COMMENTARY

Jill M. Mateo · Robert E. Johnston

Kin recognition by self-referent phenotype matching: weighing the evidence

In their commentary, Hare et al. (2003, this issue; hereafter Hare et al.) critique two recent publications reporting self-referent phenotype matching. Self-matching occurs when an animal learns some aspect of its own phenotype and later uses it as a referent to identify its relatives (kin recognition) or conspecifics (species recognition; Holmes and Sherman 1982). Self-matching has potential advantages over using nestmates or parents as referents for recognition. For example, self-matching can mediate kin recognition when multiple paternity occurs within litters, so that animals can discriminate between equally familiar maternal full- and half-siblings. It can also operate when individuals encounter unfamiliar kin for the first time, such as when paternal half-siblings initially meet or when older siblings are not encountered until after dispersal (Holmes and Sherman 1982). Self-matching may also operate in other functional contexts such as species recognition in brood parasites (e.g. Hauber et al. 2000; see Hauber and Sherman 2003, this issue, for more). Finally, self-matching should provide a more accurate means of assessing relatedness than using parents or siblings as referents for discrimination (see also Mateo 2002), as an animal's own cues more closely reflect its genotype than those of close kin.

To demonstrate self-matching for kin recognition, animals must be raised so that they do not experience kin cues, other than their own, during development. If they can later recognize unfamiliar kin, this indicates they used their own cues as a referent for that recognition. In our study (Mateo and Johnston 2000), we cross-fostered female golden hamsters (*Mesocricetus auratus*) on their day of birth, and then studied the kin-discrimination abilities of these fostered animals as adults. Hare et al. comment on selected portions of issues we raised in our "Discussion," and we take the opportunity to respond to those comments here. Hare et al.'s main disagreement is with our suggestion that hamsters did not learn the cues of their mother or littermates prior to fostering. Although no single experiment can rule out all possible alternative explanations, we feel that our experiment is the best example to date of self-referent phenotype matching in a mammal. We agree with Hare et al. that we did not entirely eliminate the possibility of the pups learning about kin odors in utero or during the first 3–12 h after birth, but for several reasons we think that such learning is unlikely. We will first address the issue of pre-natal learning and then discuss the potential for post-natal learning during the few hours prior to fostering.

Pre-natal neural development

Golden hamsters are born after one of the shortest gestations known for placental mammals, 15–16 days, and their nervous system at this time is consequently not as well developed as in other altricial rodents (Clancy et al. 2001). With regard to the sense of smell, we are not aware of any information regarding what projections exist on the day of birth or earlier. By 2 days after birth there are some connections from the olfactory bulb to some areas in the rest of the brain, but not to all areas that will eventually receive projections (Leonard 1975). The cells in the olfactory bulb that have made connections to the brain, however, originate from just one quadrant of the olfactory bulb (the medial quadrant), indicating that most areas of the bulb have not yet established connections to the brain (Grafe and Leonard 1982). Second, recent evidence suggests that the ventral part of the main olfactory bulb of mice is the area primarily involved in processing social odors and, specifically, information about individual differences in odors (Schaefer et al. 2001). At 2 days after birth the ventral area of the hamster bulb has not yet made

J. M. Mateo (✉) · R. E. Johnston
Department of Psychology, Cornell University,
Ithaca, NY 14853-7601, USA
e-mail: jmateo@uchicago.edu

Present address:
J. M. Mateo
Committee on Human Development, University of Chicago,
5730 S. Woodlawn Avenue, Chicago, IL 60637, USA
e-mail: jmateo@uchicago.edu

connections to the brain (Grafe and Leonard 1982). Hare et al. cite Leonard's (1975) summary that all structures receiving projections from the bulb are present at birth, yet her ablation and labeling studies indicate that the *projections* themselves are poorly developed at birth and that the olfactory bulb and lateral olfactory tract undergo significant development for the first 7 days after birth (Leonard 1975; Grafe and Leonard 1982). Unfortunately, we have found no studies that examine whether the projections are *functional* at birth (assumed by Hare et al.), thus making interpretation of these anatomical studies difficult. However, given our present knowledge it appears that the level of neural development at birth in hamsters is insufficient for learning and memory of complex social odors.

Potential for early learning of complex odors

Although some animals can learn odors pre-natally from amniotic fluid and show preferences for these familiar odors soon after birth (e.g. Hepper 1987; Hudson et al. 1999), it is unknown whether hamsters can learn specific aspects of family cues prior to birth and remember these cues months later. Most demonstrations of in utero odor learning have used stimuli that are quite different from those involved in individual or kin recognition. These experiments typically used single chemical compounds with strong, distinctive odors or foods with distinctive flavors that may also be dependent on one or a few highly odorous compounds (e.g. juniper, peppermint, citrus, Hudson et al. 1999; cf. Hepper 1987). Thus, the learning that takes place is of a relatively simple type, in which a simple stimulus becomes familiar. In contrast, individual discrimination and kin discrimination often depend on subtle discriminations between highly similar, complex mixtures of a large number of compounds that differ in the relative proportions of these compounds (not, as implied by Hare et al., by single compounds or "discriminators"; e.g. Gamboa et al. 1996; Singer et al. 1997; Smith et al. 2001). This type of discrimination necessarily depends on a large number of receptor-cell types and integration of many inputs in the nervous system (e.g. Schaefer et al. 2001). Further, odors that are learned in utero may not generalize to other body odors. For example, on their day of birth rat pups prefer the amniotic fluid of their mother over that of another mother but do not show a preference for their own mother's body odors (Hepper 1987; Polan and Hofer 1998). We therefore suggest that the olfactory system of hamster fetuses or pups less than 12 h old is not capable of forming memories of the odor quality of individuals because the olfactory system is not developed enough to characterize the subtle differences between individuals. Indeed, the odors of full siblings are so similar in quality that hamsters do not spontaneously notice differences among the odors unless they have had social interactions with the scent donors (Todrank et al. 1999).

Potential for post-natal olfactory learning

Hare et al. contend that we "did not control for post-partum familiarization with close kin," such as mother and siblings. We did not transfer immediately after birth because we often needed to wait until another female gave birth, and because we wanted the mother to rest briefly after birth to minimize cannibalization after the fostering process. As a result, transfers took place 3–12 h after birth, which potentially gave pups an opportunity to learn their family's cues. We suggested that this learning was minimal for several reasons, including the level of neural development on the day of birth in this highly altricial mammal (see above). To explore the influence of time spent in the natal nest prior to fostering on subsequent odor-discrimination abilities, we examined whether there was a correlation between time until fostering and investigation of the various odor sources. We found no significant correlations, which suggests that differential exposure to kin odors did not significantly influence later discrimination abilities. Again, we did not cite this as *proof* against pre-transfer learning, but as suggestive that time in the nest did not allow learning of family odors. Indeed, Samantha Larimer has recently investigated whether and how long pups remember odors to which they were exposed soon after birth. Pups exposed to an artificial odor (such as vanillin) during the first 24 h after birth showed no evidence of recognizing this odor when tested at 1 month of age (e.g. discriminating it from a novel odor; S.C. Larimer and R.E. Johnston, unpublished data). These results are consistent with the lack of correlation between time spent in the natal nest and discrimination abilities and provide further support that hamster pups did not learn their family's odor (from the flank gland or any other source) during that short window of time prior to fostering.

We suggested that, mechanistically, altricial animals are unlikely to learn their siblings' odors at birth if these cues change during early development. Hamsters can form representations of individuals (Johnston and Jernigan 1994), yet it is unknown at what age this learning ability develops. The flank gland, secretions of which were used in our odor-discrimination tests to examine kin-recognition abilities, does not begin secreting until 1 month of age (Algard et al. 1966), thus precluding learning of siblings' flank odors prior to fostering. Hare et al. suggested that had cross-fostered hamsters learned the odors of their siblings prior to fostering (again, unlikely given that pups are not producing flank-gland odors at this time), they could track developmental changes in those odors for later recognition of these siblings. However, this is not the case for Belding's ground squirrels (*Spermophilus beldingi*). Their odors are not individually distinct until after young are weaned (around 30 days of age), and their odors continue to change for at least 2 weeks after weaning. The quality of the odors also appears to change during development, as *S. beldingi* treat differently the odors of an individual collected when it was 28 days old and when it was 38 days old (J.M. Mateo, unpublished data).

We tested the kin-recognition abilities of adult hamsters using the flank-gland secretions of donor animals. We did so because this gland is important in social communication, its odor is individually distinct, and it reliably indicates kinship (Johnston 1990; Johnston et al. 1993; Heth et al. 1998). Furthermore, hamsters do not generalize from this odor to other odors of the same unfamiliar individual, indicating that odors from different places on the body have different qualities (Johnston and Jernigan 1994). In addition, this gland is not functional until sexual maturity (Algard et al. 1966). Therefore subjects had no experience with the flank-gland odors of their siblings during the first 3–12 h after birth, and thus, contrary to the claim by Hare et al., we did eliminate the possibility of recognizing these siblings by direct familiarity with this odor. These authors also claim that, since hamsters form multi-odor representations of individuals (e.g. Johnston and Jernigan 1994), pups could "track individual identity even in the face of developmental changes within one or more olfactory cues." We agree that, given repeated experience with individuals over time, subjects could track changes in the odors of these individuals. It is not clear how this is relevant to our study, however, because our subjects had no experience with the relevant odors (see preceding paragraph).

The arguments we presented in our "Discussion" (Mateo and Johnston 2000, and elaborated upon here) were suggestive, rather than presented as "proof," as intimated by Hare et al. Yet the available data cast doubt on the possibility that hamsters learned their siblings' odors prior to fostering. Taken together, the results provide converging indications that neonatal hamsters did not learn their relatives' cues prior to cross-fostering. However, it remains an empirical question to what extent hamsters are able to learn about social odors during the perinatal period. We welcome the pursuit of this question using embryo transfers to rule out late pre-natal and immediate post-natal learning.

We also question the interpretations Hare et al. make of the literature in support of their position. With regard to pre-natal learning, Hare et al. refer to the work of vom Saal, who showed that hormones secreted by embryos can affect adjacent embryos and that these interactions can affect the behavior of adults. These hormones, however, do not exert their effects through the olfactory system, but rather they have their effects by direct action on the brain of the developing embryos (vom Saal 1989; DeVries and Simerly 2002). We believe that Hare et al. are also incorrect in their interpretation of studies of recognition in mice. They claim that there is ample evidence for genetic-similarity detection in mice, by which they seem to mean detection of relatedness without relevant social experience (citing Yamazaki et al. 1976; Manning et al. 1992). But, all of these mice grew up with their mother and siblings, so the distinctions that the mice made between mice or odors of mice with MHC types different from their own were potentially influenced by learning odors of relatives and/or their own odor.

Finally, Hare et al. agreed with us that it is difficult to interpret the function of kin-recognition abilities in *M. auratus*, because so little is known about the behaviors of free-living hamsters. We suggested that self-matching may be especially important in nepotistic contexts, to identify most-closely related individuals, whereas use of other individuals as referents (e.g. mother, siblings) would be sufficient for inbreeding avoidance. Hare et al. doubt the utility of nepotistic behaviors in a solitary species, yet this is an empirical question, and depending on the costs of such behaviors even infrequent nepotism may confer a fitness advantage. It is possible that females allow kin access to their burrow systems or caches of food (as seen in more social rodents), or share nests during certain seasons, despite a generally solitary nature. Unfortunately, we just do not have the behavioral data from free-living hamsters to dismiss this possibility. In our article, we reported preliminary data on mate-choice behaviors of female hamsters. We tested females when they were either behaviorally receptive or 24 h prior to estrus (when captive females appear to choose their mates; Lisk et al. 1983) and found that females mate indiscriminately with their brothers and non-brothers in these laboratory settings. In contrast, females housed in a large arena are significantly less agonistic toward their sisters compared with non-kin, and sisters choose to sleep together in nestboxes more often than do non-kin. Together, these unpublished data are consistent with our prediction of self-matching abilities in nepotistic contexts.

References

Algard FT, Dodge AH, Kirkman H (1966) Development of the flank organ (scent gland) of the Syrian hamster. Am J Anat 118:317–326

Clancy B, Darlington RB, Finlay BL (2001) Translating developmental time across mammalian species. Neuroscience 105:7–17

DeVries GJ, Simerly RB (2002) Anatomy, development, and function of sexually dimorphic circuits in the mammalian brain. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT (eds) Hormones, brain and behavior. Academic Press, New York, pp 137–191

Gamboa GJ, Grudzien TA, Espelie KE, Bura EA (1996) Kin recognition pheromones in social wasps: combining chemical and behavioural evidence. Anim Behav 51:625–629

Grafe MR, Leonard CM (1982) Developmental changes in the topographical distribution of cells contributing to the lateral olfactory tract. Dev Brain Res 3:387–400

Hare JF, Sealy SG, Underwood TJ, Ellison KS, Stewart RLM (2003) Evidence of self-referent phenotype matching revisited: airing out the armpit effect. Anim Cogn DOI 10.1007/s10071-002-0142-y

Hauber ME, Sherman PW (2003) Designing and interpreting experimental tests of self-referent phenotype matching. Anim Cogn DOI 10.1007/s10071-003-0164-0

Hauber ME, Sherman PW, Paprika D (2000) Self-referent phenotype matching in a brood parasite: the armpit effect in brown-headed cowbirds (*Molothrus ater*). Anim Cogn 3:113–117

Hepper PG (1987) The amniotic fluid: an important priming role in kin recognition. Anim Behav 35:1343–1346

Heth G, Todrank J, Johnston RE (1998) Kin recognition in golden hamsters: evidence for phenotype matching. Anim Behav 56: 409–417

Holmes WG, Sherman PW (1982) The ontogeny of kin recognition in two species of ground squirrels. Am Zool 22:491–517

Hudson R, Schaal B, Bilko A (1999) Transmission of olfactory information from mother to young in the European rabbit. In: Box HO, Gibson KR (eds) Mammalian social learning. Cambridge University Press, New York, pp 141–157

Johnston RE (1990) Chemical communication in golden hamsters: from behavior to molecules and neural mechanisms. In: Dewsbury DA (ed) Contemporary issues in comparative psychology. Sinauer, Sunderland, Mass., pp 381–409

Johnston RE, Jernigan P (1994) Golden hamsters recognize individuals, not just individual scents. Anim Behav 48:129–136

Johnston RE, Derzie A, Chiang G, Jernigan P, Lee H-C (1993) Individual scent signatures in golden hamsters: evidence for specialization of function. Anim Behav 45:1061–1070

Leonard CM (1975) Developmental changes in olfactory bulb projections revealed by degeneration argyrophilia. J Comp Neurol 162:467–486

Lisk RD, Ciaccio LA, Catanzaro C (1983) Mating behaviour of the golden hamster under semi-natural conditions. Anim Behav 31: 659–666

Manning CJ, Wakeland EK, Potts WK (1992) Communal nesting patterns in mice implicate MHC genes in kin recognition. Nature 360:581–583

Mateo JM (2002) Kin-recognition abilities and nepotism as a function of sociality. Proc R Soc Lond B 269:721–727

Mateo JM, Johnston RE (2000) Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. Proc R Soc Lond B 267:695–700

Polan HJ, Hofer MA (1998) Olfactory preference for mother over home nest shavings by newborn rats. Dev Psychobiol 33:5–20

Saal FS vom (1989) Sexual differentiation in litter-bearing animals: influence of sex of adjacent fetuses in utero. J Anim Sci 67:1824–1840

Schaefer ML, Young DA, Restrepo D (2001) Olfactory fingerprints for major histocompatibility complex-determined body odors. J Neurosci 21:2481–2487

Singer AG, Beauchamp GK, Yamazaki K (1997) Volatile signals of the major histocompatibility complex in male mouse urine. Proc Natl Acad Sci U S A 94:2210–2214

Smith TE, Tomlinson AJ, Mlotkiewicz JA, Abbott DH (2001) Female marmoset monkeys (*Callithrix jacchus*) can be identified from the chemical composition of their scent marks. Chem Senses 26:449–458

Todrank J, Heth G, Johnston RE (1999) Social interaction is necessary for discrimination between and memory for odours of close relatives in golden hamsters. Ethology 105:771–782

Yamazaki KE, Boyse EA, Mike V, Thaler HT, Mathieson BJ, Abbott J, Boyse J, Zayas ZA, Thomas L (1976) Control of mating preferences in mice by genes of the major histocompatibility complex. J Exp Med 144:1324–1335