Chapter 16
Do Spotted Hyena Scent Marks Code for Clan Membership?

Nicole Burgener, Marion L East, Heribert Hofer and Martin Dehnhard

Abstract The spotted hyena (*Crocuta crocuta*) is a territorial carnivore that lives in highly structured social groups called clans. Individuals of both sexes produce scent in a prominent anal scent gland. Gas-chromatographic analysis of 13 fatty acids and esters in scent profiles from 45 individuals belonging to three social groups demonstrated sufficient variation to suggest that odour may permit individual olfactory recognition. Further, anal scent secretions from members of the same clan are more similar to each other than those from different clans, consistent with the idea of a social group odour. We describe a mechanism involving both scent pasting and dry-pasting behaviour to explain how a group odour label may be concocted from individual scent secretions and how this group label is spread among members of a clan.

16.1 Introduction

Animals that live in societies need effective means of communication, and selection has favoured a wide variety of visual, acoustic and olfactory signals that convey information between members of social groups (Eisenberg and Kleiman 1972; Halpin 1986; Bradbury and Vehrencamp 1998). Social coherence of a group, at a basic level, requires group members to discriminate between individuals that are members of their group and those that are not, and an unambiguous mechanism to achieve this would be a group membership label. Evidence for such group identity labels have been found in a variety of taxa (e.g. mammals: Mykytowycz 1968; O'Riain and Jarvis 1997; Sun and Müller-Schwarze 1998; Bloss, Acree, Bloss, Hood and Kunz 2002; Safi and Kerth 2003; social insects: Gamboa, Reeve, Ferguson and Wacker 1986; Venkataraman, Swarnalatha, Nair and Gadagkar 1992; Breed, Diaz and Lucero 2004). Scent membership labels are likely to be honest and reliable signals because group odours are thought to be acquired through the transfer of

Nicole Burgener
Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany and Freie Universität, Berlin, Germany
burgener@izw-berlin.de

scent among members of a group (Halpin 1986). For example, in the naked mole-rat (*Heterocephalus glaber*), a distinctive colony odour is composed of scent that is distributed among and learnt by colony members (O'Riain and Jarvis 1997).

The spotted hyena is a crepuscular/nocturnal social carnivore that lives in fission-fusion groups termed clans (Kruuk 1972; Mills and Hofer 1998). Clan members use a wide spectrum of visual and acoustic signals for both short and long distance communication with both group and non-group members (Kruuk 1972; East and Hofer 1991; East, Hofer and Wickler 1993; Holekamp, Boydston, Szykman, Graham, Nutt, Birch and Piskiel 1999). Nocturnal activity and a fission-fusion social structure would be expected to also favour olfactory communication and spotted hyenas have both a prominent anal scent gland and inter-digital scent glands (Kruuk 1972; Mills and Gorman 1987).

Although clans defend communal territories, clan members may undertake short distance excursions (Höner, Wachter, East, Runyoro and Hofer 2005) or long distance foraging trips up to 70 km from their territory boundaries that necessitate movement through or foraging within territories belonging to other clans (Hofer and East 1993a,b,c). Therefore, spotted hyenas not only need a reliable means to distinguish members of their social group from those of other groups, they also need to identify owners of the territories into which they intrude so that they can respond appropriately when challenged (Hofer and East 1993b).

When clan members meet another group member of the same sex, they normally perform a ritualized greeting ceremony in which greeting partners stand head to tail, raise their hind legs and tail while investigating the anal genital region of their greeting partner (Kruuk 1972; East et al. 1993). The format of greetings suggests that odour plays a central role in reaffirming associations among clan members. Furthermore, during aggressive encounters subordinate individuals often protrude their anal scent gland (Kruuk 1972; East et al. 1993), suggesting that scent within the anal gland may confirm an individual's membership of the group thereby decreasing the probability of escalated aggression.

Scent from the anal gland is deposited on vegetation in a process known as pasting (Kruuk 1972). Pasting may serve as an olfactory means to communicate ownership of a particular area and the resources the area contains (Gorman and Mills 1984; Mills and Gorman 1987). It has also been suggested that pasted scent may signal the recent presence of a group member in an area (Hofer, East, Sämmang and Dehnhard 2001; Drea, Vignieri, Kim, Weldele and Glickman 2002).

In a preliminary analysis of scent from the anal gland of individually known spotted hyenas from several clans in the Serengeti National Park (NP), Tanzania, Hofer et al. (2001) presented evidence of a group odour based on gas-chromatography (GC) of fatty acids in the scent. In this study, we examine samples from the anal scent gland of a larger sample of individuals from three clans using solid-phase microextraction (SPME) of volatile compounds followed by gas-chromatography—mass spectrometry (GCMS). We outline details of scent pasting behaviour in the Serengeti NP that may explain how a group odour label is concocted from individual scent secretions from group members and how this group label is spread among members of a clan.

16.2 Methods and Materials

16.2.1 Study Animals and Collection of Scent

Between 2000 and 2003, a total of 45 anal gland scent samples were collected from spotted hyenas that were members of three clans in the Serengeti NP, Tanzania. Individuals were recognized by their spot patterns (East and Hofer 1991). Females were philopatric and males normally dispersed from their natal clan (East and Hofer 2001). Animals were classified as adults (> 24 mo old), yearlings (12 — 24 mo), or cubs (< 12 mo). The three study clans held territories at the woodland / plain boundary in the centre of the Serengeti NP. Scent marking behaviour was monitored during focal follows of individuals, and during dawn and dusk observations at the communal den (East and Hofer 1991), food resources and resting sites. Focal follows were conducted both within and outside the territory of the clan to which the individual belonged. All study clans were habituated to the presence of a vehicle (East and Hofer 1991).

Scent was collected immediately after an individual pasted on an individual post (see results), following the procedure described by Hofer et al. (2001). All samples were stored in 20 ml glass headspace vials (Shimadzu) at –20 °C until analysed.

16.2.2 Chemical and Statistical Analysis

Samples were analysed using a Shimadzu GCMS-QP 5050 fitted with a 50 m SE-54 capillary column (0.32 i.d. and 1 μm film thickness, CS, Langerwehe, Germany). Volatile compounds were desorbed upon exposure of the SPME fibre in the heated injector port of the GC. SPME was carried out with a CTC combi pal system autoinjector at 70 °C for 60 min using a fibre with 85 mm polyacrylate coating. SPME sampling was done in the headspace above the collected scent sample. GC analyses were conducted using ultrapure helium as carrier gas, with a column head pressure setting of 41.2 kPa. Injector temperature was 300 °C, the interface temperature was maintained at 300 °C and ionizing voltage was at 1.2 kV. Splitless injection mode was used and the purge valve was turned on 15 min after the injection with a split flow of 8 ml min^{-1} during the GC run. The GC oven was kept at 45 °C for 2 min, then increased to 105 °C at 15 °C min^{-1}, from 105 °C to 165 °C at 10 °C min^{-1} and then from 165 °C to 290 °C at 4 °C min^{-1}. Mass spectrometer (MS) acquisition was performed in a total ion chromatogram (TIC). Compounds were identified by their retention times and comparison of mass spectra to those in the NIST 1998 mass spectral library.

To compare different profiles, peaks were matched by their retention times and mass spectra. The contribution of each peak to the overall area of the profile was calculated as a percentage (% area) and used for analyses. For the current study we focussed our analyses on fatty acids and esters (Poddar-Sarkar and Brahmachary 1999; Hofer et al. 2001).

Statistical analyses were carried out using PRIMER 5.2.9 and SYSTAT 10. Because data were not normally distributed we applied a non-parametric test. The similarity of 13 key substances was compared using the Bray-Curtis-Similarity index and the statistical significance was tested using analysis of similarities (ANOSIM) with clan membership as a factor (Clarke and Green 1988). A 3-D multidimensional scaling plot of the similarity indices was used to display the greater level of similarity between individuals within a clan in comparison to individuals between clans. “Stress” is a measure of “goodness of fit” that evaluates how well a particular configuration reproduces the observed distance matrix.

16.3 Results

16.3.1 Chemical Composition

In the 45 scent profiles we analysed, 13 compounds that were either fatty acids or esters were identified, and these were used as the key components in our analysis. There was sufficient variation in these 13 components between the scent profiles of different individual members of a social group to suggest that each group member may have a unique profile that would permit individual odour recognition (Fig. 16.1).

Scent profiles of different individuals within a clan were more similar to each other than to the profiles of individuals from other clans ($N = 8$, $N = 23$, $N = 14$, Global $R = 0.177$, $P = 0.007$, Fig. 16.2).

16.3.2 Pasting and Dry-pasting Behaviour

Both adults and yearlings of both sexes pasted mostly in the vicinity (within 50 m) of the communal den, at sites along tracks frequently used by spotted hyenas, and at communal latrines, where clan members also deposited faeces. Individuals either scent marked on individual scent posts or on communal scent posts. An individual scent post was created when a spotted hyena pasted on a stalk of grass or herb that had not been pasted with scent by another spotted hyena and thus the scent post only presented the odour from one individual. A communal scent post was created either when individuals pasted on a stalk of vegetation that already had scent pasted on it by one or several other clan members, or when several spotted hyenas pasted on stalks that were spatially close together (within less than 0.5 m). Communal scent posts thus presented scent from several individuals. Both individual and communal scent posts were investigated (sniffed) by clan members that passed by the post. Some scent posts elicited either pasting by the investigating individual, or dry-pasting in which the animal performed the normal behaviour associated with pasting except that the anal gland was not protruded, and scent was not deposited.

Communal scent posts were established at the communal den, at communal latrines, and close to frequently used hyena paths and resting sites. Individuals that

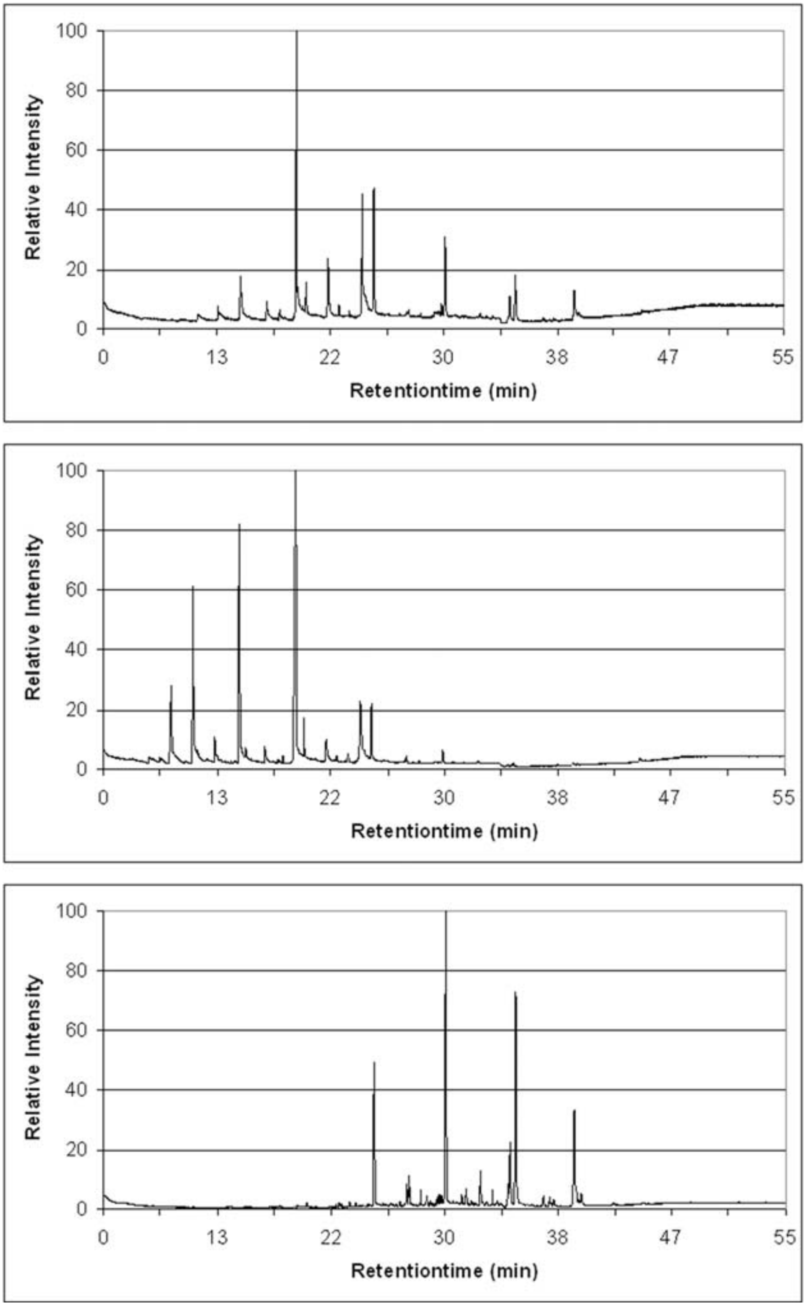

Fig. 16.1 Gas-chromatograph mass spectrometer profile of scent from the anal scent gland of three adult female spotted hyenas from the same social group

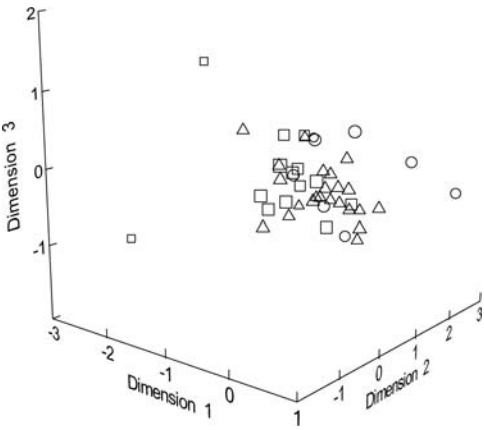

Fig. 16.2 A multidimensional scaling (MDS) plot in three dimensions for scent profiles of individuals from three spotted hyena clans (○ Isiaka clan, N = 8; □ Pool clan, N = 14; Δ Mamba clan, N = 23). Similarities between samples were calculated with the Bray-Curtis coefficient. MDS Stress: 0.08

pasted on communal scent posts not only deposited their own scent secretions on the post, but also rubbed the scent secretions previously deposited by other individuals into their scent gland and onto the fur of their ventral side.

16.4 Discussion

We found considerable individual variation between the scent profiles of individual clan members in spotted hyenas, but we also demonstrated a general similarity of odour among individual members of a clan, suggesting the existence of a clan scent signature. This result is consistent with that of Hofer et al. (2001) who also found evidence for a clan odour when comparing, by means of SPME and GC, the fatty acid content of anal scent secretion from members of the same three clans. Group odour has been proposed as a mechanism by which members of a group can distinguish between group and non-group members (e.g. Gorman, Nedwell and Smith 1974; O'Riain and Jarvis 1997; Safi and Kerth 2003). In honey bees (*Apis mellifera*), colony guards are thought to use a colony template, rather than individual templates, when discriminating colony members from intruders (Breed et al. 2004).

Our behavioural observations suggested that spotted hyenas actively acquired scent from other clan members when they pasted over stalks of vegetation that held scent deposited by another clan member. During this process the scent of other clan members may be taken into the protruded scent gland as well as being rubbed onto the pasting individual's fur. As clan membership is not static over time, there is probably a need for clan members to continuously anoint themselves with the scent of other clan members to retain the current clan odour.

Although an intruder in a clan territory could potentially use communal scent posts to acquire the group odour of the territory owning clan, we have no evidence that intruders do so. As scent is relatively persistent, there may be costs to such behaviour if acquisition of an alien clan odour results in increased aggression from group members when an intruder returns "home". For example, in house mice (*Mus musculus domesticus*), the absence of the group odour led to aggressive behaviour (Hurst, Fang and Barnard 1993).

It could be expected that a persistent honest signal of group membership would be favoured in a species, such as the spotted hyena, that lives in a fission-fusion society in which individuals may not meet for long periods. In the Serengeti NP, spotted hyenas undertake long distance foraging trips, and as these commuting trips are not coordinated between clan members there is the potential for clan members not to meet for several weeks or more (Hofer and East 1993b).

In addition to scent pasting as described by Kruuk (1972), we have observed dry-pasting when individuals do not protrude their gland or deposit scent. We suggest that dry-pasting over individual or communal scent posts is an important mechanism by which spotted hyenas acquire a general clan odour. Dry-pasting is most noticeable among cubs and yearlings, and it is likely that the acquisition of the group odour by these young age classes is important for their social integration into the clan.

Bacteria have been suggested to be external vectors that produce volatiles in scent glands (bacterial fermentation hypothesis: e.g. Gorman et al. 1974; Albone and Perry 1975; Voigt, Caspers and Speck 2005) even though this idea has been questioned (e.g. Svendsen and Jollick 1978). In several mammalian species, bacteria are active in scent glands (e.g. *Herpestes auropunctatus*: Gorman et al. 1974, *Vulpes vulpes*: Gosden and Ware 1976; *Castor canadensis*: Svendsen and Jollick 1978; *Saccopteryx billineata*: Voigt et al. 2005). Currently it is not known whether bacteria play any role in the production of group odour in spotted hyenas, but if so, we suggest that the use of communal scent posts would serve as a mechanism for the transfer of a common bacterial mix among members of a clan.

Acknowledgments We are grateful to the Tanzanian Commission for Science and Technology (COSTECH) for permission to conduct the study and the Director Generals of the Tanzania Wildlife Research Institute and Tanzania National Parks for cooperation. We acknowledge M. Hilker for ideas and discussion and we thank B. Caspers, O. Höner, B. Kostka, K. Paschmionka, J. Streich, D. Thierer, B. Wachter and K. Wilhelm for assistance. The Leibniz Institute for Zoo and Wildlife Research funded the study.

References

Albone, E.S. and Perry, G.C. (1975) Anal sac secretion of the red fox, *Vulpes vulpes*: volatile fatty acids and diamines: Implications for a fermentation hypothesis of chemical recognition. J. Chem. Ecol. 2, 101–111.

Bloss J., Acree, T.E., Bloss, J.M., Hood, W.R. and Kunz, T.H. (2002) Potential use of chemical cues for colony-mate recognition in the big brown bat, *Eptesicus fuscus*. J. Chem. Ecol. 28, 819–834.

Bradbury, J.W. and Vehrencamp, S.L. (1998) *Principles of Animal Communication*. Sinauer Associates, Sunderland, Massachusetts.
Breed, M.D., Diaz, P.H. and Lucero, K.D. (2004) Olfactory information processing in honey bee, *Apis mellifera*, nestmate recognition. Anim. Behav. 68, 921–928.
Clarke, K.R and Green, R.H. (1988) Statistical design and analysis for a "biological effects" study. Mar. Ecol. Trog. Ser. 46, 213–226.
Drea, C.M., Vignieri, S.N., Kim, H.S., Weldele, M.L. and Glickman, S.E. (2002) Responses to olfactory stimuli in spotted hyenas (*Crocuta crocuta*): II. Discrimination of conspecific scent. J. Comp. Psychol. 116, 342–349.
East, M.L. and Hofer, H. (1991) Loud calling in a female-dominated mammalian society: I. Structure and composition of whooping bouts of spotted hyaenas (*Crocuta crocuta*). Anim. Behav. 42, 637–649.
East, M.L., Hofer, H. and Wickler, W. (1993) The erect "penis" is a flag of submission in a female-dominated society: greetings in Serengeti spotted hyenas. Behav. Ecol. Sociobiol. 33, 355–370.
East, M.L. and Hofer H. (2001) Male spotted hyenas (*Crocuta crocuta*) queue for status in social groups dominated by females. Behav. Ecol. 12, 558–568.
Eisenberg, J.F. and Kleiman, D.G. (1972) Olfactory communication in mammals. Annu. Rev. Ecol. Syst. 3, 1–32.
Gamboa, J.G., Reeve, H.K., Ferguson, I.D. and Wacker, T.L. (1986) Nestmate recognition in social wasps: the origin and acquisition of recognition odours. Anim. Behav. 34, 685–695.
Gorman, M.L., Nedwell, D.B. and Smith, R.M. (1974) An analysis of the contents of the anal scent pockets of *Herpestes auropunctatus*. J. Zool. 172, 389–399.
Gorman, M.L. and Mills, M.G.L. (1984) Scent marking strategies in hyaenas (Mammalia). J. Zool. 202, 535–547.
Gosden, P.E. and Ware, G.C. (1976) The aerobic baterial flora of the anal sac of the red fox. J. Appl. Bacteriol. 41, 271–275.
Halpin, T.Z. (1986) Individual odors among mammals: origins and functions. Adv. Stud. Behav. 16, 40–70.
Hofer, H. and East, M.L. (1993a) The commuting system of Serengeti spotted hyaenas: how a predator copes with migratory prey. I. Social organization. Anim. Behav. 46, 547–557.
Hofer, H. and East, M.L. (1993b) The commuting system of Serengeti spotted hyaenas: how a predator copes with migratory prey. II. Intrusion pressure and commuters' space use. Anim. Behav. 46, 559–574.
Hofer, H. and East, M.L. (1993c) The commuting system of Serengeti spotted hyaena: how a predator copes with migratory prey. III. Attendance and maternal care. Anim. Behav. 46, 575–589.
Hofer, H., East, M.L., Sämmang, I. and Dehnhard, M. (2001) Analysis of volatile compounds in scent-marks of spotted hyenas (*Crocuta crocuta*) and their possible function in olfactory communication. In: A. Marchlewska-Koj, J.J. Lepri and D. Müller-Schwarze (Eds.), *Chemical Signals in Vertebrates 9*. Kluwer Academic / Plenum Publishers, New York, pp. 141–148.
Holekamp, K.E., Boydston, E.E., Szykman, M., Graham, I., Nutt, K.J., Birch, S., Piskiel, A. and Singh, M. (1999) Vocal recognition in the spotted hyaena and its possible implications regarding the evolution of intelligence. Anim. Behav. 58, 383–395.
Höner, O.P., Wachter, B., East, M.L., Runyoro, V.A. and Hofer, H. (2005) The effect of prey abundance and foraging tactics on the population dynamics of a social, territorial carnivore, the spotted hyena. Oikos 108, 544–554.
Hurst, J.L., Fang, J. and Barnard, C. (1993) The role of substrate odours in maintaining social tolerance between male house mice, *Mus musculus domesticus*: relatedness, incidental kinship effects and the establishment of social status. Anim. Behav. 48, 157–167.
Kruuk, H. (1972) *The Spotted Hyena*. University of Chicago Press, Chicago.
Mills, M.G.L. and Gorman, M.L. (1987) The scent-marking behaviour of the spotted hyaena *Crocuta crocuta* in the southern Kalahari. J. Zool. 212, 483–497.
Mills, G. and Hofer, H. (1998) *Hyaenas – Status Survey and Conservation Action Plan.* IUCN/SSC Hyaena Specialist Group. IUCN, Gland and Cambridge.

Mykytowycz, R. (1968) Territorial marking in rabbits. Sci. Am. 218, 116–126.
O'Riain, M.J. and Jarvis, J.U.M. (1997) Colony member recognition and xenophobia in the naked mole-rat. Anim. Behav. 53, 187–198.
Poddar-Sarkar, M. and Brahmachary, R.L. (1999) Can free fatty acids in the tiger pheromone act as an individual finger print? Curr. Sci. India 76, 141–142.
Safi, K. and 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.
Sun, L. and Müller-Schwarze, D. (1998) Anal gland secretion codes for family membership in the beaver. Behav. Ecol. Sociobiol. 44, 199–208.
Svendsen, G.E. and Jollick, J.D. (1978) Bacterial contents of the anal and castor glands of beaver (*Castor canadensis*). J. Chem. Ecol. 4, 563–569.
Venkataraman, A.B., Swarnalatha, V.B., Nair, P. and Gadagkar, R. (1992) The mechanism of nestmate discrimination in the tropical social wasp *Ropalidia marginata* and its implications for the evolution of sociality. Anim. Behav. 43, 95–102.
Voigt, C.C., Caspers, B. and Speck, S. (2005) Bats, bacteria, and bat smell: sex-specific diversity of microbes in a sexually selected scent organ. J. Mammal. 86, 745–749.