

# A First Case Report Of A Wolf Pup's Responses To A Mirror

Matheus de Mesquita Silveira<sup>1,\*</sup>, Lori Jean Schmidt<sup>2</sup>, Heloísa Allgayer<sup>3</sup>, and Shannon Michelle Barber-Meyer<sup>2</sup>

<sup>1</sup>Vale do Rio dos Sinos University, Graduate Program in Philosophy, São Leopoldo, RS, Brazil.
<sup>2</sup>International Wolf Center, Ely, MN 55731, USA
<sup>3</sup>Custom Code Management BTP ABAP, SAP Labs Brazil, São Leopoldo, Brazil

\*Corresponding author (Email: mdm.silveira@gmail.com)

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Abstract – Mirrors are an efficient tool for assessing a wide range of cognitive abilities in different species, either as a problem-solving tool or for self-recognition. Research on mirrors with canids is limited, with the existing studies focusing on domestic dogs (*Canis lupus familiaris*) and adult wolves (*Canis lupus*). Despite the central role of visual communication for the species, a recent study showed that wolves over 14 years old do not react to mirrors. Reports exist of dogs under three months old reacting to their reflection, but there are no data on how wolf pups react to mirrors. Given the close genetic relationship between dogs and wolves, it is relevant to document whether wolf pups will respond to their reflection. Therefore, the response to a mirror of a captive wolf (*C. l. occidentalis*) less than three months old was observed over four acclimation sessions with a covered reflective surface and six sessions with an uncovered mirror. The pup initially reacted to her reflection with a *flight* response. A behavioral change was documented over the sessions, the intensity of her negative reactions decreased, and her interest and relaxation. Throughout the observations, the intensity of her negative reactions, presenting a more confident stance with focused paw testing and sniffing at the reflective surface. Further replication with a larger sample will certainly provide a better understanding of how wolves react to mirrors, but the present case report is the first to document a wolf pup's reaction to its mirror image.

Keywords - Behavioral response, Mirror reactivity, Visual stimuli, Wolf pup.

The analysis of animal responses to mirrors is central to discussions in cognitive sciences. Initially associated with mark test experiments on mirror self-recognition with primates (Gallup, 1970), mirrors have been used as a methodological tool in research with distinct species (Calmette & Meunier, 2023; Horn et al., 2016; Reiss & Marino, 2001; Vanhooland et al., 2020). Beyond the initial scope of such research, mirrors have been valuable in comparative and developmental studies for eliciting and documenting relevant self-induced behaviors (e.g., self-directed reactions, social responses) in various species that typically do not show self-recognition (Bekoff, 2014; Catellan et al., 2017; Henry et al., 2008; Miklósi, 1999). Therefore, the use of mirrors in experimental and ethological observations has proven pivotal to understanding behavioral reactions triggered by visual stimuli (Bekoff et al., 1994; Frommen, 2021; Josi & Ristau, 2013).

Previous studies have demonstrated a wide range of reactions of different species to mirrors. When initially exposed to their reflections, animals tend to react to their mirrored image as a conspecific (Plotnik et al., 2006). For example, primates (*Primates*) have demonstrated a wide range of initial reactions, such as

fear-avoidance (Spencer-Booth & Hinde, 1969), anxiety (Buss, 1980), submission (Anderson, 1984; Anderson & Gallup, 2015), and interest (Lethmate & Dücker, 2010). Although self-recognition has been documented in different primate species, the initial reactions were as if the reflection was a conspecific or a "strange object." Corvids (*Corvidae*) presented initial aggressive displays to mirrors by jumping toward their reflection aggressively, and the agonistic social displays did not decrease in frequency over time (Medina et al., 2011; Prior et al., 2008). Giant pandas (*Ailuropoda melanoleuca*) of all ages also presented aggressive reactions toward their self-images, indicated by threatening, foot scraping, and backward walking behaviors when exposed to the mirror. However, their interest was quite limited, with subjects spending 80% of their time in non-mirror-related behaviors (Ma et al., 2015). In relation to canids, both adult dogs (*Canis lupus familiaris*) and pups demonstrated initial playful behaviors (jumping, barking, or trying to catch their mirrored reflection) with fast habituation to the mirror (Anderson & Gallup, 2015; Siwak, 2001; Zazzo, 1979). However, observations with adult wolves (*Canis lupus*) have not documented any behavioral reaction to their reflections (Barber-Meyer & Schmidt, 2020), and there are no reports of mirror studies with wolf pups.

The use of mirrors to observe behavioral reactions (e.g., selective attention, the interpretation of visual stimuli, proprioception, etc.) is methodologically relevant in the study of animal minds (Reiss & Morrison, 2017), but its application to wolves has raised questions (Barbar-Meyer & Schmidt, 2020). However, whereas different species exhibit positive (playful behaviors in dogs) and negative (fear and anxiety in primates, or aggression in corvids) reactions to their reflections, there are no reports of wolves exhibiting mirror reactivity in any manner (Barber-Meyer & Schmidt, 2020)<sup>1</sup>. The present study extends prior research by observing how a wolf pup reacts to a mirror, focusing on ethological behaviors in a naturalistic environment (see Barber-Meyer & Schmidt, 2020, for details on juvenile and adult wolf behavioral reactivity to a mirror).

The present study aimed to document a wolf pup's reaction to a mirror and its potential change over time, focusing on behavioral categories associated with positive (interest, relaxation, playfulness) and negative (fear-avoidance, anxiety, submission) responses (Prinz, 2004; Silveira, 2019; Silveira & Sulich, 2021), as initial reactions to mirrors in various species consist of either positive or negative behaviors (Anderson, 1984; Anderson & Gallup, 2015; Buss, 1980; Lethmate & Dücker, 2010; Ma et al., 2015; Medina et al., 2011; Prior et al., 2008; Siwak, 2001; Spencer-Booth & Hinde, 1969; Zazzo, 1979). It was noted that, based on the present report that only assessed one animal, it is not possible to infer any conclusions about the existence or absence of self-recognition in wolves (Anderson, 1994) or to generalize the observed behaviors as the species' standard. However, documenting such interactions contributes to a better understanding of the role of mirror reactivity in wolves (Bekoff & Sherman, 2004; Brandl, 2016), thus enhancing the understanding of how self-directed visual stimuli influence their behavior. In this sense, the present report may assist future research on mirror reactivity in wolves.

## Methods

## **Ethics Statement**

Wolf management at the International Wolf Center (IWC) is authorized under the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) under Exhibit Permit number 41-C-0077. The IWC is not a breeding facility; therefore, any pups are acquired via permit from a cooperative USDA APHIS-licensed facility or Canada's Accredited Zoos and Aquariums (CAZA). Animal managing protocols, including noninvasive research procedures, are authorized under the guidelines of the Animal Welfare Act and detailed in the IWC's Wolf Care Manual. USDA APHIS officials

<sup>&</sup>lt;sup>1</sup> The study was conducted with five adult wolves aged between 5 and 13 years old and two juvenile wolves aged 1 year (six males and one female total). As mentioned in the research, the yearlings and adults had been exposed to reflective window surfaces before the study. Therefore, the authors acknowledge they could not observe the wolves' responses upon their *first* experience with reflective surfaces.

conduct annual spot inspections and review all manuals, medical records, behavioral logs, research protocols and facilities related to the captive wolf program. The current study was strictly observational and did not require formal ethical review, as no invasive procedures or manipulations were involved in the observations. Research activities adhered to the ethical standards outlined in the Animal Welfare Act and the IWC's Wolf Care Manual, ensuring the welfare of the subject throughout the study.

# Subject

The wolf pup (*C. l. occidentalis*) was born on May 23, 2021, at the Wildlife Science Center (WSC) in Stacey, Minnesota, USA. The pup, Rieka, was a singleton in the litter and was one generation removed from the wild in British Columbia, Canada. She was brought to the International Wolf Center (IWC) to begin her socialization protocol and later introduction to a pack with two adult male arctic wolves (*Canis lupus arctos*). The acclimation period featured interactions through a fence with two adult dogs and two adult wolves, and 24 hours per day, 7 days each week, interactions with the wolf care staff from the neonatal period until her relocation to the pack when she was 12 weeks old.

During this period, she was bottle-fed with Esbilac milk-replacer as her primary diet, and continued with part-time Esbilac supplements until 79 days of age. Meat was later added to her diet to attend to her nutritional needs.

For the socialization, two protocols were conducted through the fence: (i) periodic interactions with adult dogs to develop positive associations with novelty and facilitate her handling as adult; and (ii) daily interactions with the two adult wolves, in order to enable spontaneous approaches to develop pack bonding.

# Procedure

In the summer of 2021, starting when Rieka was 10 weeks old, we documented her reaction to her mirror reflection at the IWC, prior to which point she had never been exposed to a mirror or any reflective surface. Observations were conducted in her home environment to which she had been acclimated since the neonatal period. The mirror presented to Reika measured 87.6 cm tall × 58.4 cm wide reflective surface.

Rieka was acclimated to the testing environment, and to two adult wolves present at the IWC and all personnel since she was 19 days old. Cameras were introduced as novel stimuli at 30 days of age, when Rieka showed significant reactivity to a camera operator, with some bark-howling towards the photographer. At 37 days old she was no longer presenting reactions to either the photographer or the camera. The wolf care team decided to delay the mirror exposure until other novel stimuli had been introduced and she was acclimated with observation instruments (namely, facilities, cameras, and personnel). On August 2nd, 2021, at 72 days old, Rieka was exposed to the covered mirror and frame in the yard adjacent to the fence panels for the first time.

Four acclimation sessions (sessions 1 to 4) were conducted with a covered mirror behind a fence from August 2nd to August 4th, 2021 (Table 1). Each session lasted around 10 minutes, allowing Rieka to explore the observation area as specified by IWC management protocols to detect possible behavioral changes caused by personnel, artifacts, or the environment. From August 5th to August 8th, 2021 (sessions 5 to 10), six observation sessions were conducted with the mirror uncovered (behind the fence on the first day and in front of the fence on the following days). Sessions started when Rieka was guided to the mirror and ended when she stopped interacting with it. The mirror was removed after each session to prevent uncontrolled interactions. Rieka's reactions to the mirror were recorded using two video cameras and two cell phones positioned to capture behaviors from multiple angles (Table 1).

## Table 1

Outline of the Sessions

Session	Date and Time of Session	Condition	Video Recording Equipment (cameras and cellphones)	Frame Rate	Outline
1	August 2, 2021 - Morning		Panasonic HC-	60 frames / s	Mirror behind the fence, two cameras placed parallel to the mirror, one mounted on a tripod and the other held by staff sitting opposing the main - camera tripod with both less than 1 meter high. Three staff members stood by on the far-left side of the mirror, ready to intervene if needed for Rieka's safety
2	August 3, 2021 - Morning	Non- mirror	X1000		
3	August 3, 2021 - Afternoon		Canon EOS 6D mark II	60 frames / s	
4	August 4, 2021 - Morning				Salety.
5	August 5, 2021 - Morning	Mirror	Panasonic HC- X1000	60 frames / s	Mirror behind the fence in session 5 and in front of the fence in sessions 6-10, two cameras placed parallel to the mirror, one mounted on a tripod and
			Canon EOS 6D mark II 60 frames / s		the other held by staff sitting opposing the main camera tripod with both less than 1 meter high, two cellphone cameras held by staff diagonally to of the
			Samsung S9	30 frames / s	mirror. Three staff members stood by on the far-left side of the mirror, ready to intervene if needed for Rieka's safety.

*Note.* Frame rate measures the speed at which a number of frames that appeared in 1 s. The analysis used a rate of 60 frames per s to classify Rieka's reactions.

## **Behavioral Coding and Analysis**

Video analysis for behavior classification was performed frame by frame at 60 frames per s using Adobe Premiere Pro, ensuring precise classification of each behavioral response (Conway et al., 2021; Cucchiara et al., 2005; Evans et al., 1993). Time intervals were not used for behavior classification; instead, the analysis relied on identifying specific bodily reactions coded in a behavioral ethogram (Table 2), with the start and end of each reaction pinpointed at the exact frame where a change in the set of bodily movements was detected.

The analysis focused on six wolf behaviors grouped into three categories: negative (anxiety, fear, submission), positive (playfulness, interest, relaxation), and neutral (disinterest) (Goodman et al., 2002; Packard, 2003; Silveira & Sulich, 2021; Smith & Kirby, 2009). Behaviors were classified based on bodily signals like ear and tail positions, hackle intensity, walking stance, and resting posture, interpreted alongside environmental context (Carbyn, 1975; Fox et al., 1974; Goodman et al., 2002; Hobkirk & Twiss, 2024; Schenkel, 1947). For example, ears pricked (EP) with a tail between the legs (T4) and hackles raised (H1 to H4) signal fear-avoidance, while ears pricked (EP), tail held high (T1) to relaxed (T3) position, and exploratory behavior (EXP) indicate interest (Carbyn, 1975; Fox et al., 1974; Goodman et al., 2002; Schenkel, 1947).

# Table 2

## Classification of Wolf Behavioral Responses to a Mirror

Behavioral Response	Description	Ethogram code
Interest	The wolf makes an investigation of a subject, object, or environment, usually presenting ears pricked in an alert posture while showing an intense fixed gaze, often with wide-open eyes, with tail varying from held high above the plane of the back to around 45° to the ground depending on its confidence.	Explore (EX), Ears Pricked (EP), Stare (STA), Tail held high above the plane of the back (T1), Tail held with the back (T2), Tail carried below the level of the back (T3).
Playful	The wolf might appear aggressive but lack any threat or anxious display, usually presenting a bow shape by lowering the front part of its torso while keeping the hind part upright. It can display soft bites, stand up on its hind legs, and put its front legs on a subject or object, usually quiet and with an open mouth, or drop altogether to the ground from a moving, standing, or sitting position without external physical enforcement.	Bow (BW), Bite, Muzzle – Soft (BMS), Play sequence (PLSeq).
Relaxation	The wolf might smile broadly, showing the teeth in a relaxed, non-threatening manner, while presenting a tail either relaxed, wagging, perpendicular to the ground, brushing the hocks, or simply resting or sleeping.	Grin (GN), Tail carried perpendicular to the ground and brushing the hocks (T3.5), Tail wag (TW), Rest (RT), Rest, back (RB), Rest, curl (RC), Rest, side (RSD), Rest, sphinx – sprawled (RSS), Rest, sphinx (RSX), Yawn (YN).
Anxiety	The wolf yawns by opening its mouth wide while showing the white of the eyes, usually as an involuntary reaction. It can also show a tail perpendicular to the ground brushing the hocks, with ears pricked turned sideways or between the legs and touching the belly.	Ears pricked, turned sideways (EPTS), Tail carried perpendicular to the ground and brushing the hocks (T3.5), Tail tucked between the legs or touching the belly (T4), Whale eyes (WE), Yawn (YN).
Fear- avoidance	The wolf might jump back with its tail tucked between the legs and possibly touching the belly or wag quickly and tensely, showing raised hackles, fixed gaze with wide-open eyes while snarling, with ears alternating from pricked, pricked turned sideways to a ponytail or turned sideways slanted back.	Ears pricked (EP), Ears pricked, turned sideways (EPTS), Ears ponytail (EPT), Ears turned sideways, slanted back (ETSB), Hackles, low intensity (H1), Hackles, medium-low intensity (H2), Hackles, medium-high intensity (H3), Hackles, high intensity (H4), Jump (JP), Tail tucked between the legs or touching the belly (T4), Tail wag (TW).
Submission, Active	The subdominant wolf crouches, whines, paws, and licks at the muzzle of the dominant subject; the tail may wag weakly while tucked, often touching the belly.	Chin rest (CR), Lick (LK), Parallel walk (PW), Whine (WH), Tail tucked between the legs or touching the belly (T4), Tail wag (TW).
Submission, Obnoxious	The subdominant wolf shows elements of threat, often testing the rank and patience of a more dominant subject by whining, inhibited biting, pawing at, or even bumping, usually with ears either on an airplane, back or ponytail position, and tail between the legs and touching the belly.	Bite, inhibited (BI), Ears, airplane (EA), Ears, back (EB), Ears, ponytail (EPT), Tail tucked between the legs or touching the belly (T4), Whine (WH).
Submission, Passive	The dominant wolf initiates the submissive behavior in the subdominant, often growling or snarling to make it lie down, roll over on its back, flatten its ears, and raise a hind leg for inguinal presentation; it may stand over, snarl, or execute inhibited bites on the subdominant subject.	Bite, inhibited (BI), Ears, airplane (EA), Growl (GL), Inguinal presentation (IP), Snarl (SL), Roll (RL), Roll on back (ROB), Stand over (SOVR).
Disinterest	Standing, sitting, lying passively with no attention to particular stimuli, or leaving the environment where a subject or object was presented.	Grin (GN), Ignore (IG), Leave (LV), Kest, back (RB), Rest, curl (RC), Rest, side (RSD), Rest, sphinx – sprawled (RSS), Rest, sphinx (RSX), Sit (ST), Tail carried below the level of the back (T3), Wander (WN).

*Note.* The behaviors described and identified by the ethogram codes are the most observed. However, it should be noted that the respective behavioral reactions are not restricted to the ethogram codes presented and may include other combinations, which may vary in different ways, with the observation context being fundamental to the classification of behavioral responses (Goodman et al., 2002).

Only Rieka's spontaneous behaviors directly related to the mirror were considered in the analysis. Positive (interest, playful, and relaxation), negative (anxiety, fear-avoidance, and submission—either active, obnoxious, or passive), and neutral (disinterest) reactions were classified only when directly related to the mirror. Thus, reactions that have direct artifacts or wolf care staff influence (for example, personnel approaching the mirror or directly interacting with Rieka, feathers, and carcass stimuli in front or near the mirror) were discarded. If the influence of a personnel or artifact on her behavior were identified after its removal from the environment, then the following behaviors were also considered non-spontaneous and discarded. Also, behaviors in which Rieka showed disinterest in the mirror due to being interested in personnel or artifacts were excluded from the final analysis.

The duration of Rieka's reactions was calculated relative to the total session time rather than just the behaviors directed toward the mirror. Each behavior's duration was converted into a percentage related to the total session time, standardizing the data for cross-session comparisons. Non-spontaneous interactions were excluded from the analysis as it is unclear if the mirror specifically triggered these reactions, but they were included in the total session time to calculate the percentage of spontaneous mirror engagement. The tabulated data included corresponding percentages for each behavioral category, facilitating the analysis of Rieka's reactivity patterns concerning the valence of reactions and responses to the mirror across sessions.

#### **Description of Acclimation and Mirror Sessions**

During sessions 1 to 4 when the mirror was covered, Rieka briefly gazed at the back of the mirror, which displayed a glass surface similar to a window. Depending on the angle of light incidence, a small percentage of light could be reflected back (Ou, 2014). During this period, she presented no interest in the mirror's structure or investigated its surrounding area. The observations showed that her focus was on other stimuli (namely, personnel, branches, turkey feathers, bones, and grids) rather than the mirror's surface, structure, or the fence in front of it (Table 3). In sessions 5 to 10, when the mirror was not covered, Rieka's behavioral reactions to the mirror transitioned from anxiety and fear avoidance to interest and relaxation, with no signs of either submission or playfulness (Table 3). Notably, her reactiveness ranged from hesitation to engaging with strong anxiety to increasing confidence with focused interest (Goodman et al., 2002; Morrison & Reis, 2018).

# Table 3

Behavioral Classification of The Acclimation and Mirror Sessions

Session	Session duration	Behavior duration	Video	Ethogram code	Behavior interpretation	
1		01m48s	<u>1</u>	EBTS, RSX, GW	Neutral posture with no anxiety or fear-avoidance behavior and no interest in the glass surface.	
	10m47s	05m31s	<u>2</u>	EPTB, GW, ST, RSX, ET	Neutral posture with no anxiety or fear-avoidance behavior and no interest in the glass surface.	
		10m05s	<u>3</u>	EP, GB, T3.5, PW, ST, CO, EBTS	Neutral posture with no anxiety or fear-avoidance behavior, with little interest in the glass surface.	
2		00m14s	<u>4</u>	EBTS, T3.5, WK, WD, LV	Neutral posture with no anxiety or fear-avoidance behavior, exploratory disposition with no interest in the glass surface.	
	07m45s	02m47s	<u>5</u>	EBTS, T3.5, GB, GW, PL, EP, ST	Neutral posture with no anxiety or fear-avoidance behavior, focused disposition with no interest in the glass surface.	
		05m54s	<u>6</u>	EBTS, GW, PL, GB, CO, LV	Neutral posture with no anxiety or fear-avoidance behavior, focused disposition with no interest in the glass surface.	
3	07m01s	02m19s	<u>7</u>	EBTS, RSX, SF, ST, PW, LV, EP, T3.5	Neutral posture with no anxiety or fear-avoidance behavior, curious disposition with some interest in the border of the fence and a little in the glass surface.	
		06m22s	<u>8</u>	EBTS, T3.5, SN, WK	Neutral posture with no anxiety or fear-avoidance behavior, with little interest in the border between the fence and the mirror's frame.	
4	16m51s	01m04s	<u>9</u>	EP, T3.5, GB, PL, EBTS, LK, GW	Neutral posture with no anxiety or fear-avoidance behavior, focused disposition with no interest in the glass surface.	
		06m43s	<u>10</u>	EP, T3, LK, SN, LV	Neutral posture with no anxiety or fear-avoidance behavior and no interest in the glass surface.	
		11m24s	<u>11</u>	EBTS, T3.5, GB, CO, LV	Neutral posture with no anxiety or fear-avoidance behavior and no interest in the glass surface.	
5	18m45s	00m10s	<u>12</u>	JP, ETS, T4, H4	Strong fear-avoidance response to the reflective surface.	
		07m00s	<u>13</u>	DG, EP, T3.5, PA	Shifted between fear-avoidance response and engaging hesitation to increased interest in the reflective surface.	
6	14m19s		14m30s	<u>14</u>	WK, EX, T4, EA, EP	Shifted between fearful response and engaging hesitation to increased interest in the reflective surface.
		00m25s	<u>15</u>	STA, PA, EA, EP, SF, T4	Fear-avoidance response with anxiety, and increased interest in the reflective surface.	
		06m00s	<u>16</u>	РА, ЕР, Т4	Fear-avoidance response with anxiety and standing interest in the reflective surface.	
		12m24s	<u>17</u>	STA, ETS, T3.5, SF, EX, STRHB, WM	Moderate levels of anxiety with increased interest in the reflective surface.	
7	10m14s	00m24s	<u>18</u>	STA, EP, WK, T4, SF, PA	Anxiety with increased interest in the reflective surface.	
		06m00s	<u>19</u>	STA, EP, BT, RSX, EB, EP	Relaxed stance without interest in the reflective surface.	
		09m55s	<u>20</u>	STA, PA, EP, SF	Relaxed stance with increased interest in the reflective surface.	
		00m03s	<u>21</u>	WK, T3, ETS, SF, EX, EP	Relaxed stance with increased intensity and interest in the reflective surface.	
8	10m03s	06m38s	<u>22</u>	EX, STA, T3, ETS	Relaxed stance with increased intensity and interest in the reflective surface.	
		09m28s	<u>23</u>	WK, STA, EP, ETS, T3	Relaxed stance with low intensity and interest in the reflective surface.	

		00m03s	<u>21</u>	WK, T3, ETS, SF, EX, EP	Relaxed stance with increased intensity and interest in the reflective surface.
9		01m24s	<u>24</u>	STA, WK, EP, T3.5, ST	Lower signs of anxiety and a moderate level of interest in the reflective surface.
	09m21s	06m23s	<u>25</u>	SF, EX, WK, T3, EP, ST	Relaxed stance with interest in the reflective surface.
		08m21s	<u>26</u>	STA, SF, EP, ETS, T3.5, T3	Lower signs of anxiety and small interest in the reflective surface.
		01m42s	<u>27</u>	WK, T3.5, EA, EP, STA, ST, YN, RTW	Relaxed posture with diminished anxiety and increased interest in the reflective surface.
10	14m39s	08m38s	<u>28</u>	SF, PA, EP, ETS, YN, WK	Increased interest in the reflective surface with a lower level of anxiety.
		13m12s	<u>29</u>	RSS, EB, MO	Relaxed posture with no interest in the reflective surface.

In session 1, Rieka presented a neutral behavior with no signs of fear-avoidance (Table 3, <u>video 3</u>). During session 2, she only glanced once at the reflective surface without exhibiting signs of either positive or negative engagement (Table 3, <u>video 5</u>). Although Rieka presented a curious disposition towards the fence's border in session 3, directing a quick stare at the mirror's glass surface (Table 3, <u>video 7</u>), she did not show any further reaction to the stimuli. The behaviors observed in session 4 indicated a neutral posture, without anxiety, fear-avoidance, or interest in the mirror's glass surface or its structure (Goodman et al. 2002).

Rieka's first mirror reactions in session 5 indicated a fear response (Table 3, video 12) followed by increasing anxiety (Table 3, video 13) with interest in the mirror. Her tail was tucked while the ears alternated between pricked and airplane position (Table, video 14), indicating interest with hesitation to engage in interactions. Notably, by the end of the session, she presented a significantly more relaxed body posture than in her previous interactions with the mirror (Goodman et al. 2002). In session 6, Rieka's reaction continued to be fearful (Table 3, video 15) with an intense anxiety response (Table 3, video 16) to the reflective surface, highlighting an engagement hesitation associated with a fear-avoidance response. Her body posture by the end of the session (Table 3, video 17) showed moderate levels of anxiety with increased interest in the mirror (Goodman et al., 2002). For the rest of session 6, her interest turned to other elements of the environment (a grid placed next to the mirror and a bone used by the wolf care staff to recover the grid), which were not triggered by her reflection.

In session 7, Rieka initially showed a tendency to physically investigate the reflective surface with her paw (Table 3, video 18) before alternating between non-mirror behaviors and inspection of the mirror and its structure (Table 3, video 19). It was the first time she presented a relaxed stance while standing in front of her mirrored image. She kept a relaxed disposition while staring at the mirror in the final part of the session (Table 3, video 20), showing an increased interest in the reflective surface (Goodman et al., 2002). In the first part of session 8, Rieka presented an exploratory drive, demonstrating interest in her reflection while inspecting the area around the frame (Table 3, video 21) in a more relaxed posture than noted in prior observations. Her interest in the mirror dropped significantly in the second part of the session, and she needed encouragement from the wolf care staff to return to the observation area. Her bodily stance indicated a relaxed posture (Table 3, video 22) and a lower interest in the mirror (Table 3, video 23) when compared to sessions 5 to 7, without signs of anxiety or fear-avoidance (Goodman et al., 2002).

Rieka's first reaction in session 9 was to explore the observation area before sitting without paying attention to the mirror (Table 3, <u>video 24</u>). Although her stance indicated signs of anxiety, the intensity was significantly lower than the ones presented in previous sessions. Her main focus was to physically inspect the reflective surface, either by sniffing the frame (Table 3, <u>video 25</u>) or investigating its surroundings (Table 3, <u>video 26</u>), showing low signs of anxiety with little interest in the reflective surface (Goodman et al. 2002). At the beginning of session 10, Rieka leaned her face against the mirror before yawning (Table 3, <u>video 27</u>), which can indicate either anxiety or sleepiness. Given the observational context, her behavior was interpreted as transitioning from relaxed with a low level of anxiety towards hesitation to engagement and interest. As observed in previous sessions, she physically inspected the mirror (Table 3, <u>video 28</u>)

before resting in front of the reflective surface (Table 3, <u>video 29</u>), shifting from curiosity with low levels of anxiety in her mirrored reflection to a relaxed posture without any interest in the reflective surface (Goodman et al. 2002). Rieka stayed laid down and did not exhibit any other mirror-related reactiveness for the remainder of the last session.

## The Valence of Rieka's Behavioral Responses

Examining the valence of Rieka's behavioral responses is critical to interpreting her engagement with the mirror. The positive valence of Rieka's reaction to the mirror unveiled the changing nature of her response to this visual stimulus. The behavioral data indicate a low positive valence engagement in session 5, that increased in subsequent sessions until it reached its peak at session 10 (Figure 1). Although in session 8 there was a slight decrease, in sessions 9 and 10 there was a resurgence in Rieka's positive reactions to the mirror. The pattern identified in positive valence reactions suggests a shift in her behavioral responses and how she apprehended the visual stimuli throughout the sessions.

# Figure 1



Spontaneous Behavioral Valence in Acclimation and Mirror Sessions

*Note.* In sessions 1-4 the mirror was covered, but in sessions 5-10 the mirror was not covered. The percentages are relative to the total session duration. For the remaining time of the sessions, Rieka displayed non-spontaneous behaviors; however, as specified in the methodology, only her spontaneous reactions to the mirror were analyzed.

Rieka's negative response in session 5 indicated her initial discomfort towards the mirror when she was first exposed to it. However, data show that negative reactivity declined in session 6 and remained stable in subsequent sessions (Figure 1). The observations indicate a habituation to the visual stimuli, as evidenced by the decrease in aversive responses. Therefore, the contrast indicates a shift in her responses from initial stress to relaxation, highlighting the adaptive nature of her reactions to the mirror.

In the acclimation sessions, a decrease in neutral valence from session 1 in relation to the latter sessions was observed (Figure 1). The variation can be attributed to Rieka's increased engagement with personnel and artifacts in sessions 2 to 4. The data indicate the neutral valence percentages are relatively close in sessions 5, 6, and 9 (Figure 1). Although such consistency can be related to the similar proportion of positive valence behaviors observed in sessions 6 and 9, session 5 is characterized by a higher percentage of negative valence behaviors. Therefore, the relationship between Rieka's interest and disinterest in the mirror was not directly influenced by the positive or negative valence of her reactions.

The acclimation sessions revealed only a subtle interest of Rieka in the covered mirror (Figure 2). She presented a neutral posture with no signs of discomfort. The data also indicate that Rieka's interest was

consistently low, as shown by her spontaneous disinterest during the acclimation sessions. Therefore, she was comfortable with the environment and did not perceive the mirror-related apparatus as relevant stimuli.

#### Figure 2



Spontaneous Behavioral Responses in Acclimation and Mirror Sessions

*Note.* In sessions 1-4 the mirror was covered, but in sessions 5-10 the mirror was not covered. The percentages are relative to the total session duration. For the remaining time of the sessions, Rieka displayed non-spontaneous behaviors; however, as specified in the methodology, only her spontaneous reactions to the mirror were analyzed.

Rieka did not show any initial positive interest in the mirror, or playfulness, in sessions 5 and 6, indicating a stressful initial first response to the mirror. The presence of anxiety and fear-avoidance behaviors were prominent throughout her early exposure to the mirror, while disinterest constituted a significant part of her response. These data indicate that her engagement with the reflective surface was initially marked by anxiety and fear-avoidance. However, as sessions progressed, her responsiveness showed a decline in anxious and fear-avoidance behavior with a discernible upward calmer stance (Figure 2).

Session 7 revealed a shift in Rieka's engagement with the mirror, as shown by her increased interest in her reflection. Also, her relaxed disposition signals a calmer stance during her interactions with the reflective surface. While her disinterest remained substantial, it was the lowest of the mirror sessions. Although negative reactions were still present, pointing to a brief reactiveness of discomfort towards the visual stimuli, the data show an overlap of interest and relaxation, indicating a positive behavioral disposition (Figure 2). The same pattern was observed in session 8, as interest represented the majority of Rieka's interactions with her reflection. Though lower than sessions 5, 6, and 9, Rieka's disinterest in session 8 was higher than that in session 7 and represents a significant portion of time spent near the mirror. Negative reactions were present to a lesser degree than in sessions 5 to 7, and the data also indicate a shorter relaxation period compared to the previous session. This suggests that active interactions were only partially mediated by a calm stance (Figure 2). The reduction of fear-avoidance behaviors and a decrease in anxiety reactions also suggest a habituation towards the mirror and an adaptative trend in her responses. While session 8 showed a slight decrease in Rieka's relaxation reactions, the following sessions presented a growing curve of positive interactions. Her disinterest increased from sessions 8 to 9 but was lower in session 10. These data indicate an active investigation, while her relaxed stance points out that the mirror no longer triggered strong anxiety or fear-avoidance behaviors (Figure 2). Such a continuum suggests that she no longer perceived her reflection as threatening, signaling that her discomfort was not related to perceiving the reflection as a threat to her physical integrity. Although disinterest was still present, data from session 10 highlight an active investigation of the reflective surface with a calm disposition, intercalated by short periods of anxious-driven responses in which Rieka remained close to the mirror (Figure 2). It is plausible to consider that the presence of anxiety combined with a standing disposition to stay in front of the reflective surface might indicate mental stress associated with contextual and visual apprehension. The observations substantiated the nature of Rieka's habituation to the visual stimuli, highlighting the importance of considering the time of exposure when investigating behavioral reactions triggered by mirrored images in canids.

Rieka's increased interest and relaxation responses were inversely correlated to anxiety and fearavoidance behaviors over time, with spontaneous disinterest varying over the sessions. Considering that only spontaneous disinterest is quantified in the analysis, the variation may be attributed to her nonspontaneous interest in personnel or artifacts. The data indicate that a decline in anxious responses from sessions 5 to 10 was correlated to an increase in relaxation, and as fear-avoidance diminished, her investigative drive increased (Figure 2). As she became more acclimated with her reflection, positive approaches emerged in opposition to initial negative withdrawals. Therefore, a relaxed engagement with the mirror emerged as fear-avoidance reactions diminished, underscoring the potential role of investigative behaviors in mitigating anxious responses through the sessions.

The absence of playfulness and submission might provide an insight into Rieka's perception of her mirrored image. Playful behaviors, typically associated with interactions among pack mates (Goodman et al., 2002), were absent during the entire observation period, as Rieka did not display any such behaviors (Figure 2). Submission in wolves is associated with recognizing a higher-ranking pack member or showing deference to an authority figure (Silveira & Sulich, 2021). Although submissive reactions are not found in all pups' interactions with each other, she presented a complete absence of submissive responses (Figure 2). Given that wolves have a narrow socialization window and show avoidance of non-related canids as they mature (Osada et al., 2015), the lack of submission does not exclude the possibility that Rieka did not perceive her reflected image as a dominant conspecific. Indeed, Rieka initially responded with *flight* or avoidance reactions (Goodman et al., 2002), but the data are not conclusive if her reaction is due to her perception of the image as a conspecific or to other factors. Therefore, the present study could not determine whether the hierarchy structure presented in wolf packs influenced her positive or negative interactions with the mirror.

# Discussion

The observation of Rieka's reactions to the mirror unveils insights into her behavior dynamics and contributes to the understanding of mirrored visual stimuli in non-domesticated canids. Three hypotheses are posited to account for Rieka's behavioral change: (i) habituation to the mirror, (ii) recognition that the mirrored image does not represent a real wolf, and (iii) development of self-confidence towards the unfamiliar canid reflection. The absence of playful and submissive responses undermines the third hypothesis. However, the exact perceptions and triggers for Rieka's behavioral shifts in relation to hypotheses (i) and (ii) remain undetermined. Her engagement with the reflection could stem from wolves' tendency to respond to visual cues (Range & Virányi, 2014) and their significant reactivity towards novel stimuli (Hansen Wheat et al., 2019). Her initial negative response might be attributed to the neophobic nature of wolves (Lurz et al., 2018), which would explain her anxious and fear-avoidance behaviors. Throughout the sessions, she repeatedly focused on the mirror, transitioning from negative reactions to observing the reflective surface with interest and, eventually, without evident fear.

It is plausible to assume that the primary factor likely influencing Rieka's transitional responses was acclimation, which might have alleviated her neophobic initial reactions. The data point to a behavioral variation from initial fear-avoidance and heightened anxiety to a gradual transformation marked by increased relaxation and focused interest, suggesting an adaptive curve in her responses to the reflective surface. However, it is prudent to interpret the data with caution due to the absence of previous research with wolf pups and the lack of significant controls in the present observations. In this regard, one possible pathway is to analyze Rieka's reactions in relation to other species to understand the similarities and differences in her responses compared to studies where such controls were employed.

Rieka's initial reactions were marked by fear-avoidance, in contrast to the playful behavior of dog pups and the investigative reactions observed in adult wolves. The difference between this wolf pup and dog pups may be attributed to the non-domesticated nature and early ontogeny of wolves (Feddersen-Petersen, 1991; Moretti et al., 2015; Woolpy & Ginsburg, 1967). Over time, Rieka's reactions changed from fear-avoidance and anxiety to interest and relaxation, while adult wolves tested by Barber-Meyer and Schmidt (2020) did not exhibit negative reactions and quickly transitioned from investigative behavior to disinterest. Similarly, dog pups tested previously demonstrated more reactivity to their mirrored reflections compared to adult dogs (Anderson & Gallup, 2015), paralleling the difference in behavioral responses reported for Rieka and adult wolves (Table 4). It is plausible that Rieka's initial fear-avoidance and anxiety behavior resulted from not being acclimated to the mirror, in contrast to adult wolves that might have habituated to reflections seem in water or glass windows. While further research is necessary to confirm these conjectures, the observations seemed to go against the hypothesis that prolonged exposure to reflective surfaces could increase canids' reactivity to mirrors (Gallup, 1970), with age and repeated exposure potentially influencing their responses.

#### Table 4

Species studied	Mirror Reactions Evolution	Factors Influencing Reactivity	Reference
Wolf (C. l. occidentalis), pup	From fear-avoidance and anxiety to interest and relaxation	Artifacts and personnel	Present study
Wolf ( <i>C l. arctos</i> and <i>C. l. occidentalis</i> ), adult	Sniffing, staring, and touching to disinterest and occasional glances	Not specified	Barber-Meyer & Schmidt, 2020
Dog (C. l. familiaris), beagles	Playful, cautious behaviors to habituation and disinterest.	Cognitive function and age	Anderson & Gallup, 2015

Reactions of Different Species to the Mirror

# Limitations

The IWC management protocols are designed to maintain a cohesive unit of wolves on display at the Center's Interpretive facility. Thus, pups are added approximately every four years, allowing enough time for the pack to be cohesive but not aging so much that their arrival would test the weaknesses of older animals. With this protocol, wolf care staff can keep the pack more cohesive and delay the retirement of the older wolves until 10 to 12 years of age (Cordoni, 2009; Rabb et al., 1967; Van Hoof & Wensing, 1987)<sup>2</sup>. Given that Rieka was a singleton, she was the only pup at the IWC during the study. Therefore, replicating the observations with other subjects was not feasible in the present study.

Wolf pups show a wide range of reactions to visual stimuli within the first 3-4 months (Lord, 2012), from interest and curiosity to fear-avoidance and anxiety (Boissy, 1995; Fentress, 1967). Reactions to visual

<sup>&</sup>lt;sup>2</sup> Leadership disputes are uncommon in wild wolf packs (Mech, 1999) but may occur in captivity (Cafazzo et al., 2016). Wild wolves disperse from their natal pack around age two to establish new territories. In captivity, wolves do not disperse, so stress and conflict may arise as younger wolves challenge older ones. Introducing pups every four years helps mitigate conflicts by shifting the pack's focus toward pup care and integration.

stimuli can begin at 13 days, though puppies only start recognizing shapes at 25 days (Fox, 1964; Scott & Fuller, 1965). From this point, visually guided behaviors gradually increase until around 6 weeks, when the central nervous system fully develops (Fox, 1968; Fox & Weisman, 1969). Intense manifestations of fear and anxiety in wolves occur because they are non-domesticated animals, and their *fight-or-flight* responses are tied to early ontogeny (Feddersen-Petersen, 1991; Moretti et al., 2015; Woolpy & Ginsburg, 1967). For instance, wolf pups display more intense anxious and fearful responses compared to dogs (Hansen Wheat et al., 2019; Lord, 2012; Marshall-Pescini et al., 2017). It is relevant to consider that, although Rieka interacted with dogs and adult wolves through a fence, her lack of exposure to other wolf pups as a singleton may have influenced her responses to the mirror.

Wolf pups between 8 to 10 weeks weigh 9 to 13 kilograms, enabling them to jump and run with considerable strength and intensity from a young age (MacNulty et al., 2009; Peterson et al., 1998). Wolves are neophobic (Moretti et al., 2015), and fear-avoidance behaviors can trigger fight-or-flight responses, so aggressive interactions with the mirror were a possibility. Such a response could break the mirror, potentially resulting in glass shards that could injure her before the wolf care staff could intervene. Considering that it was not possible to anticipate the nature and intensity of Rieka's initial reactions, the wolf care staff left the mirror behind the fence for safety reasons on the first day.

Observations on the first mirror session showed that Rieka's fear-avoidance reactions did not trigger aggressive responses but high levels of anxiety. Frustrated exploration can be stressful for investigatory species, and the provision of opportunities to explore tends to mitigate anxiety (Carlstead & Shepherdson, 2000; Mason, 2010). In this sense, exposure to novelty results in long-term familiarity, reducing stress triggered by non-threatening stimuli (Feddersen-Petersen, 1994; Hess, 1959; Scott, 1967). Thus, concerned that Rieka could develop a negative association with mirrored reflections, the personnel repositioned the mirror inside of the observational environment to allow a direct exploration for the rest of the study.

It is acknowledged that changes in the setup can influence the results. Considering Rieka's psychological health and future socialization (Frank & Frank, 1982; 1985), free-range ethological observations in a familiar environment were preferred to maintain her standard behavior. However, this approach presents a limitation, as the lack of controls and absence of direct comparisons to other stimuli make it challenging to draw definitive conclusions about Rieka's reactions to the mirror. The naturalistic setting introduced artifacts and interferences, highlighting the need for future studies with stricter experimental control protocols to determine the nature and causes of the observed behaviors.

Wolves are known to respond to visual, auditory, and olfactory stimuli from conspecifics, such as urination, growls, and bodily postures (Hobkirk & Twiss, 2024; Janik & Slater, 2000; Peters & Mech, 1975). Mirror reflections lack sound or smell, which could have triggered behavioral confusion in Rieka's perceptions: stress to unfamiliar stimuli contrasted to investigative drive. While the valence of her reactivity changed as sessions progressed (Figure 1), the frequency of interest in the mirror was related to a reduction in the intensity of her reactions in the final sessions (Figure 2). However, Rieka's behavior continued to indicate interest, even if for specific moments and not the overall session. Thus, the fact that she was a pup and more likely to respond to visual stimuli is relevant to our findings (Packard, 2003). Although the present study documented a wolf pup less than 3 months of age reacting to its reflection in the mirror, previous research indicates that wolves do not respond to their mirrored image by 14 months of age (Barber-Meyer & Schmidt, 2020). These findings do not rule out a potential developmental phase between 3 and 14 months, during which such responses may diminish or undergo significant behavioral changes. However, alternative explanations beyond age, such as the potential for habituation to mirror reflections over time. Therefore, further comparative studies on the evolution of mirror-related behaviors in both wolf pups and adults, as well as other canids, are recommended to provide a deeper understanding of this phenomenon.

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