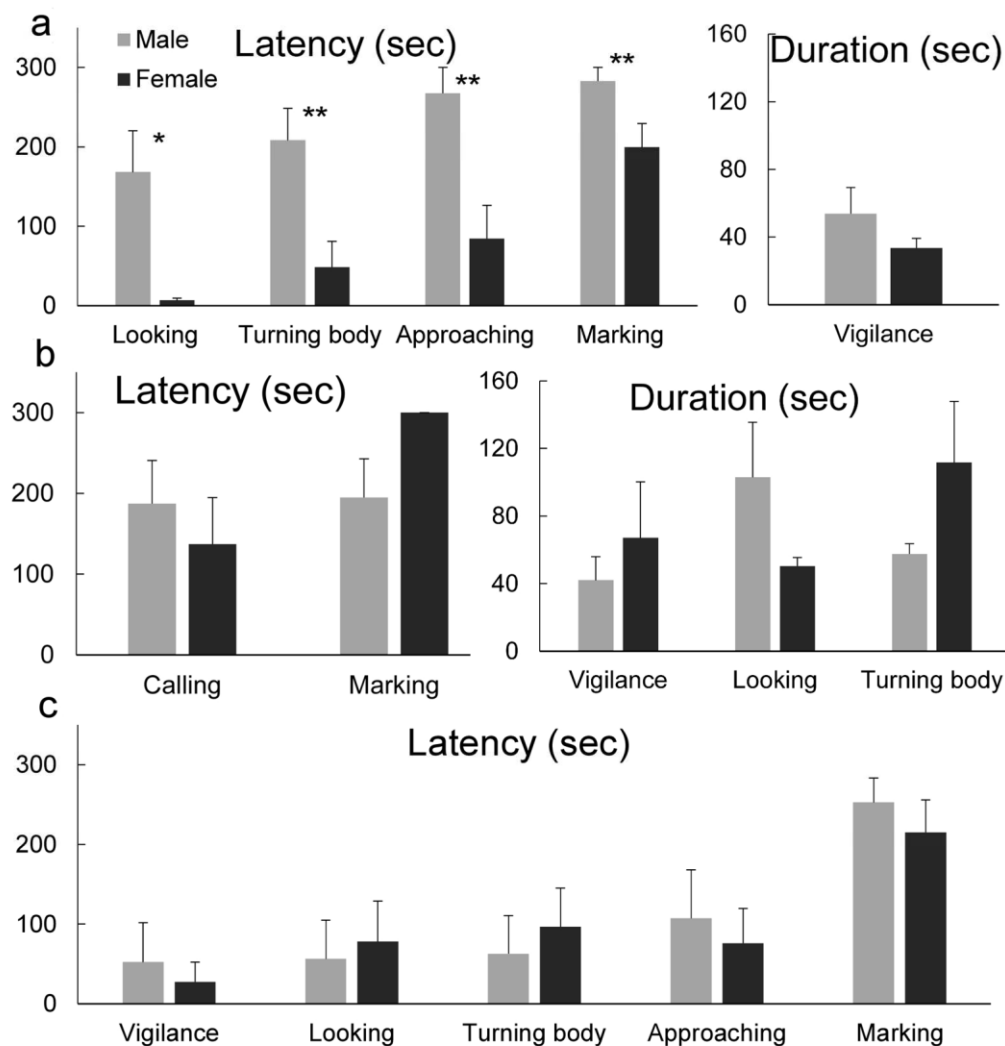
Fig. 5 Reactions of the bulls towards the male and female pant calls (mean ± SE) included in PC analyses in experiment 1 (a), 2 (b) and 3 (c). ** $P < 0.05$, * $P < 0.10$

DISCUSSION

We provide the first evidence of cognitive abilities in any rhinoceros species in terms of discriminating information from vocalizations of conspecifics and closely related allopatric species. Adult southern white rhinoceros males in isolation from other rhinos in experiment 1 discriminated sex from the contact pant calls of conspecifics and reacted more intensively to the calls of females than territorial males, showed a shorter latency to turn body towards the speaker and approach it and a shorter latency to mark their territory. This suggests that pant calls are less important communication channel for interactions between territorial males than between a male and a female. Adult males mark their territory with dung and urine (Owen-

Smith 1973) and react intensively to the olfactory signals in dung of adult males including walking or running around and attempting to find the depositor (Cinková and Policht 2015). Pant calls were therefore probably not selected in the context of territorial male-male communication, which can preferentially occur via the olfactory signals as in certain situations, one communication channel can be favoured at the expense of other ones (Endler 1993).

Duration of a call or its parts is in mammals often longer in males than females as they usually have a bigger size and therefore larger lungs with greater air volume available for calling (reviewed by Ey et al. 2007; Lemasson et al. 2009). However, males of some primate species without sexual size dimorphism have shorter calls than females (reviewed by Ey et al. 2007) and females were found to prefer males with the shortest calls (Miller et al. 2004). Height of the white rhinoceros males largely overlaps with that of females (Hillman-Smith et al. 1986b; Groves et al. 2010) although they are heavier (Owen-Smith 1973) and we found that males have a shorter duration of exhalation and of shortest inhalation in contact calls than females. These call characteristics might allow a male to better intercept other animals' movements or vocalizations between particular call elements. Several temporal parameters of calls including call duration and number of elements in call depended on the sex, age-class and context at the same time. Calls of adult males in isolation were shorter and contained less elements than calls recorded in visual contact (=hic calls, see Introduction) in contrast to the calls of adult females and subadult males (see Fig. 2). Longer call duration and higher number of calls per bout have been shown to be associated with increased arousal (Rendall 2003; Charlton et al. 2010) and more frequent repetition of syllables could help other animals locate the caller (Aubin and Jouventin 2002). The adult white rhinoceros males are solitary (Owen-Smith 1973, 1975) and they therefore do not experience in isolation any strong need to re-associate with a group like females or subadults. On the contrary, adult males can experience higher arousal while in visual contact with a female since hic calls are most often uttered when a female is in oestrus. Interestingly, calls of subadult females did not differ in duration between contexts and number of elements was highly variable.

Bulls in isolation discriminated between the calls of female and male northern white rhinos in experiment 2 and responded to them strongly and with the same intensity. Bulls in the same social context therefore reacted intensively to the female calls of both northern and southern white rhinos and to the calls of northern male, but their reactions to the southern males were weak. This suggests that males indeed attend to the differences in vocalizations between male calls in both species and since they are naturally allopatric (Hillman-Smith

1986a), discrimination between them cannot result from learning. The role of communication signals in recognition between conspecifics and closely related species may be complicated and animals can show a greater reaction to a heterospecific signal (reviewed by Ord and Stamps 2009). If territorial southern males prefer an olfactory channel for male-male communication (see above) and do not react strongly to the pant calls of conspecific males, a similar vocal signal of a northern male, which contains different and unknown call characteristics could cause more intensive reaction due to a novelty effect (Tulving and Kroll 1995).

Bulls also marked their territory sooner after the playbacks of southern females than males, but later after the playbacks of northern females than male (however, the differences between both sexes of northern white rhinos were not significant, see Fig. 5). The asymmetries in reactions to the female and male calls of both species could also suggest that the differences between the calls of both sexes are distinct between the northern and southern species, which has also been shown in other closely related mammalian species (e.g. Siemers et al. 2005). Playbacks of calls of both white rhinoceros species to the females are necessary to determine if there is any indication of reproductive isolation between them based on the vocalizations as female reactions to the male calls are more relevant to assess this (Searcy 1992). For instance, red deer and sika deer naturally live in allopatry, but sometimes hybridize when in sympatry after introductions and females of both species display different reactions to the calls of conspecific and heterospecific males. Although red deer females show a variability in their reactions, they generally prefer roars of conspecific males (Wyman et al. 2011), but sika females do not show any preference for roars of sika versus red deer males (Wyman et al. 2014).

We did not find any differences in the reactions of bulls in visual contact with a female to the female and male calls of southern white rhinos in experiment 3. In contrast to the experiment 1, bulls often approached the speaker or a female in reaction to the pant calls of both sexes and were also calling or marking their territory. We suppose that their reactions were influenced by the presence of a female as the attitude of the animals towards acoustic signals can change depending on the audience (Vignal et al. 2004). Intensities of the reactions of males were variable, particularly to the male calls, which could have been caused by a concrete social situation with a female or by a different position of speaker in relation to a female and a subject male in particular trials. Experiment 3 contained the lowest sample size from all three experiments and a larger sample size would therefore be necessary to determine which factors influence males' perception of signals in the presence of a female.

Stimulation of the animals using vocal and olfactory signals has been found to affect physiology of domestic and farmed mammals. Red deer females conceive earlier when exposed to playbacks of male roars during the pre-rut period (McComb 1987), dairy cows increase milk production when exposed to playbacks of calf vocalizations (McCowan et al. 2002), the success of artificial insemination is greater when dairy cows are continuously exposed to bull urine than steer urine (Tauck and Berardinelli 2007) and male's urine, wax and wool have a stimulating effect on ovulation in sheep (reviewed by Rekwot et al. 2001). Intensive reactions of the territorial bulls to the pant calls in our study suggest that pant call can stimulate the social and territorial behaviour of rhinos. It might therefore find together with olfactory signals in dung (Cinková and Policht 2015) use in behavioural manipulation techniques and help optimize the management of rhinos and improve chances for their breeding in captivity.

ACKNOWLEDGEMENTS

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ELECTRONIC SUPPLEMENTARY MATERIAL

Table S1: Differences between sexes, age-classes and contexts in the acoustic parameters of pant calls. Results of univariate general linear models are shown. N = 33, df $_{error}$ = 25.

	Call parameter	df	Mean Square	F	Sig.
Sex	Number of elements	1	0.476	0.106	0.747
	Call duration	1	0.194	1.001	0.327
	Duration of longest exhalation	1	0.003	0.397	0.534
	Duration of shortest inhalation	1	0.006	5.316	**0.030**
	Duration of shortest exhalation	1	0.006	2.006	0.169
	E: duration	1	0.027	6.012	**0.022**
	Order of longest exhalation	1	5.750	3.445	0.075
	I: peak freq (max)	1	1049.741	0.048	0.829
	I: ampratio 1	1	0.072	3.460	0.075
	I: fp1amax	1	4.874	6.497	**0.017**
	I: entropy (std)	1	0.001	1.120	0.300
	E: pftrmean	1	6.325	0.286	0.598
Age-class	Number of elements	1	24.276	5.408	**0.028**
	Call duration	1	1.773	9.175	**0.006**
	Duration of longest exhalation	1	0.038	4.377	**0.047**
	Duration of shortest inhalation	1	0.000	0.034	0.855
	Duration of shortest exhalation	1	0.003	0.939	0.342
	E: duration	1	0.007	1.617	0.215
	Order of longest exhalation	1	0.336	0.201	0.657
	I: peak freq (max)	1	41253.930	1.880	0.183
	I: ampratio 1	1	0.175	8.432	**0.008**
	I: fp1amax	1	0.005	0.007	0.934
	I: entropy (std)	1	0.000	0.138	0.713
	E: pftrmean	1	18.625	0.842	0.368
Context	Number of elements	1	1.066	0.237	0.630
	Call duration	1	0.019	0.098	0.757
	Duration of longest exhalation	1	0.000	0.037	0.850
	Duration of shortest inhalation	1	0.002	1.284	0.268
	Duration of shortest exhalation	1	0.000	0.149	0.703
	E: duration	1	0.003	0.699	0.411
	Order of longest exhalation	1	0.311	0.186	0.670
	I: peak freq (max)	1	2633.355	0.120	0.732
	I: ampratio 1	1	0.005	0.230	0.635
	I: fp1amax	1	1.910	2.545	0.123
	I: entropy (std)	1	0.004	4.205	0.051
	E: pftrmean	1	3.012	0.136	0.715
Sex * age-class	Number of elements	1	8.072	1.798	0.192
	Call duration	1	0.001	0.003	0.957

	Duration of longest exhalation	1	0.013	1.441	0.241
	Duration of shortest inhalation	1	0.007	5.448	**0.028**
	Duration of shortest exhalation	1	0.002	0.647	0.429
	E: duration	1	0.006	1.331	0.259
	Order of longest exhalation	1	0.223	0.133	0.718
	I: peak freq (max)	1	33871.967	1.544	0.226
	I: ampratio 1	1	0.018	0.881	0.357
	I: fp1amax	1	0.328	0.437	0.515
	I: entropy (std)	1	0.000	0.259	0.615
	E: pftrmean	1	6.612	0.299	0.590
Sex * context	Number of elements	1	3.173	0.707	0.408
	Call duration	1	0.382	1.977	0.172
	Duration of longest exhalation	1	0.014	1.617	0.215
	Duration of shortest inhalation	1	0.000	0.000	0.994
	Duration of shortest exhalation	1	0.000	0.091	0.765
	E: duration	1	0.000	0.045	0.833
	Order of longest exhalation	1	0.576	0.345	0.562
	I: peak freq (max)	1	6941.030	0.316	0.579
	I: ampratio 1	1	0.007	0.351	0.559
	I: fp1amax	1	0.021	0.028	0.867
	I: entropy (std)	1	0.000	0.262	0.613
	E: pftrmean	1	2.283	0.103	0.751
Age-class * context	Number of elements	1	4.382	0.976	0.333
	Call duration	1	0.190	0.982	0.331
	Duration of longest exhalation	1	0.009	1.031	0.320
	Duration of shortest inhalation	1	0.003	2.527	0.124
	Duration of shortest exhalation	1	0.025	8.691	**0.007**
	E: duration	1	0.050	10.884	**0.003**
	Order of longest exhalation	1	7.150	4.283	**0.049**
	I: peak freq (max)	1	85991.737	3.919	0.059
	I: ampratio 1	1	0.063	3.061	0.092
	I: fp1amax	1	2.191	2.921	0.100
	I: entropy (std)	1	0.004	4.272	**0.049**
	E: pftrmean	1	127.229	5.749	**0.024**
Sex * age-class * context	Number of elements	1	62.411	13.903	**0.001**
	Call duration	1	1.542	7.977	**0.009**
	Duration of longest exhalation	1	0.023	2.629	0.117
	Duration of shortest inhalation	1	0.005	4.406	**0.046**
	Duration of shortest exhalation	1	0.001	0.193	0.664
	E: duration	1	0.000	0.084	0.775
	Order of longest exhalation	1	13.166	7.887	**0.010**
	I: peak freq (max)	1	23336.308	1.063	0.312
	I: ampratio 1	1	0.049	2.359	0.137
	I: fp1amax	1	0.009	0.012	0.913
	I: entropy (std)	1	0.000	0.012	0.914
	E: pftrmean	1	57.196	2.584	0.120

Table S2: Effects of potentially confounding factors on the intensity of reaction of males to the playbacks of pant calls.

	Spearman's coefficient	Exact p*	N
Speaker-subject distance			
Experiment 1	0.233	0.348	18
Experiment 2	0.153	0.600	14
Experiment 3	-0.353	0.258	12
Volume of playback			
Experiment 1	0.185	0.457	18
Experiment 2	-0.024	0.943	14
Experiment 3	-0.453	0.140	12
Order of a call in an experiment			
Experiment 1	0.412	0.094	18
Experiment 2	-0.147	0.642	14
Experiment 3	-0.173	0.613	12

* Statistical analysis was performed using StatXact 7.0.0 (Cytel Inc., Cambridge, MA, USA).

Table S3: Eigenvalues, explained variances and rotated loadings of behavioural measures on the first two factors of the PCAs in playback experiments 1, 2 and 3.

Behavioural measures	Experiment 1 SWR calls in isolation		Experiment 2 NWR calls in isolation		Experiment 3 SWR calls in visual contact	
	PC1	PC2	PC1	PC2	PC1	PC2
L vigilance					0.829	0.373
D vigilance	-0.025	0.972	-0.592	-0.41		
L looking	0.867	-0.222			0.900	0.040
D looking			0.955	0.011		
L turning body	0.955	0.002			0.815	0.226
D turning body			0.957	0.092		
L approaching	0.805	0.399			0.217	0.844
D approaching						
L calling with contact call			0.405	0.843		
L marking the territory	0.693	0.011	-0.089	0.855	0.143	0.864
Eigenvalue	2.79	1.15	2.35	1.62	2.23	1.65
% Variance	55.85	23.08	47.03	32.38	44.57	33.04

SWR = southern white rhinoceros, NWR = northern white rhinoceros, L = latency, D = duration.

Curriculum vitae

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Vzdělání

2009–nyní   Katedra zoologie a Ornitologická laboratoř, PřF UP v Olomouci
Prezenční doktorské studium, obor Zoologie

2006–2009   Katedra zoologie a Ornitologická laboratoř, PřF UP v Olomouci
Mgr. v oboru Zoologie, absolvovala s vyznamenáním
Diplomová práce: Behavioural study of captive northern white rhinoceros (*Ceratotherium simum cottoni*) and free-ranging southern white rhinoceros (*C. s. simum*).

2003–2006   Katedra zoologie a Ornitologická laboratoř, PřF UP v Olomouci
Bc. v oboru Systematická biologie a ekologie, absolvovala s vyznamenáním
Bakalářská práce: Rozmnožovací, sociální a hravé chování nosorožce tuponosého (*Ceratotherium simum*) v zoologické zahradě.

Výzkumná činnost a zahraniční stáže

2005 (4 měsíce)	Výzkum sociálního a reprodukčního chování nosorožce Cottonova v zoo Dvůr Králové pro bakalářskou práci.
2008 (5 měsíců)	Výzkum sociálního chování a preference habitatu volně žijících nosorožců tuponosých v Leadwood bushveld reserve a Lichtenburg biodiversity conservation centre v Jihoafrické republice pro diplomovou práci.
2009, 2010, 2014 (3 měsíce)	Výzkum vokální komunikace nosorožců Cottonových a tuponosých v zoologických zahradách Dvůr Králové, Salzburg, Bratislava a Zlín.
2011–2012 (13 měsíců)	Výzkum vokální a olfaktorické komunikace volně žijících nosorožců tuponosých v rezervacích Lapalala Wilderness, Welgevonden Game Reserve, Mthethomusha Nature Reserve a Lichtenburg biodiversity conservation centre v Jihoafrické republice.
Zahraniční stáže	Veškerý výzkum v Jihoafrické republice byl prováděn za podpory University of the Witwatersrand v Johannesburgu (Prof. Norman Owen-Smith).

Publikace v odborných impaktovaných časopisech

Cinková I., Bičík V. (2013) Social and reproductive behaviour of critically endangered northern white rhinoceros in a zoological garden. Mammalian Biology 78: 50–54.
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Cinková I. (v recenzi) Sociální chování a komunikace nosorožců tuponosých a Cottonových. Živa.

Účast na mezinárodních konferencích

7th International Conference on Behaviour, Physiology and Genetics of Wildlife, Berlín 2009:

Cinková I., Ganslosser U., Kretzschmar P. (2009) Social behaviour of southern white rhinoceros (*Ceratotherium simum simum*) in game reserves in South Africa. Conference proceedings, s. 47. (poster)

International Elephant & Rhino Conservation & Research Symposium, Pittsburgh 2013:

Cinková I., Policht R. (2013) Contact calls of the northern and southern white rhinoceros: Source of information on individuality and species of the caller? Conference proceedings, s. 960–961. (přednáška)

Cinková I., Bičík V. (2013) Social and reproductive behaviour of critically endangered northern white rhinoceros (*Ceratotherium cottoni*) in a zoological garden. Conference proceedings, s. 1203. (přednáška)

87th Annual Meeting of the German Society of Mammalogy, Praha 2013:

Cinková I., Policht R. (2013) Contact calls of the northern and southern white rhinoceros contain information about individual and species identity. Mammalian biology, special issue to volume 78, s. 7–8. (přednáška)

Cinková I., Policht R. (2013) Wild southern white rhinos (*Ceratotherium simum*) are able to recognize information about familiarity and sex in the dung of their conspecifics. Mammalian biology, special issue to volume 78, s. 8. (poster)

9th International Conference on Behaviour, Physiology and Genetics of Wildlife, Berlín 2013:

Cinková I., Policht R. (2013) Identity, species and sex-specific information is contained in the contact calls of northern and southern white rhinoceros. Conference proceedings, s. 46. (poster)

Cinková I., Policht R. (2013) Wild southern white rhinos (*Ceratotherium simum*) are able to recognize information about familiarity and sex in the dung of their conspecifics. Conference proceedings, s. 47. (poster)

Účast na tuzemských konferencích

Zoologické dny, Brno 2013:

Cinková I., Policht R. (2013) Kontaktní hlasy nosorožců tuponosých a Cottonových: zdroj informací o identitě a druhu volajícího jedince? Sborník abstraktů, s. 40–41. (přednáška)

Cinková I., Bičík V. (2013): Sociální a reprodukční chování kriticky ohroženého nosorožce Cottonova (*Ceratotherium cottoni*) v zoologické zahradě. Sborník abstraktů, s. 41. (poster)

Zoologické dny, Ostrava 2014:

Cinková I., Policht R. (2014) Vokální a olfaktorická komunikace volně žijících nosorožců tuponosých. Sborník abstraktů, s. 43–44. (přednáška)

Kurzy a praktické zkušenosti

2009   Kurz angličtiny na úrovni C1 (= Proficient user), Hampstead school of English, Londýn (šest týdnů)

2009   Získání Certificate in Advanced English (= CAE, úroveň C1), Londýn

2011–2012   Účast na příležitostných operacích managementu během terénní práce v jihoafrických rezervacích: imobilizace, ošetření, odchyt zvířat.

2013   7th Berlin Summer School: Non-invasive Monitoring of Hormones, Leibniz Institute for Zoo and Wildlife Research, Berlín (jeden týden). Vedoucí kurzu: Dr. M. Dehnhard, Dr. M. Heistermann, Dr. A. Ganswindt

2013   The Elephant and Rhino Immobilization Workshop (28. srpen) během International Elephant & Rhino Conservation & Research Symposium v Pittsburghu. Vedoucí kurzu: Dr. Jeffery Zuba

2014–2015   Kurz angličtiny na úrovni C2 (= velmi pokročilý), příprava ke složení zkoušky CPE (= Certificate of Proficiency in English), Britské centrum Univerzity Palackého, Olomouc (38 lekcí)

Další činnost:

- Pořádání přednášek pro veřejnost.
- Popularizace výzkumu nosorožců v televizi, rádiu a v tisku.

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Behaviorální studie nosorožce tuponosého a Cottonova v zoologických zahradách a ve volné přírodě, se zaměřením na vokální komunikaci

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Autoreferát dizertační práce k získání titulu Ph.D.

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Dizertační práce byla vypracována formou prezenčního studia doktorského studijního programu na Katedře zoologie a Ornitologické laboratoři Přírodovědecké fakulty Univerzity Palackého v Olomouci.

Autoreferát dizertační práce
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Studijní program:	P1527 Biologie
Studijní obor:	Zoologie
Název práce:	Behaviorální studie nosorožce tuponosého a Cottonova v zoologických zahradách a ve volné přírodě, se zaměřením na vokální komunikaci

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Obhajoba dizertační práce se koná dne………. v …... hod. na učebně č. …..
Katedry zoologie a ornitologické laboratoře PřF UP, 17. listopadu 50, Olomouc. S dizertační prací a posudky se bude možné seznámit nejméně 14 dní před konáním obhajoby ……………………………………….........

OBSAH

1. Úvod

Všechny důležité projevy sociálního chování jsou zprostředkovávány komunikačními signály (McGregor a Peake 2000). Vokální komunikace je výhodná pro přenos informací na delší vzdálenost (Kondo a Watanabe 2009) a na rozdíl od olfaktorických signálů (Eisenberg a Kleiman 1972; Endler 1993) je mnoho vokálních projevů přímo namířeno ke konkrétnímu příjemci, ačkoli může být vokalizace dostupná mnoha dalším jedincům díky odposlouchávání (McGregor a Peake 2000). Olfaktorické signály mají zase velkou výhodu v tom, že v prostředí přetrvávají po delší dobu i v absenci signalizujícího jedince (Eisenberg a Kleiman 1972).

Vokální a olfaktorické signály různých druhů savců obsahují informace o identitě jedince (Linklater et al. 2013; Proops et al. 2009), jeho pohlaví, věku, velikosti (Charlton et al. 2009), druhu (Schneiderová a Policht 2012), emočním stavu (Fichtel et al. 2001), sociální situaci (Tallet et al. 2013), příbuznosti (Bates et al. 2008), fázi reprodukčního cyklu u samic (Swaisgood et al. 2000; Charlton et al. 2010), úrovni hladiny androgenů u samců (Charlton et al. 2011) a tyto signály hrají roli i při výběru partnera (Johansson a Jones 2007). Příjemci tak mohou tyto informace v signálech rozlišovat a rozhodovat se na základě chování, fyziologie nebo morfologie ostatních jedinců (Endler 1993).

Mnoho druhů živočichů používá kontaktní hlasy k zachování soudržnosti skupiny a k oznámení identity jedince (Kondo a Watanabe 2009) a chemické složení pachových značek, jejich umístění, hustota výskytu v dané oblasti nebo čerstvost přispívají k posouzení signalizujícího jedince příjemcem ještě předtím, než se potkají (Gosling a Roberts 2001). Nosorožci mají výborný sluch a čich, ale jejich zrak je slabší (Penny 1987). Nosorožci

tuponosí a Cottonovi mají nejvíce rozvinuté sociální chování ze všech druhů nosorožců (Goddard 1967; Owen-Smith 1973, 1975; Laurie 1982; van Gyseghem 1984; Penny 1987) a dobře vyvinutý komunikační systém může být tedy pro ně zvláště důležitý. Oba druhy jsou blízce příbuzné a byly odlišeny teprve nedávno (Groves et al. 2010). Vokální signály také mohou sloužit jako prezygotický reprodukčně izolační mechanismus a omezit nebo zabránit křížení příbuzných druhů (Ryan a Kime 2003).

Nosorožci tuponosí i Cottonovi se v zajetí velmi špatně rozmnožují (Hermes et al. 2005, 2006; Swaisgood et al. 2006) a jedním z důvodů může být změna jejich sociálního chování v zajetí a nedostatek sociálních podnětů (Lindemann 1982; Swaisgood et al. 2006; Metrione et al. 2007; Cinková a Bičík 2013). Behaviorální výzkum a znalost komunikace jsou velmi významné v ochraně a managementu živočichů a mohou pomoci nalézt a vyřešit problémy s jejich rozmnožováním v zajetí (Lindburg a Fitch-Snyder 1994; Swaisgood et al. 2003; Campbell-Palmer a Rosell 2011). Komunikace nosorožců byla ale donedávna studována jen velmi málo a experimentální studie, které by zkoumaly, jestli nosorožci dokáží rozlišit informace ze svých hlasových a olfaktorických signálů, chyběly.

2. Cíle práce

Cílem mé práce bylo zjistit, jestli hlasy nosorožců kódují informace o vokalizujícím jedinci a jestli jsou volně žijící nosorožci schopni rozpoznat nějaké informace z vokálních a olfaktorických signálů. Mými studijními druhy byli nosorožec tuponosý a Cottonův, kteří jako jediní z nosorožců používají kontaktní hlas sípání (angl. pant), který se skládá ze série nádechů a výdechů (Owen-Smith 1973; Policht et al. 2008). Oba druhy se také

vyprazdňují na společných hnojištích a samci si značí své teritorium močí a trusem (Owen-Smith 1973; van Gyseghem 1984).

3. Přehled výsledků

Hlavní výsledky této práce jsou:

- Kontaktní hlas sípání nosorožců tuponosých a Cottonových umožňuje identifikaci jedince i jeho druhu.
- Kontaktní hlasy nosorožců tuponosých obsahují informace o pohlaví, věkové třídě a sociální situaci jedince.
- Volně žijící samci nosorožců tuponosých rozpoznají pohlaví z kontaktního sípání jedinců vlastního druhu i nosorožců Cottonových.
- Volně žijící samci nosorožců tuponosých jsou schopni rozpoznat některé rozdíly v kontaktním sípání vlastního druhu a nosorožců Cottonových.
- Volně žijící nosorožci tuponosí poznají familiaritu a pohlaví jedinců vlastního druhu pouze na základě pachu jejich trusu.

4. Abstrakty prací

Cinková I., Policht R. (2014) Contact Calls of the Northern and Southern White Rhinoceros Allow for Individual and Species Identification. PLoS ONE 9 (6): e98475. (doi: 10.1371/journal.pone.0098475)

Inter-individual relationships particularly in socially living mammals often require a well-developed communication system. Vocal and olfactory signals are the most important for the communication of rhinos, however, their vocal

communication has been investigated to a very limited extent so far. White rhinos have the most developed social system out of all the rhinoceros species and vocal signals might therefore play an important role in their social interactions. We recorded repetitive contact pant calls from six captive northern white rhinos (*Ceratotherium cottoni*) and 14 captive and free-ranging southern white rhinos (*Ceratotherium simum*) and examined if they transmit information about individual identity, species, social context and age class. Discriminant analyses revealed that a high percentage of the pant calls of both species could be classified to a correct individual. We calculated signature information capacity of pant calls recorded from adult animals in isolation at 3.19 bits for the northern white rhinos and at 3.15 bits for the southern white rhinos, which can potentially allow for a vocal discrimination of nine individuals of both species. We found that pant calls varied by species. Northern white rhinos had longer calls and also differed from the southern white rhinos in several frequency parameters of their calls. We also analysed the pant calls of southern white rhinos for the differences between the age classes and between social contexts in which they were recorded. Our results show that pant calls carry information about individual, species, age class and context. The ability to recognize this information would allow rhinos, in addition to olfactory cues, to communicate with highly increased accuracy. A better understanding of communication of white rhinos has potential practical use in their management and conservation particularly because of the low breeding success of white rhinos in captivity.

Communication in rhinos is primarily mediated by the vocal and olfactory signals as they have relatively poor eyesight. White rhinos are the most social of all the rhinoceros species, they defecate at common dungheaps and the adult bulls use dung and urine to mark their territory. Chemical communication may therefore be particularly important in the social interactions of white rhinos, and its knowledge could be very helpful in their management and conservation. However, no studies have investigated up until now the olfactory discrimination in any rhinoceros species in the wild. We have experimentally studied the reactions of the wild southern white rhinos (*Ceratotherium simum*) to the dung of familiar and unfamiliar adult females and adult territorial males. We registered the number of sniffing events, the duration of sniffing and the latency of the vigilance posture from the onset of sniffing. The dung of unfamiliar rhinos was sniffed longer than that of familiar rhinos. The rhinos showed a shorter latency of vigilance posture to the familiar dung of males than that of females. For unfamiliar dung, they displayed a shorter latency of vigilance posture to female than male dung. Our results indicate that the rhinos are able to discriminate the familiarity and sex of conspecifics from the smell of their dung. Olfactory cues could therefore play an important role in the social relationships and spatial organization of the southern white rhinoceros.

Cinková, I., Policht, R. (submitted) Sex and species recognition by wild male southern white rhinoceros using contact pant calls.

Recognizing information from acoustic signals is crucial in many animals and individuals are under strong selection pressure to discriminate between the signals of conspecifics and closely related species. The role of communication signals in species recognition may be complicated. Here, we first report that rhinos use information encoded in their calls to assess conspecifics and individuals of closely related species. The southern (*Ceratotherium simum*) and critically endangered northern (*C. cottoni*) white rhinos are the most social out of all the rhinoceros species and use a unique contact call pant. We studied the structure of pant calls in southern white rhinos and found that certain temporal and frequency parameters carry information about sex, age-class and social situation of the caller. To test whether the rhinos attend to the sex and species differences in pant calls, we conducted playback experiments on wild territorial southern white rhinoceros males. Males recognized sex from the calls of conspecifics and responded more strongly to the calls of females than territorial males, which suggests that pant calls are more important in male-female than male-male communication. We also found that southern males discriminated between the female and male calls of northern species and reacted more intensively to the calls of northern than southern males, which could have been caused by a novelty effect as both species naturally live in allopatry and discrimination between them therefore cannot result from learning. Better understanding of vocal communication in white rhinos has potential management and conservation implications particularly because of their low reproduction in captivity.

Keywords Southern white rhinoceros, Northern white rhinoceros, Vocal communication, Vocalization, Sex recognition, Species recognition, Playback experiment

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Výzkumná činnost a zahraniční stáže

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2008 (5 měsíců) Výzkum sociálního chování a preference habitatu volně žijících nosorožců tuponosých v Jihoafrické republice.

2009, 2010, 2014 Výzkum vokální komunikace nosorožce tuponosého a
(3 měsíce)   Cottonova v zoologických zahradách.

2011, 2012   Výzkum vokální a olfaktorické komunikace volně žijících
(13 měsíců)   nosorožců tuponosých v Jihoafrické republice.

Zahraniční stáže: Veškerý výzkum v Jihoafrické republice probíhal za podpory University of the Witwatersrand v Johannesburgu (Prof. Norman Owen-Smith).

Další činnost

- Pořádání přednášek pro veřejnost.

- Popularizace výzkumu nosorožců v televizi, rádiu a v tisku.

7. Summary

Behavioural study of the captive and free-ranging southern and northern white rhinos with focus on their vocal communication

Well-developed communication system can be particularly important in socially living species and allow for an establishment of a large network of social relationships. Mammals often use vocal and olfactory signals to obtain information about conspecifics. Vocalizations are mostly directed to concrete individual/s and can be used for communication over a long distance while olfactory signals are transmitted slowly, but can remain in the environment for a long time. Rhinos have an excellent sense of hearing and smelling, but their eyesight is poor. Vocal and olfactory signals are therefore very important in their social behaviour, but communication in rhinos has only been studied to a limited extent until recently and experimental studies from the wild have been missing.

This study describes for the first time the information encoded in the calls of rhinos and the ability of wild rhinos to recognize information from the vocal and olfactory signals. The southern (*Ceratotherium simum*) and critically endangered northern (*C. cottoni*) white rhinos have the most developed social system out of all the rhinoceros species, use contact pant calls and defecate at common dungheaps. Both species reproduce poorly in captivity and better knowledge of their communication could be very useful in their management and conservation.

The first aim of this study was to analyse contact calls of the southern and northern white rhinos to determine what information they encode. Discriminant function analyses revealed that contact calls allow for the identification of individual identity and the calculation of information capacity in calls showed that they contain sufficient information for the recognition of nine individuals in both species. Pant calls can also be assigned with a high probability to a correct species. The northern white rhinos have

longer calls and also differ from the southern species in several frequency parameters of their calls. The calls of the southern white rhinos also vary according to the sex, age-class and social situation of the caller.

Playback experiments and manipulations with dung were conducted to determine if rhinos are able to discriminate the information about other animals from their contact pant calls and dung. Wild territorial southern white rhinoceros bulls discriminated between the calls of adult male and female conspecifics and reacted significantly more intensively to the calls of females, which suggests that contact calls are more important for the communication between a male and a female than between two territorial males. Bulls also discriminated between the calls of both sexes of northern white rhinos and reacted more intensively to the calls of northern than southern males. This could be caused by a novelty effect as both species are allopatric and discrimination between them therefore cannot result from learning. Experiments with dung showed that wild southern white rhinos are able to discriminate familiarity and sex of conspecifics based only on the smell of their dung. The rhinos sniffed the dung of unfamiliar animals more than twice as long as the dung of familiar rhinos. The latency of vigilance posture was shorter in reaction to the dung of familiar than unfamiliar males and longer in reaction to the dung of familiar than unfamiliar females.

This study shows that the contact calls of southern and northern white rhinos encode a lot of information about a vocalizing animal and that the southern white rhinos use the information encoded in contact calls and dung to assess other individuals. These signals therefore play an important role in the social behaviour of rhinos. The intensive reactions of the animals to the playbacks and to the dung samples show that they stimulate their social and territorial behaviour. Manipulation of vocal and olfactory signals could therefore be used in the management and conservation of rhinos and improve the chance for their reproduction in captivity.