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African penguins utilize their ventral dot patterns for individual recognition

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Birds are known to be highly social and visual animals. Yet no specific visual feature has been identified to be responsible for individual recognition in birds. Here, using a differential looking paradigm across five experiments, we demonstrated that African penguins, *Spheniscus demersus,* spontaneously discriminated between life-size photographs of their monogamous, lifelong partner and a nonpartner colonymate using their ventral dot patterns. Our findings challenge the assumption of limited visual involvement in penguin communication and suggest a rather complex and flexible recognition process in these birds. The combination of our current results and previous findings, which showed cross-modal (visual/auditory) recognition in these animals, suggests that African penguins use their ventral dot patterns to individually recognize their colonymates. Our results provide the first evidence of a specific visual cue responsible for spontaneous individual recognition by a bird, and highlight the importance of considering all sensory modalities in the study of animal communication.

The ability to recognize others as specific individuals is considered to be a building block for the evolution of sociality and the maintenance of cooperation (Tibbetts & Dale, 2007; Wiley, 2013). Individual recognition can be helpful for many complex social behaviours, such as understanding the relative position of a conspecific within a dominance hierarchy (Paz-y-Miño et al., 2004), allocating efficient parental care (Jouventin & Aubin, 2002) and discriminating neighbours from intruders in territorial defence (Godard, 1991; Temeles, 1994). Individual recognition has been shown across the animal kingdom (Brecht & Nieder, 2020; Gherardi et al., 2012; Tibbetts & Dale, 2007; Wiley, 2013; Yorzinski, 2017) and the different sensory cues that are used (primarily or solely) for identity vary widely (Chung & Wang, 2020), including visual (e.g. Pascalis & Kelly, 2009; Tibbetts, 2002), olfactory/chemical (e.g. D'Ettorre & Heinze, 2005; Pitcher et al., 2011) and auditory cues (Charrier et al., 2001; Lovell & Lein, 2005).

Birds are highly visual and social animals. Yet only a handful of studies have attempted to investigate their visual recognition abilities, and of those, few can speak to visual individual recognition (Brecht & Nieder, 2020).

Some earlier experimental studies (Guhl & Ortman, 1953; Izawa & Watanabe, 2008; Shimizu, 1998) had taken for granted the presence of individual visual recognition because of the formation of hierarchies in the birds studied. The aim was then to determine which visual cues were responsible; for example, by measuring aggressive behaviour between individuals as a result of overt artificial changes to a bird's appearance. However, class recognition (Wiley, 2013; Yorzinski, 2017) can be sufficient to form hierarchies; for example, by simply distinguishing conspecifics as subordinate or dominant (Guhl & Ortman, 1953). Therefore, it is unclear whether these previous works' findings relate to individual visual recognition.

Several studies used extensive training procedures to determine which visual features birds could learn to use to differentiate images of individual conspecifics (Brecht et al., 2017; Brown & Doolong, 1992; D'Eath & Stone, 1999; Jitsumori et al., 1999; Ryan & Lea, 1994; Trillmich, 1976). Although these works highlighted birds' visual discrimination abilities, they did not provide evidence

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of what birds actually use for individual recognition. To do so, we must rely on an animal's spontaneous reactions.

Several studies have measured birds' spontaneous behaviours (e.g. courtship displays or feeding proximity) to determine specific visual features responsible for those behaviours (D'Eath & Stone, 1999; Nash et al., 1989; Patton et al., 2017). However, these designs examined class recognition, such as familiar versus unfamiliar conspecifics, or simply males' responses to images of females, and thus not individual recognition.

Perhaps the most convincing way to show visual individual recognition has been to test for cross-modal recognition. Only two bird studies so far have used this design, one with the large-billed crow, *Corvus macrorhynchos* (Kondo et al., 2012) and one with African penguins, *Spheniscus demersus* (Baciadonna, Solvi, et al., 2021), both of which employed a 'violation of expectation' paradigm. After a pair of birds spent a short time where they could see each other, visual access was then blocked and a call from either the same or different familiar conspecific was played on a speaker. Crow and penguin subjects looked faster towards calls that did not match the just previously seen conspecific, suggesting that these birds are able to individually identify colonymates across the vocal and visual domains. However, neither of these studies attempted to test which specific visual features were involved.

Another study with a design that could speak to individual visual recognition compared the spontaneous reactions of rooks, *Corvus frugilegus*, to videos of their partner or of a nonpartner (Bird & Emery, 2008). Because rooks are a monogamous species (Emery et al., 2007), this design measured responses to a specific individual (i.e. a rook's partner) and therefore could measure spontaneous individual recognition. Although rooks responded differently to their partner compared to a nonpartner, no specific visual feature was found to be responsible. In fact, because the difference in response disappeared when using static images, one interpretation is that the rooks responded to differences in movement patterns rather than specific visual features (Brecht & Nieder, 2020).

Although the vocal cues birds use to recognize each other have been well studied (Carlson et al., 2020), which specific visual features are responsible for individual recognition in birds has remained elusive. To be effective as identity cues, visual features need to fulfil multiple criteria (Dale et al., 2001; Tibbetts, 2002; Tibbetts & Dale, 2007). They must be salient, have a high degree of phenotypic variability across individuals, and have at least a high polymodal distribution across a large population (if not unique to each individual). Their distinctiveness should also be independent of the individual's fitness, and they should be relatively stable over the animal's life span (Dale et al., 2001).

African penguins have salient ventral dot patterns on their lower bodies (Fig. 1) that provide ample opportunity for individual recognition as they meet all the requirements for a potential individual identifier. This distinctive pattern of dots is present within the ventral feathers of each penguin, appears at the age of 3–5 months and does not change throughout their lives (Tilo et al., 2004). The pattern does not seem to be reliant on the individual animal's living conditions (e.g. amount of food, temperature during development) and is unique to each individual penguin. These patterns have been exploited to develop an artificial intelligence system to identify penguins from images or video sequences (Sherley et al., 2010), and are often used by zookeepers to recognize individual penguins. Penguins' vision is adapted for water and air (Hadden & Zhang, 2023; Howland & Sivak, 1984; Sivak et al., 1987); hence, we hypothesized that African penguins may utilize their ventral dot patterns to recognize each other.

Figure 1. A few members of the Zoomarine Italia penguin colony. Unique ventral dot patterns are visible on each penguin's chest. Photo: Cristina Pilenga.

Penguins are an excellent example of a social animal utilizing unique cues to identify individuals. Their vocalizations are understood to play a strong role in individual recognition (Aubin et al., 2000; Aubin & Jouventin, 2002; Clark et al., 2006; Favaro et al., 2015, 2016; Jouventin & Aubin, 2002; Jouventin et al., 1999; Robisson et al., 1993), and for communication in general (Dobson & Jouventin, 2018). Penguins belonging to the genus *Aptenodytes* (two non-nesting species), for example, have a two-voice system which provides a unique vocal signature for each bird enabling them to localize and recognize a parent or a mate within large, noisy and crowded colonies (Aubin et al., 2000; Aubin & Jouventin, 2002). Although visual features of penguins, such as the coloured beak spots and auriculars of the king and emperor penguins, have been examined for their potential role in mate choice (Dobson et al., 2011; Jouventin et al., 2008; Keddar et al., 2015; Nolan et al., 2010), whether penguins use any specific visual features for individual recognition is unknown.

Here, using a 'differential looking time' paradigm (Pfefferle et al., 2014) over a series of experiments, we tested whether African penguins spontaneously discriminate between their partner and a nonpartner colonymate by using their ventral dot patterns. Note that partner recognition by an animal that has multiple partners may only indicate a type of class recognition (Steiger & Müller, 2008; Tibbetts & Dale, 2007; Tibbetts et al., 2008), unless the animal can also distinguish between multiple partners. However, if an animal has only one partner, then this same behaviour can be taken to be indicative of individual recognition (Steiger & Müller, 2008; Tibbetts et al., 2008). African penguins are a monogamous species, and stay with one partner their entire lives, creating a special social bond (Borboroglu & Boersma, 2013). However, penguins are unlikely to be more familiar with their partner than with any other penguin in their colony. This is because during the breeding season, they often leave their partner at the nest for extended periods of time to forage with nonpartner colonymates. Further, especially with zoo-kept colonies, penguins are always able to see (and hear) all their colonymates as often as their partner. Therefore, we were able to examine whether these birds' ventral dot patterns are responsible for their ability to visually recognize each other.

METHODS

Subjects and Housing

The African penguins in our study were housed at the Zoomarine Marine Park, Torvaianica, Rome, Italy. The colony consisted of

16 adult individuals and six young birds (age range 2 months—19 years). Each penguin can be identified by a uniquely coloured plastic ring (flipper band) attached to one of their wings.

For the study, 12 individuals were tested from September 2021 to March 2022. These 12 penguins (five females, F, and seven males, M) were stable partners (five F/M pairs and one M/M pair). According to the veterinary book records, the penguins did not have hearing or eyesight problems and were comfortable with, and habituated to, being around humans.

Preparation of Stimuli and Testing Design

Two months before testing began, each individual penguin within the colony was photographed multiple times (Canon EOS 250D camera, using an EF-M 55-200 mm F4.5—6.3 IS STM lens). Of these images, 37 were digitized and printed (TASKalfa 2554ci laser printer, Kyocera Document Solutions) on an opaque white cardboard (250 g/m², 170/CIE, 29.7 × 42 cm; i.e. approximately real-life size of African penguins) to be used in the experiments. The original background of all images was replaced with a white uniform background (Adobe Photoshop Lightroom). The 37 images (see Table 1 for the images used for each test) comprised three images of each of the test subjects, plus one image of a nontest subject colonymate (Kowalsky). The images of Kowalsky and Chicco were used for the nonpartner conditions. Kowalsky was used for nonpartner images because he was the only remaining adult penguin in the colony that was not a test subject, since he did not have a partner. Chicco was also used for nonpartner images, in addition to Kowalsky, to ensure reactions were not specific for a particular nonpartner.

In tests 1 and 2, two different sets of images were used. In test 3 we used a new set of images except for that of Kowalsky which was taken from test 1. However, the image of Kowalsky was presented only to the subjects that in test 1 experienced the image of Chicco. This reduced the impact of seeing the same image more than once. In tests 4 and 5 we reused portions (head or lower body portion) of 13 images that were used in the first three tests. In particular, the images of Bianca, Fiorella, Gerry, July and Kowalsky were reused from test 1. The images of Africa, Nemo and Nino were reused from test 2. Finally, the images of Akira, Chicco, Rico, Soldato and Yve were reused from test 3. Although in tests 4 and 5 portions of some images were reused from tests 1—3, we expected that the addition of the novel wooden panel and the change (missing portion) of the earlier image would reduce any potential habituation to the stimuli.

Experimental Set-up and General Procedure

Each penguin was tested using an opportunistic method to adhere to the hosting facility regulations for handling and separating the birds. Each daily experimental session was performed as follows: before one of the daily feedings, two keepers entered the main exhibit area, called the attention of the entire colony, led them all into a secluded area and began the normal daily feeding session. While feeding the penguins, the experimenter gradually herded them all, except the one selected to be tested, back into the outdoor exhibit. The experimenter switched on two video cameras (one was a Sony HDR-CX140, 1280 × 720 resolution with 25 fps and the other was a Nilox 4K naked, 1920 × 1080 resolution with 60 fps) to record the test, while a second experimenter herded the focal penguin towards the entrance of the arena (Fig. 2). The test began when the test subject entered the arena and ended 90 s later, at which point the keeper entered the experimental area and herded the focal penguin to the main exhibit area.

For each test, penguins were allocated two different images which were placed in the arena as shown in Fig. 2b. Each penguin experienced test 1 twice so that presentation of the images on left and right sides of the set-up were counterbalanced for each subject over the two tests (Supplementary Material, Video S1). Given that side had no effect on penguins' behaviour, each penguin experienced each of tests 2—5 once, and the presentation of images on left and right sides was counterbalanced across penguins. The same subject was never tested twice on the same day, and always had a minimum of 2 days between their tests.

Image Types Used for Each Test

Each test used the images described below (see Table 1 for individual subject details).

Test 1 assessed whether penguins spontaneously discriminate between their partner and a familiar nonpartner by presenting one image of their partner (P) and one image of a nonpartner (NP; Fig. 3a).
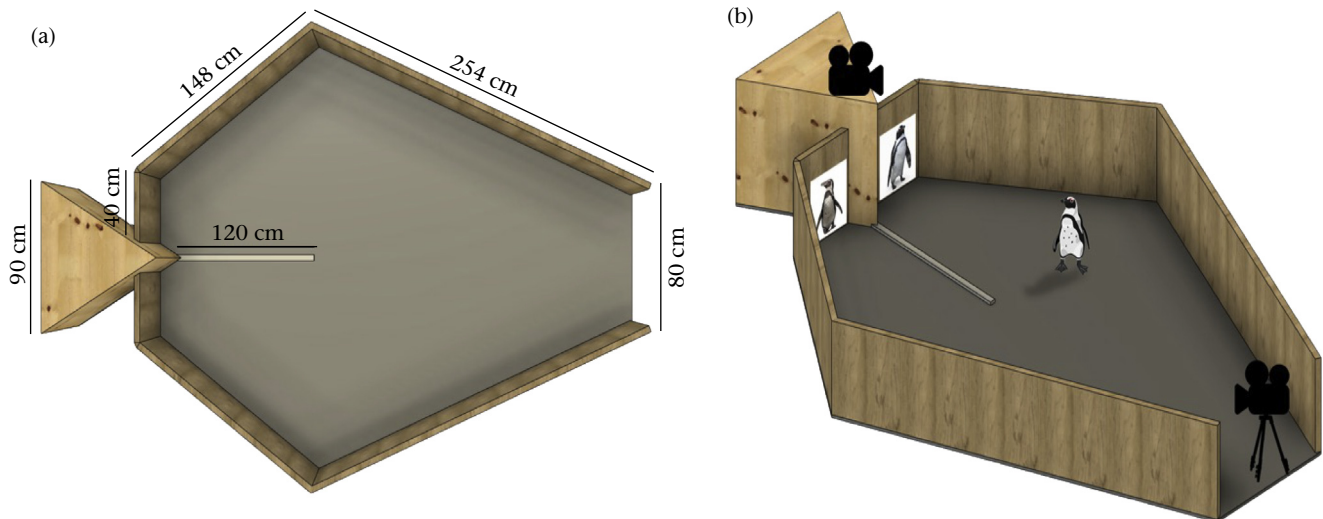
Test 2 assessed whether penguins' ventral dot patterns play a role in individual recognition by presenting one image of their partner (P) and one image of their partner without the ventral dot pattern (P(−dots); Fig. 3d). The dots were removed by overlaying each of them with an extracted small section of white colour plumage using Adobe Photoshop Lightroom. The two images were identical except for the absence of the dots.

Table 1
Image types used for all tests

Name	ID	Sex	Partner	Test 1		Test 2		Test 3		Test 4		Test 5	
				P	NP	P	P(−dots)	P(−dots)	NP(−dots)	P(head)	NP(head)	P(body)	NP(body)
Africa	12048	Female	Akira	Akira[1]	Kowalsky[1]	Akira[2]	Akira[2]	Akira[3]	Chicco[3]	Akira[3]	Chicco[3]	Akira[3]	Kowalsky[1]
Akira	13055	Male	Africa	Africa[1]	Chicco[1]	Africa[2]	Africa[2]	Africa[3]	Kowalsky[1]	Africa[2]	Chicco[3]	Africa[2]	Kowalsky[1]
Bianca	12257	Female	Nino	Nino[1]	Kowalsky[1]	Nino[2]	Nino[2]	Nino[3]	Chicco[3]	Nino[2]	Chicco[3]	Nino[2]	Kowalsky[1]
Nino	13252	Male	Bianca	Bianca[1]	Kowalsky[1]	Bianca[2]	Bianca[2]	Bianca[3]	Chicco[3]	Bianca[1]	Kowalsky[1]	Bianca[1]	Chicco[3]
Chicco	13255	Male	Nemo	Nemo[1]	Kowalsky[1]	Nemo[2]	Nemo[2]	Nemo[3]	Kowalsky[1]	Nemo[2]	Kowalsky[1]	Nemo[2]	Kowalsky[1]
Nemo	19158	Male	Chicco	Chicco[1]	Kowalsky[1]	Chicco[2]	Chicco[2]	Chicco[3]	Kowalsky[1]	Chicco[3]	Kowalsky[1]	Chicco[3]	Kowalsky[1]
Fiorella	10824	Female	Gerry	Gerry[1]	Chicco[1]	Gerry[2]	Gerry[2]	Gerry[3]	Kowalsky[1]	Gerry[1]	Chicco[3]	Gerry[1]	Kowalsky[1]
Gerry	3852	Male	Fiorella	Fiorella[1]	Chicco[1]	Fiorella[2]	Fiorella[2]	Fiorella[3]	Chicco[3]	Fiorella[1]	Chicco[3]	Fiorella[1]	Kowalsky[1]
July	10101	Female	Rico	Rico[1]	Kowalsky[1]	Rico[2]	Rico[2]	Rico[3]	Chicco[3]	Rico[3]	Chicco[3]	Rico[3]	Kowalsky[1]
Rico	10092	Male	July	July[1]	Kowalsky[1]	July[2]	July[2]	July[3]	Chicco[3]	July[1]	Kowalsky[1]	July[1]	Chicco[3]
Soldato	9061	Male	Yve	Yve[1]	Chicco[1]	Yve[2]	Yve[2]	Yve[3]	Kowalsky[1]	Yve[3]	Kowalsky[1]	Yve[3]	Chicco[3]
Yve	9048	Female	Soldato	Soldato[1]	Chicco[1]	Soldato[2]	Soldato[2]	Soldato[3]	Kowalsky[1]	Soldato[3]	Kowalsky[1]	Soldato[3]	Chicco[3]

The name, ID (penguin's European Association of Zoos and Aquaria (EAZA) code), sex and partner of the tested penguin are given. Image type indicates the image used across all tests. NP: nonpartner; P: partner. (−dots): ventral dot pattern removed; (head): only the head was visible on the image; (body): only the lower body was visible on the image. The superscript indicates the specific image used across all tests.

Figure 2. Individual visual recognition in African penguins. (a) Graphical representation of the test area from an aerial view. (b) Graphical representation of the experimental procedure.

Test 3 assessed whether penguins discriminate between their partner and a nonpartner both without ventral dot patterns by presenting one image of their partner without the ventral dot pattern (P(−dots)) and one image of a nonpartner without the ventral dot pattern (NP(−dots); Fig. 3g).

Test 4 assessed whether penguins discriminate between their partner and a nonpartner when only their heads are visible by showing one image of their partner (P(head)) and one image of a nonpartner (NP(head); Fig. 4a), each with their body covered by a wooden panel (36 × 40 cm).

Test 5 assessed whether penguins discriminate between their partner and a nonpartner when only their body is visible by presenting one image of their partner (P(body)) and one image of a nonpartner (NP(body); Fig. 4d), each with their head covered by a wooden panel (10 × 40 cm).

Video Coding

One of the coauthors (F.T.), blind to conditions, analysed all videos using the software BORIS (version 7.10.7; Friard & Gamba, 2016). The 'duration of looking' and 'duration of being in proximity' of each image were scored. The duration of looking was defined as the total time a penguin spent with its beak directed at the image. This method was used because behavioural evidence suggests that African penguins normally use forward-facing binocular vision when attending to objects (Hadden & Zhang, 2023). Further, in our experience, these birds point their beak at what they are attending to (e.g. experimenters and keepers; also see Supplementary Material, Video S1). The duration in proximity was defined as the total time the penguin spent on the side of the arena for that image (separated by a wooden post buried and level with the ground in the arena; Fig. 2), within 120 cm from and facing the image.

Of the videos, 25% were scored by a second observer (A.M.C.I., see Acknowledgments), also blind to the study design and conditions. The interclass correlation coefficients calculated for all the behaviours considered were 0.84 for duration of looking, 0.96 for duration of being in proximity, and total agreement (1) for first choice.

Statistical Analyses

Using the lme4 package (Bates et al., 2015) in R version 3.6.1 (R Development Core Team, 2020), we created two models for each test. Generalized linear mixed models (GLMMs) were used for test 1 and generalized linear models (GLMs) were used for tests 2−5. For all tests except for test 2, the fixed factors included the two image types used (described above for each specific test), the side on which the partner image was located (left or right) and the identity of the nonpartner penguin (Chicco or Kowalsky). For test 2, only image type (P and P(−dots)) and side (left and right) were included as fixed factors. For test 1, the identity of the test subject was set as a random factor to control for repeated measures over the two repetitions of test 1. For test 1, the significance of the full model was established by comparing the full model (all factors) with the null model (only the random factor) using the likelihood ratio test (Zuur et al., 2009). The repetition of test 1 was originally planned to control for side bias and to increase the number of data points for each penguin. However, as expected, when the penguins were retested, the overall duration of looking and duration in proximity decreased (Appendix Table A1). Therefore, for each of tests 2−5, penguins were tested only once and the side on which the partner image was placed was counterbalanced across penguins. For tests 2−5, the significance of each fixed factor for all models was obtained using the ANOVA function. For all tests, the model fit and the overdispersion were checked using the package DHARMa 0.3.3.0. The estimate, *t* ratio and *P* value are reported for all tests.

Ethical Note

All procedures were conducted in accordance with the ASAB/ABS guidelines for the care and use of animals in research (ASAB Ethical Committee & ABS Animal Care Committee, 2021) and were approved by the Ethics Committee of the University of Turin (approval number 0623075).

RESULTS

Using a differential looking paradigm over a series of tests, we examined whether African penguins spontaneously discriminate between their partner and a nonpartner colonymate by using exclusively visual information and whether penguins' ventral dot patterns play a role in individual recognition. We expected that each penguin should look longer at, and spend more time close to, their partner's image over a nonpartner's image since their partner
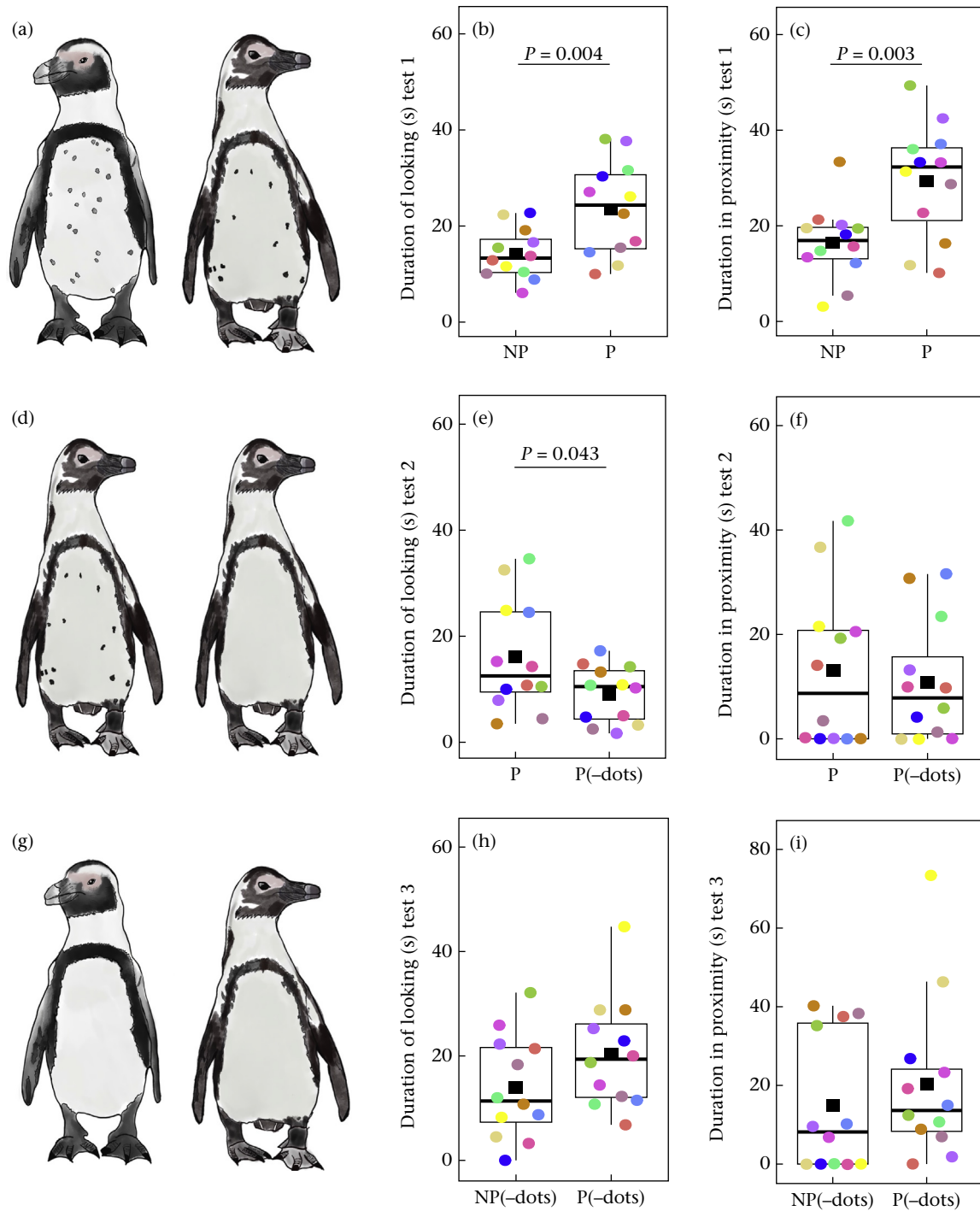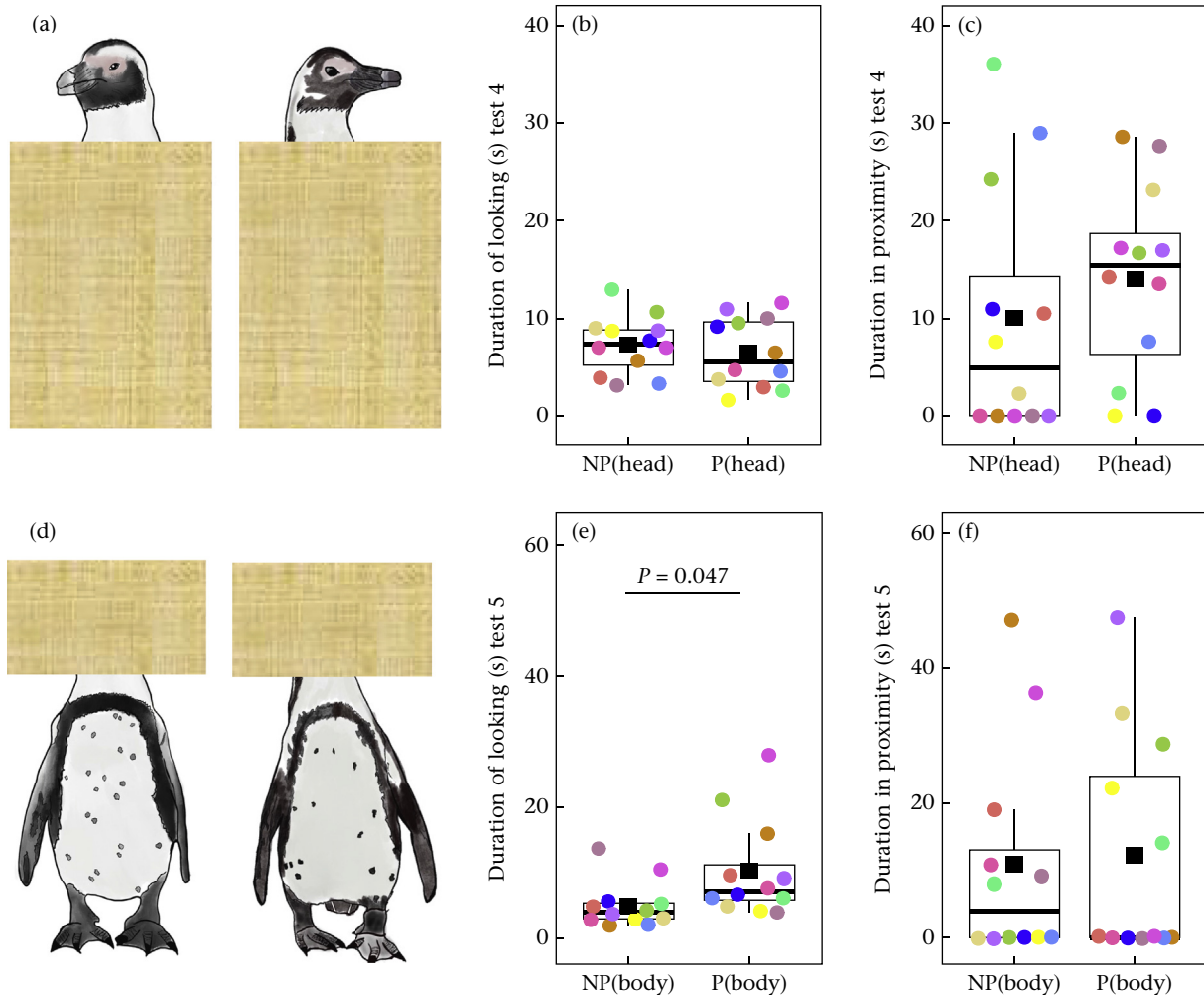
Figure 3. Role of ventral dot patterns, tests 1–3. (a) Graphical representation of test 1 images: a penguin's partner and a nonpartner. (b) Duration of looking towards and (c) duration in proximity of each image in test 1. (d) Graphical representation of image types used in test 2: a penguin's partner, P, and its partner without the ventral dot pattern, P(−dots). (e) Duration of looking towards and (f) duration in proximity of each image in test 2. (g) Graphical representation of images used in test 3: a penguin's partner and a nonpartner, both without their ventral dot pattern, P(−dots) and NP(−dots), respectively. (h) Duration of looking towards and (i) duration in proximity of each image in test 3. Box plots: horizontal lines = median; black squares = mean; boxes extend from lower to upper quartile and whiskers indicate interquartile range above the upper quartile (maximum) or below the lower quartile (minimum). Coloured filled circles represent each individual penguin.

should be the most important, salient and interesting penguin to them.

If penguins consistently looked more at their partner's image over the nonpartner image (test 1; Fig. 3a), this would suggest they can discriminate between the two images purely on visual cues. In test 1, we found that the full model differed significantly from the null model for duration of looking and for duration in proximity (Appendix Table A1). Penguins looked longer at their partner's (P) image (23.52 ± 3.12 s) than the nonpartner (NP) image (14.16 ± 2.11 s; Fig. 3b, Appendix Table A1). Penguins also spent more time in proximity of their partner's image (P: 29.37 ± 3.52 s) than the nonpartner image (NP: 16.37 ± 3.15 s; Fig. 3c, Appendix

Figure 4. Role of penguins' heads and lower bodies, tests 4—5. (a) Graphical representation of images used in test 4: a penguin's partner's head, P(head), and a nonpartner's head, NP(head). (b) Duration of looking towards and (c) duration in proximity of each image in test 4. (d) Graphical representation of images used in test 5: a penguin's partner's lower body, P(body), and a nonpartner's lower body, NP(body). (e) Duration of looking towards and (f) duration in proximity of each image in test 5. Box plots: horizontal lines = median; black squares = mean; boxes extend from lower to upper quartile and whiskers indicate interquartile range above the upper quartile (maximum) or below the lower quartile (minimum). Coloured filled circles represent each individual penguin.

Table A1). Neither side nor nonpartner identity predicted duration of looking or duration in proximity (Appendix Table A1). These results provide compelling evidence that penguins can visually discriminate between their partner and a nonpartner. It also shows that the images of their partner were highly relevant for the tested penguins; that is, they represented their partner well.

If penguins' ventral dot patterns act as a primary visual identity cue, we would expect a preference towards a complete image of the partner compared to the partner's image without the ventral dot pattern (test 2; Fig. 3d). Note that we might expect a preference for the unusual image because all the penguins within the colony have some dots on their ventral body. However, the duration of looking was predicted by image type (Appendix Table A2), and penguins looked for longer at the complete partner image (P: 16.09 ± 3.04 s) than the one without the ventral dot pattern (P(−dots): 9.04 ± 1.55 s; Fig. 3e, Appendix Table A2). The side (left or right) on which the partner-with-dots image was located did not affect penguins' choices (Appendix Table A2). The duration in proximity was not predicted by image type (Fig. 3f) or the side on which the partner-with-dots image was located (Appendix Table A2). The results of test 2 indicate that the ventral dot patterns play a role in penguins' visual recognition of their partners.

If individual visual recognition relied heavily on their ventral dot patterns (over other visual features), we would also expect an impediment in penguins' ability to discriminate between a partner without ventral dot patterns (P(−dots)) and a nonpartner without ventral dot patterns (NP(−dots)). In test 3, we examined this question (Fig. 3g). The duration of looking was not predicted by image type (P(−dots): 20.41 ± 3.04 s; NP(−dots): 13.96 ± 2.87 s; Fig. 3h), side or nonpartner identity (Appendix Table A3). Likewise, the duration in proximity was also not predicted by image type (P(−dots): 20.36 ± 6.02 s; NP(−dots): 14.82 ± 5.03 s; Fig. 3i), side or nonpartner identity (Appendix Table A3). These results, in combination with those of test 2, suggest that the ventral dot pattern plays a major role in penguins' ability to visually recognize their partners.

Tests 4 and 5 were done to examine whether penguins need to see the entire bodies of other penguins to recognize them. In test 4 (Fig. 4a), when penguins could only see other penguins' heads, duration of looking was not predicted by image type (P(head): 6.48 ± 1.03 s; NP(head): 7.31 ± 0.86 s; Fig. 4b), side or nonpartner identity (Appendix Table A4). The duration in proximity was also not predicted by image type (P(head): 14 ± 2.85 s; NP(head): 10.06 ± 3.70 s; Fig. 4c), side or nonpartner identity (Appendix

Table A4). In test 5 (Fig. 4d), when penguins could only see other penguins' lower bodies, penguins looked for longer at their partner's image (P(body): 10.31 ± 2.17 s) than the nonpartner image (NP(body): 5.04 ± 1.02 s; Fig. 4e, Appendix Table A5). Neither side nor nonpartner identity predicted the duration of looking (Appendix Table A5). Duration in proximity was not predicted by image type (P(body): 12.16 ± 4.86 s; NP(body): 10.89 ± 4.56 s; Fig. 4f), side or nonpartner identity (Appendix Table A5).

DISCUSSION

Our results suggest that African penguins strongly rely on their ventral dot patterns for individual visual recognition of their partners. Penguins clearly preferred their partner over a nonpartner when the entire body was visible (test 1), preferred their partner with dots to without dots (test 2), showed no preference when the ventral dot pattern was removed (test 3), and lacked a preference when only the head was visible (test 4). Although penguins showed a preference when they could only see others' lower bodies (test 5), this preference was less strong than when the entire body could be seen (compare Fig. 4e with Fig. 3b). This difference in behaviour may have been a result of penguins' reactions to the novel wooden panels, or perhaps to a habituation effect because parts of some images were reused in tests 4 and 5 (Methods). However, it is very likely that under certain circumstances, penguins can use different parts of others' bodies to help recognize each other. Together, these observations suggest that African penguins may rely on a more holistic visual representation of their partner, which includes both the ventral dot patterns and their partner's facial features. Notably, these abilities are not dependent on the ability of a pair to produce offspring together. Nemo and Chicco, male partners, showed the same preference for each other as other penguin partner pairs, suggesting that the ability to distinguish the partner from others is driven simply by the special bond developed between nesting partners.

Could it be that penguins simply recognize their partner from all other penguins, and cannot distinguish between other colonymates? Alone, our current results leave open the possibility that African penguins are unable to distinguish nonpartner colonymates from each other, and only make the distinction between partner and nonpartner. Confirmation of this might require a paradigm more sensitive to subtler differences in saliency (i.e. nonpartner colonymates may only be slightly more salient than each other), or comparing penguins' responses to individuals belonging to a different colony. However, as mentioned above, we previously showed that African penguins can identify different nonpartner colonymates cross-modally (visually/vocally), showing that they can visually recognize nonpartner colonymates (Baciadonna, Solvi, et al., 2021). It might be that penguins use their ventral dot patterns to recognize their partner, while using some other visual cue to individually recognize nonpartner colonymates. However, we argue that the most parsimonious interpretation of these two studies is that penguins use their ventral dot patterns for individual recognition of any colonymate.

It is pertinent to ask how African penguins evolved to use their ventral patterns for individual recognition. Various hypotheses have been proposed to explain how animals develop systems of recognition, and which types of cues they use to recognize others (Wiley, 2013). One possible explanation is that variation in recognition evolves to match the minimum recognition requirements of each species and that more complex forms of recognition evolve only when simpler mechanisms do not provide enough information to distinguish individuals (Tibbetts & Dale, 2007; Wiley, 2013; Yorzinski, 2017). For example, in non-nesting penguins, the necessity to localize and recognize important individuals (their partner and offspring) among thousands of colonymates, in the absence of reliable and/or distinct visual cues, might have provided the necessary driver for more complex and individually distinct vocalizations (Jouventin & Aubin, 2002; Jouventin & Dobson, 2018). In contrast, African penguins build nests which they defend from others. Visual cues, like their ventral dot patterns, would have ultimately been a more efficient modality, and perhaps were selected for, to distinguish both partner from nonpartners, close neighbours from distant neighbours, and familiar from nonfamiliar penguins. Note, however, that perhaps African penguins simply took advantage of a visual cue which evolved for other reasons (i.e. by chance) and this in and of itself drove its use alongside vocalizations as concomitant cues for recognition (Freeberg et al., 2021). It may also be that individual recognition abilities are a simple by-product of a species' general learning ability. For example, corvids' remarkable cognitive skills (Baciadonna, Cornero, et al., 2021) are proposed to explain their ability to combine visual and auditory cues to recognize others (Kondo et al., 2012). The results of the current study and previous work (Baciadonna, Solvi, et al., 2021) support the idea that penguins have a rather complex and flexible ability to reliably identify others in their colony. Our results challenge the idea that penguins use only acoustic information to identify each other, and that the only visual information they rely on is the spatial location of their nest (Aubin & Jouventin, 2002; Brecht & Nieder, 2020; Jouventin & Aubin, 2002; Jouventin & Dobson, 2018). We provide the first behavioural evidence of a specific visual feature that is at least partially responsible for spontaneous individual recognition in a bird species.

An intriguing question arising from our results is what perceptual aspects of the ventral dot patterns are driving penguins' recognition of other individuals. Future studies with African penguins that could help address this question might be to manipulate dot number, position and/or spatial configuration. These manipulative studies could shed light on whether penguins' ventral dot patterns play a role in the social dynamics and cooperation within a colony, and the interplay with vocalizations. The receiver's responses to these changes could also be measured. If the ability to individually recognize other penguins is adaptive, we should expect selective pressure on the receiver to have evolved an ability to assess and discriminate that specific signal and ignore other costly or unreliable signals.

We speculate that, given their high visual acuity, many other bird species also probably use visual features for individual recognition. It may be difficult to isolate and manipulate the most important specific features, especially as easily as the ventral dot patterns of African penguins. If a bird uses holistic representations of others, it may also be that various features play relatively equal roles. This would result in either no or a similar difference in behaviour with any feature manipulation. However, we believe that as long as a bird has a particularly special individual in its life (e.g. long-term partner), combining a cross-modal recognition expectancy violation paradigm with a differential looking paradigm can be a powerful approach for investigating specific features responsible for spontaneous individual recognition in any species.

Author Contributions

Luigi Baciadonna: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - Original draft. **Cwyn Solvi**: Methodology, Writing - Original draft. **Francesca Terranova**: Formal analysis. **Camilla Godi**: Investigation, Data curation. **Cristina Pilenga**: Investigation, Data curation. **Livio Favaro**: Conceptualization, Writing - Review & editing.

Data Availability

The data used for this study are available in the Supplementary Material.

Declaration of Interest

The authors have no competing interests to declare.

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Supplementary Material

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Appendix

Table A1
Summary of the GLMM examining the influence of the fixed factors on response variables for test 1

Response variable	Fixed factor	Estimate	SE	t	P
Duration of looking					
	(intercept)	20.41	3.65		
	Image type	9.35	3.14	2.80	0.004
	Session	−13.15	3.14	−4.23	< 0.0001
	Side	−1.54	3.14	−0.52	0.62
	Nonpartner identity	1.89	3.19	0.63	0.55
Duration in proximity					
	(Intercept)	19.61	4.89		
	Image type	12.99	4.21	3.08	0.003
	Session	−12.41	4.21	−2.94	0.004
	Side	0.28	4.21	0.06	0.94
	Nonpartner identity	4.86	4.27	1.13	0.25

Results of the reduced model when the duration of looking was considered: full versus null: $X^2_4 = 21.36, P < 0.0001$. Image type and session predicted the duration of looking and neither side nor nonpartner identity predicted duration of looking. Results of the reduced model when the duration in proximity was considered: full versus null: $X^2_4 = 16.35, P = 0.003$. Image type and session predicted the duration of looking and neither side nor nonpartner identity predicted duration of looking.

Table A2
Summary of the GLM examining the influence of the fixed factors on response variables for test 2

Response variable	Fixed factor	Estimate	SE	t	P
Duration of looking					
	(Intercept)	15.51	3.02		
	Image type	−7.05	3.48	−2.02	0.043
	Side	1.16	3.48	0.33	0.73
Duration in proximity					
	(Intercept)	14.14	4.86		
	Image type	−2.28	5.61	−0.40	0.68
	Side	−2.02	5.61	−0.36	0.71

Image type ($X^2_1 = 4.08, P = 0.043$) predicted the duration of looking, whereas side ($X^2_1 = 0.11, P = 0.73$) did not. Image type ($X^2_1 = 0.16, P = 0.68$) and side ($X^2_1 = 0.12, P = 0.71$) did not predict the duration in proximity of either image.

Table A3
Summary of the GLM examining the influence of the fixed factors on response variables for test 3

Response variable	Fixed factor	Estimate	SE	t	P
Duration of looking					
	(Intercept)	10.30	5.62		
	Image type	6.45	4.29	1.50	0.13
	Side	1.75	4.98	0.35	0.72
	Nonpartner identity	4.77	5.05	0.94	0.34
Duration in proximity					
	(Intercept)	4.49	10.37		
	Image type	5.53	7.93	0.69	0.48
	Side	7.16	9.20	0.77	0.43
	Nonpartner identity	11.57	9.33	1.24	0.21

None of the fixed factors predicted the duration of looking (image type: $X^2_1 = 2.25$, $P = 0.13$; side: $X^2_1 = 0.12, P = 0.72$; nonpartner identity: $X^2_1 = 0.89, P = 0.34$) or the duration in proximity (image type: $X^2_1 = 0.48, P = 0.48$; side: $X^2_1 = 0.60, P = 0.43$; nonpartner identity: $X^2_1 = 1.53, P = 0.21$).

Table A4
Summary of the GLM examining the influence of the fixed factors on response variables for test 4.

Response variable	Fixed factor	Estimate	SE	t	P
Duration of looking					
	(Intercept)	6.11	1.29		
	Image type	−0.95	1.36	−0.69	0.48
	Side	0.71	1.36	0.52	0.59
	Nonpartner identity	1.79	1.34	1.33	0.18
Duration in proximity					
	(Intercept)	8.25	4.67		
	Image type	3.86	4.92	0.78	0.43
	Side	0.45	4.92	0.09	0.92
	Nonpartner identity	3.22	4.85	0.66	0.50

None of the fixed factors predicted the duration of looking (image type: $X^2_1 = 0.48$, $P = 0.48$; side: $X^2_1 = 0.27, P = 0.59$; nonpartner identity: $X^2_1 = 1.78, P = 0.18$) or the duration in proximity (image type: $X^2_1 = 0.61, P = 0.43$; side: $X^2_1 = 0.008, P = 0.92$; nonpartner identity: $X^2_1 = 0.44, P = 0.50$).

Table A5
Summary of the GLM examining the influence of the fixed factors on response variables for test 5

Response variable	Fixed factor	Estimate	SE	t	P
Duration of looking					
	(Intercept)	7.30	2.85		
	Image type	4.93	2.49	1.98	0.047
	Side	−1.99	2.49	−0.79	0.42
	Nonpartner identity	−1.63	2.60	−0.62	0.52
Duration in proximity					
	(Intercept)	14.72	7.98		
	Image type	0.28	6.96	0.04	0.96
	Side	−5.97	6.96	−0.85	0.39
	Nonpartner identity	−0.52	7.28	−0.07	0.94

Image type ($X^2_1 = 3.92, P = 0.047$) predicted the duration of looking, whereas side ($X^2_1 = 0.68, P = 0.42$) and nonpartner identity ($X^2_1 = 0.39, P = 0.52$) did not. None of the fixed factors (image type: $X^2_1 = 0.001, P = 0.96$; side: $X^2_1 = 0.73, P = 0.39$; nonpartner identity: $X^2_1 = 0.005, P = 0.94$) predicted the duration in proximity.