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Olfactory discrimination between two cryptic species of bats *Pipistrellus pipistrellus* and *P. pygmaeus*

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Abstract. Absence of accuracy in species recognition can lead to inter-specific mating and hybridization. Aside from acoustic signals, bats can also use olfactory signals to search for mates. We compared the level of attractiveness of facial glands scents and urinary scents for discrimination in two cryptic bat species. Both sexes of two sympatric bat species *Pipistrellus pipistrellus* and *P. pygmaeus* were used as model. Possible sexual preferences using two olfactory signals were studied in a dual choice experiment in a glass Y-maze. Both sexes, without reference to the species, performed lower searching activity in tests with urinary scents than in the case of signals composed of scents of facial glands. Males of both species were able to discriminate and prefer the odour of conspecific females, with small proportion of disassortative choices. Females of both species did not have species-specific preferences. Absence of females' odour preferences and small proportion of males' disassortative choices can provide theoretical background for the existence of inter-species hybridization or point at more important role of acoustic signals in pre-mating behaviour.

Key words: olfactory signal, species-specific recognition, pipistrelles, Vespertilionidae, Chiroptera

Introduction

One of the critical steps in the understanding the process of speciation is determining the factors that promote and maintain isolated species in sympatry or parapatry. These factors can be represented as diverged mate recognition signals (e.g. premating courtship). Absence of accuracy in species recognition can lead to inter-specific mating and hybridization. Pheromone signals serve as the primary basis for species-specific recognition and sexual selection in most mammalian species (see Ptacek 2000 and references therein, Beauchamp & Yamazaki 2003, Hurst et al. 2005, Milinski 2006).

The use of chemical signals by animals may represent the oldest form of communication and especially in bats, due to their nocturnal activity; olfactory cues are likely to be an important mode of gathering information. Until now, however, information about the use of olfactory signals in bats is very limited

(Brown & Macdonald 1985, Bloss 1999, Burger 2005, Dechmann & Safi 2005, Caspers et al. 2009). The few field studies dealing with olfactory communication in bats emphasize the general importance of this communication channel (Brooke & Decker 1993, Voigt 2002, Safi & Kerth 2003) and confirm the ubiquitous role of odours in bats social life e.g. in social communication within maternity colonies or foraging groups (e.g. Loughry & McCracken 1991, McCracken & Gustin 1991, Bloss 1999, Bouchard 2001, Nielsen et al. 2006) and roost recognition (De Fanis & Jones 1995, Bloss et al. 2002). Use of olfactory cues for mate choice and sexual selection has only been studied thoroughly in one bat species (*Saccopteryx bilineata*; Voigt & von Helversen 1999, Voigt et al. 2001, Voigt 2002, Caspers et al. 2008). But, as studies such as those on the scent production of bat-pollinated plants have shown (Pettersson et al. 2004), odours can play an important role even

beyond signalling within and between bat species (Voigt & von Helversen 1999, von Helversen et al. 2000, Dechmann & Safi 2005, Voigt et al. 2008). The differences in male advertising calls are important for females and serve as inter-individual recognition among males before mating. However female bats do not only choose their sexual partners based on the vocalization of males, but also on olfactory cues (Bouchard 2001, Voigt 2002).

Two cryptic bat species, common pipistrelle (*Pipistrellus pipistrellus*) and soprano pipistrelle (*Pipistrellus pygmaeus*), occur in sympatry in most of Europe (Mayer & von Helversen 2001) where they often exploit the same foraging areas (e.g. Bartonička et al. 2007). However, Park et al. (1996) never found mixed inter-species harems in boxes during the mating period. The differences in acoustic characteristics of advertising calls become just one of the most robust arguments supporting the distinct species status of the two cryptic species, *P. pipistrellus* and *P. pygmaeus* (Barlow & Jones 1997). During the mating period, males of both species very often vocalize in the same locations, usually at a mutual distance of only tens of metres. Advertising calls are emitted during flight around a fixed path and are believed to both attract females and repel rival males. There is a presumption that females are able to immediately recognize conspecific males according to their advertising calls and fly to the male roost (cf. Barlow & Jones 1997). However, across very small distances olfactory cues in mammals including bats could play a more important role than vocalization (Geyer 1979, Bouchard 2001). Regarding the common occurrence of both pipistrelle species with males advertising their territory by calls emitted in close proximity, therefore we assume that olfactory recognition of conspecific males can be crucial to avoid mischoice and potential interspecific mating. The goals of our study were i) to compare the level of attractiveness of facial glands and urinary scents for species discrimination in two closely related cryptic species *P. pipistrellus* and *P. pygmaeus* and ii) to analyse the level of disassortative mate choice based on olfactory signals.

Material and Methods

Study subject

For odour preference tests, we used 14 females and 12 males of *P. pipistrellus* and 14 females and 15 males of *P. pygmaeus*. All the experiments were performed during the mating season for pipistrelle bats (September 2006). Only visibly sexually active males with reduced pigmentation of *cauda epididymis*

accompanied by distension of the *epididymis* and enlarged testes were used in the experiments. Males of *P. pygmaeus* were caught in bat-boxes in Milovická obora game-park. Adult females of *P. pygmaeus* were netted in a nursery colony in Vranovice close to the males' collecting site. Females and males of *P. pipistrellus* were mist-netted in a park in Brno city or caught in the day roost in 30 km distant Březník village (all localities, N 48°48'40"–49°10'24", E 16°11'33"–16°43'40", south Moravia, Czech Republic). Acoustic studies show that both species are widely distributed in all studied localities in southern Moravia (Bartonička et al. 2007). All bats were transported by car to a laboratory with the experimental aviary (3 × 3 × 2.7 m) up to 50 km from the original site and habituated by feeding with mealworm larvae (*Tenebrio molitor*) for two days. Samples of wing membrane tissues for DNA analysis were taken from all tested animals and species identification was confirmed by species-specific PCR amplification of partial mtDNA (Kaňuch et al. 2007). Before experimental sessions the bats were kept in clean linen bags for 24 h to separate experimental animals from odours of conspecifics or other species. The bats were fed, before the first choice experiment, and had access to water (present *ad libitum*) enriched by vitamins. During captivity, the light regime was natural and air conditions stable. The bats were captured and temporarily kept in captivity under the permit of the Ministry of Environment of the Czech Republic (No. 922/93-OOP/2884/93). The authors have been authorized to manipulate free-living bats according to the certificate of competency by § 17 of the law No. 246/1992 (No. 104/2002-V4).

Experimental design

We estimated preferences for olfactory stimuli in a dual choice system, where a tested animal had a choice between a pair of conspecific and heterospecific signal stimulus. In the first set of tests (5 males and 4 females of *P. pipistrellus*, 4 males and 4 females of *P. pygmaeus*; in total 17 bats) a pair of urinary scents was used as signal targets. In second set of tests, a pair of signal targets was presented by scents of facial glands (10 males and 10 females of *P. pipistrellus*, 11 males and 12 females of *P. pygmaeus*; in total 43 bats). Bouchard (2001) showed by histological work that the muzzle and interaural area can contain sebaceous glands with sexually dimorphic tissues. Washed cotton swabs were used to collect odour samples directly from the muzzle and face area for 30 sec (Bouchard 2001, Safi & Kerth 2003)

and were immediately inserted to the ends of Y-maze arms. Urine was frozen (-20°C) immediately after sampling by micropipettes. Prior to each experiment, the urine was defrosted and 10 µl were spotted in the middle of a sterile strip of filter paper (1.5 × 20 cm). Possible sexual preferences in our tests were achieved by the presence of opposite sex stimuli to the tested individual. One urinary or facial glands scent sample was collected from three individuals of each species and sex to reduce the effect of individuality. We used only one swab during the whole collection process of three individuals. In total we used 30 urinary samples collected from 30 individuals and 80 facial glands scent samples from 65 individuals. Bats were never tested with their own odour/urine or tested more than once with the same stimuli type.

The testing apparatus consisted of a Y-maze (glass tube 5 cm in diameter; stem 35 cm long, side arms 23 cm long) connected to a starting plastic roller (10 cm long and 5 cm in diameter) as a place to habituate bats before entering into the tube (cf. Bímová et al. 2005). Constant air current in the Y-maze from the arms ends to the roller ensured that the scent signals were continuously present at the branching point of the arms during the whole experiment (cf. Kraemer & Apfelbach 2004). Air current was forced by an electric valve placed in a neighbouring room. To avoid cross contamination, the Y-mazes and roller were cleaned with 70% ethanol and thoroughly rinsed with hot tap water after each experiment. All sessions were performed during the night under infrared light at 25°C in an air-conditioned and sound-proofed laboratory room. At the beginning of each test, the bat was placed in the starting box where it was allowed to habituate for 10 min. The swabs with scent of glands or paper-strips with urine were placed on the bottom of each side arm immediately before each test began. The position of the stimuli in the left and right arms was changed between each test. After habituation, the swabs or strips were placed, the door between the starting box and the Y-maze was opened and the bat was allowed to enter the Y-maze. When the bat left the starting box, it entered the stem of Y-maze and video recording of its behaviour started. For 300 seconds the animal was free to explore the Y-maze. All video recordings and time measurements were analysed using Observer software (Noldus et al. 2000).

Data analyses

We considered a bat to be in the arm with the presented stimulus when it crossed the junction of the side arm (the

zone of decision) with half of its body. Contact with the stimulus was recorded when the bat sniffed or licked the swab or urine spot in the middle of the paper-strip. We scored the total time and number of activity periods spent exploring the side arms, sniffing, grooming (licking, pulling, chewing fur or wings) or without movement (sitting, no movements). These behavioural elements were exclusive and thus the total time of one session (300 s) was equal to the sum of all behavioural time elements. In most experiments, bats repeatedly entered both arms of the Y-maze and the times represent the sum of these multiple visits. All variables showed a non-normal distribution (Kolmogorov-Smirnov test) and in consequence medians were used. Behavioural preferences were tested by Mann-Whitney U-test and Wilcoxon Matched Pairs Test (Zar 1984). Statistica for Windows 7.0 was used for all data analyses. When the difference in time spent in each arm of the maze was less than 5 sec, or the bat did not move from the stem of the maze, the choice was treated as a tie and excluded from subsequent analysis (Loughry & McCracken 1991). In tests of conspecific mate choice we used the following two estimators to assess preferences: the coefficient of preference, and the latency of conspecific and heterospecific choice of signal. The latency of choices represents the signal investigated after the subject first crossed the zone of decision and the coefficient of preference was calculated as: time spent in the conspecific arm – time spent in the heterospecific arm / total time of the experiment (300 s). Data sets from both species were pooled, but males and females were analysed separately, because we expected different strategies in mate choice (Panhuis et al. 2001).

Results

Attraction of urinary and facial glands scents

No differences in the time spent in exploring activity between the urine (U) and facial glands scents (O) experiments were found (Mann-Whitney U-test, $Z = -0.48$, NS, $n_U = 17$, $n_O = 43$) in the pooled data set for all males and females (Fig. 1). When we tested the two species separately there were no differences as well (*P. pygmaeus*, $Z = -0.72$, NS, $n_U = 8$, $n_O = 21$; *P. pipistrellus*, $Z = 0.38$, NS, $n_U = 9$, $n_O = 22$). Total time without movement (no activity, sitting and grooming, altogether in proximity of both conspecific and heterospecific signals) was found significantly higher for urinary signals than in the case of facial glands scents ($Z = -2.34$, $P = 0.019$, $n_U = 15$, $n_O = 40$) (Fig. 1). However, the exploring and sniffing (pooled data) activity had a significantly higher number of activity periods when the facial glands scents were

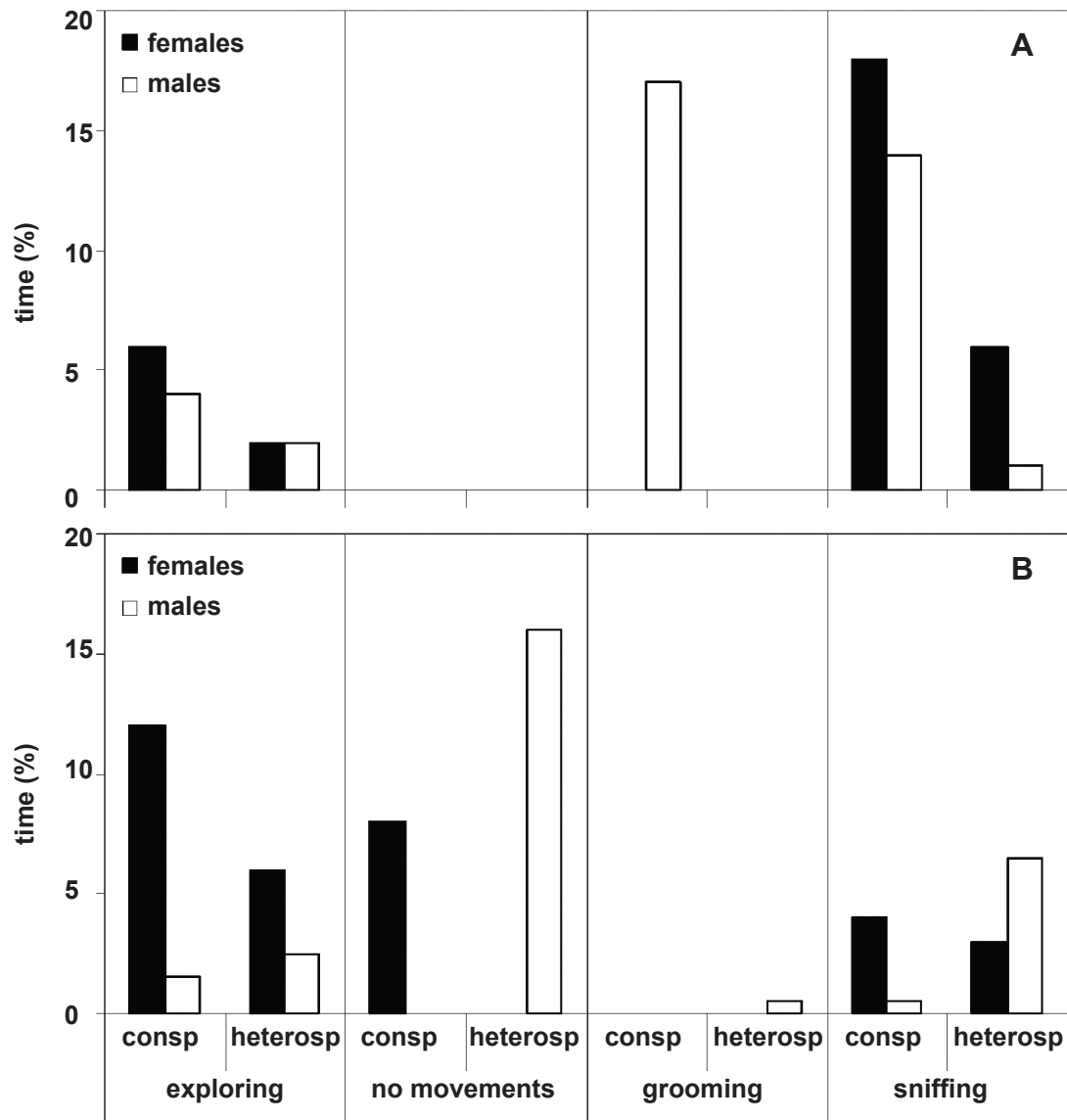


Fig. 1. Relative activity (medians) spent by exploring, no movements (bats usually sit without any movement), grooming and sniffing under different stimulus (A – facial glands, B – urine) in the arms of the Y-maze with conspecific or heterospecific scents.

presented than in the case of urinary signals ($Z = -3.37$, $P = 0.008$, $n_U = 15$, $n_O = 40$) (Fig. 2). Samples with urine spots were less attractive (only few exploring periods) for bats and no differences were found between both sexes (*P. pygmaeus*, $Z = -0.252$, NS, $n_F = 4$, $n_M = 4$; *P. pipistrellus*, $Z = -0.324$, NS, $n_F = 4$, $n_M = 5$). Bats did not explore the arms with urinary scents as intensively as they did with the samples of scent of facial glands. No conspecific grooming was found in proximity to urinary scents. Contrarily, high levels of grooming found in facial glands scents experiments shows special activity of males connected with a slicing of glandular secretion on their body, mainly wings (Fig. 1).

Reaction to conspecific vs. heterospecific sexual partner
We used only swabs with scents of facial glands to test discrimination ability for a species-specific signal of conspecific sexual partner, because of higher number of exploring periods than in urinary scents, they are presumed to be more important signals in species-specific discrimination. Males spent a higher number of activity periods by sniffing and also the total time for sniffing was significantly longer in the arm of Y-maze with conspecific female scent than heterospecific female scent (Wilcoxon Matched Pairs Test, $Z = 2.93$, $P = 0.003$, $n = 19$) (Fig. 2). Discrimination ability of females based on the time spent exploring and sniffing (pooled data) was not significant ($Z = 0.44$,

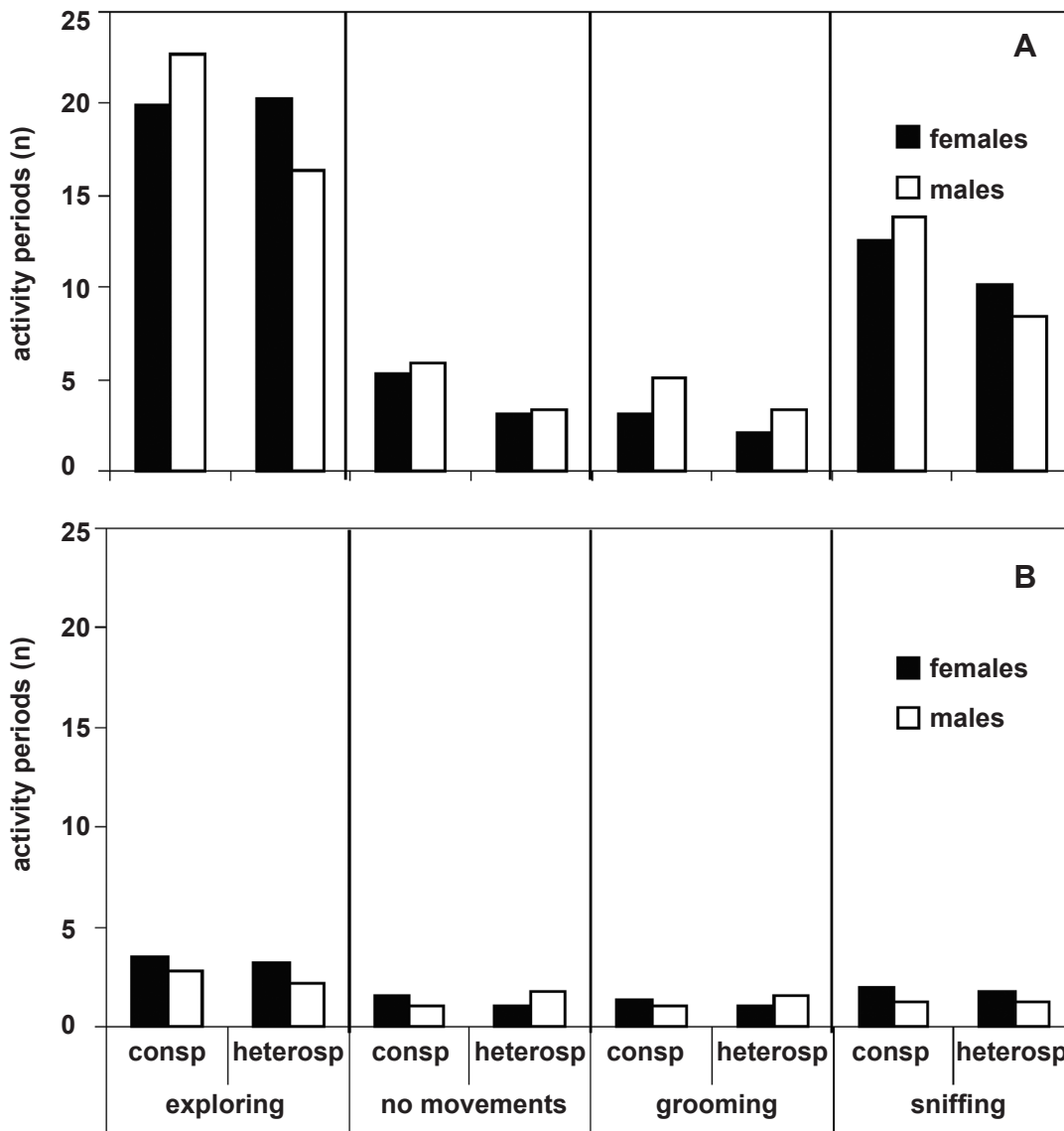


Fig. 2. Reaction to conspecific and heterospecific urine and smells (A – facial glands, B – urine) presented in number of activity periods (medians) found in particular experiments.

NS, $n = 21$) (Fig. 1). The coefficient of preference was significantly higher for males than females (U-test, $Z = -2.55$, $P = 0.011$, $n_F = 21$, $n_M = 19$). Males visited the arms with conspecific odours significantly earlier than the arms with heterospecific scents (Wilcoxon signed-rank test, $Z = 2.20$, $P = 0.028$, $n = 23$). In females, there was no significant difference in the latency of first choice between conspecific and heterospecific signal ($Z = 1.10$, NS, $n = 25$).

Discussion

Olfactory signals in bats

Discrimination ability to recognize olfactory stimuli is based on the chemical structure of these signals.

Recognition of a conspecific roost based on urine and faeces as the stimuli has been already tested in bats, but only marginally with ambiguous results (Bouchard 2001, Nielsen et al. 2006). We expected that bats would differ in their sensitivity to the stimuli of odour of facial glands and urine, despite of huge variety of type of glands produce chemically different scents (Dechmann & Safi 2005). Facial glands are pronounced mainly in the breeding season and thus are presumed to be more important signals in mate-recognition. Moreover, no single study on bats contains data that were obtained by applying both these stimuli. Odour differences at individual, family, hierarchical and/or other levels are usually determined

in the laboratory without further identification of the specific molecules (Safi & Kerth 2003). The weakness of such an approach is that differences at a statistical level do not prove that animals perceive and use these differences (Gralapp et al. 2001). Similarly, the inability to detect differences by chemical analysis does not imply the inability of the animal to distinguish between scents in natural circumstances (Kazial & Masters 2004). When planning sampling design it is important to consider the fact that scents may only occur seasonally or may be affected by the emotional state of animals including stress caused by handling. To minimize the influence of habituation and stress we performed just one-shot short-term tests (we found high level of habituation in repetitive experiments with the same individual; data not shown) during the autumn mating period when the gland secretion of muzzle and face area is the most intensive. Because only the scented swabs and strips of filter paper with urinary scents were presented to the bats as signal targets, their preferences could not be based on auditory or visual cues and thus exclusively the role of olfactory signals was observed in our study.

The investigation of the role and character of such signals is crucial for the understanding of sociality in bats (Bloss et al. 2002). Chemical communication is also commonly involved in the recognition of closely related species (cf. Crowley et al. 1996). As a target for natural selection promoting divergence in specific mate recognition, signals can play an important role in speciation and behavioural isolation of species in sympatry or parapatry (see Coyne & Orr 2004). Such analyses have not yet been applied to bats. It should be rewarding to relate new knowledge to the large and diverse amount of knowledge on the neurobiology and ecology of this mammalian order (Bloss 1999). Our results show very low searching and sniffing activity when urinary scents were used. Therefore, it is possible that species-specific signals are expressed in our model species more in facial glands than in the urine. It means that the role of the urine in signalling is not excluded but if it exists it is probably restricted to the intra-specific level and may indicate lower complexity of signal comparing to the facial glands secretions.

Discrimination in cryptic species

In our experiments, a mischoice of species-specific olfactory signals found in a small proportion of males could show incomplete behavioural pre-mating barrier and provide the opportunity for hybridization among the studied cryptic species. It is noticeable in

connection with the fact that the females did not show any preferences in species-specific signals (neither from urine nor from facial glands scents). We can conclude that females did not discriminate between conspecific and heterospecific olfactory male signals. In central Europe (southeastern Moravia, Czech Republic) both cryptic species occur in sympatry (Hulva et al. 2004, Bryja et al. 2009, Kaňuch et al. 2010) and their male territories occur in high densities and largely overlap. Trajectories of vocalizing males of different species are often separated only by several tens of metres. Lundberg & Gerell (1986) observed false landings of males towards tree trunks in the vicinity of the day roosts and interpreted that behaviour as scent marking. In this case an olfactory allurement is the most important way to recognize conspecific partners. Mischoice could be possible when one or more non-territorial males of different species are in the vicinity of dominant male territory and they mark an area near the entrance of their own roosts only by scent.

Current genetic analyses found that colonies of the same species are more similar to each other than to those from the other species, thus supporting the hypothesis that the cryptic species do not hybridize (Racey et al. 2007, Bryja et al. 2009). In that case pipistrelle species have to be separated by another type of isolation barrier such as postzygotic isolation and/or in the case of pre-mating isolation, it thus seems that different social (territorial) calls may be more important as ultimate pre-mating isolating mechanisms. Detailed analysis of the possible existence of heterospecific mating based on social calls, olfactory signals, mtDNA, and nuclear DNA variation in other parts of the species distribution ranges is required for understanding of isolation barriers in both pipistrelles.

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Literature

- Barlow K.E. & Jones G. 1997: Differences in songflight calls and social calls between two phonic types of the vespertilionid bat *Pipistrellus pipistrellus*. *J. Zool. Lond.* 241: 315–324.
- Bartonička T., Řehák Z. & Gaisler J. 2007: Can pipistrelles, *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825), foraging in a group, change parameters of their signals? *J. Zool. Lond.* 272: 194–201.
- Beauchamp G.K. & Yamazaki K. 2003: Chemical signalling in mice. *Biochem. Soc. Trans.* 31: 147–151.
- Bímová B., Karn R.C. & Piálek J. 2005: The role of salivary androgen-binding protein in reproductive isolation between two subspecies of house mouse: *Mus musculus musculus* and *Mus musculus domesticus*. *Biol. J. Linn. Soc.* 84: 349–361.
- Bloss J. 1999: Olfaction and the use of chemical signals in bats. *Acta Chiropterol.* 1: 31–45.
- Bloss J., Acree T.E., Bloss J.M., Hood W.R. & Kunz T.H. 2002: Potential use of chemical cues for colony-mate recognition in the big brown bat, *Eptesicus fuscus* using olfactory cues: Behavioral and chemical analysis. *J. Chem. Ecol.* 28: 799–814.
- Bouchard S. 2001: Sex discrimination and roostmate recognition by olfactory cues in the bats, *Mops condylurus* and *Chaerephon pumilus*. *J. Zool. Lond.* 254: 109–117.
- Brooke A.P. & Decker D.M. 1993: Scent marking and the use odorous secretions by the fishing bats, *Noctilio leporinus*. *Bat Res. News* 34: 103.
- Brown R.E. & MacDonald D.W. 1985: Social odours in mammals. *Clarendon Press, Oxford*.
- Bryja J., Kaňuch P., Fornůsková A., Bartonička T. & Řehák Z. 2009: Low population genetic structuring of two cryptic bat species suggests their migratory behaviour in continental Europe. *Biol. J. Linn. Soc.* 96: 103–114.
- Burger B.V. 2005: Mammalian semiochemicals. In: Schulz S. (ed.), *Chemistry of pheromones and other semiochemicals II*. Springer-Verlag, Berlin: 231–278.
- Caspers B., Franke S. & Voigt C.C. 2008: The wing-sac odour of male greater sac-winged bats *Saccopteryx bilineata* (Emballonuridae) as a composite trait: Seasonal and individual differences. *Chem. Signals Vertebr.* 11: 151–160.
- Caspers B., Schroeder F., Franke S., Streich J. & Voigt C.C. 2009: Odour-based species recognition in two sympatric species of sac-winged bats (*Saccopteryx bilineata*, *S. leptura*): Combining chemical analyses, behavioural observations and odour preference tests. *Behav. Ecol. Sociobiol.* 63: 741–749.
- Coyne J.A. & Orr H.A. 2004: Speciation. *Sinauer Associates, Inc. Sunderland, MA*.
- Crowley P.H., Provencher L., Sloane S., Dugatkin L.A., Sophn B. & Rogers L. 1996: Evolving cooperation: The role of individual recognition. *Biosystems* 37: 49–66.
- De Fanis E. & Jones G. 1995: The role of odour in the discrimination of conspecifics by pipistrelle bats. *Anim. Behav.* 49: 835–839.
- Dechmann D.K.N. & Safi K. 2005: Studying communication in bats. *Cognition, Brain, Behavior* 9: 479–496.
- Geyer L.A. 1979: Olfactory and thermal influences on ultrasonic vocalization during development in rodents. *Am. Zool.* 19: 419–431.
- Gralapp A.K., Powers W.J. & Bundy D.S. 2001: Comparison of olfactometry, gas chromatography, and electronic nose technology for measurement of indoor air from swine facilities. *Trans. ASAE* 44: 1283–1290.
- Hulva P., Horáček I., Strelkov P.P. & Benda P. 2004: Molecular architecture of *Pipistrellus pipistrellus* / *P. pygmaeus* (Vespertilionidae) complex: Further cryptic species and Mediterranean origin of the divergence. *Mol. Phylogenet. Evol.* 32: 1023–1035.
- Hurst J.L., Thom M.D., Nevison C.M., Humphries R.E. & Beynon R.J. 2005: MHC odours are not required or sufficient for recognition of individual scent owners. *Proc. R. Soc. Lond. B* 272: 715–724.
- Kaňuch P., Fornůsková A., Bartonička T., Bryja J. & Řehák Z. 2010: Do two cryptic pipistrelle bat species differ in their autumn and winter roosting strategies within the range of sympatry? *Folia Zool.* 59: 102–107.
- Kaňuch P., Hájková P., Řehák Z. & Bryja J. 2007: A rapid PCR-based test for species identification of two cryptic bats *Pipistrellus pipistrellus* and *P. pygmaeus* and its application on museum and dropping samples. *Acta Chiropterol.* 9: 277–282.
- Kazial K.A. & Masters W.M. 2004: Female big brown bats, *Eptesicus fuscus*, recognize sex from a callers echolocation signals. *Anim. Behav.* 67: 855–863.
- Kraemer S. & Apfelbach R. 2004: Olfactory sensitivity, learning and cognition in young, adult and aged male Wistar rats. *Physiol. Behav.* 81: 435–442.

- Loughry W. & McCracken G. 1991: Factors influencing female-pup recognition in Mexican free-tailed bats. *J. Mammal.* 72: 624–626.
- Lundberg K. & Gerell R. 1986: Territorial advertisement and mate attraction in the bat *Pipistrellus pipistrellus*. *Ethology* 71: 115–124.
- Mayer F. & von Helversen O. 2001: Sympatric distribution of two cryptic bat species across Europe. *Biol. J. Linn. Soc.* 74: 365–374.
- McCracken G.F. & Gustin M.K. 1991: Nursing behaviour in Mexican free-tailed bat maternity colonies. *Ethology* 89: 305–321.
- Milinski M. 2006: The major histocompatibility complex, sexual selection, and mate choice. *Annu. Rev. Ecol. Evol. Syst.* 37: 159–186.
- Nielsen L.T., Eaton D.K., Wright D.W. & Schmidt-French B. 2006: Characteristic odors of *Tadarida brasiliensis mexicana* Chiroptera: Molossidae. *J. Cave Karst Stud.* 68: 27–31.
- Noldus L.P.J.J., Trienes R.J.H., Hendriksen A.H.M., Jansen H. & Jansen R.G. 2000: The Observer Video-Pro: New software for the collection, management, and presentation of time-structured data from videotapes and digital media files. *Behav. Res. Meth. Instr. Comp.* 32: 197–206.
- Panhuis T.M., Butlin R., Zuk M. & Tregenza T. 2001: Sexual selection and speciation. *Trends Ecol. Evol.* 16: 364–371.
- Park K.J., Altringham J.D. & Jones G. 1996: Assortative roosting in the two phonic of *Pipistrellus pipistrellus* during the mating season. *Proc. R. Soc. Lond. B.* 263: 1495–1499.
- Pettersson S., Ervik F. & Knudsen J.T. 2004: Floral scent of bat-pollinated species: West Africa vs. the New World. *Biol. J. Linn. Soc.* 82: 161–168.
- Ptacek M.B. 2000: The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. *Behav. Proc.* 51: 111–134.
- Racey P.A., Barratt E.M., Burland T.M., Deaville R., Gotelli D., Jones G. & Piernney S.B. 2007: Microsatellite DNA polymorphism confirms reproductive isolation and reveals differences in population genetic structure of cryptic pipistrelle bat species. *Biol. J. Linn. Soc.* 90: 539–550.
- Safi K. & Kerth G. 2003: Secretions of the interaural gland contain information about individuality and colony membership in the Bechstein's bat. *Anim. Behav.* 65: 363–369.
- Voigt C.C. 2002: Individual variation in perfume blending in male greater sac-winged bats. *Anim. Behav.* 63: 907–913.
- Voigt C.C., Behr O., Caspers B., von Helversen O., Knörnschild M., Mayer F. & Nagy M. 2008: Songs, scents, and senses: Sexual selection in the greater sac-winged bat, *Saccopteryx bilineata*. *J. Mammal.* 89: 1401–1410.
- Voigt C.C. & von Helversen O. 1999: Storage and display of odour by male *Saccopteryx bilineata* (Chiroptera, Emballonuridae). *Behav. Ecol. Sociobiol.* 47: 29–40.
- Voigt C.C., von Helversen O., Michener R. & Kunz T.H. 2001: The economics of harem maintenance in the sac-winged bat, *Saccopteryx bilineata* (Emballonuridae). *Behav. Ecol. Sociobiol.* 50: 31–36.
- von Helversen O., Winkler L. & Bestmann H.J. 2000: Sulphur-containing “perfumes” attract flower-visiting bats. *J. Comp. Physiol.* 186: 143–153.
- Zar J.H. 1984: Biostatistical analysis. *Simon and Schuster, Englewood Cliffs, New Jersey*.