

Supplemental Information

Application of insects to wounds of self and others in chimpanzees in the wild

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Supplemental Experimental Procedures

Methods and Selected Event Descriptions

Methods. All events involving insect application were observed whilst conducting behavioural observations on chimpanzees of the Rekambo community in the Loango National Park, Gabon (2°04'S and 9°33'E). The habituation of this community began in 2005 and the majority of individuals were habituated to human presence in 2017. During the study period considered here, November 2019 – February 2021, the community consisted of approximately 42-45 individuals including eleven adult males, 15 adult females, adolescents, juveniles and infants (following the classification of Goodall^{S1}). Focal animal sampling² was used during daily follows on a rotational basis of all adolescent and adult individuals. However, total focal hours varied between male and female individuals due to a tendency for researchers to follow males more frequently, given the variability in daily party composition and the lower habituation levels of females. The total observation hours for the period considered here were 2614 hours for males and 927 hours for females. The documentation of wounds was recorded using all occurrence sampling^{S2} across all visible community members (see Table S1). Data collection was operationalized via 4K video cameras (SONY AX53), and CyberTracker software (CyberTracker version 3.507; <https://www.cybertracker.org/>) on water-resistant smart phones (Cyrus CS45).

We attribute the differences in the rates of male versus female open wounds (63:13) to the fact that wounds are acquired mostly during aggressive interactions which are more frequent amongst males^{S3}. All reported observations of insect capture and insect application were made by different teams of our Ozouga Loango Chimpanzee Project comprising seven eco-guides, Christopher Igoumounamendet, Fredy Makaya, Ulrich Bora Moussouami, Gael Mweniambiet, Stephane Nzienge, Darlhy Rendjogo, and six researchers, Louise Ducroix, Harmonie Klein, Laura Martínez-Íñigo, Alessandra Mascaro, Lara Southern, and Tanguy Tanga. All events were filmed whenever possible and were later transcribed into detailed written reports. In the case that no footage was recorded, a detailed case report was written the same day of the event. The video footage available for 14 application events (n=14) had an average length of 1.8 minutes (111 seconds, SD = 76 seconds) per video. The average time applied insects spent on the wounds was 0.9 minutes (56 seconds, SD = 29 seconds) per event. However, these are minimum estimates and multiple factors may have influenced these

estimates. For example, filming often began after the initial application, the view on the wound and insect were often temporarily obscured by other chimpanzees and in the majority of cases, following the termination of the application sequence, it was unclear whether the insect remained inside the wound or had been discarded.

Table S1. Observation of wounds and insect applications

Date	Individual	Sex	Age class	Wound location	Insect application	Applicator	Video footage
11/13/19	Sia	male	adolescent	left foot	1	Suzee ¹	X
11/25/19	Freddy	male	adult	left arm	2	Freddy	
11/26/19	Freddy	male	adult	left arm	1	Freddy	X
11/28/19	Freddy	male	adult	left arm	1	Freddy	
11/24/19	Pandi	male	adult	back	0	N/A	
11/30/19	Ngonde	male	adult	back	0	N/A	
12/09/19	Louis	male	adult	left foot	1	Louis	
01/05/20	Moana	male	juvenile	back	0	N/A	
01/21/20	Pandi	male	adult	lower back	0	N/A	
01/30/20	Orian	male	adult	upper back	0	N/A	
01/30/20	Freddy	male	adult	left hand	0	N/A	
02/02/20	Gia	female	adolescent	right arm	0	N/A	
02/17/20	Ngonde	male	adult	right wrist	1	Ngonde	
03/14/20	Thea	male	adult	right side	1	Thea	
03/16/20	Thea	male	adult	right side	1	Thea	X
03/15/20	Freddy	male	adult	left hand	0	N/A	
03/15/20	Littlegrey	male	adult	right leg	0	N/A	
03/27/20	Gump	male	adult	left arm	0	N/A	
04/09/20	Chinois	male	adult	left foot	0	N/A	
05/18/20	Carol	female	adult	right arm, back, right leg	0	N/A	
05/19/20	Cesar	male	adolescent	left hand	0	N/A	
05/26/20	Freddy	male	adult	right arm, lower back	1	Freddy	
05/29/20	Freddy	male	adult	left arm	0	N/A	
06/06/20	Freddy	male	adult	right arm	1	Freddy	X
06/25/20	Freddy	male	adult	left wrist	1	Freddy	X
07/03/20	Chenge	male	adult	back of neck	0	N/A	
07/11/20	Arnold	male	adult	back	0	N/A	
07/22/20	Carol	female	adult	back, shoulder, head	0	N/A	
07/22/20	Chenge	male	adult	left leg	0	N/A	
07/23/20	Chenge	male	adult	right arm	0	N/A	
08/02/20	Chinois	male	adult	back	0	N/A	
08/05/20	Moana	male	juvenile	left leg	0	N/A	
09/01/20	Littlegrey	male	adult	lower back	0	N/A	
09/01/20	Thea	male	adult	back, lip	0	N/A	
09/02/20	Carol	female	adult	genital swelling	0	N/A	
09/02/20	Littlegrey	male	adult	right leg	0	N/A	
09/03/20	Littlegrey	male	adult	left hand	0	N/A	
09/06/20	Carol	female	adult	left leg	0	N/A	
09/10/20	Spock	female	adult	back	0	N/A	
10/03/20	Chenge	male	adult	back, left arm	0	N/A	
10/06/20	Roxy	female	adult	left arm	0	N/A	
10/10/20	Freddy	male	adult	head	0	N/A	
10/12/20	Queliba	female	adolescent	left hand	0	N/A	
10/18/20	Olive	female	juvenile	left hand	0	N/A	

Date	Individual	Sex	Age class	Wound location	Insect application	Applicator	Video footage
10/19/20	Littlegrey	male	adult	left foot, left leg	1	Littlegrey	X
10/20/20	Littlegrey	male	adult	left foot, left leg	3	1.Littlegrey Carol ² , Thea ³ , Ngonde ⁴	X X X
10/24/20	Chenge	male	adult	chest	0	N/A	
10/24/20	Quelibaba	female	adolescent	left leg	0	N/A	
10/27/20	Chenge	male	adult	Back	0	N/A	
10/28/20	Thea	male	adult	left hand	0	N/A	
10/28/20	Orian	male	adult	left leg	0	N/A	
10/28/20	Chinois	male	adult	left arm	0	N/A	
10/28/20	Moana	male	adolescent	right leg	0	N/A	
11/01/20	Gump	male	adult	lower back	0	N/A	
11/03/20	Thea	male	adult	left arm	0	N/A	
12/04/20	Freddy	male	adult	left side	0	N/A	
12/09/20	Quelibaba	female	adolescent	right leg	0	N/A	
12/12/20	Cesar	male	adolescent	right foot	0	N/A	
12/28/20	Ngonde	male	adult	back	0	N/A	
12/29/20	Pandi	male	adult	left arm	0	N/A	
01/01/21	Chinois	male	adult	right arm	0	N/A	
01/13/21	Pandi	male	adult	left foot	0	N/A	
01/16/21	Freddy	male	adult	penis	1	Freddy	X
01/20/21	Freddy	male	adult	penis	1	Freddy	X
01/23/21	Chenge	male	adult	right/left arm	0	N/A	
01/28/21	Mimi	female	adult	shoulder	1	Mimi	X
01/29/21	Littlegrey	male	adult	left finger	1	Arnold	X
02/01/21	Littlegrey	male	adult	left finger	1	Littlegrey	X
02/02/21	Ngonde	male	adult	right back	0	N/A	
02/03/21	Chenge	male	adult	right/left leg	0	N/A	
02/04/21	Ivindo	female	adolescent	left arm	1	Ivindo	
02/07/21	Littlegrey	male	adult	right arm	0	N/A	
02/26/21	Chenge	male	adult	right leg	0	N/A	
Total	22			76	22		14

Table S1 shows all observations of wounds and insect applications of individuals of the Rekambo community between November 2019 and February 2021 as a function of date of observation, name of individual with wound, sex, age class, wound location, and whether insect application was observed. In cases where another individual was the applicator of an insect to the wound, the name is depicted with superscript numbers: ^{1,2, 3 and 4}. The total counts depict the total number of different individuals, the total number of different wounds observed, the total number of insect-catching events with at least one application to an open wound and the total number of events with video footage. All observations of insect application were observed by research teams composed of one guide and one researcher, except in one case on the 29/01/21 where the observation was made by a team composed of two guides (further credits are provided in the methods section of the SI).

Selected Event Descriptions. The event descriptions below were selected to provide further detail on one self-application event (event 1) and the three instances of allo-application within the study period (event 2, 3 and 4).

Event 1 (see Video S1 on xxx): Capture and self-application of an insect by an adult male, Freddy, to his own open wound

This video was recorded on November 26th, 2019. It involved an adult male, Freddy, who had a day-old wound, approximately five cm in length in the elbow crook of his left arm. He was resting in proximity (one armlength) to another adult male, Chenge. At 11:37 a.m., Freddy pulled a shrub towards himself with his left foot, reached out with his right hand and then made a quick catching movement thereby grabbing a leaf with his right hand. He then moved the leaf towards his mouth and used his lips to pick up an unidentified insect from it. He took the insect from his mouth with the thumb and index finger of his right hand and applied it to the wound on his left arm using his thumb to move the insect on the surface of the open wound. He then moved his head quickly towards the wound and picked up the insect again with his lips. Next, he again took the insect with the thumb and index finger of his right hand from his mouth/lips and applied it again to the wound. He pressed it softly with his thumb against the surface of the wound. Subsequently, he moved his fingers of his right hand back to his mouth and seemed to move and press the insect between his lips. He then applied it again to the wound with the thumb and index finger of his right hand. Overall, this video sequence shows a total of three applications of an unidentified insect to a wound.

Event 2 (see Video S2 on xxx): Capture and allo-application of an insect from an adult female, Suzee, to a wound of her adolescent son, Sia

This video footage was recorded on November 13th, 2019. It involved an adult female, Suzee, who had previously been resting with her infant, Sassandra, and her adolescent son, Sia. Sia had a day-old open wound of approximately three cm on his left foot and was lying on his back in proximity (one armlength) to her. Sassandra was swinging playfully around her. At 08:34 a.m., Suzee sat up and reached out with her right hand to catch an unidentified insect from under a leaf of a low hanging branch. She looked at it between her fingers and then placed it carefully between her lips. Sassandra directly stopped swinging, and jumped towards her mother and observed her behaviour. She stretched out her arms to be picked up but Suzee parried her off and moved closer to Sia whilst in parallel grabbing his left foot with her

right hand. Sia moved the foot up in the air and disengaged Suzee's grip while Suzee was still parrying off Sassandra. She then grabbed Sia's foot with her left hand, handed it over to her right hand, took the insect out of her mouth with the fingers of her left hand and applied it to the wound. Subsequently, she picked up the insect from the wound with her mouth and appeared to keep it tightly pressed between her lips. She then moved the fingers of her left hand to her lips, took the insect and applied it again to Sia's wound. An adult female, Joy, approached Suzee, Sassandra and Sia to observe the behaviour and her positioning reduced the visibility of the observers. Suzee then again moved the fingers of her left hand from the wound back to her lips and then back to the wound. An adult male, Littlegrey, who had been in proximity approached them and positioned himself quadrupedally to look over Joy's shoulder and observe Suzee's behaviour. Overall, this video sequence shows a total of three applications of an unidentified insect to the wound of a related individual.

Event 3: Capture and hand-over of an insect from an adult female, Carol, to an adult male, Littlegrey, application and allo-application by two other adult males

This video footage was recorded on October 20th 2020. It involved an adult female, Carol, her adolescent son, Cesar, and three adult males, Littlegrey, Ngonde and Thea. Littlegrey had been observed with a large open wound of approximately five cm on the inner calf of his left leg on October 19th 2020. Across two days, we observed a total of four events where unidentified insects were applied to the wound. Three of these events involved the capture and application of an insect into the open wound by himself. In the specific event described in detail here, an adult female, Carol, had groomed the injured male, Littlegrey, around his wound. At 01:13 p.m., she stood up, turned away from him and walked two meters to a small bush, paused and then reached out with her right hand to catch an unidentified insect from under a leaf and put it between her lips. Littlegrey had been observing Carol grooming around his wound and getting up and catching the insect without moving or changing his position. Carol then moved her fingers to her lips and took the insect out of her mouth using the index finger and thumb of her right hand. Littlegrey then immediately stood up, approached her and reached out to take the insect from her fingers with his right hand. Carol did not object to his behaviour.

Littlegrey then moved two meters, sat down and put the insect directly between his lips. Subsequently, he applied it with his lips to the open wound on his left calf with his foot

propped against a small tree. Carol had moved with him and now stood quadrupedally in front of him and watched his behaviour. While Cesar and Ngonde were watching from a distance of approximately five meters, Thea stood up and approached Littlegrey. Carol moved her hand towards the wound and moved the insect on the surface of the wound with the fingers of her right hand. Without hesitation, Thea also touched the wound with the fingers of his right hand and moved the insect on the wound. Whilst moving the insect on the surface of the wound, Thea was lip-bobbing⁵⁴. The subsequent behaviours could not be seen, because Cesar and Ngonde approached and blocked the view of the human observers. When the observers regained sight of the wound approximately six sec later, all adult chimpanzees were sitting around Littlegrey and looked closely at his wound. Cesar stood quadrupedally watching the adults. It was now Ngonde, who re-applied the insect to the wound of Littlegrey with his fingers and specifically the index finger of his right hand. Cesar approached closer and moved between Ngonde and Littlegrey. He observed Ngonde's behavior while staying in a quadrupedal standing posture. Ngonde moved his fingers carefully on the wound, returned them to his mouth and placed them again on the surface of the wound. Littlegrey still had his left foot propped against the small tree and observed Ngonde's behavior. Ngonde stopped touching the insect and the wound and Cesar sat down. Littlegrey now engaged in a behavioural sequence where he put the fingers of his right hand in his mouth and on his tongue, and then returned them back to the wound. He did this sequence seven times. Cesar then also touched near the wound with his left hand. Following this, Littlegrey engaged in self-grooming his wound and the skin and hair around it. The event ended with Carol, Cesar, Ngonde and Thea moving out of proximity from Littlegrey whilst he continued to groom around the wound. In this event it was not clear whether the insect remained in the wound or was discarded on the ground.

Event 4: Capture and application of an insect from an adult male, Arnold, to a wound of another adult male, Littlegrey

This video footage was recorded on January 29th, 2021. It involved an adult male, Arnold and an adult male, Littlegrey, who had been wounded that day, with a one cm open flesh wound on his right thumb. Arnold, had been grooming Littlegrey on his arm and then began inspecting the wound on his thumb. At 03:13 p.m., Arnold then turned away from Littlegrey and looked toward a nearby shrub, after a short pause he reached out and grabbed an insect

from under a low-hanging leaf with his right hand. Arnold moved the insect to his mouth, and held it between his lips. Littlegrey observed these actions but did not make any movements. Arnold repositioned himself in front of Littlegrey and moved to take Littlegrey's right hand with his left hand. He then held Littlegrey's thumb and moved the fingers of his right hand to his mouth and took the insect between his thumb and index finger. Next, he applied the insect to the open wound of Littlegrey with the thumb and index finger of his right hand, using his index finger to move the insect on the open wound. Arnold held the tree trunk next to him with his left hand for support and then began lip-smacking whilst moving the insect on the surface of the wound with the fingertips of his right hand. Arnold then released his grip from the tree trunk and used both the fingers of his left and right hands to touch the surface of Littlegrey's wound. When Arnold stopped inspecting and touching the insect on the wound, he started to groom Littlegrey again and it was unclear whether the insect remained inside the wound or had been discarded. Littlegrey inspected his thumb and casually groomed around it with the fingers of his left hand.

References

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