Current Zoology, 2022, 1–9
https://doi.org/10.1093/cz/zoac013
Advance Access Publication Date: 23 February 2022
Article

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Don't stop me now, I'm having such a good time! Czechoslovakian wolfdogs renovate the motivation to play with a bow

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Handling Editor: Zhi-Yun Jia

Received on 29 December 2021; accepted on 20 February 2022

Abstract

Dogs engage in play behavior at every age and the play bow is their most iconic playful posture. However, the function of this posture is still under debate. Here, we selected the Czechoslovakian Wolfdog (CWD) as a model breed to clarify the function of the play bow. We analyzed frame-by-frame 118 sessions of 24 subjects and recorded 76 play bow events. We found that all the play bows were performed in the visual field of the playmate suggesting that the sender takes into account the attentional state of the receiver when releasing the signal. By drawing survival curves and using log-rank test we found that play bow was mainly performed during a short pause in an ongoing session and that its performance triggered the playmate's reaction again. These findings show that play bow functions in restoring the partner motivation to play. Finally, by using a sequential analysis and a generalized mixed model, we found no evidence supporting the metacommunicative function of the play bow. The signal did not necessarily precede a contact offensive behavior (e.g., play biting and play pushing) and it was not affected by the level of asymmetry of the play session. In conclusion, in CWDs play bow can be considered a visual signal useful to maintain the motivation to play in the receiver. Therefore, we suggest that the mismatched number of play bows emitted by the 2 players in a given session can be predictive of their different motivations to play.

Key words: *Canis lupus familiaris*, first-order intentionality, metacommunication, motivation, play fighting, wolf-like traits.

Play is a widespread behavior among mammals and it represents a fertile field in the study of animal communication (Palagi et al. 2016). We can distinguish different kinds of social play according to the presence of behavior patterns typical of other "serious" behavioral domains (e.g., nurturing offspring/play mothering; courtship and mating/sexual play; and real aggression/play fighting; Fagen 1981; Burghardt 2005; Pellis and Pellis 2009). Play fighting is the most common form of social play and it can involve competitive motor patterns that are borrowed by other behavioral spheres (Burghardt 2005; Palagi et al. 2019; Nolfo et al. 2021a). For this reason, play fighting needs sophisticated communicative skills to make the session successful and avoid escalation of aggression. To increase the agreement between players, animals have evolved identifiable signals that pervade the interaction (Palagi et al. 2016; Pellis and Pellis 2017).

Dogs, *Canis lupus familiaris*, frequently engage in play fighting at all ages (Bradshaw et al. 2015). The Relaxed Open Mouth (hereafter ROM) facial expression and the Play Bow posture (hereafter PBOW) are the most well-known signals reported in dogs (Bekoff 1995; Ward et al. 2008; Smuts 2014; Palagi et al. 2015; Byosiere

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et al. 2016a; Maglieri et al. 2022). The first report of the canid PBOW dates back to Darwin (1872), who recorded this posture while observing his dog playing. Darwin (1872) noticed that the same posture was also present in species closely related to dogs, such as wolves, coyotes, and foxes. More than a century later, Bekoff (1995) observed that cubs of many canid species play bowed in association with play biting and shaking. The author suggested that this peculiar and stereotyped posture could act as a play signal anticipating subsequent actions that could be misinterpreted during play fighting. In contrast, Pellis and Pellis (1996) stated that PBOW may not be a full-fledged signal, debating that the bow posture may simply put the performer in an advantageous position to attack the playmate or flee from it. In addition, other researchers did not find any evidence indicating that PBOW is either a signal used to clarify behaviors that could be misinterpreted or a posture to achieve an advantageous position in either dog puppies, wolf, and dingo cubs (Byosiere et al. 2016b, 2018), or adult dogs (Byosiere et al. 2016a). However, there is evidence that dogs tend to perform PBOW in the visual field of the playmate, suggesting that this posture may act as a visual signal (Horowitz 2009; Palagi et al. 2015; Byosiere et al. 2016a). Moreover, when the PBOW was performed out of the visual field of the playmate, or when the playmate was inattentive, attention-getting behaviors such as barking were concurrently emitted with the PBOW (Bekoff 1995; Horowitz 2009; Palagi et al. 2015).

Finally, some studies propose that adult dogs and puppies engage in PBOW to initiate a new play session or after a "brief pause" (Horowitz 2009; Byosiere et al. 2016a, 2016b). This seems to be not true for wolves and, at least in part, for dingo cubs (Byosiere et al. 2018). The "pause behaviors" included stationary positions, sitting and lying down, and all those behavioral patterns involving little movements. Byosiere et al. (2016a, 2016b) found that both bowers and partners were often in stationary positions before the performance of PBOWs and active after them.

To clarify the function of PBOW in dogs, we selected the Czechoslovakian Wolfdog (CWD) as a model breed. This recent breed derives from the crossing between the German Shepherd and the wild Carpathian Wolf in the second half of the twentieth century. The aim of this crossing was to select healthy and strong hard-working dogs with enhanced night and acuity vision. After the end of the Cold War the second phase of artificial selection that favored wolf-like morphological features began (Smetanová et al. 2015; Caniglia et al. 2018). Different from what occurred during the early wolf domestication phases (Range and Viranyi 2014), the CWD has never been selected for the expression of neotenic behavioral traits (e.g., low aggressive propensity and high levels of confidence). For this reason, the CWD is a valuable candidate to investigate the role of PBOW as a playful signal in a breed of dogs characterized by wolf-like behavioral traits.

Being that CWDs were mainly selected for their visual abilities (Smetanová et al. 2015; Caniglia et al. 2018), their communication should strongly rely on visual cues. Different from mixed-breed dogs, where the bower can bark when out of the receiver's field of view (Horowitz 2009), CWDs never bark during playful interactions thus suggesting that acoustic cues may not be effective in this context (Maglieri et al. 2022). Therefore, CWDs cannot enrich their PBOWs with acoustic cues to maximize the detection probability of this playful posture. For this reason, if PBOW is an effective visual signal (*Visual Signal Hypothesis*), we expect CWDs to perform it almost exclusively within the visual field of the playmate (Prediction 1).

Metacommunication hypothesis

If PBOW has a metacommunicative function and serves to change the meaning of a subsequent pattern, we expect that it mostly precedes contact offensive patterns (the riskiest playful patterns; Pellis and Pellis 2017) thus maintaining the playful mood of the partner (Prediction 2). If PBOW is effective in improving communication between players, we expect that those fighting sessions characterized by a high reciprocation of offensive patterns (i.e., balanced sessions) contain the highest number of PBOWs (Prediction 3).

Motivation hypothesis

If PBOW is used to invite a partner to start a play session, we expect PBOW to be mainly performed at the beginning of a session (Prediction 4). Moreover, if PBOW has a role in restoring the motivation to play in the partner during an ongoing session, we expect that PBOW is produced as a consequence of a delay of the playmate's reaction (indicating a loss of motivation in the partner) (Prediction 5) and that its emission triggers the playmate's reaction again (Prediction 6).

Materials and Methods

Video collection

Video data were collected by 2 observers on 24 subjects (12 females and 12 males) of pure-breed CWD (3 months to 6.5 years of age) from March 2016 to June 2017. The dogs were selected thanks to personal contacts, websites, and dog-expos. All the videos were recorded in environments familiar to the dogs (i.e., backyards and public gardens regularly frequented). A familiarization phase preceded the video collection. During this phase, the dogs were free to sniff the observers and interact with them. The observers took advantage of the familiarization period to interview the owners and collect information on the dogs (i.e., habits, possible ill-treatment, age, and cohabitation with other dogs). The video collection started when the dogs began to ignore the observers and spent at least 10 consecutive minutes without interacting with them.

All the dogs were pets, lived with families, and did not undergo any specific training. All the subjects were healthy dogs that were adopted at the same age (i.e., 2-months-old) and spent most of their time with their owners. For safety reasons, our sample included exclusively dogs sharing a certain degree of familiarity. Therefore, not all dogs had the opportunity to play together: 12 dogs could meet only one playmate (6 playing dyads), 6 dogs formed 2 groups of 3 subjects (5 playing dyads, because not all dogs engaged in dyadic play sessions), and 6 dogs formed 1 group (8 playing dyads, because not all dogs engaged in dyadic play sessions). This allowed having 19 dyads of dogs playing together.

The owners had to not interact with their dogs during data collection to avoid them influencing the activities engaged by the animals. The observers video-recorded in continuum the social interactions, including play. A total of 15 h of video recordings were gathered with the aid of 2 cameras (Sony HANDYCAM® DCR-SR32E and Canon EOS® 1100D).

Video analysis

PotPlayer® was employed to analyze the videos. The program allows catching the time of each behavioral pattern with an accuracy of 0.02 s. Before commencing the analysis, VM and a field assistant underwent a training period to acquire the competence for frame-by-frame video analysis (trainer EP). During video analysis, about

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20% of randomly selected videos were scored at regular intervals to calculate the inter-observer reliability (Kaufman and Rosenthal 2009). For each of the behavioral item included in the ethogram (Table 1), Cohen's kappa values never scored <0.85.

Following previous literature (Horowitz 2009; Palagi et al. 2015), we defined the duration of each play session. All the sessions analyzed were dyadic. A spontaneous play fighting session (hereafter, playful session) started when a dog engaged in a playful pattern toward another dog who responded in a congruent way. The end of the session corresponded with one of the dogs moving away or with the interruption/interference of a third subject on the ongoing session (in only 2 occasions we had >2 dogs present at the same time). A session was considered as new, if it began after 10 s from the end of the previous one. These scoring criteria make our data comparable with those of previous studies. For each playful session analyzed, we recorded the identity of the players, the exact sequence of each behavioral item (Table 1), and the exact duration (seconds) of each behavioral pattern. We were able to record 118 spontaneous playful sessions that never escalated into real aggression.

Based on the literature (Bekoff and Allen 1998; Smuts 2014), we defined PBOWs as follows: a dog crouches on its forelimbs, remains standing on its hind legs, and may wag its tail and sometimes bark. The bow is a stable posture from which the animal can move easily

Table 1. List of the behavioral items observed during play in the study group of CWDs

Behavioral pattern	Description
	Play signals
Full Play Bow	The dog crouches on its forelimbs with the elbow in contact with the ground and stands on its hind legs (Figure 2A)
Half Play Bow (*first report*)	The dog folds its forelimbs halfway with the elbow raised off the ground and stands on its hind legs (Figure 2B)
ROM	The mouth is relaxed and kept open at different gradients; the mouth can be opened (1) just a little revealing only the upper parts of the most forward teeth of the lower jaw and (2) in a wider way completely revealing the lower and upper jaws. The dog never closes its mouth even though it reaches the body/skin of the playmate. In most cases, the signaler does not close its mouth either when a body part of the playmate is in its mouth. The subject avoids touching with the mouth any part of the playmate's body
	Offensive patterns
Attempt to bite[C]	The dog (actor) moves its open mouth toward the playmate (receiver) closing it fast and touching or not the playmate's skin which, however, is never bitten
Jump over[C]	The dog (actor) jumps on another individual (receiver) by starting from a semi-crouching position (jump attempt) and making contact with both front paws on the body of the playmate, which can or cannot leap away; during the jumping attempt, the dog crouches on its forelimbs and remains standing on its hind legs for a while
Knock down[C]	The dog pushes down another individual on the ground
Play ambush[C]	The dog attacks (e.g., by jumping or pushing) suddenly and unexpectedly a fellow in a playful manner from a concealed position
PBIT[C]	The dog (actor) moves its open mouth toward the playmate (receiver) and gently grasp it without producing any injury
Play hold[C]	The dog holds another individual in place with its anterior paws
Play paw[C]	The dog (actor) paws on another's individual (receiver) body part
Play push[C]	The dog (actor) pushes another individual (receiver) with its body
Play run[L]	The dog chases another animal, usually with ears forward and not pilo-erect
Standing over[C]	The dog stands over the body of supine recipient with all 4 paws on the ground and the tail held high. The supine dog may have either the whole body or just the forepaws under the standing dog's belly/side; the 2 dogs do not move
	Self-handicapping patterns
Laying on back	The dog is laying on its back
Play squirming[C]	The dog shakes the body when in contact with the playmate
Rolling[L]	The dog rolls from side to side with its back on the ground
Turn on themselves[L]	During a Play run, the dog performs a twirl
	Neutral patterns
Jump[L]	The dog jumps during the play session
Object play	The dog plays with an object
Play confrontation[C]	The dog and its play partner stand on their hind legs and start pushing/hitting by using their forelimbs or snout. In this pattern, the offensive actions are performed by the 2 players concurrently in a bi-directional way thus making the pattern perfectly balanced and reciprocated.
Tug of war	The dog engages in "a tug of war" interaction with another individual. An object (e.g., ropes and balls) is pulled concurrently by the 2 subjects. This is a dyadic pattern. It is worth to note that when one of the 2 dogs releases the grip, the other does immediately the same.
Watching back[L]	While walking or running, the dog turns its head to watch the other animal
Play object sharing	The dog shares or makes another individual take an object (or part of it) during play
Play turn around[L]	Twirling with object
Play chewing	The dog chews an object
Go and return[L]	The dog approaches another individual and then flees back
Rough and Tumble[C]	This behavior involves physical contact between partners and may include motor actions typical of real fighting (e.g., biting, pulling, and knocking down). Being R&T a mixed interaction of patterns difficult to disentangle and categorize because not always clearly visible, we considered an R&T interaction as neutral pattern. It is worth noting that in this study, we had only one case in which it was not possible to clearly discern each single pattern

L = locomotor pattern; C = contact pattern.

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in many directions, allowing the individual to stretch its muscles and places the head of the bower below another animal in a non-threatening position (Bekoff and Allen 1998). If the face of the receiver was directed toward the bower, we considered that PBOW was performed in the visual field of the receiver (Figure 1A illustrates an example of this condition). The PBOWs occurring in all the other conditions were classified as performed out of the visual field of the receiver (Figure 1B reports an example of this condition).

Operational definitions

In our sample, we could identify 2 degrees of familiarity. We labeled those dogs that lived with the same family together as "cohabitants" and those dogs that regularly met and played together but did not live with the same family as "non-cohabitants." We identified 2 age classes (immature subjects <1 year; adult subjects ≥ 1 year). This choice relies on the fact that dogs reach their full size at $\sim$1 year of age (Howell et al. 2015; Geiger et al. 2016). When subjects belonging to the same age-class played together, the dyad was classified as "age-matched." The "age-mismatched" dyads involved an adult and an immature subject.

The play patterns were categorized into 3 categories: offensive, self-handicapping, and neutral (Table 1). This allowed to calculate the Play Asymmetry Index (PAI). Although play fighting patterns can be classified as both offensive and defensive according to the contexts in which they occur (Pellis and Pellis 1997; Norman et al. 2015), for the PAI calculation we needed to evaluate the direction of each "offensive" pattern. So, if a dog tries to push a playmate (offensive) and the playmate tries to defend itself by counterattacking with a bite (offensive), the outcome of the interaction is considered balanced, which means that the 2 subjects have a similar opportunity to gain advantage during their playful encounters thus producing reciprocity. Similarly, if both subjects engaged in self-handicapping patterns in a balanced way, they have a similar opportunity to have the lower hand during an encounter. Following this reasoning, we calculated PAI for each session involving A and B players as follows:

$$\frac{(\text{offensive A} + \text{self}_{\text{handicapping B}}) - (\text{offensive B} + \text{self}_{\text{handicapping A}})}{(\text{offensive A} + \text{self}_{\text{handicapping B}}) + (\text{offensive B} + \text{self}_{\text{handicapping A}}) + \text{NEUTRAL}}.$$

The absolute values of PAI were used to assess the level of the session asymmetry ($|\text{PAI}|$ ranges from $0_{\text{symmetric session}}$ to $1_{\text{asymmetric session}}$).

For each play session, we checked for the presence of ROM. During a ROM, the mouth is open in a relaxed way often revealing both the upper and the lower teeth (Maglieri et al. 2022). Two criteria were used to distinguish a ROM from a play bite (PBIT). First, the dog does not close its mouth even if it reaches the playmate's body/skin, so that the ROM is maintained even when the receiver's body part is inside the performer's mouth. Second, while engaging in an attempt to bite or PBIT, the dog rapidly opens and closes its mouth while lunging at the partner trying to reach its body/skin (see Table 1 for a detailed description of ROM).

Data analysis and statistics

Preliminary analysis

To investigate if full PBOW and half PBOW had different durations, we ran a linear mixed model (LMM; *glmmTMB* R-package; Brooks et al. 2017; R Core Team 2020; version 1.4.1717). The response variable was the logarithm of the duration of the pattern (Gaussian

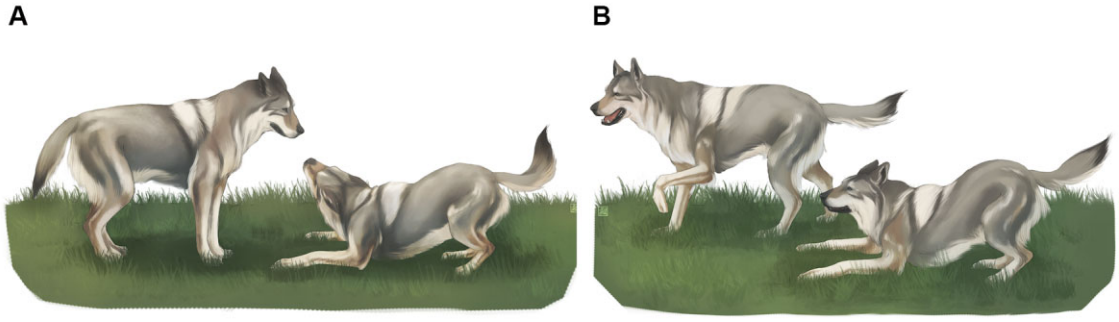

Figure 1. Illustrations showing the emission of the PBOW. PBOW was considered as detected when the face of the receiver was directed toward the bower (**A**) and not detected when it was performed out of the receiver's field of view (**B**). Credits Fosca Mastrandrea.

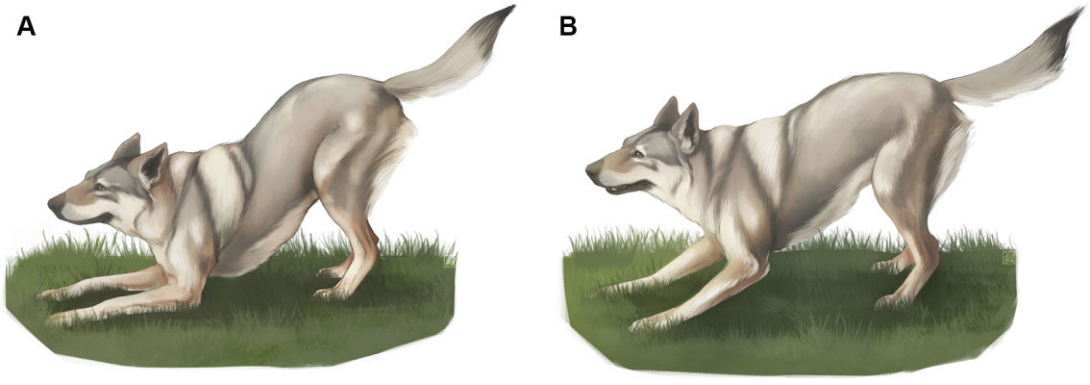

Figure 2. The 2 variants of PBOW. (**A**) Full Play Bow and (**B**) Half Play Bow. See Table 1 for a detailed description. Credits Fosca Mastrandrea.

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error distribution). We verified the normal distribution and homogeneity of the model's residuals by looking at the *Q*–*Q* plot and plotting the residuals against the fitted values (Estienne et al. 2017). We used age (<1 year/>1 year), sex (male/female), and type of pattern (full PBOW/half PBOW) as fixed factors. The identity of the subject was the random factor. No collinearity has been found between the fixed factors (range $VIF_{min} = 1.02$; $VIF_{max} = 1.04$).

Metacommunication hypothesis

Using the software Behatrix version 0.9.11 (Friard and Gamba 2020), we conducted a sequential analysis to evaluate which category of playful patterns (offensive, self-handicapping, and neutral) was more likely to be performed by the actor after the emission of a PBOW. We created a string for each PBOW event that represented the ordered concatenation of patterns as they occurred after a PBOW (PBOW|ContactOffensive, PBOW|LocomotorOffensive, PBOW|self-handicapping, and PBOW|neutral). Via Behatrix version 0.9.11 (Friard and Gamba 2020), we generated the flow diagram with the transitions from PBOW to the following pattern, with the percentage values of relative occurrences of transitions. Then, we ran a permutation test based on the observed counts of the behavioral transitions ("Run random permutation test" Behatrix function). We permuted the strings 10,000 times (allowing us to achieve an accuracy of 0.001 of the probability values), obtaining *P*-values for each behavioral transition.

To understand which factors could influence the number of PBOW performed, we ran a generalized linear mixed model (GLMM; *glmmTMB* R-package; Brooks et al. 2017; R Core Team 2020; version 1.4.1717). The response variable was the number of PBOW performed (with a Poisson error distribution). We used |PAI|, age (matched/mismatched), sex combination (male–male/male–female/female–female), level of familiarity (non-cohabitants/cohabitants), and the ROM as fixed factors. The playing-dyad identity and the duration of the session were included as random factors. The variable ROM was obtained by dividing the duration of all the ROMs performed within a session by the duration of such play session. No collinearity has been found between the fixed factors (range $VIF_{min} = 1.12$; $VIF_{max} = 2.20$).

For both models, we used the likelihood ratio test (Anova with argument test "Chisq"; Dobson 2002) to verify the significance of the full model against the null model comprising only the random factors (Forstmeier and Schielzeth 2011). Then, the *P*-values for the individual predictors were calculated based on the likelihood ratio tests between the full and the null model by using the R-function "drop1" (Barr et al. 2013).

Motivation hypothesis

To compare the number of PBOWs performed to start a new session with those performed during an ongoing session, we applied a randomization paired *t* test (https://www.uvm.edu/~statdhtx/StatPages/ResamplingWithR/ResamplingR.html by David Howell et al. 2015).

To understand if PBOW was actually performed after a pause during an ongoing play session, we calculated the amount of time needed to define a "pause". For those sessions including at least one PBOW, we calculated the time-lag separating the beginning of a PBOW of the player B and the beginning of the play pattern performed immediately before by the player A ($\text{time-lag}_1 = t_{PBOW_B} - t_{pattern_A}$). Similarly, within the same session, we also calculated the time-lag separating the beginning of 2 subsequent patterns enacted by the 2 playmates ($\text{time-lag}_2 = t_{pattern_B} - t_{pattern_A}$). From the calculation of time-lag_2, we excluded the first pattern performed after a PBOW. The same calculation was also applied to those sessions, not including PBOW ($\text{time-lag}_3 = t_{pattern_B} - t_{pattern_A}$). Finally, we determined the time-lag separating the beginning of a PBOW performed by A and the beginning of the subsequent pattern performed by B ($\text{time-lag}_4 = t_{pattern_B} - t_{PBOW_A}$).

By using the time-lag values of each category (time-lag_1; time-lag_2; time-lag_3; time-lag_4), we drew 4 survival curves based on Kaplan–Meier estimates using R package "*survminer*" (Kassambara et al. 2021). Then, we compared all the survival curves by using the function "pairwise_survdiff" (R package *survminer*, Kassambara et al. 2021).

Results

Preliminary results

Eighteen out of the 24 playing dogs emitted at least a PBOW. The analysis of the 118 playful sessions revealed that dogs can perform the PLAY BOW in 2 different variants (see also Table 1 for the definitions): the full PBOW (Figure 2A, $N = 27$) and the half PBOW (Figure 2B, $N = 49$). Dogs emitted the variant half PBOW more frequently than the variant full PBOW (Exact Wilcoxon Signed Rank $t = 31.00$; $N = 18$; ties = 4; $P = 0.010$). The full model built to investigate if these 2 PBOW variants vary in durations did not differ from the null model including only the random factor (likelihood ratio test: $\chi^2 = 1.84$, df = 3, $P = 0.61$). For this reason, we decided to pool the data of the 2 variants.

Visual signal hypothesis

All the 76 PBOWs punctuating the play sessions were performed within the receiver's field of view (Figure 1A; Prediction 1 supported).

Metacommunication hypothesis

Contrary to the expectations, the sequential analysis revealed that the offensive contact patterns, which are considered the riskiest playful actions (Pellis and Pellis 2017), were not the most likely to occur after the emission of a PBOW (Prediction 2 not supported). All the behavioral transitions considered were significant ($P < 0.001$) and are reported in the flow diagram with their percentages in Figure 3.

The full model built to investigate which factor could influence the number of PBOW punctuating each session did not significantly differ from the null model including only the random factors (likelihood ratio test: $\chi^2 = 4.44$, df = 6, $P = 0.618$) indicating that the emission of PBOW was not affected by any of the variables we included as fixed factors (|PAI|, age, sex, level of familiarity, and emission of ROM) (Prediction 3 not supported).

Motivation hypothesis

The randomization paired *t* test showed that PBOWs were performed significantly less at the beginning than during the course of the session ($t = 2.420$; $N = 35$; $P = 0.034$; $N_{beginning} = 14$; $N_{during} = 104$) (Prediction 4 not supported).

An overall survival plot for the 4 curves built on the values of the time-lag calculations was made based on Kaplan–Meier estimates (Figure 4). The results of the pairwise comparisons using log-rank test are reported in Table 2 (*P*-value adjusted using Bonferroni correction). Specifically, the time-lag_1 separating a pattern and a PBOW (median $t_{PBOW_B} - t_{pattern_A} = 2.759$ s) was significantly longer compared with the time-lag_2 separating 2 consequent patterns

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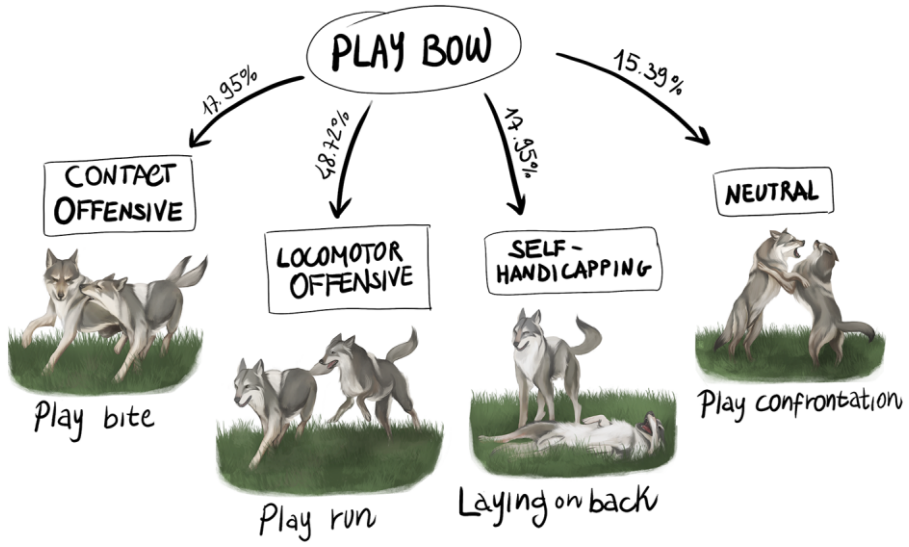

Figure 3. Transition PBOW→Contact Offensive play pattern (e.g., play bite); transition PBOW→Locomotor Offensive play pattern (e.g., play run); transition PBOW→Self-handicapping play pattern (e.g., laying on back); transition PBOW→Neutral play pattern (e.g., play confrontation). The percentage of occurrence of each transition is reported. Credits Fosca Mastrandrea.

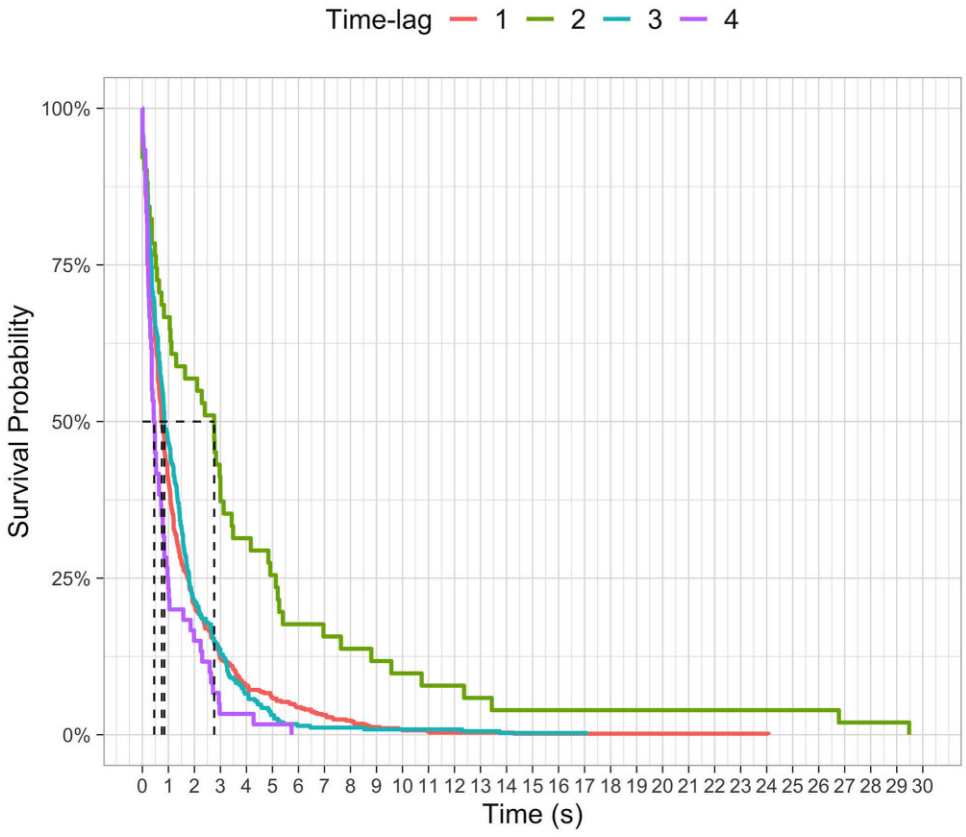

Figure 4. Kaplan–Meier analysis and survival plot for the 4 survival curves. Time-lag$_1 = t_{pattern_B} - t_{pattern_A}$ in session with at least one PBOW (red line); time-lag$_2 = t_{PBOW_B} - t_{pattern_A}$ (green line); time-lag$_3 = t_{pattern_B} - t_{pattern_A}$ in session lacking PBOW (blue line); time-lag$_4 = t_{pattern_B} - t_{PBOW_A}$ (purple line). The dashed lines represent the medians of the survival curves. The results of the Log-rank test are reported in Table 2.

(median $t_{pattern_B} - t_{pattern_A} = 0.748$ s) (Prediction 5 supported). Moreover, the time-lag$_4$ separating the pattern performed by the receiver immediately after the perception of a PBOW (median $t_{pattern_B} - t_{PBOW_A} = 0.143$ s) was shorter compared with all the other time-lags (Table 3, Prediction 6 supported). Seventy four out of the 76 PBOWs recorded triggered a playful reaction from the receiver, and in these cases, the sender stopped performing the PBOW as soon as the receiver began its playful reaction.

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Finally, the time-lag$_3$ separating 2 consequent patterns of the sessions lacking PBOW (median $t_{pattern_B} - t_{pattern_A} = 0.843$ s) was comparable with the time-lag$_2$ separating 2 subsequent patterns of the sessions including at least a PBOW.

Discussion

The PBOW is historically considered the most iconic playful posture of dogs (Darwin 1872; Fox 1970; Bekoff 1975; Manning and Stamp Dawkins 2012). Yet, its function is still under debate. Here, we found that CWDs adjusted their PBOWs by emitting them exclusively when the playmate was engaging in a face-to-face interaction with the sender (Figure 1A). This result strongly supports the *Visual Signal Hypothesis* of the PBOW (Prediction 1 supported, Table 3). Horowitz (2009) reported that in mixed-breed groups of dogs PBOWs were mainly directed toward attentive receivers, a pattern that has been confirmed in subsequent studies (Palagi et al. 2016; Byosiere et al. 2016a). It may be worth noting that some postures, including play bow, performed during play fighting may be also for combat purposes and not for signaling (Pellis and Pellis 2015). However, our results do not seem to support the *Combat Hypothesis* (Pellis and Pellis 2015) because in that case play bow should be randomly emitted and not always performed in the receiver's field of view.

Outside the canid family, a similar finding has also been reported for wild spotted hyenas *Crocuta crocuta*, a highly competitive carnivore species. The authors found that, during play fighting, animals emitted their playful signals (ROM and head bobbing) only when the sender was in direct visual contact with the playmate (Nolfo et al. 2021b).

Table 2. Statistical results (*P*-values) of each pairwise comparison by using the log-rank test

	Time-lag$_1$	Time-lag$_2$	Time-lag$_3$	Time-lag$_4$
Time-lag$_1$	N/A	—	—	—
Time-lag$_2$	**<0.001**	N/A	—	—
Time-lag$_3$	1.000	**<0.001**	N/A	—
Time-lag$_4$	**0.040**	**<0.001**	**0.027**	N/A

The significant *P*-values are in bold. Time-lag$_1$ = $t_{pattern_B} - t_{pattern_A}$ in session with at least one PBOW; Time-lag$_2$ = $t_{PBOW_B} - t_{tpattern_A}$; Time-lag$_3$ = $t_{pattern_B} - t_{pattern_A}$ in session lacking PBOW; Time-lag$_4$ = $t_{pattern_B} - t_{PBOW_A}$.

During the emission of a signal, being attentive to others' attention is one of the prerequisites to be satisfied for intentional communication to evolve (Ben Mocha and Burkart 2021).

To be effective, a visual signal needs to be dynamically adjusted by the sender according to timing, physical environment, and social circumstances. For example, to evoke an appropriate response in the receiver the signal has to be released at the right time during a specific interaction (Ben Mocha and Burkart 2021). This seems to be supported by our findings showing the precise timing of PBOW emission. Although PBOWs were not preferentially performed to start a new play session (Prediction 4 not supported, Table 3), they often punctuated the pauses during an ongoing session (Prediction 5 supported, Table 3). A delay in the behavioral response clearly indicates a decrease in motivation in the responder (Lorenz and Leyhausen 1973). When one of the 2 dogs started losing its motivation to play, the emission of a PBOW by the playmate led to the regeneration of the session thus inducing a change in the behavior of the receiver. The presence of the PBOW not only triggered the partner's response but it also reduced the reaction time that was much lower than that usually occurring between 2 play patterns exchanged by the playmates. Referring to the first-order intentionality of the signal (*sensu* Townsend et al. 2017), the goal of the sender is evident in the receiver's behavioral changes that have to be followed by a cessation of signaling. Our data clearly show that once the goal of resuming the session is achieved, the dog stopped bowing and immediately engaged in a playful pattern with the playmate. Considering all our findings, we suggest that the mismatched number of PBOWs emitted by the 2 players could be predictive of their different motivation to play and can also explain why some playful sessions do not need such kind of signal.

Via the sequential analysis we found that after a PBOW, offensive contact patterns were not the most likely to occur (Figure 3) thus indicating that PBOW does not strictly anticipate a possible misinterpretable behavior in CWDs. This finding is in agreement with data obtained by Pellis and Pellis (1996) who did not find any strict temporal association between PBOW and bite shaking, one of the most aggressive play patterns observed in puppies. A similar result was also obtained by Byosiere et al. (2016a) who found that PBOW did not anticipate patterns that could be misinterpreted.

In wild populations of spotted hyenas, both immature and mature subjects bobbed their heads to engage in affiliative or playful locomotor patterns but were not found to predict the occurrence of

Table 3. Summary of the hypotheses, predictions, and outcomes presented in the study

Hypotheses	Predictions	Outcomes
Visual signal hypothesis: The Play Bow is a visual signal	(P1)—Play Bow is almost exclusively performed within the visual field of the playmate	Supported
Metacommunication hypothesis: The Play Bow has a metacommunicative function and serves 1) to change the meaning of the following pattern and 2) to improve communication between players	(P2)—Play Bow mostly precedes contact offensive patterns thus maintaining the playful mood of the partner	Not supported
	(P3)—The most balanced and even play fighting sessions contain the highest number of PBOWs	Not supported
Motivation hypothesis: Play Bow is used to invite a partner to start a play session and it is effective in renovating the motivation to play in the partner	(P4)—Play Bow is mainly performed at the beginning of a session	Not supported
	(P5)—Play Bow is frequent during an ongoing session and coincides with a delay of the playmate's reactions	Supported
	(P6)—The emission of PBOW triggers the playmate's reaction again after a pause	Supported

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an offensive contact behavior (Nolfo et al. 2021b). A further important piece of information in CWDs comes from the analysis showing that the number of PBOWs was not affected by the degree of asymmetry characterizing the playful session thus suggesting that this signal does not have a role in fine-tuning the balance between competitive and cooperative patterns performed by the 2 players. As a whole, these findings lead us to conclude that the PBOW does not have a metacommunication function, at least in this breed of dogs (*Metacommunication Hypothesis* not supported).

The number of PBOWs was not influenced by the rate of ROM emitted in a session thus highlighting a difference in the potential functions of these signals. In the same group of CWDs, a previous study focused on ROM and its potential metacommunicative function revealed that the facial expression frequently anticipated an offensive pattern and was affected by the degree of asymmetry in the session (Maglieri et al. 2022). It appears evident that a dichotomy between PBOW and ROM exists and that these signals have different, but complementary functions in renovating the motivation to play ("don't stop me now") and in modulating the session ("I am not cheating"), respectively. Hence, for a play fighting session to be successful in terms of providing both immediate and long-term rewards, animals need to flexibly emit and interpret every single signal in a proper and rapid way by reading the behavior of the playmate moment by moment.

It would be interesting to expand the comparative approach in the study of playful facial expressions and body gestures across different breeds of dogs and wolves. This would allow understanding of the functional dichotomy characterizing these 2 different communicative patterns is consistent across different lineages, and robustly persistent despite domestication process and ongoing artificial selection.

Acknowledgments

We would like to thank Dr Sandra Fontana and the Hashtag Formazione e Benessere for providing economic support (360-HASHTAG-200213-D043-24000). We would also like to thank all the dog owners and Erica Tommasi for helping in data collection. We are grateful to 3 anonymous reviewers for their helpful comments on an earlier version of the manuscript.

Funding

Hashtag Formazione e Benessere grant number: 360-HASHTAG-200213-D043-24000.

Ethical Approval Statement

This study was purely observational and did not involve any manipulation of animals. No special permission was needed to carry out the study. All the owners gave consent to video-record their dogs and provided the information required.

Conflict of Interest Statement

None declared.

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