1 **PLAY FIGHTING IN WILD SPOTTED HYENAS: LIKE A BRIDGE OVER THE TROUBLED WATER OF A**

2 **HIERARCHICAL SOCIETY**

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23   **Abstract**

24   Play fighting, the most iconic form of social play, has been hypothesised to serve multiple evolutionary

25   roles as a function of the age of the players. Although widely practiced by youngsters, in some mammal

26   species this form of play can be also present in the adult phase. Here, we aim to test these hypotheses by

27   looking at the play fighting behaviour of spotted hyenas (*Crocuta crocuta*) by analyzing the behavior across

28   the different age classes. Spotted hyenas live in fission-fusion societies characterized by a rigid, nepotistic

29   system of dominance hierarchy. Yet, the species is also characterized by social flexibility, which is evident

30   from the high levels of support, cooperative behaviours and alliances. All these social features make

31   spotted hyenas a valuable model to explore play fighting at every age. We found that both immature

32   individuals and adults invested a comparable amount of time in playful activities and showed a similar

33   motivation in initiating and maintaining their playful interactions. By play fighting, immatures can improve

34   their motor and physical skills as predicted by the motor training hypothesis and, in agreement with the

35   social assessment hypothesis, adults can gain information on social partners with whom they will have to

36   interact in the future. Finally, contrary to our expectations, we found that those playful interactions

37   characterized by strong competition (measured via play asymmetry index) also had the longest durations

38   independently from the age of the players involved. Due to the absence of escalation into real fighting,

39   both immatures and adults appear to be able to manage the playful sessions despite their unbalanced

40   nature. All these findings suggest that play fighting in spotted hyenas can function as a "safe social bridge"

41   navigating both immatures and adults into the real future competitive challenges of the clan.

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44   **Keywords** – *Crocuta crocuta*; Immature and adult play; Motor training hypothesis; Play asymmetry index;

45   Play ethogram; Social assessment hypothesis; Training for the unexpected hypothesis

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Highlights

- Wild spotted hyenas (*Crocuta crocuta*) are highly playful
- Both adults and immatures are motivated to initiate playful sessions
- Play fighting is highly unbalanced between players
- Play fighting sessions never escalate into overt aggression
- Spotted hyenas are able to fine-tune their playful interactions

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Introduction

Play behaviour occurs sporadically in the animal kingdom, and has been observed in many species of mammals (Fagen, 1981; Burghardt, 2005; Pellis & Pellis, 2009; Palagi et al., 2016a), several species of birds, and few species of reptiles, fish and invertebrates (Diamond & Bond, 2003; Wenig, Boucherie & Bugnyar, 2021; Burghardt, 2005; Dapporto, Turillazzi & Palagi, 2006; Zylinski, 2015). As play lacks characteristics that are typical of the so-called functional behaviours (e.g., predation, reproduction), it has been often defined as a non-functional behaviour with no obvious immediate benefits (Bekoff & Allen, 1998; Martin & Caro, 1985; Graham & Burghardt, 2010). Recently, Burghardt (2005, 2011) described five criteria that a behaviour must meet to be categorized as play: i) it is not fully functional in the context in which it is expressed, ii) it is spontaneous and autotelic, iii) it is structurally and temporally modified respect to the functional behaviours, iv) it is performed repeatedly and, v) it is more likely to occur during relaxed contexts.

Play has been traditionally divided into three main categories: solitary locomotor-acrobatic play (an animal engaging in running, jumping, pirouetting and somersaulting) (Nishida & Inaba, 2009), solitary object play (an animal carrying, throwing, splitting or generally manipulating an inanimate object) (Cangiano & Palagi, 2020) and social play (two or more group mates fighting, chasing, or otherwise manipulating one another) (Fagen, 1981; Smith, 1982). Social play can include different behavioural patterns derived from different behavioural domains such as rearing of offspring, courtship and reproduction, and aggression (Burghardt, 2005; Cordoni, Gioia, Demuru & Norscia, 2021; Fagen, 1981; Vankova & Bartos, 2002; Pellis & Pellis, 2009). Play fighting, the most iconic form of social play, may be a combination of behavioural patterns involving competitive interactions from many different functional contexts. By engaging in play fighting, subjects compete to reach specific body parts typically contacted during agonistic interactions, courtship, or grooming (Pellis & Pellis, 2017, 2018). For play fighting to remain playful, the partners need to negotiate between cooperative and competitive elements, ensuring that "playful competition" does

96   not escalate to aggression. Such fairness can be achieved by fine-tuning movements, inhibiting

97   some forms of offensive playful patterns and by avoiding the performance of behaviours that might

98   be interpreted as cheating (Palagi et al., 2016b).

99   Generally, play fighting begins in infancy, reaches its peak during the juvenile phase, and tends to

100   decrease at puberty (Fagen, 1993). In mammals, although widely practised by youngsters (Fagen,

101   1993; Burghardt, 2005), play fighting can be also present during adulthood. Examples of this

102   include some species of rodents (for an extensive review see Pellis & Pellis, 2009), carnivores

103   (wolves, *Canis lupus*, Cordoni, 2009, Cordoni & Palagi, 2016; dogs, *Canis familiaris*, Bauer &

104   Smuts, 2007, Cordoni, Nicotra & Palagi, 2016; meerkats, *Suricata suricatta*, Sharpe, 2005, Palagi,

105   Marchi, Cavicchio & Bandoli, 2019) and primates (Pellis & Iwaniuk, 2000; bonobos, *Pan paniscus*

106   and chimpanzees, *Pan troglodytes*, Palagi, Paoli & Borgognini Tarli, 2006; geladas, *Theropithecus*

107   *gelada*, Mancini & Palagi, 2009; sifaka, *Propithecus verreauxi*, Antonacci, Norscia & Palagi, 2010;

108   ring-tailed lemurs, *Lemur catta*, Palagi, 2009).

109   In the last two decades, studies have revealed that play fighting may have different evolutionary

110   functions depending on the species, sex, and age of players (Burghardt, 2005; Pellis & Pellis, 2009;

111   Palagi et al., 2016a; Smith & Roopnarine, 2019). Due to the apparent absence of immediate

112   benefits, hypotheses on the functions of play fighting have often focused on long-term rather than

113   immediate advantages gained (Graham & Burghardt, 2010; Palagi et al., 2016a).

114   Developing/refining motor skills (motor training hypothesis, Smith, 1982; Byers & Walker, 1995)

115   and the skills needed to control emotional reactions (training for the unexpected hypothesis, Špinka,

116   Newberry & Bekoff, 2001) are two of the main hypotheses advocated to explain the potential long-

117   term benefits of play fighting in immature subjects. However, play fighting seems to have important

118   roles at an immediate level as well, and these may explain the presence of this behaviour in adults.

119   The social assessment hypothesis (Pellis & Iwaniuk, 2000) predicts that playful contact helps

120   individuals to i) gain information about the physical/social skills of players with whom they share a

121   low degree of familiarity (Antonacci et al., 2010) and ii) maintain/reinforce alliances that have been

already established (Palagi & Paoli, 2007; Palagi, 2008; Cafazzo, Marshall-Pescini, Lazzaroni, Viranyi & Range, 2018; Cordoni & Palagi, 2015). Of course, while all these hypotheses are commonly accepted, they are neither mutually exclusive nor are they sufficient to explain the presence and role of play fighting in every species, age, or sex.

Play fighting is considered one of the several behavioural domains that is epigenetically correlated with the competitive/cooperative pattern of inter-individual relationships across species (Thierry et al., 2000; Hare, Wobber, & Wrangham, 2011). For example, in those species, whose relationships rely on rigid and crystallized hierarchical ranking, play fighting rarely involves adults and is characterized by a high degree of asymmetry in its performance. In contrast, when the inter-individual relationships are characterized by social flexibility thanks to the possibility of circumventing rank-rules, subjects of different ages are often involved in play fighting, which is characterized by higher balanced exchanges of offensive/defensive patterns (Reinhart et al., 2010; Palagi, 2006, 2018; Beltrán Francés et al., 2020).

Spotted hyenas (*Crocuta crocuta*), like some species of primates, live in fission-fusion societies (Smith, Memenis & Holekamp, 2007; Drea & Frank, 2003). The spotted hyena is characterized by a rigid, nepotistic system of dominance hierarchy (Frank, 1986; Kruuk 1972; Tilson & Hamilton 1984; Mills, 1990; Wahaj et al., 2004) in addition to social flexibility, which is evident from the high levels of support, cooperative behaviour and alliances (Vullioud et al., 2019; Stratford & Périquet, 2019). In the *Crocuta* nepotistic system, individuals of each matriline occupy adjacent ranking positions in the clan. Before reaching reproductive maturity, both male and female juveniles inherit rank similar to that of their mothers (Kruuk, 1972; Tilson & Hamilton, 1984; Frank, 1986; Mills, 1990; Engh, Esch, Smale & Holekamp, 2000). However, social support is an important tool to manipulate power asymmetries among individuals (Vullioud et al., 2019). The social support can take different forms such as helping in rearing offspring (König, 1997), in cooperative hunting and in defence of their territory (Holekamp, Sakai & Lundrigan, 2007). All

147 these social features make *Crocuta crocuta* a valuable model to test hypotheses on the potential role

148 of play fighting according to the different age-class of the players.

149 By playing, infants and juveniles can refine and develop their physical skills and improve their

150 kinematic and emotional responses to unexpected events. The experiences thus gained through play

151 can translate into greater skill in real competitive interactions engaged in at later ages (Turner et al.,

152 2020). In spotted hyenas the first two weeks of life are characterized by high levels of aggression,

153 followed by a shift into play fighting in their subsequent weeks (Drea et al., 1996). Play fighting is

154 the main behaviour through which young hyenas interact with conspecifics once the mothers

155 introduce them to their peers and other clan members (Kruuk 1972; Mills 1990; East, Hofer, &

156 Wickler, 1993). If play fighting affords young hyenas the opportunity to train/improve their motor

157 skills (motor training hypothesis, Smith, 1982; Byers & Walker, 1995) and to navigate the uncertain

158 interactions with the adults of the clan with which they are not familiar (training for the unexpected

159 hypothesis, Špinka et al., 2001), we predict that immature subjects are equally likely to initiate play

160 with peers and adults (Prediction 1).

161 For the adult clan members, engaging in play fighting with immature group mates could serve

162 several functions. Since cubs gain ranking positions immediately below those of their mothers

163 (Holekamp & Smale 1993, Smale, Frank & Holekamp, 1993), for adult clan members playing with

164 the young would not only allow them to become acquainted, but may also help lay the foundation

165 for future alliances and reinforce existing relationships with the mothers (Vullioud et al., 2019). If

166 play fighting has a role in the regulation of social relationships among clan members (social

167 assessment hypothesis, Pellis & Iwaniuk, 2000), we predict that adult-adult play is present in the

168 species and that adults are also motivated to initiate playful sessions with immature subjects

169 (Prediction 2).

170 In spotted hyenas, aggression is used to reinforce an individual's rank, gain access to food, and

171 defend offspring (Kruuk 1972; Frank 1986; Mills 1990; Holekamp & Smale 1991; Engh et al.

172 2000). Aggression is a pervasive trait at all ages in this species, with both adult and immature

animals frequently engaging in severe agonistic interactions (Smale et al., 1996). If, as suggested by Drea and co-workers (1996), play fighting has a role in testing/modulating social relationships throughout the life cycle (social assessment hypothesis, Pellis & Iwaniuk, 2000), we predict that individuals will initiate play with clanmates outside their age class and engage in asymmetric (unbalanced) playful interactions (Prediction 3).

In hierarchical species, asymmetrical playful interactions can easily escalate into overt aggression (Bekoff, 1995; Pellis & Pellis, 2009; Wright et al., 2018; Reinhart et al., 2010). A strategy to limit the risk of escalation is to reduce the duration of the playful interaction, so being able to sustain prolonged interactions would indicate that the animals have other tactics to mitigate the risk of escalation (Gallo et al., 2021). If spotted hyenas are able to maintain their playful mood by fine-tuning their playful interactions, we predict that the duration of play interactions would not decrease as the degree of asymmetry between playmates increases (Prediction 4).

Methods

Ethical note

This is non-invasive research compliant with the ASAB/ABS Guidelines for the Use of Animals in Research, the current South African law and University regulations. Thus, no permit from the Bio-Ethical Committee was needed.

The reserve

Observations took place at the Siyafunda Wildlife & Conservation research base (S -24.15029; E 30.65742), at the Greater Makalali Private Game Reserve (GMPGR, Limpopo, South Africa) that is located within the savannah biome, is characterized by herbaceous plants, tall trees and bushes (Low & Rebelo, 1996). The reserve is crossed by the Makhutswi River, a tributary of the Olifants.

197 Spotted hyenas (*Crocuta crocuta*) were introduced in 1995. The number of hyenas constituting the

198 population of the Greater Makalali Private Game Reserve is unknown.

199

200 Data Collection

201 Data were collected from June to October 2019. During the observation period, the researchers and

202 the rangers counted and individually recognized 64 subjects (14 cubs, 5 juveniles, 45 sub-

203 adult/adults). Due to the difficulty in recognizing the sex of hyenas in the wild (Frank 1990), no

204 data on the number of males and females are available. The identity of each individual was

205 established based on peculiar morphological markings, such as scars, patches of missing fur and the

206 pattern of spots on the fur (Holekamp, Smale & Szykman, 1996; Holekamp & Smale, 1998).

207 Individuals were identified by patrolling the various areas and dens known to be frequented by

208 hyenas. Tracking walks were organized by the rangers to follow the animals tracks to the dens.

209 During the study, four dens in active use were identified and used as observation spots for

210 collecting videos on the lactating females, their cubs and all the subjects visiting the dens.

211 The observers collected data from the safety of vehicles to which the animals were well habituated.

212 The observation sessions ranged from two to three per day (06.00-10.00 pm; 05.00-11.00 am;

213 03.00-06.00 pm). To limit the disturbance as much as possible, nocturnal data (06.00-10.00 pm)

214 were collected with the aid of red illumination that was never directed towards animals but on the

215 ground around the animals (Finley, 1959; Spoelstra et al., 2017). Video-recordings were taken with

216 a video camera (Canon ® EOS 110D). If the subjects were scattered around the observation spot, a

217 second camera (Full HD Panasonic Lumix DC-FZ82) was used to allow the entire group to be

218 recorded. The use of 50x optical zooms and tripods ensured accurate video-recording at long

219 distances (up to 50 meters). The concurrent use of the two cameras permitted the continuous video-

220 recording of all the activities of the subjects and the quantification of the exact amount of time each

subject was present in each video. A total of about 26 hours of videos was directly recorded by the observers.

Data were also collected with the aid of camera traps provided by the Siyafunda Wildlife & Conservation research base (Ranger digital trail, BN056). These were located in front of the dens, approximately 10-meters from the entrance and covered a range of five meters around the den hole. The camera traps were secured in metal cases to avoid being crushed by animals and were tied on trees 1.50 meter above the ground. Cameras were active 24 hr/day, with no delay between consecutive videos (lasting from 40 to 60 seconds), and the sensitivity of the motion sensor was set to high. A total of 12 hours of videos was collected using the camera traps.

Only the subjects (n=24) with at least 30 min of video-recordings were included in the analyses (individual mean 109 ± 19SE minutes). Among the 24 subjects, eight were cubs, two were juveniles and 14 were subadults/adults. The cubs and the juveniles were clustered as 'immature subjects'; the subadults and adults as 'mature subjects'.

Data on temperature were extracted from a website (https://www.timeanddate.com) that provided the mean temperatures of the Limpopo region calculated per 3-hour time slots.

Video analyses

The videos were analysed by VLC 2.1.5 Rincewind software and Jump-to-Time extension with an accuracy of 0.02 seconds. A.P.N. and G.C. analysed the videos. Before starting the video scoring, they underwent a 30-hour training period by E.P. Inter-observer reliability in characterizing and scoring the behavioural patterns was checked by E.P., who randomly selected some sections of the dataset (corresponding to 10 minutes of videos) and verified whether the behavioural items were correctly classified. Such a check was done every two hours of analysed videos. The Cohen's kappa values for the playful behavioural items (see Figure 1 for the definitions) were never less than 0.93.

245 Via the all occurrences sampling method (Altmann, 1974), we analysed all the playful interactions

246 recorded in the videos. For each playful session we registered i) the exact duration (0.02 seconds

247 accuracy), ii) the identity of the initiator, iii) the identity of the receiver, iv) the sequence of the

248 playful behavioural actions performed (items listed in Figure 1), and v) the time of the day.

249 The list of the playful behavioural items observed and used for this study (Figure 1) has been

250 defined on the basis of playful items previously described for spotted hyenas (Drea et al., 1996)

251 and other social carnivores (coyotes, *Canis latrans*, Bekoff, 1974; wolves, *Canis lupus*, Bekoff,

252 1974, Cordoni, 2009; Cordoni & Palagi, 2016; dogs, *Canis familiaris*, Bekoff, 1974, Bauer &

253 Smuts, 2007, Ward, Bauer & Smuts, 2008, Cordoni et al., 2016, Cafazzo et al., 2018; meerkats,

254 *Suricata suricatta*, Palagi et al., 2019).

255 A dyadic playful session began when one of the subjects directed a play pattern (listed in the Figure

256 1) towards a receiver. If the receiver did not react to the playful pattern, the interaction was not

257 considered as a successful play session. The session ended when one of the two players moved

258 away from their partner or if a third individual interfered during the session, either by starting a

259 polyadic interaction or interrupting the previous one (Llamazares-Martín, Scopa, Guillen-Salazar &

260 Palagi, 2017).

261 To calculate the Play Asymmetry Index (PAI) for each entire dyadic session, we classified the

262 patterns performed by each individual (including both physical and non-physical contact) as either

263 offensive and defensive actions (Bauer & Smuts, 2007; Palagi et al., 2019; Llamazares-Martín,

264 2017). The items not included in the previous classification were defined as neutral actions. See

265 Figure 1 for the definition and the classification of the play behavioural items. For each dyadic play

266 session, we calculated the PAI relative to a given dyad (AB) (Ward et al., 2008; Palagi, Norscia &

267 Spada, 2014; Gallo, Caselli, Norscia & Palagi, 2021) as follows:

268

$$PAI = \frac{(offensive_{A \to B} + defensive_{B \to A}) - (offensive_{B \to A} + defensive_{A \to B})}{(offensive_{A \to B} + defensive_{B \to A}) + (offensive_{B \to A} + defensive_{A \to B}) + neutral_{A+B}}$$

269

270   This formula takes into account the relative contribution of both players (animal A and animal B)

271   during a single session in terms of offensive and defensive patterns (numerator). This is divided by

272   the total number of patterns (offensive + defensive + neutral) constituting the session

273   (denominator). The absolute PAI values (|PAI|) range from 0 (completely symmetrical/balanced

274   session) to 1 (completely asymmetrical/unbalanced session, perfectly skewed towards one of the

275   two players). The |PAI| makes it possible to arrange the sessions along with a gradient of

276   asymmetry.

277

278   Statistics

279

280   To compare the overall amount of time spent in playing (calculated as seconds spent in playing per

281   minute of observation) across the age-class combinations (immature→immature;

282   immature→mature; mature→immature; mature→mature), we applied the Kruskal-Wallis One-Way

283   ANOVA (non-normal distribution). To determine which among the six pairs of age-combinations

284   significantly differed in the time spent to play over the entire period of observation, we applied the

285   Tukey's post hoc test (Bonferroni's correction).

286   Focussing on the mixed-age playing dyads, we applied Chi-square test to compare the observed

287   frequency (number of times) of initiation to play by adults (mature→immature) to the frequency

288   (number of times) expected by chance if initiation was performed by either individual randomly.

289

290   To evaluate which factor affected the play asymmetry index (response variable = play asymmetry

291   index values transformed by logarithmic function, log|PAI|) and the length of each playful session

292   (response variable = length of each session in seconds transformed by logarithmic function,

293   LOGduration), we ran two Linear Mixed Models (LMM) with a normal distribution by using the R-

294   package glmmTMB 1.2.5042 (Brooks et al., 2017).

For the first model (log|PAI| as response variable), we considered the following fixed factors: the age combination of the players taking into account the age class of the subject inviting to play (immature→immature; immature→mature; mature→immature; mature→mature), Day/Night; Temperature (T °C: 5-10; 11-15; 16-20; 21-25; >25); Lactating Female (presence/absence); observations (cameratrap/observer).

For the second model (LOGduration of each session as response variable), the fixed factors considered were: the logarithm of the values of play asymmetry index (log|PAI|), the age combination of the players (immature→immature; immature→mature; mature→immature; mature→mature), Day/Night; Temperature (T °C: 5-10; 11-15; 16-20; 21-25; >25); Lactating Female (presence/absence); observations (cameratrap/observer). In both models, the identity of the dyad involved in each play session was entered as a random factor.

The Likelihood Ratio Test (LRT; Dobson, 2002) was applied to compare the full model (including all the fixed factors and the random factors) with the null model (including only the random factors) (Forstmeier & Schielzeth, 2011). The LRT was also calculated to test the significance of the fixed factors by using the function Anova (R-package car 3.0-10) (Fox & Weisberg, 2019). To exclude the occurrence of collinearity among fixed factors, we examined the variance inflation factors (VIF; X Fox, 2015) by the R-package performance 0.4.4 (Lüdecke, Makowski & Waggoner, 2020). Model fit and overdispersion were verified by using the R-package DHARMa 0.3.3.0 (Hartig, 2020). The marginal R^2 (representing the variance explained by fixed factors only) and the conditional R^2 (representing the variance explained by the entire model including both fixed and random factors) (Nakagawa et al. 2017), were calculated via the R-package MuMIn 1.43.17 (Bartoń, 2020). All analyses were performed using R 4.0.3 (R Core Team 2020).

Results

Play ethogram

320 We observed, identified and defined 18 playful behavioural patterns (18 observed in immature

321 subjects; 14 observed in mature subjects). In the immature subjects, but not in the adults, we

322 observed instances of social play involving the manipulation of an object for which the two players

323 competed, comprising about 6% of the total amount of time they spent playing socially.

324 Except for social play with objects, all the other encounters involved play fighting, with alternating

325 contact and locomotor patterns. The presence of 14 out of 18 patterns in mature individuals suggests

326 that in adults as well, play was characterized by a certain level of variability.

327 On the total of play bites directed to specific target body parts, the percentage of the play bites

328 directed to the neck (or throat) was 58% in mature and 27% in immature subjects, a possible

329 qualitative indication of the diverse playful tactics used by different age classes. The detailed

330 descriptions and graphical illustrations of the playful items involved in both play fighting and social

331 object play are reported in Figure 1.

332

333 The total amount of time spent in playing across the different age-combinations

334

335 The time spent in playing (seconds of play/minute of observation) differed across the age

336 combinations (Exact Kruskal-Wallis One way Anova Chi-square = 16.383, $N_{mature-mature}$=25; $N_{mature-immature}$=29; $N_{immature-mature}$=9; $N_{immature-immature}$=13, P=0.001) (Figure 2). The Tukey's post hoc test

338 revealed that the amount of time spent playing between immature subjects was higher than between

339 mature subjects (q=26.775; P=0.001) and that mature subjects spent a larger amount of time playing

340 with immature subjects than with other mature group mates (q=17.332; P=0.017) (see Table 1). The

341 amount of play recorded between immature subjects did not differ from that recorded between

342 immature and adult subjects (Prediction 1 supported). Focussing on the mixed-age playful

343 interactions, we did not find any difference between the number of invitations performed by adult

344 and immature subjects (Chi-square=0.725; P>0.05) (Prediction 2 supported). Lactating females,

who are always present at the den, were never observed to interrupt the playful interactions involving their offspring.

Factors influencing the play asymmetry index of each playful session

Only the playful sessions (N=174) that included at least two patterns were used in the following analyses. We calculated the absolute values of Play Asymmetry Index (|PAI|) for each age-class combination (|PAI| mean$_{immature-immature}$=0.448 ± 0.038SE; mean $_{mature-immature}$=0.496 ± 0.046SE; mean $_{mature-mature}$ =0.314 ± 0.103SE). The LMM (log|PAI| as response variable) did not reveal any significant difference between the full model, including all the fixed factors (see Methods for the definitions) and the null model, comprising only the random factors (likelihood ratio test: χ^2_{10} = 10.144, P = 0.428) thus indicating that none of the fixed variables, including the age class of the players, had an effect on the response variable (log|PAI|) (Prediction 3 supported).

Factors influencing the duration of each playful session

There was a significant difference between the full model, including all the fixed factors (see Methods for the definitions), and the null model, comprising only the random factors (likelihood ratio test: χ^2_{11} = 29.789, P = 0.0017). No collinearity was found between the fixed factors (low correlation, range VIFmin=1.03; VIFmax=2.05). The model was not over dispersed (P=0.968, dispersion parameter=1.001). The fixed factor "log|PAI|" (Table 2; Figure 3) and "age-combination" (immature-immature; immature-mature; mature-immature; mature-mature) had a strong significant effect on the duration of each play session (Table 2; Figure 4).

As for the effect of log|PAI| on the duration of each playful interaction, we found that those sessions characterized by strong asymmetry lasted longer compared to those sessions that were more balanced (Table 2; Figure 3) (Prediction 4 supported).

370   As for the age-combinations, the Tukey test revealed that the play sessions between mature subjects

371   were shorter than those involving at least an immature subject (t-ratio$_{immature-immature\ vs\ mature-mature}$ =

372   3.370, df=160, P = 0.005; t-ratio$_{immature-mature\ vs\ mature-mature}$ = 3.728, df=160, P = 0.0015; t-ratio $_{mature-}$

373    $_{immature\ vs\ mature-mature}$ = 4.221, df=160, P = 0.0002) (Table 2; Figure 4). We did not find any difference

374   in the play duration of each session between those interactions involving at least one immature

375   subject (t-ratio$_{immature-immature\ vs\ immature-mature}$ = 0.170, df=160, P = 0.998; t-ratio$_{immature-immature\ vs\ mature-}$

376    $_{immature}$= -0.567, df=160, P = 0.942; t-ratio$_{immature-mature\ vs\ mature-immature}$= -0.736, df=160, P = 0.882)

377   (Figure 4). Hence, the duration of each mixed-age play session did not differ as a function of the

378   age of the initiator. None of the age-class combinations, play fighting escalated into real fighting.

379   A summary of the hypotheses, predictions and outcomes is shown in Table 3.

380

381   **Discussion**

382   This study provides new findings on play behaviour and its modalities in wild spotted hyenas

383   demonstrating that the behaviour is well represented not only in immature animals but also in

384   adults. Our observations on object play revealed that, while cubs occasionally manipulated objects

385   with playmates (see Walking Play Object Sharing/Attempt Object Steal in Figure 1), adults were

386   never observed engaging in this activity. This finding is in agreement with previous observations

387   reported by Holekamp & Smale (1990) and with the motor training hypothesis (Smith, 1982; Byers

388   & Walker, 1995). For immature subjects, object play could provide training for competing with

389   peers and strengthen the jaw muscles that, in this species, are fundamental to gain access to food

390   resources such as carcasses comprised largely of bones and skins (Holekamp & Smale, 1990).

391   Moreover, compared to primates and other more dexterous carnivore species, spotted hyenas show

392   limited forelimb manipulative abilities (Glickman & Sroges, 1966), for this reason, object play

393   involving the mouth may represent a valuable tool for gaining information about the physical

394   environment and exploring novel objects (Tanner, Smale & Holekamp, 2007). The pups and cubs of

several other carnivore species are commonly observed to play with objects (cats, Bradshaw et al., 2012; dogs, Burghardt et al., 2016; bears, Fagen & Fagen, 2004). The patterns observed during object play are often seen in prey handling and hunting (an extensive review is provided in Burghardt, 2005), thus suggesting that object play can help in developing motor skills, although experimental/empirical evidence is still lacking (Martin & Caro, 1985). To explore the motor training hypothesis, further steps in the study of spotted hyena object play are required. It would be important to trace the development of this activity covering the period from lactating to food independence and evaluate if the beginning of the independent phase coincides with the decrease of object play as suggested by Rogers et al. (2020).

Different from object play, play fighting was observed both in immature and adult subjects. Adults spent a larger amount of time in playing (seconds of play/minutes of observation) with immature subjects compared to with other adults (Table 2; Figure 2). Moreover, immature subjects played with peers and adults at comparable levels (Tables 2 and 3; Figure 2; Prediction 1 supported). Interestingly, the analyses did not reveal any differences either in the direction of the play invitations or the total time spent in playing between mature and immature subjects (Table 4; Prediction 2 supported). All these findings reveal that adults, as well as immature hyenas, are motivated to engage in play fighting.

In agreement with Tanner et al. (2007), our data indicate that play fighting in spotted hyenas goes well beyond that typical of the immature phase, expanding into adulthood. This hypothesis seems to be corroborated by the results emerging from the analysis of the play asymmetry index, a valuable analytical tool reflecting the balance of cooperation/competition characterizing a play session (Bauer & Smuts, 2007; Essler et al., 2016; Llamazares-Martín et al., 2017; Palagi et al., 2019, Kottferová et al., 2020; Gallo et al., 2021). The age of the players did not affect the level of asymmetry during play, suggesting that it can have a role in modulating future competitive interactions not only in the immature (Drea et al., 1996) but also in the adult phase (Table 4; Prediction 3 supported). Contrary to our findings, Essler et al. (2016) found that in wolves play

asymmetry changed according to the age of the players. In the mixed-age dyads, the play session was more competitive with the adult wolves performing more offensive behaviours than their play partners.

In spotted hyenas, playful asymmetry seems to be so prominent that, contrary to any expectations, it positively correlated with the duration of the session (Figure 3). So far, no previous study on play fighting in mammals has ever shown a positive relation between the level of competitive elements (measured via PAI) and the length of a play session. It is possible that in the spotted hyena an amalgam of playful tactics such as self-handicapping, role reversal, cheating avoidance, and meta-communicative signals can ensure the maintenance of fairness, thus avoiding escalation into serious fighting, that in this species, can be particularly severe (Bekoff, 1995; Bekoff & Allen, 1998; Burghardt, 2005).

The motivation of immature subjects to initiate play with both peers and adults is in agreement with both the motor training (Smith, 1982; Byers & Walker, 1995; Carter et al., 2019) and the training for the unexpected hypothesis (Špinka et al., 2001). These two hypotheses may blend together and so, their relative contribution to explaining the play performed can be difficult to disentangle (Carter et al., 2019; Pellis, Pellis & Bell, 2010). For the immature subjects, playing with peers and siblings can help improve their motor skills, which have been already trained in the natal den through inter-sibling aggressive interactions to gain feeding priority and dominance (Drea et al., 1996). The linkage between play fighting and the development of physical strength has been also reported in young chimpanzees, whose play fighting appears to be particularly rough (Cordoni & Palagi, 2011) and positively correlated with reciprocal aggression (Paquette, 1994). By inviting adults to engage in play fighting, immature individuals can acquire the ability to cope with challenging and novel situations thus developing their emotional and physical resilience in response to new stimuli deriving from playing with less familiar, older, larger partners that are mismatched. A study on brown hyenas (*Parahyena brunnea*) reveals that juveniles engaged in muzzle-wrestling with adults and that with such behaviour the younger animals sometimes went to great lengths to

447   test the adults' reactions (Mills, 1990). For immature spotted hyenas, play fighting could be a social

448   bridge fostering their integration into the clan (Drea et al., 1996), and, at the same time, allowing

449   youngsters to measure themselves both physically and emotionally with other clan members.

450   During the mixed-age playful interactions, lactating mothers (always in proximity during the

451   recorded playful activities) never intervened to interrupt the interaction, suggesting that the playful

452   mood maintained by the adults was clearly perceived by bystanders.

453   According to the economic models of behavioural ecology (Bekoff & Byers, 1985; Fagen, 1993,

454   Fagen & Fagen, 2004), the motivation of adults to invite immature playmates suggests that the

455   benefits linked to this activity can outweigh the costs for adults as well. We can only speculate

456   about the benefits of play in adulthood, as empirical demonstration of such benefits is extremely

457   difficult (Palagi, 2006; 2009). However, through play, adults can gain information about social

458   partners with whom they will have to cope in the future (social assessment hypothesis, Cafazzo et

459   al., 2018; Cordoni & Palagi, 2015; Pellis & Iwaniuk, 2000). Moreover, playing with immature

460   subjects can help adults create a social bridge with the mothers thus increasing their tolerance to the

461   close proximity of the adult players and, possibly, reinforcing their social relationships. In social

462   primates, play can be considered as a gateway to the social environment (Palagi & Paoli, 2007). In

463   chimpanzees (*Pan troglodytes*) and geladas (*Theropithecus gelada*), play between adults and

464   infants, allows those adults to contact the mothers, gaining social support and friendship (Mancini

465   & Palagi, 2009; Palagi, Cordoni, & Borgognini, 2004). For example, gelada females belonging to

466   different one-male units (OMU) engage in play with immature subjects from other OMUs, thus

467   creating an inter-OMU tolerant playful network (Mancini & Palagi, 2009). Additional examples of

468   the role of adult play as a gateway to establish or renovate social relationships can also be found in

469   solitary species. For example, in wild brown bears, play fighting seems to be used as an icebreaker

470   between males and females during courtship and immediately before copulation (Herrero & Hamer,

471   1977).

In spotted hyenas, the adult-adult play sessions had the shortest durations (Table 3; Figure 4). When one playmate uses unequal force or fails to comply with the rules ensuring turn-taking (role-reversal) and/or self-handicapping, the probability of an escalation into overt aggression increases (Palagi et al., 2016b; Pellegrini, 2009; Pellis & Pellis, 1998; Pellis, Pellis & Reinhart, 2010). Hence, especially in those species in which play is strongly competitive (coyotes, Bekoff, 1974; black bears, Henry & Herrero, 1974; wolves, Essler et al., 2016), adult-adult play requires high levels of inhibitory control (e.g., self-handicapping) and fine-tuning (Cordoni, 2009). In this view, shortening the play session could be a tactic used by adult spotted hyenas to manage their play fighting contacts and limit the risk of an escalation. A similar result was also found in juvenile chimpanzees that, due to their high level of roughness during play and low level of tolerance, tend to shorten the sessions to avoid serious risks of physical injuries (Palagi & Cordoni, 2012).

In conclusion, social play in spotted hyenas is well represented in both immature and adult animals. Unfortunately, we were not able to explore the role that sex and kinship can have on play fighting across the different age phases. Future studies will be necessary to explore such issues in this monomorphic species. Due to the total absence of interventions by the lactating females interrupting play involving their young, and the absence of play fights escalating into real fighting, play in spotted hyenas appears to be fair. The animals seem to be able to manage these interactions despite their highly competitive nature. An important next step will be to identify the mechanisms by which spotted hyenas fine-tune their playful contacts and whether/how they recruit meta-communicative signals to convey messages of playful motivation thus limiting the risk of misunderstanding.

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746 Ethical note

This is a non-invasive research compliant with the ASAB/ABS Guidelines for the Use of Animals in Research, the current University regulations. Bio-Ethical Committee

AGE-COMBINATION	Tukey (q)	P
imm/imm > mat/mat	26.775	0.001
mat/imm > mat/mat	17.332	0.017
mat/mat ~ imm/mat	8.160	1.000
mat/imm ~ imm/mat	-9.172	1.000
mat/imm ~ imm/imm	9.443	1.000
imm/mat ~ imm/imm	18.615	0.260

South African law and Thus, no permit from the was needed.

Table 1 – Tukey's post hoc test (q values; *P* = probability, Bonferroni's correction)

771 Statistical results comparing the amount of time spent to play between the different age-class combinations.

772 imm=immature subjects; mat=mature subjects

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785 **Table 2** – Results of the Linear Mixed Model analysis (response variable LOGplayduration, normal

786 distribution).

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Fixed Effects	Coeff	SE	2.5% CI	97.5% CI	χ^2	df	*P*
Intercept	1.075	0.167	0.747	1.403			
Log|PAI|	0.072	0.025	0.022	0.121	7.824	1	**0.005**
AC					17.895	3	**0.0001**
AC [immature→mature][b,c]	-0.024	0.143	-0.305	0.256			
AC [mature→immature][b,c]	0.070	0.123	-0.172	0.312			

	Coeff	SE	2.5% CI	97.5% CI	LRT	df	p
AC [mature→mature][b,c]	-0.657	0.195	-1.040	-0.275			
Day/Night	0.109	0.087	-0.062	0.279	1.548	1	0.213
T °C					1.480	4	0.830
T °C [11-15][b,d]	0.125	0.115	-0.101	0.350			
T °C [16-20][b,d]	0.072	0.158	-0.238	0.381			
T °C [21-25][b,d]	0.038	0.106	-0.170	0.246			
T °C [>25][b,d]	0.140	0.158	-0.169	0.450			
LF [presence/absence]	0.155	0.118	-0.077	0.387	1.707	1	0.191
OBS [cameratrap/observer]	0.062	0.130	-0.193	0.318	0.229	1	0.632

Estimated parameters (Coeff), Standard Error (SE), 95% Confidence intervals (2.5% - 97.5% CI), and results of the likelihood ratio tests (LRT) of the best Linear Mixed Model (with a normal distribution) investigating the effect of the following variables on the LOGplayduration: logarithm of the values of Play Asymmetry Index (**log|PAI|**): Age-Combination (**AC**: immature→immature; immature→mature; mature→immature; mature→mature), **Day/Night**; Temperature (**T °C**: 5-10; 11-15; 16-20; 21-25; >25); Lactating Female (**LF**: presence/absence); OBServations (**OBS**: cameratrap/observer). marginal R2=0.164; conditional R2=0.164; Ncases = 174; Ndyads=63. Variance for the random factor dyads = 0.203 (±0.451 SD).

[b]Estimate parameters ± SE refer to the difference of the response between the reported level of this categorical predictor and the reference category of the same predictor.
[c]These predictors were dummy coded, with the "AC [immature→immature]" being the reference category.
[d]These predictors were dummy coded, with the "T °C [5-10]" being the reference category.

Hypotheses	Predictions	Outcomes
Play fighting affords young hyenas the opportunity to train/improve their motor skills and to navigate the uncertain interactions with the unfamiliar adults of the clan	Immature subjects are equally likely to initiate play with peers and adults (P1)	Supported
Play fighting has a role in the regulation of social relationships among clan members	Adult-adult play is present and adults are also motivated to initiate playful sessions with immature subjects (P2)	Supported
Play fighting has a role in testing/modulating social relationships throughout the life cycle	Individuals will initiate play outside their age class and so engage in asymmetric (unbalanced) playful interactions (P3)	Supported

| Spotted hyenas are able to maintain their playful mood by fine-tuning their playful interactions | Duration of play interactions do not decrease as the degree of asymmetry between playmates increases (P4) | Supported |

802   **Table 3** – Summary of the Hypotheses, Predictions and Outcomes presented in the study.

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819   **Figure legends**

820   **Figure 1 –** Graphical illustrations and definitions of the playful behavioural patterns observed in the

821   group of spotted hyenas under study. Legends: I=Immature; M=Mature; C=contact; NC=non-

822   contact; O=offensive; D=defensive; N=neutral; ✓ presence; ✗ absence; * This item was codified

823   when it was not possible to detect a specific body part as target. Credits: Fosca Mastrandrea

| Pattern	Definition	Presence √/Absence X			Pattern	Definition	Presence √/Absence X			Pattern	Definition	Presence √/Absence X		
		II	IM/MI	MM			II	IM/MI	MM			II	IM/MI	MM
Reciprocal Mouth Bite C - N	Two individuals simultaneously grasp the jaw of the partner	√	√	√	Side play bite	The target is the side of the body	√	√	√	Rough & Tumble C - N	Two individuals play entwined, rolling and grasping each other. General term used when it was not possible discerning the directionality of each contact pattern.	√	√	√
Attempt to Bite NC - O	An individual moves its open mouth towards another animal without reaching the skin of the partner	√	√	√	Play Push C - O	An individual A shoves B via forelimbs or the side part of the body	√	√	X	Laying Back NC - D	An individual lays supine on the ground	√	√	X
Play Bite C - O	The bite is inhibited and inflicted without excessive pressure*	√	√	√	Play Run NC - O	An individual chases the playmate while trotting, jumping, bouncing	√	√	X	Play Ambush NC - O	An animal hides itself and waits the playmate, then, suddenly jumps towards or chases it	√	X	X
Muzzle play bite	Bite target: the muzzle of the playmate	√	√	X	Muzzle Rubbing C - N	An individual repeatedly passes from side to side the lateral part of its muzzle on a body part of the playmate	√	√	X	Walking Play Object Sharing C - N	Two individuals hold the same object in their mouths while walking side-by-side	√	X	X
Neck play bite	Bite target: the ventral part of the neck (throat)	√	√	√	Nose-Body Play C - N	An individual touches with its nose the body of another individual	√	√	√	Attempt Object Steal C - O	An individual tries to rip out an object from the playmate's mouth or catch an object that is close to the holder	√	X	X
Nape play bite	Bite target: the nape of the playmate	√	√	√	Nose-to-Nose Play C - N	Two individuals touch reciprocally their noses	√	√	√					
Back play bite	Bite target: the back of the playmate	√	√	√	Jump Over C - O	An individual jumps on the body of the playmate	√	√	√					
Tail/Paw play bite	The target is one of the body extremities (tail or paws)	√	√	X										

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Figure 2 – Box plots showing the total amount of time spent in playing (seconds/minute of observation) across the age-class combinations over the entire period of observation. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the inter-quartile range, IQR. The open dots indicate outliers more than 1.5 IQR from the rest of the

835 scores. The asterisk indicates an outlier more than 3.0 IQR from the rest of the scores. Only the

836 statistical significances are reported.
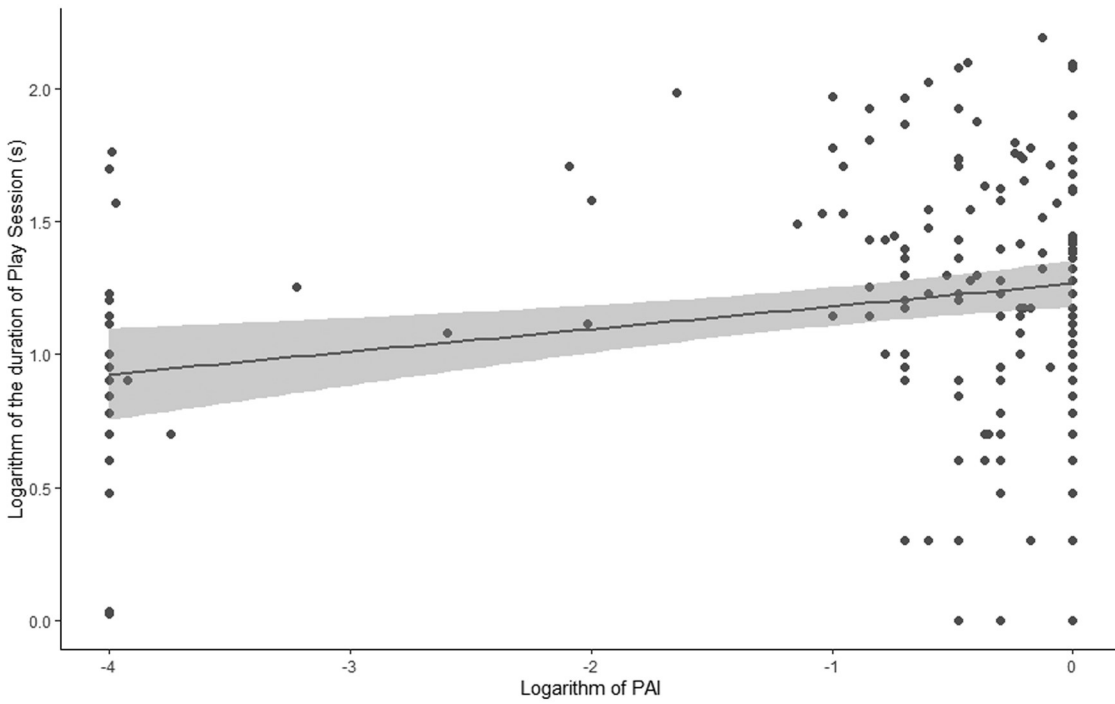
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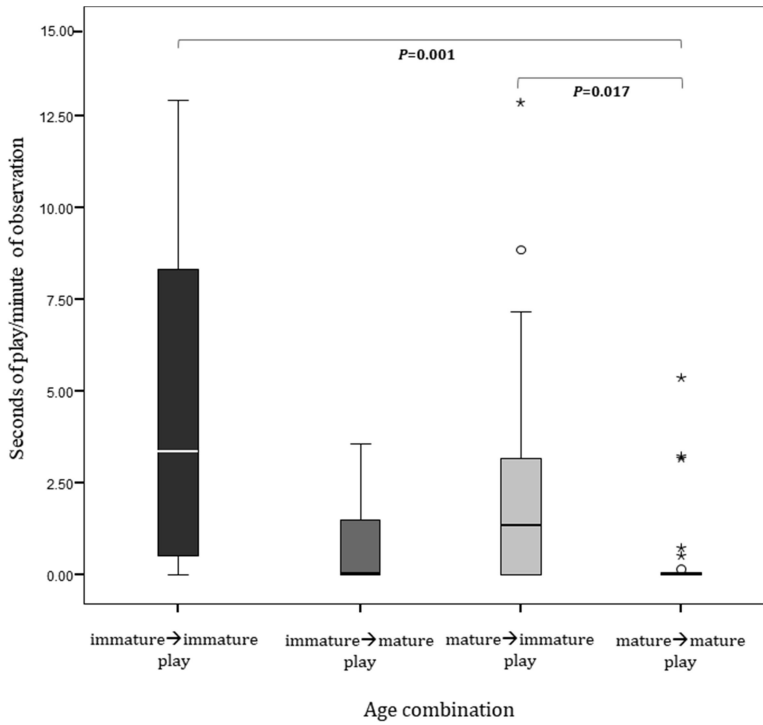
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845 **Figure 3** – Scatterplot showing the positive correlation between the logarithm of the absolute values

846 play asymmetry index (|PAI|) and the logarithm of the duration of each play session. Each dot

847 represents the length of a single play session and its relative |PAI| value. The gray band represents

848 the confidence region.
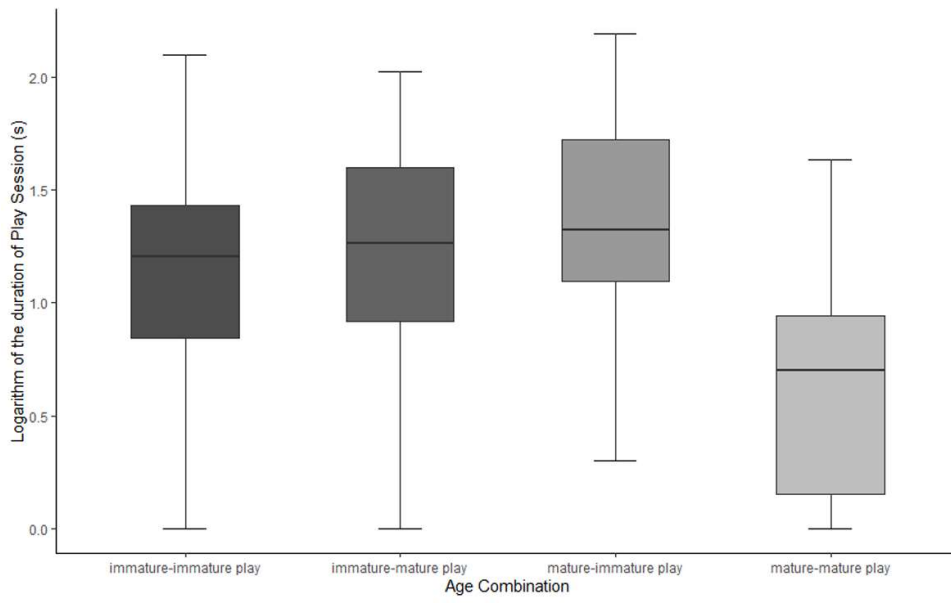
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860 **Figure 4** – Box plot showing the logarithm of the length of each play session according to the

861 different age-class combinations. The box plots show the median and 25th and 75th percentiles; the

862 whiskers indicate the values within 1.5 times the inter-quartile range, IQR.

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