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To cite this article: Roberto Cazzolla Gatti, Alena Velichevskaya, Benjamin Gottesman & Karen Davis (2021) Grey wolf may show signs of self-awareness with the sniff test of self-recognition, *Ethology Ecology & Evolution*, 33:4, 444-467, DOI: [10.1080/03949370.2020.1846628](https://doi.org/10.1080/03949370.2020.1846628)

To link to this article: <https://doi.org/10.1080/03949370.2020.1846628>



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Grey wolf may show signs of self-awareness with the sniff test of self-recognition

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Received 13 May 2020, accepted 6 October 2020

Although there are recent claims of a lack of evidence of self-consciousness in many tested species, the ability to recognize oneself in a mirror, which seems an exceedingly rare capacity in the animal kingdom, may not be the only way to check for animal self-awareness (i.e. the capacity to become the object of your own attention). A new testing approach, based on a different sensory modality (such as the sniff-test for self-recognition, STSR), recently proved to be effective with dogs. We applied this sniff test to a group of four captive grey wolves, living in male-female couples in two different enclosures at the Wolf Park in Indiana, USA. In this preliminary study, wolves showed some signs of the ability to recognize themselves through the “olfactory mirror” and exhibited some clues of mark-directed responses, particularly scent-rolling, which may shed more light on this still unclear behavior and represent a sort of olfactory equivalent to passing the original mirror test.

KEY WORDS: self-awareness, grey wolf, sniff test, mirror test, animal cognition, animal consciousness.

INTRODUCTION

The recognition of one's own self, known as self-recognition, has been tested mainly by examining the response behavior of animals and children as they look at their reflection in a mirror (Gallup 1970; see Appendix 1 for a list of studies that have employed the mirror test or alternative self-recognition tests). Until now, only great apes (including, of course, human beings) have shown convincing evidence of self-recognition by way of the mirror test (Povinelli et al. 1997; De Waal et al. 2005). However, other animals are showing interesting responses. At least one individual

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elephant (Plotnik et al. 2006) and two individual dolphins (Marten & Psarakos 1994; Reiss & Marino 2001) showed a spontaneous use of the mirror to touch or inspect the mark apposed on their body by the experimenters. Some species of birds (Prior et al. 2008), fish (Ari & D'Agostino 2016; Kohda et al. 2019), and ants (Cammaerts Tricot & Cammaerts 2015) also showed signs of self-recognition in the mirror test (Gallup & Anderson 2019), although their responses were not definitive. The idea behind the mirror test is to determine whether the subject understands the concepts of “self” and “others,” which is proof of self-consciousness. However, the comprehensiveness of this test has recently been challenged, especially with respect to animals that rely on sensory modalities other than vision to perceive their environment and themselves (Bekoff & Sherman 2004).

The controversy is: it is still unclear if failure to recognize oneself confirms that an animal lacks self-awareness (Safina 2015). Moreover, the capacity to recognize one own image in its reflection is limited to species for which vision is a well-developed sense. The capacity to touch a changed part of one's own body is considered a self-recognition prove in the mirror test. This, however, is limited to those species that possess appendices (e.g. arms, proboscides, beaks, antennas, etc.) and are, therefore, able to touch specific parts of their body. The ability to recognize oneself in a mirror, which seems an exceedingly rare capacity (Bekoff & Sherman 2004), might not be the only way to test for self-awareness, and may also be a biased test.

One of the biggest challenges is due to the difficulties in implementing and interpreting adequate controls necessary to obtain robust evidence from the mirror test in animals unable to display self-recognition by touching a marked part of the body (Taylor Parker et al. 2006). In fact, the mirror test can yield false negatives if an individual lacks the necessary visual and morphological requirements. However, this does not necessarily mean that the species is not self-conscious. For instance, in previous tests (Siwak et al. 2001), dogs showed no interest in mirrors as image-reflective objects. Instead, they usually sniff or urinate around them as general elements within the space. However, dogs and wolves, like primates, dolphins, and elephants, show a high level of behavioral and cognitive complexity (Howell et al. 2013). Therefore, new methods to investigate the levels of their behavior and cognition are needed.

Bekoff (2001) sharply observed that there were clues of self-recognition in species that were phylogenetically distant from primates (i.e. with different display and sensory modalities). To better test cognition and self-awareness in these species, alternative self-understanding tests have emerged, including the “body-awareness test” (Dale & Plotnik 2017; Khvatov et al. 2019; Lenkei et al. 2020) and the “sniff test” (Cazzolla Gatti 2016; Horowitz 2017). In particular, the original sniff-test for self-recognition (STSR; Cazzolla Gatti 2016) made use of the evidence that dogs are considerably less affected by visual events than are humans and most apes (Bekoff 2003). Therefore, it is likely that their and other animals' failure at mirror tests could be due to the sensory modality used to test self-awareness. The STSR showed some proofs of dog self-recognition. The test was employed on four individuals of different age and sex living in a group. It proved that the STSR can play a pivotal role in demonstrating that self-recognition is not a feature specific to humans and great apes (and, possibly, a few other animals), and that our current understanding into animal cognition depends largely on the way researchers have tested it. In the STSR, the researchers look at how long each animal investigates, through smelling and interacting with canisters containing urine samples from itself and another individual.

The purpose of the STSR is to check whether the animals in question can distinguish their own scent from those of the others.

After the first application of the STSR, a research study (Horowitz 2017) used the same sniff-test to evaluate the ability of a bigger sample of dogs to recognize themselves. This study seems to support the hypothesis of dog self-awareness that was proposed by a former (Cazzolla Gatti 2016). Dogs seemed capable of distinguishing between the olfactory “image” of themselves when modified. Nevertheless, Gallup and Anderson (2018) proposed at least two improvements to provide definitive evidence for self-recognition in dogs, and also to make the sniff test comparable to the mirror test. The first improvement calls for the need to check the sniffing and post-sniffing behavior towards modified familiar odors because there is a possibility that dogs would show the same response to comparable changes in other familiar odors, such as that of another companion dog (“familiarity hypothesis”). The second improvement is related to the idea that if dogs could identify the source of their own odor they should show a mark-directed behavior. Examples of such behaviors include smelling or inspecting themselves after sniffing the samples containing their odor combined with another odor. This would be the olfactory equivalent to passing the mirror mark test since the novel odor represents an analog to the mark. Unfortunately, although present in the original study with a limited number of dogs (Cazzolla Gatti 2016), these important control conditions were not incorporated into the study by Horowitz (2017).

Here we applied the STSR test to a dog-related species, the grey wolf (*Canis lupus*). In this test, we incorporated the controls suggested by Gallup and Anderson (2018) and included in the preliminary test performed in Cazzolla Gatti (2016). We applied five trials of the sniff-test for self-recognition to a group of four captive grey wolves, living in male-female couples in two different enclosures at the Wolf Park in Indiana, USA. We hypothesize that if wolves were self-aware: (i) overall, they would spend less time smelling their own pure odor compared to that of themselves with the mark and of other wolves or other species; (ii) they would be more interested in an unknown wolf's odor than the partner's one; (iii) they would not show significant differences in the interest towards their own vs partner's odors (without a mark); (iv) they would be more attracted by the marked urines than by the marker itself; (v) they should show evidence of mark-directed behavior after smelling the marked urine samples.

MATERIALS AND METHODS

Subjects and experimental location

The group of tested subjects consisted of four captive, adult wolves. These wolves lived in couples in two separate enclosures at the Wolf Park research center in Lafayette, IN, USA. The α -couple was composed of Fiona, a 7-year-old female, and Kanti, a 7-year-old male. The β -couple was composed of Timber, a 5-year-old female, and Woton, a 14-year-old male.

Testing theatre

Initially, we selected a 5 × 5 m room within the Wolf Park facility as the testing theatre. However, this indoor space proved to be uncomfortable for wolves. In a preliminary test, the wolves almost completely ignored the samples, showed signs of stress, and were focused primarily on exiting the room. Therefore, we moved the testing theater outdoors, to each wolf's own

enclosure. In their enclosures, they felt more comfortable and were not distracted by external stresses or other confounding elements.

For each trial, the experimenter randomly placed a stimulus array consisting of three unmarked canisters (metal round containers of 10 cm diameter \times 4 cm height with three holes on the lid) that contained the odor samples, which were prepared directly before the trial. The canisters were placed in a row on a non-absorbent 2 m \times 1.5 m wood platform, which was cleaned with a solution of 70% isopropyl alcohol prior to each trial. The order of canisters was randomized for each trial. One canister was placed in the middle of the platform, and the other two were placed approximately 0.75 m to either side of the center point. During the placement of the canisters, wolves were kept in a pre-enclosure in order to mitigate interactions between the subjects and presenting experimenter. The outdoor platform was big enough and no permeant to any odor. We documented that wolves did not perceive the odors under the platform because they did not sniff under it or around its borders.

Trials were recorded with two digital video cameras. One camera was placed on the wood platform in front of the canisters facing the subject. It captured a wide view of the testing theatre, including the experimenter and subject; the second camera was placed to the side of the canisters. It focused on the canisters and served as a backup if the subject's body blocked the first camera's view of the subject's nose.

Stimuli, odor collection, and other materials

Urine was selected as the stimuli for this sniff-test as in the previous tests with dogs (Cazzolla Gatti 2016; Horowitz 2017). We collected urine samples from each wolf immediately after secretion (directly during the urination events and not from the ground to avoid odour contamination) using a sterile bowl and then absorbing it with a non-reactive syringe and/or cotton and vinyl gloves. Samples were stored in a refrigerator at 4 °C until the testing session, at which time they were brought to room temperature before use.

A "mark" – a change in an animal body's element – was added to some subjects' urine to prepare the mark (M), self-modified (SM), partner-modified (KM), and unknown-modified (UM) samples (see the next section for details on the experimental design). Urine was applied to cotton swabs and then these swabs were exposed to anise essential oil (*Pimpinella anisum*), an odorant used in intermediate and advanced nose work practice (Horowitz 2017; NACSW 2019). Pieces of cotton of 0.5 cm were secured in a jar with six drops (< 1 mL) of anise (Horowitz 2017). An assistant wearing vinyl gloves prepared the samples away from the testing theatre. The jar was shaken so that each piece was in contact with the odorant.

For each trial, 1.5 mL of the subject's urine was pipetted into each of the canisters. New self and self-modified samples were prepared for each trial. Anise-cotton samples were added to the canisters with the subject's urine. The canisters were about 3 cm wide round tin containers with three air holes in their lids.

Experimental design

Subjects participated in five trials. Each trial consisted of presenting an individual wolf with the stimulus array consisting of the three canisters. Before each trial, an assistant brought the three canisters (labeled with the code of each trial) on a tray to the presenting experimenter. After placing the canisters, the experimenter left the testing theater (enclosure) and did not look in the direction of the subject or canisters and did not interact with the subject. The wolf was then allowed to enter the testing area.

Trials were 5 min in length. They began once the wolf showed directed attention toward the samples (Fig. 1) through proximity maintenance (within 1 m of the sample), body position, or engaged in indirect sniffing. In a trial, the wolf was allowed to interact with the canisters or itself



Fig. 1. — During the trials, each wolf was allowed to interact for 5 min with three canisters, randomly placed on an outdoor platform in their own enclosures, its behavior was recorded with digital cameras and the time spent inspecting each canister measured with a digital stopwatch. Other post-sniffing behaviors were observed.

without interference from any staff member. After 5 min passed, the animal keepers entered and brought the wolf outside of the enclosure, thus ending the trial.

A minimum inter-trial interval of 24 hr was established so that wolves could rest and reset between tests. In the five-trial arrangement, subjects were presented with a sequence of the following ordered comparisons (with the three canisters randomly placed in each trial):

- (1) S-SM-M, their own odor ("Self"), their own odor with added "mark" ("Self-Modified"), and the mark ("Mark");
- (2) S-SM-KM, their own odor ("Self"), their own odor with added "mark" ("Self-Modified"), and the odor of the known (partner living in the same enclosure) wolf with added "mark" ("Known-Modified");
- (3) S-SM-D, their own odor ("Self"), their own odor with added "mark" ("Self-Modified"), and the urine of a "Dog";
- (4) S-U-UM, their own odor ("Self"), the odor of an unknown (non-companion living in a different enclosure) wolf ("Unknown"), and the odor of the same unknown wolf with added "mark" ("Unknown-Modified");
- (5) S-K-KM, their own odor ("Self"), the odor of the known (partner living in the same enclosure) wolf ("Known"), and the odor of the same known wolf with added "mark" ("Known-Modified").

Trial rationale and logic

The first trials were a combination of self (S) and self-modified (SM, i.e. each wolf's own urine marked with anise essential oil) scents with a third canister containing: (i) the mark by itself (M), to check the pure interest in the mark; (ii) the urine of the know (partner) wolf modified with the mark (KM), to check the interest in a modified companion's odor; and (iii) the urine of a dog (D), to check the interest in a completely unknown and heterospecific odor.

Then, to definitively disentangle the “familiarity hypothesis and the unfamiliarity preference” suggested by Gallup and Anderson (2018), we performed a fourth and a fifth trial with a combination of self (S), known (K; urine from the partner wolf), unknown (U; urine from a non-partner wolf), and the marked modifications of known and unknown (KM and UM, respectively). These two additional trials were added as further controls to check the ability of wolves to (1) differentiate the “self” odor from “known” (familiar) and “unknown” (unfamiliar) odors, and (2) discern between marked “known” (familiar modified) and “unknown” (unfamiliar modified) odors.

Data analysis

Each trial was videotaped by a Wolf Park employee who did not know the hypothesis behind the trials. Videos were coded by two separate coders (whose compared data confirmed the reliability of the coding with the Cohen’s K metric) using frame-by-frame playback to accurately classify subjects’ behavior. The two dependent variables related to the olfactory investigation a wolf directed toward a canister, including (1) duration (how many seconds per trial a wolf was sniffing a given canister), and (2) mark-directed behavior frequency per trial (how frequently wolves displayed a behavior after sniffing the canisters with the mark). Both dependent variables, duration and frequency, could be measured effectively by video playback analysis.

Given that the wolf nose reach is approximately 10–15 cm from the nares, “sniffing” was scored as beginning when the wolf’s nose was 10 cm from the canister and ending when the wolf moved its head away from the canister. Sniffing time was calculated first as the “number of frames” with sniffing behavior, and subsequently converted into seconds. Secondly, exploratory behavior was gauged by the number of visits to the canisters. While a subject had to visit each canister in each trial for the trial to be analyzed, all additional return visits to canisters during the 5 min of the trial were also noted. A count was thus made of the number and order of visits to each canister.

Then, any mark-directed behavior (an act induced by the realization of the presence of the mark on one’s own body, for example attempts to remove or scratch the mark by primates, insistently inspection or paying more attention to that change on the body by elephants and dolphins, etc.; Gallup 1970; Plotnik et al. 2006) by a subject after it sniffed canisters containing the mark was also noted. For canids, behaviors of particular importance might be scent-rolling, scent-marking, and scent-creeping.

A pairwise comparison of investigation duration for dependent samples in each trial was performed with a Wilcoxon Signed-Rank Test for Paired Samples ($\alpha < 0.05$). An analysis of the statistical difference of the scent-rolling behavior frequency was performed with Pearson’s χ^2 test ($\alpha < 0.05$) with the Yates correction for small data sets.

RESULTS

Trials with self and self-modified odors

In the first trial (S-SM-M), wolves spent significantly more time ($T = 0$, $P < 0.05$) sniffing the canister with the self-modified urine than the one with their own odor (Fig. 2A). They did not spend significantly more time smelling the mark in comparison with their own urine ($T = 3$, $P = 0.23$). Similarly, time spent sniffing the mark was not significantly less than time spent sniffing the self-modified odor ($T = 5$, $P = 0.5$).

Three out of the four wolves, Fiona, Kanti, and Woton, showed a scent-rolling behavior over the self-modified (SM) sample after smelling it (Table 1 and Fig. 4; see Movie 1 in Supplemental data for an example of scent-rolling on the SM sample).

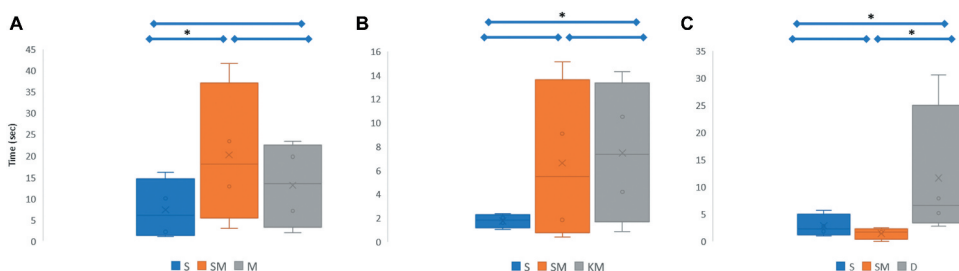


Fig. 2. — Time (in seconds) spent by the four tested wolves on each canister in trial 1 (Self S – Self-modified SM – Mark M), trial 2 (Self S – Self-modified SM – Known-modified KM), and trial 3 (Self S – Self-modified SM – Dog D). In each boxplot, the horizontal line is the median, the x is the mean, lower and upper bounds of the boxes are quartiles Q1 and Q3, and the buffers are the standard errors (SE). Stars over the connection lines between boxes represent the significance ($P < 0.05$) of the statistic test (pairwise Wilcoxon Signed-Rank Test for Paired Samples).

Timber, the 5-year-old female in the β -couple, was the only subject that did not scent-roll. Kanti, Timber, and Fiona (see Movie 2, which is published as Supplemental data) also scent-rolled on the mark (M) sample after sniffing it. None of the wolves scent-rolled on the self (S) canister. In general, the frequency of scent-rolling behavior towards non-self samples (i.e. an “other-directed” response) vs self samples was statistically significant (Pearson’s $\chi^2 = 28.36$, $df = 3$, $P < 0.01$).

In the second trial (S-SM-KM), wolves spent significantly more time ($T = 0$, $P < 0.05$) smelling the partner-modified odor than the self-odor (Fig. 2B). No significant difference in time spent sniffing was observed between the self-modified odor and the self ($T = 6$, $P = 0.14$) and between the self-modified and partner-modified ($T = 1$, $P = 0.07$) samples.

Both Kanti (see Movie 3, which is published as Supplemental data) and Fiona (see Movie 4, which is published as Supplemental data) rolled over both the self-modified and partner-modified canisters (Table 1 and Fig. 4). No other post-sniffing, mark-directed behavior was displayed. The scent-rolling behavior frequency towards non-self samples (i.e. an “other-directed” response) was statistically significant (Pearson’s $\chi^2 = 27.33$, $df = 3$, $P < 0.01$).

In the third trial (S-SM-D), the time spent sniffing the dog (D) sample was significantly higher than the time directed to sniff their own urine canister ($T = 0$, $P < 0.05$) and the self-modified one ($T = 0$, $P < 0.05$) (Fig. 2C). There was no significant difference between the smelling times of self and self-modified urines ($T = 3$, $P = 0.23$). Only Fiona rolled and urinated over the dog’s sample (Table 1). As in the first two trials, scent-rolling behavior was significantly directed towards non-self samples (Pearson’s $\chi^2 = 34.96$, $df = 3$, $P < 0.01$).

Trials with self urine compared to familiar and unfamiliar conspecific odors

In the fourth trial (S-U-UM; Fig. 3A), wolves spent significantly less time smelling their own urine than they spent smelling the urine of one of the non-partner wolf ($T = 0$, $P < 0.05$). Time spent sniffing their own urine was also significantly less than time spent the unknown wolf’s modified urine ($T = 0$, $P < 0.05$). There was no

Table 1.
Cumulative frequency (%) of post sniffing mark-directed behavior in the four tested wolves.

Behavior	Trial 1				Trial 2				Trial 3				Trial 4				Trial 5			
	S	SM	M		S	SM	KM		S	SM	D		S	U	UM		S	K	KM	
Scent-roll	0%	75%	75%		0%	50%	50%		0%	0%	25%		0%	25%	50%		0%	0%	50%	
Scent-mark	0%	0%	25%		0%	0%	0%		0%	0%	25%		0%	0.0%	0%		0%	0%	0%	
Scent-creep	0%	0%	0%		0%	0%	0%		0%	0%	25%		0%	50%	50%		0%	0%	0%	

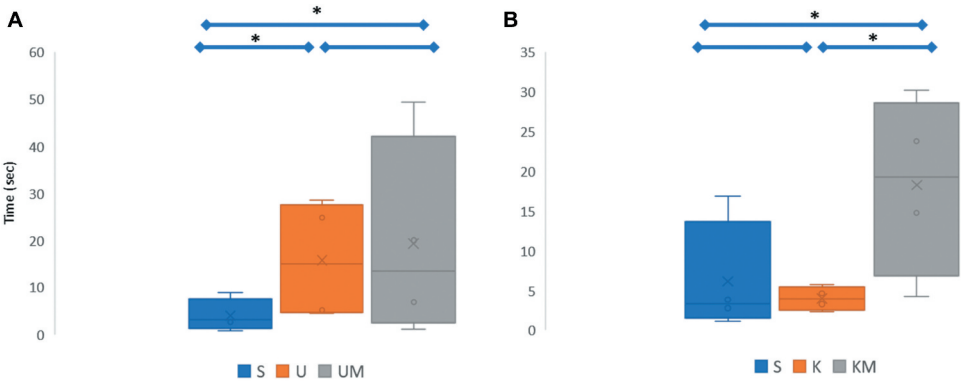


Fig. 3. — Time (in seconds) spent by the four tested wolves on each canister in trial 4 (Self S – Unknown U – Unknown – modified UM) and trial 5 (Self S – Known K – Known-modified KM). In the boxplot, the horizontal line is the median, the x is the mean, lower and upper bounds of the boxes are quartiles Q1 and Q3, and the buffers are the standard error (SE). Stars over the connection lines between boxes represent the significance ($P < 0.05$) of the statistic test (pairwise Wilcoxon Signed-Rank Test for Paired Samples).

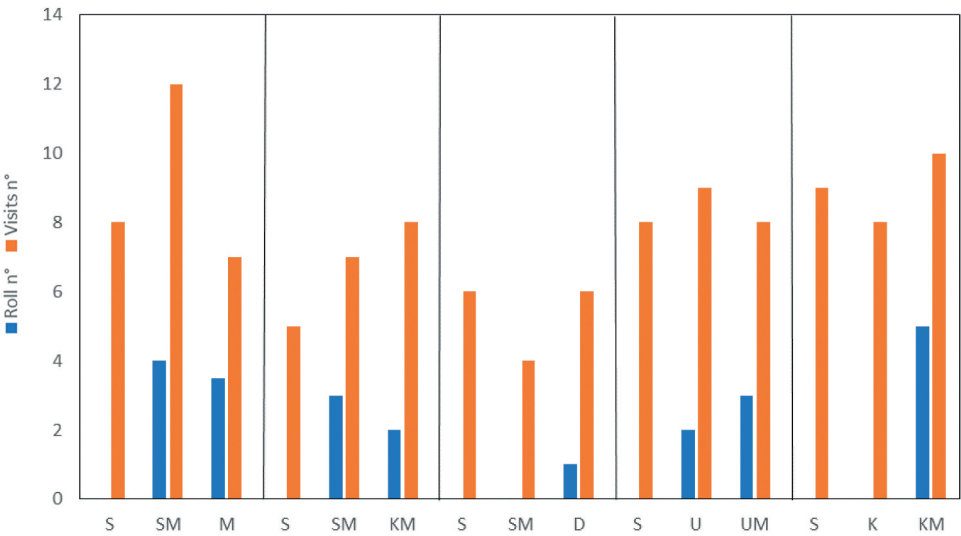


Fig. 4. — Total number, for the four tested wolves, of visits and scent-rolls over each canister in the five trials. S = Self; S = Self-Modified; M = Modified; UM = Unknown Modified; K = Known; KM = Known Modified; D = Dog.

significant difference between the time spent on the unknown and unknown-modified canisters ($T = 4$, $P = 0.36$).

Fiona, after smelling the unknown-modified canister rolled on it, while Kanti rolled on both the unknown and unknown-modified canisters and chewed the latter

(Table 1 and Fig. 4). In general, the scent-rolling behavior frequency towards non-self samples (i.e. an “other-directed” response) was statistically significant (Pearson’s $\chi^2 = 28.51$, $df = 3$, $P < 0.01$).

The fifth trial (S-K-KM; Fig. 3B) showed that smelling the self-odor was significantly lower than that of the known-modified canister ($T = 0$, $P < 0.05$), but there was no difference between time spent sniffing the self-odor and known-odor ($T = 4$, $P = 0.36$). However, there was a significant difference between the time spent on the known-odor and the known-modified odor ($T = 0$, $P < 0.05$).

Fiona and Kanti rolled over the partner-modified sample after smelling it. No other post-sniffing mark-directed behavior was displayed. In general, the frequency of scent-rolling behavior towards non-self samples (i.e. an “other-directed” response) was statistically significant (Pearson’s $\chi^2 = 31.19$, $df = 3$, $P < 0.01$).

DISCUSSION

Confirming our hypotheses (based also on previous studies on dogs) our set of five trials showed that wolves spend less time smelling their pure odor compared to its marked version. Wolves seemed also much less interested in their own odor than in that of unknown wolves and other species. They also appeared to be more interested in an unknown wolf’s odor than in the partner’s one. At the same time, they did not show a significant difference in the interest towards their own vs partner’s unmarked odors. Furthermore, wolves seemed more attracted by the marked urines than by the marker itself and showed mark-directed behaviors after smelling the marked urine samples. We discuss the importance and implications of these preliminary results following their order of presentation in the previous section.

Trials with self and self-modified odors

Wolves displayed a higher interest in the self-modified odor than their self-odor in the first trial (S-SM-M), echoing the previous results gathered from dogs (Cazzolla Gatti 2016; Horowitz 2017). As in a previous version of the STSR test (Horowitz 2017), the “modified” condition was added to the three sample trials to ensure that any investigation of the self-marked sample was not only due to interest in the mark odor, per se. Since there was no significant difference between the smelling of the mark and their odor, it appears that wolves exhibited a minimal interest in the mark. Had the subjects investigated the mark odor significantly longer than the self odor (and, proportionally, than the self-modified one), this could have been a confirmation of the hypothesis of unfamiliarity preference (i.e. subjects prefer to investigate an unfamiliar or novel odor than a familiar or a familiar-modified odor; Gallup & Anderson 2018). Instead, the significantly greater time spent sniffing the self-modified odor compared with time spent sniffing their own urine, with no significant differences with the mark by itself, shows signs of self-recognition, irrespective of unfamiliar odors.

Moreover, the post-sniffing, scent-rolling behavior showed by most of the wolves over the self-modified urine and the mark in the first trial – but not over their own odor – may represent a method analogous to investigating the “mark-directed responses” in the mirror test, such as the exploration of the mark by appendices

(Gallup 1970). Although the meaning of this behavior is still unclear, scent-rolling showed by canids over an object seems a means to collect “other’s” smells, to either camouflage themselves for predation (Allen et al. 2017) or to inform other pack’s members of the surrounding elements (Mertl-Millhollen et al. 1986). Therefore, it might be not surprising that wolves rolled on the samples with the mark, assuming that the abovementioned hypothetical reasons behind this behavior are correct.

However, while the first trial showed self-understanding, it did not completely explain whether wolves are actually self-conscious. Are they able to identify themselves from the others and have an idea of “I am” or are they just mine-conscious, able to identify their own “familiar” qualities from others’ “unfamiliar” qualities? Therefore, we performed four additional trials with urine combinations that could better shed light on the consciousness question.

In the second trial (S-SM-KM), we tested whether wolves showed the same response toward their own modified odor as with modified familiar odors, such as the urine of a companion wolf. Wolves, like dogs and some other animals, which habituate to familiar signals like their own odor, may show dishabituation to their own odor when it is combined with another odor (Nash & Gallup 1976). As suggested by Gallup and Anderson (2018), this comparison is an important control condition. In our study, wolves sniffed significantly more the companion wolf’s modified odors than their own pure urine. In contrast, the time spent sniffing self-modified odor versus their own pure urine was not significantly different. This increased fascination with the companion wolf’s modified odor relative to their own odor or self-modified odor could represent proof that the decreased interest in their own scent is not due to habituation to a familiar stimulus, but rather a conscious knowledge of “me” and the “others.” In this trial, Fiona and Kanti rolled over the two canisters with the mark but not on the one with their own pure odor. This mark-directed behavior may represent a confirmation of self-recognition or, at least, of self-understanding: “I do not roll on myself. I roll on other’s smells”. However, as in the previous trial, an explanation for some of the subjects’ behaviors may still rely on the “unfamiliarity preference”. This explanation would require the presumption that one’s own odors, even when it is modified, become “familiar” after the first time sniffing (which would, however, be indicative of recognition of “self” odors). Given that premise, one would expect that wolves investigated unfamiliar samples more than their own and self-modified odors. Wolves actually did this in the second trial and it might be due to the fact that, possessing a good memory (Mech & Boitani 2010), wolves habituated to the self-modified odor after the first trial. This possibility was further investigated in the third phase of the test.

In this third trial (S-SM-D), in the presence of a heterospecific odor, all wolves spent more time smelling the unknown than the known samples, with or without the mark. The tested subjects, thus, perhaps mindful of the mark’s odor after the first two trials, did not much mind the self-modified canister and highly dedicated their attention to the dog’s sample. Therefore, also in the presence of another species’ odor, wolves looked aware of their own smell. Yet, this does not definitively prove independence from “familiarity” because, in this trial, wolves spent significantly more time smelling the dog’s sample than the self-urine canister, which might have become either a self-conscious or a familiar element which warranted less attention. Fiona was the only wolf who showed a mark-directed response over the dog’s urine and this might be due to sex differences with the heterospecific animal that discouraged the α -male to roll over it (Mech & Boitani 2010).

Trials with self-urine compared to familiar and unfamiliar conspecific odors

The fourth phase of the test (S-U-UM) confirmed that wolves can distinguish their own odor not only from a heterospecific one but also from a conspecific, non-companion smell. In this trial, wolves spent less time sniffing the self odor than the non-companion and the modified non-companion odors. Although they could have become habituated to the mark after the first trials (in fact, there is no significant difference between the time spent to smell the unknown and the marked-unknown samples), wolves still clearly show a higher interest in “other” odors. Both Fiona and Kanti showed a mark-directed response over the non-companion’s odor. Fiona also rolled on the unknown marked sample. As before, none of the wolves rolled on their own odor.

Emerging from the fifth trial (S-K-KM) was the last piece of evidence towards wolf self-understanding, which – if future studies will confirm this preliminary result – may be considered as a sign of proper self-recognition. This last trial seemed to refute the “unfamiliarity preference”. Even though wolves might have become familiar with the mark in the first trials, they still showed significantly higher interest in the partner-modified canister than in the unmarked self and companion’s odors. The sustained interest in the partner-modified canister differed from trial 3 in which there was potential habituation and disinterest (“familiarity”) toward the self-modified canister after the first sniffing. The relevant evidence, in this case, is that both the partner and the mark were familiar odors after four trials but wolves still spent significantly higher time sniffing only the marked partner’s sample. This means that the ability to self/other recognition, more than familiarity, directs the interest towards the canisters. A mark-directed response was shown by Kanti and Fiona only on the partner-modified sample, which provides additional support for this interpretation.

Scent-rolling: a mark-directed sign of wolf self-recognition for the olfactory mirror?

We documented a common (i.e. displayed in all the five trials by a minimum of one wolf) mark-directed behavior: the rolling on non-self and non-partner’s odors. This could represent a potential proof, like the ones claimed by Gallup and Anderson (2018), that wolves have a sort of self-understanding, which may be a proper sign of self-awareness: wolves never displayed a scent-roll on self-urine canisters after smelling them, showing that they are aware of the “not me/other” odors that they want to collect from (or cover in) their environment (Fig. 5). Furthermore, had the mark been the only attractive element, they should not have rolled on the unmarked dog and non-partner wolf’s odors.

Recognizing a change in one’s own and well-known “reflection” (both visual and olfactory) represents an important clue of self-understanding, which could be a proxy of actual self-awareness (for instance, Cattelan et al. 2017, while testing guppies with a mirror, added the odor of a conspecific to the stimulus tank in order to improve the effectiveness in simulating a live conspecific and, thus, measure the ability to discern themselves from the others in a social group). Dogs spend more time smelling themselves and their companions after the olfactory test (Cazzolla Gatti 2016), which is similar to how other animals do with their own and other individuals’ image after the visual mirror test (Reiss & Marino 2001; Plotnik et al. 2006; De Waal 2008). Wolves – by scent-rolling – may have showed an evident mark-directed behavior (Fig. 4) that

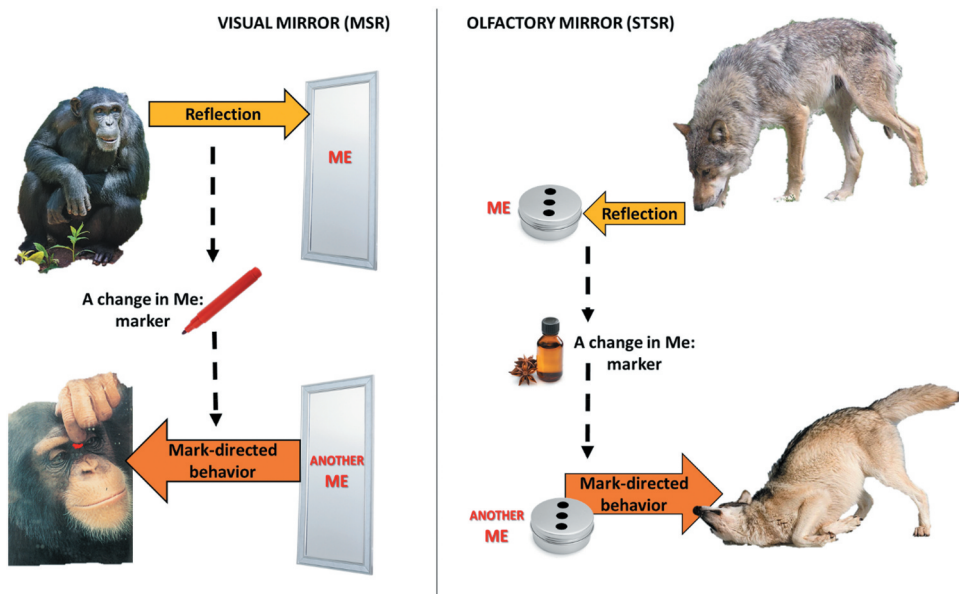


Fig. 5. — The analogies for the mark-directed behavior between the mirror (MSR) and sniff (STSR) tests. The physical touch of a “change” in one’s own image in the visual test may correspond to the rolling over a “change” in one’s own smell in the sniff test. Both mark-directed behaviors represent self-investigations where the animal interacts with its own body after collecting information about itself through species-specific sensory modalities. To be a valid proof of self-recognition, the mark-directed act in the smell test is mainly displayed on self-modified “olfactory reflections” (until the subject loses interest in this change after a certain interaction time). It may also be shown on partner-modified or unknown subjects’ “olfactory reflections” but never on self and well-known partner’s “olfactory reflections”.

could be interpreted as an “other-directed” (including “another-me-directed”; Fig. 5) response, possibly analogous to the “self-directed” act shown with other animal species in the mirror test. This similarities seems reasonable in an olfactory test because, after sniffing the canisters, a “handless” animal has only two ways to respond with a mark-directed act: (1) smelling itself (as dogs do) or (2) taking the smell of others on itself/leaving its smell on others (as wolves do). However, the mark/other-directed scent-rolling with this sample of four wolves is just preliminary evidence based on a still unclear behavior showed by canids and other predators and should be interpreted with cautions until further investigated with a larger number of animals and statistical analysis appropriate for bigger samples. Differently from dogs, wolves do not usually smell themselves, even in natural conditions but they often roll over carcasses, feces and urine of conspecifics or other animals (Harrington et al. 2003). Some authors believe that small canids use the scent left by large predators as a form of odor camouflage, to hide from other big predators (Allen et al. 2017). However, this does not explain why larger canids, such as wolves, also rub themselves in the scent left by others, particularly other predators. One explanation is that wolves and other predators are instead depositing their own scent, rather than picking up the scent. Or, this behavior might be a way to gather information about where they have been and

bring it to the rest of the pack (Mertl-Millhollen et al. 1986). This seems to suggest a social function for the scent-rolling and may explain why tested wolves in this study never rolled on their own or companion wolves' urine unless they were modified with the mark. Some authors have noticed that in the wild some alpha canids tended to be the first to roll in a strong scent followed by the others (Gadbois & Reeve 2014), which suggests that scent-rolling could be useful to establish a sort of "group smell", something to share with all the others in the group. This could be the reason why only the α -male (Kanti) and α -female (Fiona), in the group of four tested wolves in our study, rolled over "stranger" odors in the full set of trials, whereas β -wolves (Woton and Timber) rolled only on their own modified odor and the mark by itself. This odor-sharing behavior, which seems to increase the sense of "togetherness" in other canids (Sillero-Zubiri & Macdonald 1998), offers evidence for the kind of "social-awareness" coming from our study.

Other studies have suggested that scent-rolling may also be used by canids to collect pungent odors, analogous to human perfume (Fox 2007). For instance, a group of captive wolves studied in Canada was less interested in rolling over the feces of herbivores or over food than over artificial odors like perfume or motor oil (Ryon et al. 1986). This is in line with the tendency shown in our experiment of post-sniffing scent-rolls over the urine samples marked with anise oil (Table 1 and Fig. 4). However, it seems an odd behavior for animals seeking either to camouflage from their prey or to increase social interconnection, to adopt the smell of something uncommon in their habitat.

May wolves have multiple reasons for scent-rolling? The fact that, in our study, wolves rolled not only on marked samples but also on those unmarked of the dog and the non-companion wolves (Table 1 and Fig. 4) may be considered a preliminary indication that the scent-rolling behavior might have multiple functions: mimicry, sociality, and identity.

In any case, when more light will be shed on this interesting behavioral manifestation and if the abovementioned hypotheses about its function will be confirmed, the display of a scent-rolling in the sniff test of self-recognition (STSR) with some species of canids might be considered an equivalent of the mark touching in the mirror test because our results indicate that wolves may be able to recognize something that is "non-self, non-familiar and non-social". In other words, they seem aware of what is "me" and what is "you", in addition to differentiating from what is "mine" from what is "yours" and what is "familiar" from what is "unfamiliar". In this way, they may be capable of thinking about themselves and use their own experience to make inferences about comparable experiences in others (Gallup & Anderson 2019).

Besides the scent-rolling over specific canisters, the two female wolves, Fiona and Timber, displayed some other post-sniffing behaviors that, although difficult to interpret at this stage, represent interesting patterns that it is worth discussing them (Table 1). For instance, scent-marking was shown only on the mark and the dog samples. This might not be accidental since both the mark and the dog represented heterospecific odors to wolves. This behavior might be a signal of species specific-awareness (Sillero-Zubiri & Macdonald 1998; Bekoff 2002). Differently, creeping leaves and soil over the samples was shown only on the dog canister, the unknown wolf's odor and its marked sample. Again, it might not be fortuitous because only the dog and the non-partner wolf's odor represented unknown odors. Instead, it might be an evidence of social-awareness, which seems an additional consciousness level besides self-awareness (Packard 2012).

CONCLUSIONS

In this study, we adopted a similar paradigm to previous approaches using a sniff-test (Cazzolla Gatti 2016; Horowitz 2017) but we included additional controls to shed light on the fact that, if wolves could identify the source of their own odor, they should show a mark-directed behavior. We also checked whether wolves showed the same response to comparable changes in other familiar odors, such as that of another companion wolf and displayed mark-directed responses after smelling those samples. We reported some common patterns: (i) the longer time spent smelling their own odor modified compared to their pure urine, on the first instance; (ii) the improved ability to recognize the self-modified odor after smelling it once; (iii) the higher interest in the partner-modified odor than in both their own and the partner's pure urines despite habituation to them in subsequent trials; (iv) the general inflated attention towards non-companion conspecific and heterospecific odors compared to that of themselves and partners; (v) the evident mark-directed behavior towards all other-than-self odors after smelling them.

Although the sample of tested wolves in this research is limited to four adult individuals, this represents the first-ever attempt to test self-awareness in wolves with a different sensory approach. Our relatively small sample size was due to the impossibility to apply the sniff-test on a wild pack, the limitation in the availability of a high number of captive wolves in wolf centers and zoos worldwide, and the difficulties to collect urine samples from young wolves because of the absence of marking behavior in the youths. Nevertheless, previous attempts to check for self-awareness with the mirror test with a few (and sometimes single) individuals of different species (Appendix 1) provided fundamental clues to better interpret animal minds. Adding other evidence to the sniff-tests for self-recognition (STSR) from canids and other species in future via replicated studies would increase our understanding of self-awareness in animals. Eventually, we may build an idea of “I think, therefore I am”, the Cartesian “*cogito ergo sum*”, with a smell rather than an image, potentially extending the notion of self-awareness beyond the primates (and a few other species) dominion.

ACKNOWLEDGEMENTS

The authors are thankful to Jacob Kose for his precious work in improving the narrative and language clarity of the manuscript sections and to three anonymous reviewers who helped us with their suggestions to better address results and discussion presentation. Authors are also grateful to the Wolf Park in Lafayette, Indiana (USA) and its animal keepers, particularly to Monty Sloan and Pat Goodmann, who supported the experimental phases with professionalism, ensuring animal safety and wellness.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

FUNDING

No funding to declare.

ETHICAL STANDARD

The authors declare that the animals used in this study have been not harmed by the experiments and have been subject to ethical treatments and that this research followed to the ASAB and ABS Guidelines for the Use of Animals in Research.

AUTHOR CONTRIBUTIONS

R. Cazzolla Gatti conceived the test, designed the study, and wrote the paper. R. Cazzolla Gatti, A. Velichevskaya and K. Davis performed the test and collected the data. K. Davis managed animals and the testing facilities and ensured animal welfare. R. Cazzolla Gatti and A. Velichevskaya analyzed the data. A. Velichevskaya reviewed previous studies to prepare the [APPENDIX 1](#). B. Gottesman revised the manuscript and provided support to the discussion of results.

SUPPLEMENTAL DATA

Supplemental data for this article can be accessed at <https://doi.org/10.1080/03949370.2020.1846628>

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APPENDIX 1.
SELF-AWARENESS TESTS (MIRROR AND SNIFF) CONDUCTED SO FAR ON DIFFERENT SPECIES

Title	Authors	Journal reference	No. of animals tested	Species	Passed test (Y/N)
Chimpanzees: self-recognition	Gallup G.G.	Science, 1970, Vol. 167, Issue 3914, pp. 86-87	4: 2 M and 2 F (preadolescent)	Chimpanzee (<i>Pan troglodytes</i>)	Y
Self-recognition in an Asian elephant	Plotnik J.M., de Waal F., Reiss D.	PNAS, 7 November 2006; 103(45), pp. 17,053-17,057	4: 2 pairs of adult F	Asian elephant (<i>Elephas maximus</i>)	Y
The responses of bonobos (<i>Pan paniscus</i>) to their mirror images: Evidence of selfrecognition	Westergaard G.C., Hyatt C.W.	Human Evolution, 1994, Volume 9, Issue 4, pp. 273-279	9: 4 M and 5 F	Bonobo (<i>Pan paniscus</i>)	Y
"Self-Awareness" in the pigeon	Epstein R., Lanza R.P., Skinner B.F.	Science, 1981, Volume 212, pp. 695-696	3	Pigeon (<i>Columba livia domestica</i>)	Y
Mirror self-recognition in the bottlenose dolphin: A case of cognitive convergence	Reiss D., Marino L.	PNAS, 2001 May, Vol. 98, N. 10, pp. 5937-5942	2 M	Bottlenose dolphin (<i>Tursiops truncatus</i>)	Y
Ontogeny of mirror behavior in two species of great apes	Robert S.	American Journal of Primatology, 1986, Vol. 10, Issue 2, pp. 109-117	2: 1 F chimpanzee and 1 M orangutan	Chimpanzee (<i>Pan troglodytes</i>); orangutan (<i>Pongo pygmaeus</i>)	N
Mirror-induced behavior in the Magpie (<i>Pica pica</i>) Evidence of self-recognition	Prior H., Schwarz A., Gunturkun O.	PLoS Biology, 2008, Vol. 6, Issue 8, pp. 1642-1650	5 adults	Magpie (<i>Pica pica</i>)	Y?
Contingency checking and self-directed behaviors in giant manta rays: Do elasmobranchs have self-awareness?	Ari C., D'Agostino D.P.	Journal of Ethology, 2016, Vol. 34, Issue 2, pp. 167-174	2	Giant manta ray (<i>Manta birostris</i>)	Y/N

Are ants (Hymenoptera, Formicidae) capable of self-recognition?	Cammaerts M.-C., Cammaerts R.	Journal of Science Zoology, 2015, Vol 5, Issue 7, pp. 521–532	1 to 4 queens, brood (larvae, nymphs) and about 500 workers	Ants (<i>Myrmica sabuleti</i> , <i>Myrmica rubra</i> , <i>Myrmica ruginodis</i>)	Y/N
Self-recognition in chimpanzees (<i>Pan troglodytes</i>): Distribution, ontogeny, and patterns of emergence	Povinelli D.J., Rulif A. B., Landau K.R., Bierschwald D.T.	Journal of Comparative Psychology, 1993, 107(4):347–72.	105	Chimpanzee (<i>Pan troglodytes</i>)	Y
Reactions of a group of pygmy chimpanzees (<i>Pan paniscus</i>) to their mirror-images: Evidence of self-recognition	Walraven V., van Elsacker L., Verheyen R.	Primates, 1995, 36(1): 145–150	7: 3 adult F, 1 adult M, 2 subadult M, and 1 infant M	Pygmy chimpanzee (<i>Pan paniscus</i>)	Y/N
An 8-year longitudinal study of mirror self-recognition in chimpanzees (<i>Pan troglodytes</i>)	de Veer M.W., Gallup G.G., Theall L.A., van den Bos R., Povinelli D.J.	Neuropsychologia, 2003, Volume 41, Issue 2, pp. 229–234	92	Chimpanzee (<i>Pan troglodytes</i>)	Y
Long-term retention of self-recognition by chimpanzees	Calhoun S., Thompson R.L.	American Journal of Primatology, 1988, Vol. 15, Issue 4, pp. 361–365	2 youths	Chimpanzee (<i>Pan troglodytes</i>)	Y
Failure to find self-recognition in Asian elephants (<i>Elephas maximus</i>) in contrast to their use of mirror cues to discover hidden food.	Povinelli D.J.	Journal of Comparative Psychology, 1989, Vol 103(2), pp. 122–131	2 adults	Asian elephants (<i>Elephas maximus</i>)	N
Self-recognition in pigeons revisited	Uchino E., Watanabe S.	Journal of the Experimental Analysis of Behavior, 2014, 102 (3), pp. 327–334.	2	Pigeon (<i>Columba livia domestica</i>)	Y

(Continued)

(Continued)

Title	Authors	Journal reference	No. of animals tested	Species	Passed test (Y/N)
Giant pandas failed to show mirror self-recognition	Ma X., Jin Y., Luo B., Zhang G., Wei R., Liu D.	Animal Cognition, 2015, 18 (3), pp. 713–721.	34: 18 F and 16 M juveniles, sub-adults and adults	Giant panda (<i>Ailuropoda melanoleuca</i>)	N
Responses of gibbons (<i>Hylobates lar</i>) to their mirror images	Hyatt C.W.	American Journal of Primatology, 1998, 45 (3), pp. 307–311	9 gibbons (<i>Hylobates lar</i>) and 1 gibbon-siamang crossbreed	Gibbon (<i>Hylobates lar</i>)	N
Rhesus monkeys (<i>Macaca mulatta</i>) do recognize themselves in the mirror: Implications for the evolution of self-recognition	Rajala A.Z., Reiningger K.R., Lancaster K. M., Populin L.C	PLoS ONE, 2010, 5 (9): e12865	5 M	Rhesus monkey (<i>Macaca mulatta</i>)	Y
Black-and-White Colobus monkeys (<i>Colobus guereza</i>) do not show mirror self-recognition	Shaffer V.A., Renner M.J.	International Journal of Comparative Psychology, 2000, 13, pp. 154–160.	5	Black and white colobus monkey (<i>Colobus guereza</i>)	N
Mark tests for mirror self-recognition in capuchin monkeys (<i>Cebus apella</i>) trained to touch marks	Roma P.G., Silberberg A., Huntsberry M.E., Christensen C.J., Ruggiero A.M., Suomi S.J.	American Journal of Primatology, 2007, 69 (9) pp. 989–1000	3	Capuchin monkey (<i>Cebus apella</i>)	N

Mirror-mark tests performed on jackdaws reveal potential methodological problems in the use of stickers in avian mark-test studies	Soler M., Pérez-Contreras T., Peralta-Sánchez J. M.	PLoS ONE, 2014, 9 (1): e86193	9 adults	Jackdaw (<i>Corvus monedula</i>)	N
Factors affecting mirror behaviour in western lowland gorillas, <i>Gorilla gorilla</i> .	Shillito D.J., Gallup G. G. Jr, Beck B.B.	Animal Behaviour, 1999, 57 (5), pp. 999–1004	2	Western lowland gorilla (<i>Gorilla gorilla</i>)	N
Failure to demonstrate self-recognition in gorillas	Ledbetter D.H., Basen J.A.	American Journal of Primatology, 1982, 2 (3), pp. 307–310	2	Gorillas	N
Mirror Self-Recognition in a Gorilla (<i>Gorilla gorilla gorilla</i>)	Allen M., Schwartz B. L.	Journal of Integrative Biosciences, 2008, Vol 5, Issue 1, pp. 19–24	1 M	Western lowland gorilla (<i>Gorilla gorilla</i>)	Y
Another gorilla (<i>Gorilla gorilla gorilla</i>) recognizes himself in a mirror	Posada S., Colell M.	American Journal of Primatology, 2007, 69 (5), pp. 576–583	1	Western lowland gorilla (<i>Gorilla gorilla</i>)	Y
Observations on the behavior of gibbons (<i>Hylobates leucogenys</i> , <i>H. gabriellae</i> , and <i>H. lar</i>) in the presence of mirrors	Ujehvi M., Merker B., Buk P., Geissmann T.	Journal of Comparative Psychology, 2000, 114 (3), pp. 253–262	3	Gibbons (<i>Hylobates leucogenys</i> , <i>H. gabriellae</i> , and <i>H. lar</i>)	Y/N
Chimpanzees recognize themselves in mirrors	Povinelli D.J., Gallup G.G. Jr, Eddy T.J., Bierschwald D.T., Engstrom M.C., Perilloux H.K., Toxopeus I.B.	Animal Behaviour, 1997, 53, pp. 1083–1088	7	Chimpanzee (<i>Pan troglodytes</i>)	Y

(Continued)

(Continued)

Title	Authors	Journal reference	No. of animals tested	Species	Passed test (Y/N)
The monkey in the mirror: Hardly a stranger	de Waal F.B.M., Dindo M., Freeman C.A., Hall M.J.	PNAS, 2005, 102 (32), pp. 11,140–11,147	14: 8 adult F and 6 adult M	Brown capuchin monkey (<i>Cebus apella</i>)	Y/N
Mirror image reactions in the oval squid <i>Septoteuthis lessortiana</i>	Ikeda Y., Matsumoto G.	Fisheries Science, 2007, Volume 73, Issue 6, pp. 1401–1403	9 adults	Oval squid (<i>Septoteuthis lessortiana</i>)	Y/N
Are horses capable of mirror self-recognition? A pilot study	Baragli P., Demuru E., Scopa C., Palagi E.	PLoS ONE, 2017, 16;12(5): e0176717	4	Horses	Y/N
A social cichlid fish failed to pass the mark test	Hotta T., Komiyaama S., Kohda M.	Animal Cognition, 2018, 21(1), 127–136	9: 5 M and 4 F	Tanganyikan cichlid (<i>Neolamprologus pulcher</i>)	N
Mirror image processing in three marine mammal species: killer whales (<i>Orcinus orca</i>), false killer whales (<i>Pseudorca crassidens</i>) and California sea lions (<i>Zalophus californianus</i>)	Delfour F., Marten K.	Behavioural Processes, 2001, 53(3), 181–190	5 killer whales: 3 F and 2 M 2 false killer whales: 1 M and 1 F 3 sea lions: 1 M and 2 F	Killer whales (<i>Orcinus orca</i>), false killer whales (<i>Pseudorca crassidens</i>) and California sea lions (<i>Zalophus californianus</i>)	Y/N
Self-consciousness: beyond the looking-glass and what dogs found there.	Cazzolla Gatti R.	Ethology Ecology & Evolution, 2016, 28(2), 232–240.	4: 1 M and 3 F	Dogs (<i>Canis familiaris</i>)	Y
Smelling themselves: dogs investigate their own odours longer when modified in an “olfactory mirror” test	Horowitz A.	Behavioural Processes, 2017, 143, 17–24	36	Dogs (<i>Canis familiaris</i>)	Y

Elephants know when their bodies are obstacles to success in a novel transfer task.	Dale R., Plotnik J.M.	Scientific Reports, 2017, 7(1), 1–10	12: 7 young and 5 old	Elephant	Y/N
That dog won't fit: body size awareness in dogs.	Lenkei R., Faragó T., Kovács D., Zsilák B., Pongrácz P.	Animal Cognition, 2019, 1–14	32: 16 M and 16 F	Dogs (<i>Canis familiaris</i>)	Y
Snakes <i>Elaphe radiata</i> may acquire awareness of their body limits when trying to hide in a shelter.	Khvatov I.A., Sokolov A. Y., Kharitonov A.N.	Behavioral Sciences, 2019, 9(7), 67	20	Snake (<i>Elaphe radiata</i>)	Y