COMMENTARY

Chemical fingerprints reveal clues to identity, heterozygosity, and relatedness

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Olfaction is a key sense for mammals, and as a result chemical signals are an important means of communication for most mammalian species. It has long been established that most mammals make, distribute, and respond to chemosignals in a range of contexts, including reproduction, parent–offspring interactions, and social relationships (1). However, most aquatic mammals are unable to use olfaction when foraging, and evidence for its role in social behavior has been equivocal. Historically, reports in the literature have ranged from describing the semiaquatic pinnipeds as microsmatic (2) to those that have observed the high prevalence of naso-nasal inspection during social interactions (Fig. 1), and so inferred an important role for olfactory recognition (3). It is only recently that we experimentally confirmed in wild Australian sea lions that olfactory cues are a reliable mechanism in offspring recognition even in the absence of other sensory cues (4). Similarly, new experimental evidence in other large, wild mammals indicates the importance of olfactory cues in discrimination of potential mates and competitors as well as kin (5–7). However, perhaps due to both the complexity of working with natural vertebrate populations and the complexity of vertebrate scents, the mechanistic basis of chemical communication has received little study (8). In PNAS, Stoffel et al. (9) provide an important advance in the understanding of chemical communication in wild mammals. They compared genetic similarity and the chemical profiles of Antarctic fur seals in two colonies. In so doing they revealed that individual-specific chemical fingerprints have both inherited and environmental components and seem to encode mother–offspring similarity, heterozygosity, and genetic relatedness. The implications of these findings for chemical communication in wild mammals are profound.

Mother–Offspring Recognition

In colonially breeding species, where parents must leave to forage and then return to provision offspring, parent–offspring recognition is vital (10). Mistaken identity can incur severe costs both in misplaced parental effort and potential loss of offspring. Further, in colonies hundreds of individuals typically communicate simultaneously, using the same sensory channels, and similar environmental conditions, imposing strong constraints on communication systems (10). To overcome these constraints many species have evolved complex signals, enabling traits such as individual identity to be encoded in parameters that transmit well in a crowded, noisy, smelly environment. By using multiple sensory modalities, for example auditory and olfactory, they may mitigate potential effects of masking or signal degradation within a single modality (11). In pinniped species with extended maternal care, both mothers and offspring produce vocalizations that encode individual identity. The complexity of individual vocal signatures seems to be influenced by factors such as the population density of colonies (12). However, whereas recognition using vocal signals alone is possible under good environmental conditions, observations of mother–pup reunions suggest that in a many species olfactory information is the confirmatory step in the multimodal recognition process (3, 4). The new information provided by Stoffel et al. (9) is the first step in revealing the complexity of individual chemical fingerprints in pinnipeds, while providing a mechanism by which to explore how chemical fingerprints are adapted to the signaling challenges imposed by environmental conditions.

Social Stability in Colonies

Altruistic behaviors, whether through kin selection or reciprocal relationships, require recognition of specific individuals or groups of individuals. Although observations of potentially altruistic female behavior toward pups, such as allosuckling, are low in Antarctic fur seals (13), the ability to recognize kin and associate with related individuals potentially allows for weakly selected cooperative behaviors to evolve. Wolf and Trillmich (14)

Fig. 1. An Australian sea lion mother–pup pair using naso-nasal inspection during reunion. Olfactory recognition seems to be the confirmatory step in individual recognition.

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identified elevated levels of genetic relatedness among “social communities” within a Galápagos sea lion colony. They suggest that, using chemical fingerprints, individuals may be able to modulate their aggressive behavior in accordance with the degree of relatedness of their opponent. Reduced aggression among related individuals may lead to increased tolerance and a reduction in chronic stress, which can positively affect survival and fitness. The chemical fingerprints identified by Stoffel et al. (9) provide the first evidence of a potential mechanism for kin recognition in pinnipeds underlying the evolution of cooperative behaviors.

Chemical Fingerprints As a Tool in Mate Choice

Female mate choice seems to be an important driver of the Antarctic fur seal breeding system. Males exhibit resource-defense polygyny, yet successful resource defense does not seem to guarantee reproductive success (15). Females show active mate choice, preferring males that are heterozygous and unrelated, but the mechanism by which this is achieved has remained elusive (16). Stoffel et al. (9) propose that chemical fingerprints may be involved in female mate choice, but they were hampered by a lack of male sampling. This raises the exciting possibility that by investigating male chemical fingerprints and undertaking an experimental assessment of female preferences for chemical signals linked to heterozygosity, an understanding of the mechanism underlying mate selection may finally be within our grasp.

Antarctic fur seals and subantarctic fur seals are not completely reproductively isolated. In some colonies around 1% of the population are F1 hybrids and at least 2.4% are backcrossed (17). However, the low level of hybridization and the occurrence of backcrossing suggest the existence of reproductive barriers. The importance of chemical signals as an inhibitor of inappropriate mate selection has heretofore been overlooked in large wild mammals (18). Given the tantalizing glimpse into the potential importance of chemical signals in individual mate selection, there is likely a role in species selection. In the face of multiple species range changes and increased interspecific interactions, understanding barriers to hybridization is ever more important (19). The link between genotype and chemical fingerprints identified by Stoffel et al. (9) opens a new avenue of investigation into the possible role of chemical information in the maintenance of species boundaries.

Identifying Genetic Linkages

The study by Stoffel et al. (9) demonstrates the new avenues that are opening up as molecular approaches become more powerful with larger arrays of, and new and more powerful, markers. Although panels of 10–15 microsatellite markers are often sufficient to detect population structure and other coarse patterns, they lack power to detect subtle effects. Their analysis of 41 markers shows that in excess of 35 markers were required to detect linkages between chemical signatures and genetic traits such as heterozygosity and relatedness. Increasing sophistication of statistical analyses supported by large panels of economically derived markers is opening up entire new avenues of study. In the near future, identifying parts of the genome under divergent selection may prove even more powerful in detecting subtle underlying processes resulting from adaptive genetic variation (20) than the application of large numbers of neutral markers such as those applied in Stoffel et al. (9).

Translating Chemical Profiles into an Understanding of Semiochemicals

An important consideration when exploring chemical profiles using methods such as those of Stoffel et al. (9) is that the results are based on chemical analysis that may not directly indicate biologically relevant compounds. The chemical profiles identified through this method are liable to differ from the semiochemical, or indeed odorant, fingerprints of individuals. It is likely that some of the chemicals identified are not salient in social recognition or mate choice, whereas others that were undetectable through these methods may play a role. Stoffel et al. (9) have endeavored to address this by identifying the substances that contribute most heavily to the similarity of mother–pup pairs and to the dissimilarity between colonies. Although not being a comprehensive list of semiochemicals involved in social recognition or mate choice, these substances should be regarded as a shortlist of potential candidates for examination to determine whether they are biologically relevant. Hurst and Beynon (8) propose a simple behavioral assay of “kin-shifting” where a sample from an unrelated animal is combined with a candidate chemical. If the chemical plays a role in kin recognition the resulting response should be more similar to one for kin than an unrelated individual. However, it is likely that social recognition or mate choice may not be encoded by a single component but a chemosensory bouquet composed of several components whose composition may vary among individuals.

Where to Next?

The study of Stoffel et al. (9) advances our understanding of mammalian chemical communication. It sets out a framework to examine the chemicals used in recognition and provides clear directions for future work. In particular, future research should aim to characterize the chemical fingerprints of both young and adult males and females of other species to build a comparative understanding of the importance of chemical communication in recognition and mate choice. Further, behavioral assays are required to translate chemical fingerprints identified through GC-MS into an understanding of functional semiochemicals. Refinement of genetic techniques will continue to reveal how genotypes influence an individual’s chemical profile. Stoffel et al. (9) have for the first time revealed the potential chemical fingerprints that underpin the chemical communication of pinnipeds and provided a robust approach for further investigations across entire taxa.

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1 Brown RE, Macdonald DW (1985) *Social Odours in Mammals* (Oxford Univ Press, Oxford), Vol 2.

2 Lowell WR, Flanigan WF (1980) Marine mammal chemoreception. *Mammal Rev* 10(1):53–59.

3 Dobson FS, Jouventin P (2003) How mothers find their pups in a colony of Antarctic fur seals. *Behav Processes* 61(1–2):77–85.

4 Pitcher BJ, Harcourt RG, Schaal B, Charrier I (2011) Social olfaction in marine mammals: Wild female Australian sea lions can identify their pup’s scent. *Biol Lett* 7(1):60–62.

5 Cinková I, Policht R (2015) Discrimination of familiarity and sex from chemical cues in the dung by wild southern white rhinoceros. *Anim Cogn* 18(1):385–392.

6 Masi S, Bouret S (2015) Odor signals in wild western lowland gorillas: An involuntary and extra-group communication hypothesis. *Physiol Behav* 145:123–126.

7 Owen MA, et al. (2015) An experimental investigation of chemical communication in the polar bear. *J Zool* 295:36–43.

8 Hurst JL, Beynon RJ (2010) Making progress in genetic kin recognition among vertebrates. *J Biol* 9(2):13.

9 Stoffel MA, et al. (2015) Chemical fingerprints encode mother–offspring similarity, colony membership, relatedness, and genetic quality in fur seals. *Proc Natl Acad Sci USA*, 10.1073/pnas.1506076112.

10 Aubin T, Jouventin P (2002) How to vocally identify kin in a crowd: The penguin model. *Adv Stud Behav* 31:243–277.

11 Stevens M (2013) *Sensory Ecology, Behaviour, & Evolution* (Oxford Univ Press, Oxford).

12 Pitcher BJ, Harcourt RG, Charrier I (2012) Individual identity encoding and environmental constraints in vocal recognition of pups by Australian sea lion mothers. *Anim Behav* 83:681–690.

13 Hoffman JI, Amos W (2005) Does kin selection influence fostering behaviour in Antarctic fur seals (*Arctocephalus gazella*)? *Proc Biol Sci* 272(1576):2017–2022.

14 Wolf JBW, Trillmich F (2008) Kin in space: Social viscosity in a spatially and genetically substructured network. *Proc Biol Sci* 275(1647):2063–2069.

15 Gemmell NJ, Burg TM, Boyd IL, Amos W (2001) Low reproductive success in territorial male Antarctic fur seals (*Arctocephalus gazella*) suggests the existence of alternative mating strategies. *Mol Ecol* 10(2):451–460.

16 Hoffman JI, Forcada J, Trathan PN, Amos W (2007) Female fur seals show active choice for males that are heterozygous and unrelated. *Nature* 445(7130):912–914.

17 Kingston JJ, Gwilliam J (2007) Hybridization between two sympatrically breeding species of fur seal at Iles Crozet revealed by genetic analysis. *Conserv Genet* 8(5):1133–1145.

18 Shurtliff QR (2013) Mammalian hybrid zones: A review. *Mammal Rev* 43(1):1–21.

19 Miller W, et al. (2012) Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. *Proc Natl Acad Sci USA* 109(36):E2382–E2390.

20 Funk WC, McKay JK, Hohenlohe PA, Allendorf FW (2012) Harnessing genomics for delineating conservation units. *Trends Ecol Evol* 27(9):489–496.