Slow lorises use venom as a weapon in intraspecific competition

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eToc Blurb

Nekaris et al. studied wounding patterns and aggressive behaviours in a venomous mammal– the Javan slow loris - for eight years in Indonesia. With high wounding rates in more than 20% of the population and extreme territoriality, loris venom unusually functions as a weapon in intraspecific competition used for resource and mate defence.

Animals have evolved an array of spectacular weapons, including antlers, forceps, proboscises, stingers, tusks and horns [1]. Weapons can be present in males and females of species needing to defend critical limiting resources, including food (rhinoceros beetles *Trypoxylus*) and territories (fang blennies *Meiacanthus*) [1,2.3]. Chemicals, including sprays, ointments and injected venoms, are another defence system used by animals. As with morphological weapons, venom can serve multiple purposes, including facilitating feeding, attacking predators, and in defence when attacked [4]. Although rare, several taxa use venom for agonistic intraspecific competition (e.g. ghost shrimp - *Caprella* spp.; sea anemones - *Actinia equina*; cone snails - Conidea; male platypus - *Ornithorhynchus anatinus*) [4,5,6]. Another group of venomous mammals are the nocturnal slow lorises (*Nycticebus*) [7]. Slow loris bites often result in dramatic diagnostic wounds characterised by necrotic gashes to the head and extremities. Although these bites are the major cause of death of lorises in captivity, the function of this aggressive behaviour has never been studied in the wild [7]. Here through an 8-year study of wounding patterns, territorial behaviour, and agonistic encounters of a wild population of Javan slow lorises (*N. javanicus*), we provide strong evidence that venom is used differentially by both sexes to defend territories and mates.

Slow lorises possess a venomous bite that can injure humans and other lorises [7]. The venom consists of oil from the brachial gland that is mixed with their saliva [6,7]. The main symptoms of the venom in slow lorises are characteristic wounds unlike any seen in other primate taxa, usually affecting the head where an animal loses large patches of fur and skin; the hands and feet that can lead to digit loss; the eye that can lead to blindness (Figure 1). Other symptoms include emaciation after wounding, anaphylactic shock, and death – symptoms also reported in humans being bitten [6,7]. Slow lorises share a number of traits exhibited by other taxa that use weapons for intraspecific competition, including aposematic face masks, social organisation of small family groups with both sexes dispersing, lack of sexual dimorphism, and a diet consisting of defendable food (tree exudates) [4,5,8]. Thus, if a function of the venom is female defence by males, we predicted that males will exhibit wounds, be territorial and will fight each other [2,9]. If a function of venom is for food resource defence, we predicted that females would also exhibit wounds and fighting behaviour [1,10].

We captured 82 slow loris individuals, including 40 females and 42 males, 338 times (females, $n=167$; males, $n=171$); 33% of females and 57% of males exhibited at least one wound. Across all captures, 20.4% of lorises exhibited fresh diagnostic bite wounds, including necrotic head wounds and loss of ears and digits (Figure 1). The presence of wounds was significantly higher in males than in females ($Z = 1.645$, $P < 0.001$), with 55 in males (32.2%) and 14 in females (8.4%). Wounds were recorded during all months. We found a peak in wound number in dispersing males at ~20-25 months old. Based on 26,751 location points for 25 neighbouring adult individuals (14 males, 11 females), we found that slow lorises were highly territorial with a mean overlap index of 0.031 ± 95%CI 0.028 for females and 0.091 ± 0.048 for males in the home range, and 0.006 ± 0.006 for females and 0.017 ± 0.014 for males in the core area. We closely observed 25 aggressive interactions: stable males defending the territory from an intruder ($n=9$); stable males defending a female from an intruder ($n=5$); collared unstable males ($n=4$); collared natally dispersing males ($n=3$); stable females rejecting a mate ($n=2$); two neighbouring stable pairs ($n=1$); unidentified animals ($n=1$). We observed two successful take-overs by males ousting the resident male, not leading to death, and one attempted male take-over, with fighting leading to death of the resident male.

Our evidence supports the hypothesis that venom is a mutual weapon in male and female slow lorises, whose use yields frequent wounds that can lead to death. This extraordinary use of venom in intraspecific competition supports theories regarding the evolution of weaponry as a mechanism for sexual selection among males and adds to the growing body of literature that weapon use by females for resource competition is a widespread and significant evolutionary selection pressure [10].

Wounding rates have been used as a proxy for aggressive behaviour in other taxa, including in the platypus [6]. The differential wound rate in our population points to the importance of controlling resources. Slow lorises lack a breeding season and have a promiscuous mating system, providing more opportunities for contests in males, which showed higher wounding rates. While dispersing animals also showed higher wound rates, highly territorial females showed the least, as seen in other taxa where females have weapons to defend territories [1,9].

To be considered territorial, species need to engage in spatial defence, including high levels of intolerance to intruders [9]. Social selection theory suggests that females compete more for ecological resources than for mates, and that weaponry in females may be selected for to compete for food, sleeping sites and offspring, as well as the quality of their care [10]. Slow loris females show all these traits [8].

Animal venoms play at least 14 distinct ecological roles, yet behavioural and ecological aspects of venom use in natural settings have been largely neglected [4]. Slow loris venom already is known potentially to function in ectoparasite control and as an anti-predator deterrent [5,6,7]. In a single taxon, venom can have multiple functions [4]. In a survey of all known independently evolved venomous lineages, only four species were identified to use venom for intraspecific competition [4]. Slow lorises can now be added to this list. With high competition for space containing a defendable resource, the possession of a toxic weapon would confer competitive advantages to male and female slow lorises in a situation of high contest competition [1]. The result is a rare example of venom as a mutual weapon whose proximate use serves for resource and mate defence.

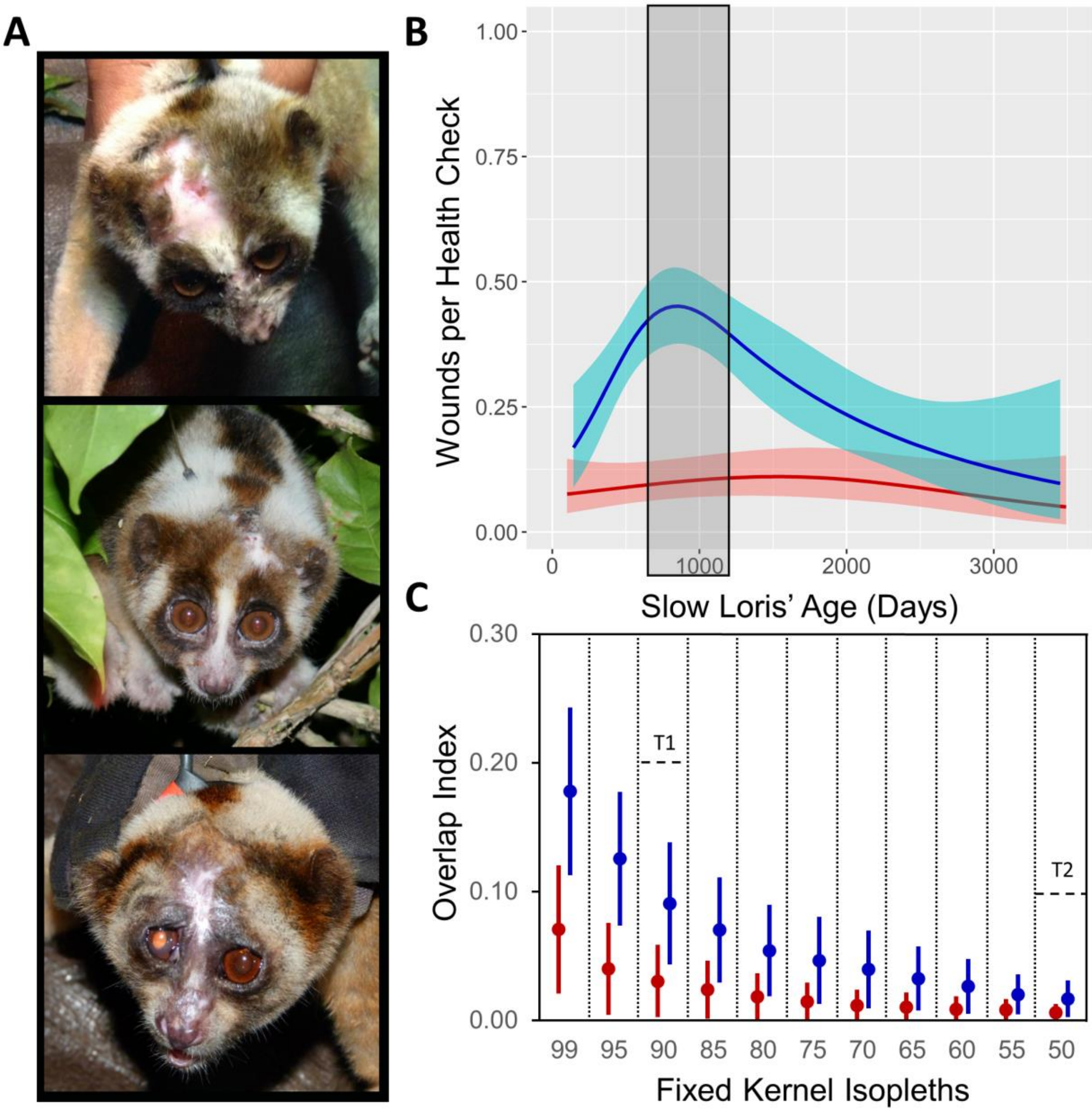

Figure 1. Javan slow lorises are territorial and use venom for intraspecific competition. a) Examples of head wounds resulting from venomous bites: dispersing male (above); dispersing female (middle); resident male after a territorial fight when he maintained his territory (below). b) Relationship obtained via a Generalised Additive Mixed Model between

presence of new wounds and age in females (red) and males (blue) based on 338 health checks on 82 individuals between April 2012 and June 2020. The grey box represents the dispersal age. c) Overlap index of neighbouring females (red) and males (blue) considering Fixed Kernel probability contours from 99% to 50%. The threshold that defines the species as territorial considering home ranges and core areas are indicated as T1 and T2. Shaded areas and line bars represent 95% confidence intervals.

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SUPPLEMENTAL INFORMATION

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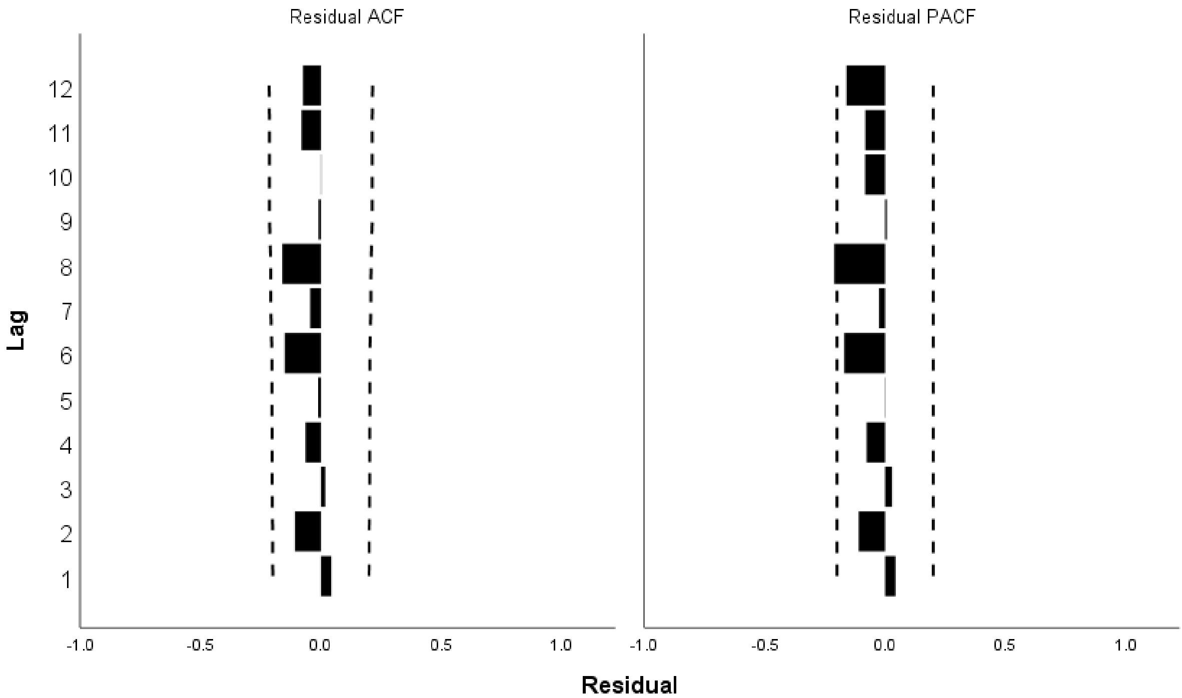

Figure S1. Time series correlation between wound presence and months of the year in Javan slow lorises in West Java, Indonesia, showing that wounds occur throughout the year. Data are from 2012 to 2020. Dark bars indicate the correlation coefficient for residual autocorrelation function (ACF) and residual partial autocorrelation function (PACF). Dashed lines indicate 95% confidence intervals. Models have been selected automatically via the function Expert Modeler in SPSS v 26, that select the best models based on the stationary R-squared value. The model automatically selected was ARIMA (0,0,1).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Subjects and Study Site

We present data from April 2012 to June 2020 collected on wild Javan slow lorises near Cipaganti, Garut District, West Java, Indonesia (S 7°6 6 - 7°7 07 E 107°46 0 - 107°46 5). The recorded animals occurred in mixed agroforest habitats at elevations ranging from 1200-2100 m a.s.l. The climate is aseasonal, with temperatures in the day averaging 22.6° C (range 12.4-28.0 °C), and 18.9° C (range 12.6-26.7 °C) at night.

To capture slow lorises, we used established methods described in detail in Nekaris et al. [S1]. We located each slow loris with red headlamps and climbed the tree or bamboo patch in which it was situated. We placed the loris into a clean cotton capture bag. We measured, weighed and collared animals with no need for anaesthesia. The process lasted around 20 minutes, after which time the animal was returned to the tree where it was captured. We captured the individuals on average every 139.1 SD 91.5 days. For 20 fully grown animals it was possible to weigh them when they had a fresh wound and compare that to their average weight during the captures where they did not show fresh wounds. We used a related-samples Wilcoxon signed rank test for comparison.

During each capture, we recorded whether or not animals showed new wounds since the last capture. We based our diagnosis on the basis of extensive previous experience with these wounds in captivity [S2,S3]. We distinguished the diagnostic slow loris bite wounds from wounds of other types, which we only recorded 9 times during captures: scars from electric shock on powerlines (n=2) and small spots from being pricked by a branch or wire (n=7). We recorded location of wound and monitored wound healing progression, during which time we noted if necrosis, hair loss, or a general change in condition of the bite area had occurred. In the field, the freshly delivered bites were observable through binoculars, and generally looked like a typical bloody animal bite. We acknowledge that not all bites may have contained venom. We should note that this population is in a human-dominated landscape where potential predators are infrequent, and we never witnessed a death due to predation during the study period, meaning a low potential of bites from non-lorises.

Behavioural Data Collection

We observed animals nightly over two shifts between 18:00 to 00:00 and 00:00 to 05:00, totalling 7629.1 h for 68 individuals. We recorded behavioural data using instantaneous sampling at 5-minute intervals and recorded all occurrences of aggressive behaviours following Altman [S4]. In addition, we continuously VHF-tracked individuals using an antenna (Yagi, Biotrack, UK) and receiver (Sika, Biotrack, UK). We followed one subject per shift unless other animals were within the vicinity of the collared focal, in which case we used instantaneous scan sampling of all visible subjects [S4]. For this study, we focussed on all occurrences of aggressive interactions [S5].

Data Analysis

We recorded the presence of loris bite wounds during capture events. For individuals captured more than once, we considered the presence of new wounds (not present in the previous capture). We then used logistic generalised additive mixed models (GAMM) with wound presence (0=no new wound, 1=new wound) as the dependent variable, age as the independent variable, and individuals as random effects via gamm” command in R 3.5.1 [S6] package mgcv” [S7]. We used full restricted maximum likelihood method for model selection, tensor product smooth and penalised regression spline [S6]. We used GAMM as they provide a flexible approach as they do not assume a linear or other parametric form of relationship *a priori* and can be used to reveal and estimate non-linear effects of the covariate on the dependent variable [S8].

We used territoriality indices and presence of wounds as measures of intraspecific competition. We calculated the size of fixed kernel estimates of slow loris home ranges using Ranges 9 software with least square cross validation as smoothing parameter by choosing probability contours from 99% to 50%. We estimated probability contours for 25 neighbouring individuals (14 adult males and 11 adult females) for a total of 26,751 location points (14,665 for females; 12,086 for males) taken every 15 min from April 2012 to June 2020. We considered neighbouring individuals as a dyad when there was overlap between the 99% probability contours. We determined territoriality based on the overlap index by Ginsberg and Young [S9] and considered the animals as territorial when the overlap index calculated with 90% (home range) and 50% (core area) probability contours was less than 0.2 and 0.1 respectively [S10]. Given the instability of social structure during the study period, we divided the dataset in periods of stability of home ranges and averaged the overlap index for each dyad. Some animals were unstable with large home ranges not clearly overlapping with an adult female (<50%), we defined them as unstable adults. We considered separately the overlap index of females and males since females had more stable home ranges than males that was dependent on habitat structure while males had more variable home ranges and overlapped with one or two females at the same time. In total, 13 female and 21 male dyads formed during the study period.

Ethics

All research was approved by the Animal Care Subcommittee of Oxford Brookes University (OBURASC0911) and adhered to the ASAB/ABS Guidelines for the Use of Animals in Research. We obtained all necessary research permits from the Indonesian government (Permit 109/SIP/FRP/SM/V/2014 –386/SIP/FRP/E5/Dit.KI/XI/2017 – 57/EXT/SIP/FRP/E5/ Dit.KI/X/2018 – 24/E5/E5.4/SIP/2019). Research adhered to the legal and ethical guidelines of the Indonesian Institute of Sciences, Department of Wildlife and Department of Forestry.

SUPPLEMENTAL RESULTS

Wounds led to a significant weight loss of around four per cent (animals with fresh wounds - median=886.0 g, IQR=73.0 g; these same animals during periods with no wounds - median=920.0 g, IQR=56.0 g) (W=39.0, p=0.024). The duration of the 25 aggressive interactions ranged from 3 minutes to 3 hours and 55 minutes (Mean: 57

minutes, ±SD 64.7). Aggressive behaviours included aggressive staring, whereby animals approached and stared at each other, usually uttering aggressive whistles. These interactions ended with one of the antagonists retreating, or one approaching leading to a fight. Fighting involved one animal actively chasing another, using chitter and growling vocalisations [S5]. Contact after a chase occurred with the combatants hanging by their two feet and grappling at or wrestling with each other with the hands or holding the arms above the head and swaying at each other trying to bite. While the arms are above the head, the animal licks the brachial gland very rapidly during the course of the fight or paused the fight on a separate branch to lick the brachial gland. As the animals swayed, they attempted to bite the top of the head, sometimes missing and biting the hands. One animal may also stand above another that is hanging, biting its feet in attempt to get it to loosen its grip on the branch. On five occasions, these bites led to animals losing their grip and falling from trees, which is significant suggesting extreme pain as the loris grip is incredibly strong. Biting can last just seconds (hands or feet) or 30-40 seconds, where one animal locks on to the other's head. We could closely observe 6 of these severe head bites, and each time, the fight was ended by the bitten animal retreating. Almost all animals we captured showed only 1-2 bite wounds. On seven occasions we caught an animal within three to seven days of a fight taking place. On five of these, the wounds were already severe. The maximum bites we could observe on two of these occasions was seven. For one of these individuals, he was ousted from his home range and died within three days. Two of the caught individuals showed no wounds at all after a fight.

Only two dyads of unstable males had an overlap index higher than 0.2, suggesting that unstable males may overlap with other males, resulting in higher aggression rates. Females home ranges were stable and were of three types: adult females that kept the same territory for the study duration (n=2); females that died from natural causes not related to intraspecific competition (n=5); females resident in a territory established after dispersal during the course of the study (n=2). Two of the females that died were replaced by their daughters that then occupied their mother's territory. Males' home ranges were more dynamic. Four males were unstable, with the other males able to overlap with the same partner for varying periods of time (mean: 21.9 SD 14.6 months, range: 6.0-57.0 months). Males tended to overlap with only one female but in rare cases could overlap with the home ranges of two females for a limited time (maximum 26 months). In 21 cases (1 dispersing female, 20 males, 11 natally dispersing, 6 unstable adults, 3 stable adults) the individuals presented severe head wounds, leading to necrosis and, twice, requiring veterinary intervention. The presence of wounds was not different between months, with no clear peaks (Figure S1). Other wounds led to the loss of ears and digits, and permanent scars.

MODEL OUTPUT OF GAMM

Model relative to the presence of wounds in males: REML = 103.650; ti(age): edf = 2.483, Ref.df = 2.885; χ^2 = 6.778, P = 0.048; model intercept: estimate = -0.841 (SE 0.177), Z = -4.948, P < 0.001. Model relative to the presence of wounds in females: REML = 46.544;

ti(age): edf = 1.987, Ref.df = 2.395; χ^2 = 2.612, P = 0.333; model intercept: estimate = -2.525 (SE 0.318), Z = -7.941, $P < 0.001$

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Author Contributions

K.AI.N. conceptualised the project, runs the research site, wrote the main text, collected field data, and procured funding; M.C. and A.W. analysed the data and contributed to writing; V.N., H.B., and E.J.RM. collected field data and contributed to the writing; W.W. and M.A.I. contributed to the writing and helped to procure research permits; B.G.F. contributed to the writing and procured funding.

Competing interests

The authors declare no competing interests.