



## Research

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## Animal behaviour

## Hygienic tendencies correlate with low geohelminth infection in free-ranging macaques

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Parasites are ubiquitous in nature and can be costly to animal fitness, so hosts have evolved behavioural counter-strategies to mitigate infection risk. We investigated feeding-related infection-avoidance strategies in Japanese macaques via field-experimentation and observation. We first examined risk sensitivity during foraging tasks involving faecally contaminated or debris-covered food items, and then investigated individual tendencies to manipulate food items during natural foraging bouts. We concurrently monitored geohelminth infection in all subjects. We ran a principal component analysis on the observational/experimental data to generate a hygienic index across individuals and found that hygienic tendencies towards faeces avoidance and food manipulation correlated negatively with geohelminth infection. Females scored higher in hygienic tendencies than males, which might contribute to the common vertebrate pattern of male-biased infection. The behavioural tendencies observed may reflect a general form of hygiene, providing a mechanism of behavioural immunity against parasites with implications for the evolution and diversification of health maintenance strategies in humans.

## 1. Introduction

Parasites and pathogens are ubiquitous in nature and can be costly to animal fitness. Animals have thus developed behavioural strategies to avoid infection [1–3], akin to the suite of behaviours we collectively refer to as ‘hygiene’ among humans [4]. Some well-known behavioural defences against parasites include their direct removal via grooming or weeding [5,6], the avoidance of infected conspecifics [7] and strategies for waste management [8], all of which function to mitigate fitness losses associated with infection.

Trophic interactions provide a particularly common pathway to infection by parasites that use intermediate hosts or infect hosts orofaecally. Regarding the latter, multiple studies have shown that grazing ungulates, for example, prefer to feed away from areas contaminated by faeces [9–11]. Given the ubiquity of parasites that use the faecal–oral pathway, and the broad range of defence strategies exhibited by humans, we also expect non-human primates to display similar behaviours. However, despite parasite avoidance strategies having been suggested in non-human primates [12,13], concrete evidence remains somewhat scarce. To our knowledge, studies have not yet directly investigated food-related hygienic behaviour under risk of infection. Thus, our foci were to investigate food-processing and faeces avoidance behaviours in a non-human primate and test whether the infection-avoidance hypothesis—that such behaviours evolved to mitigate infection risk—can explain their occurrence.

Japanese macaques of Koshima (*Macaca fuscata fuscata*) are known to use a set of food-processing behaviours, such as rubbing, rolling and/or washing food before consumption. Even the famous sweet potato washing behaviour—an innovation observed to spread through the group at Koshima in the 1950s following



**Figure 1.** Field setting and test substrates for faeces avoidance experiments. (a) Subject consuming wheat from plastic control substrate. (b) Three experimental substrates (from left to right: fresh Japanese macaque faeces, plastic replica faeces and plastic notebook cover). (Online version in colour.)

the onset of provisioning, which provided some of the first evidence for culture in animal societies [14]—may in fact be hygienic, though its specific function remains unexplored. We used experimentation and observation to investigate three sets of food-related behaviour ('hygiene proxies'), which we hypothesized might reduce the risk of infection by faecal–oral parasites. The first experiment (H1) tested whether macaques identify and avoid feeding on faecally contaminated food items. The second experiment (H2) tested whether sweet potato manipulation behaviour (e.g. washing) functioned to remove debris. In addition to these experiments, we concurrently observed natural foraging behaviour (H3) to investigate whether tendencies to rub fallen acorns before consumption were consistent with faeces avoidance and sweet potato manipulation behaviours across individuals. In parallel, we monitored geohelminth infection across subjects. We predicted that (i) individuals would behave consistently across hygiene proxies and that (ii) geohelminth infection intensity would correlate negatively with faeces avoidance and food-processing behaviour (i.e. hygienic tendencies).

## 2. Material and methods

From January 2014 to April 2014, we studied 16 adult Japanese macaques (11 females and five males) inhabiting Koshima islet, southern Japan, a free-ranging and provisioned population amenable to field-experimentation and naturalistic observation. Detailed methods and data analysis appear in the electronic

supplementary material, along with all raw data used in these analyses, videos of our experimental design and supplementary results.

### (a) Hygiene proxies

For faeces avoidance experiments (H1,  $N = 151$  trials), we aligned three substrates (fresh macaque faeces, plastic faeces replica and piece of brown plastic notebook) adjacently on the sand in a semi-confined area of beach at the provisioning site (figure 1). On each substrate, we placed a grain of wheat or half a peanut to increase motivation ( $N_{\text{wheat}} = 76$ ,  $\bar{u} \pm \text{s.d.} = 4.75 \pm 1$  trials individual<sup>-1</sup>;  $N_{\text{peanut}} = 75$ ,  $4.69 \pm 1$  trials individual<sup>-1</sup>). Subjects were baited to the experimental area, which was visually cut-off from other animals by rocks, and given 2.5 min to feed before items were removed. For sweet potato manipulation experiments (H2,  $N = 87$  trials,  $2 \pm 0.83$  tests individual<sup>-1</sup> condition<sup>-1</sup>), a piece of sweet potato, either pre-washed ( $N_{\text{washed}} = 45$ ) with tap water or covered with sand (a potential contaminant;  $N_{\text{sand}} = 42$ ), was presented to a subject at the provisioning site. We recorded all food manipulation behaviours observed before consumption. Finally, for our naturalistic observations of acorn foraging behaviour (H3), we extracted 84 bouts of acorn foraging ( $5.3 \pm 2.3$  bouts individual<sup>-1</sup>) from 500 collected 15 min focal animal samples and recorded all acorn manipulation behaviours observed.

### (b) Geohelminth monitoring

We processed 125 faecal samples ( $7.8 \pm 2.4$  samples individual<sup>-1</sup>) using faecal sedimentation, and used faecal egg counts (FECs:

**Table 1.** Factors affecting variation in hygiene tendencies. Italicized text denotes predictor variables causing significant variation in the response. Significant *p* values are marked: \*\*\*(*p* < 0.001), \*\*(*p* < 0.01), \*(*p* < 0.05).

statistical model [ <i>M<sub>x</sub></i> ]	predictor variable	est.	s.e.	stat.	<i>p</i>
[ <i>M<sub>1</sub></i> ] likelihood of wheat/peanut consumption—H1 ( <i>N</i> = 151)	(intercept)	6.823	2.279	2.99	<0.005**
	sex (male versus female)	0.551	1.029	0.54	0.592
	age	0.023	0.124	0.18	0.854
	rank	0.028	0.157	0.18	0.857
	<i>condition</i> (wheat versus peanut)	-6.370	0.699	-9.12	<0.001***
	<i>substrate</i> (fresh versus control)	-3.455	0.531	-6.51	<0.001***
	<i>substrate</i> (replica versus control)	-2.124	0.434	-4.89	<0.001***
	trial no.	-0.069	0.124	-0.56	0.579
[ <i>M<sub>2</sub></i> ] likelihood of sweet potato manipulation—H2 ( <i>N</i> = 87)	(intercept)	2.551	2.956	0.86	0.388
	<i>condition</i> (washed versus sandy)	-4.431	1.220	-3.63	<0.001***
	sex (male versus female)	-3.624	1.517	-2.39	0.017*
	age	0.003	0.169	0.02	0.988
	rank (low versus high)	-1.747	1.492	-1.17	0.242
	rank (mid versus high)	-0.624	1.523	-0.41	0.682
	trial no.	1.468	0.578	2.54	0.011*
[ <i>M<sub>3</sub></i> ] likelihood of acorn manipulation—H3 ( <i>N</i> = 84)	(intercept)	1.752	2.793	0.63	0.530
	<i>season</i> (winter versus spring)	-2.393	1.091	-2.19	0.028*
	sex (male versus female)	-3.037	1.218	-2.49	0.013*
	age	-0.419	0.168	-2.50	0.013*
	rank (low versus high)	0.110	1.081	0.10	0.919
	rank (mid versus high)	2.241	1.321	1.70	0.090

number of eggs gram<sup>-1</sup> faecal sediment) determined via microscopy as a proxy for variation in infection risk across subjects [15]. We focused on *Oesophagostomum aculeatum* (OA) and *Trichuris* sp. (cf. *trichiura*) (TT), the two most commonly observed parasites in our subjects and the only ones to be acquired during foraging via the ingestion of contaminated soil, water or food [16]. FECs of OA and TT were highly correlated across subjects (Pearson's product moment correlation,  $\rho = 0.89$ ,  $p < 0.001$ ).

### (c) Data analysis

We constructed generalized linear mixed-effects models to analyse the hygiene proxy data. For H1, models included feeding decision (consume or not) across trials as a binary response, and condition (wheat or peanut) and substrate (fresh faeces, plastic control and plastic faeces) as predictor variables. For H2, models included food manipulation decisions (manipulate or not) as a binary response and condition (pre-washed or sand-covered) as a predictor variable. In each model, we controlled for trial number to account for sensitivity/habituation effects. For H3, models included the number of acorn manipulations performed as a negative-binomial count response, offset by the number of acorns consumed during each bout. In all models, we included age, sex and dominance rank as predictor variables and individual identity as a random effect to control for pseudoreplication. We added season (winter or spring) to models of H3 to account for temporal variation in foraging behaviour and acorn abundance.

To generate an index by which to score hygienic tendencies across individuals, we ran a principal component analysis (PCA) using the three hygiene proxies expressed as the probability to (i) consume wheat on fresh faeces (H1), (ii) manipulate sweet potatoes (H2) and (iii) manipulate acorns (H3). We then used a general linear model to test whether individual attributes such as age, sex and dominance rank influenced hygienic tendencies, and generalized linear models to test whether hygienic tendencies correlated with geohelminth infection. We set the retained principal components as predictor variables in models with the negative-binomially distributed FEC of OA and TT as response variables.

## 3. Results

In experiment H1, significantly fewer macaques ate wheat from the fresh faeces (37.5%) and replica faeces (56%) than from the control substrate (100%; table 1). However, all subjects consumed peanuts from all substrates in all trials. In experiment H2, all sweet potatoes were consumed but the probability to perform manipulation behaviours was greater in the sand-covered condition (table 1). Females were also more likely than males to perform manipulations. During observations (H3), subjects manipulated acorns before consumption with a mean probability of  $0.61 \pm 0.30$  acorn<sup>-1</sup> consumed, with females manipulating more often than males (table 1).



**Table 2.** Factors affecting variation in geohelminth infection and PC1/PC2. Italicized text denotes predictor variables causing significant variation in the response. Significant *p* values are marked: \*\*\*(*p* < 0.001), \*\*(*p* < 0.01), \*(*p* < 0.05).

statistical model [ <i>M<sub>x</sub></i> ]		predictor variable	est.	s.e.	stat.	<i>p</i>
[ <i>M<sub>4</sub></i> / <i>M<sub>5</sub></i> ] variation in OA infection	<i>N</i> = 125	(intercept)	7.541	0.682	11.07	<0.001***
	<i>N</i> = 16	(intercept)	8.076	0.084	96.37	<2 × 10 <sup>-16</sup> ***
		<i>PC1</i>	-0.368	0.070	-5.22	1.75 × 10 <sup>-7</sup> ***
		<i>PC2</i>	0.190	0.082	2.33	0.020*
[ <i>M<sub>6</sub></i> / <i>M<sub>7</sub></i> ] variation in TT infection	<i>N</i> = 125	(intercept)	8.763	1.277	6.86	6.9 × 10 <sup>-12</sup> ***
	<i>N</i> = 16	(intercept)	8.864	0.174	51.03	<2 × 10 <sup>-16</sup> ***
		<i>PC1</i>	-0.473	0.146	-3.24	0.001**
		<i>PC2</i>	0.084	0.170	0.50	0.620
[ <i>M<sub>8</sub></i> ] variation in PC1	<i>N</i> = 16	(intercept)	1.018	0.846	1.20	0.25644
		sex (male versus female)	-1.444	0.416	-3.47	0.006*
		age	-0.022	0.051	-0.42	0.683
		rank (low versus high)	-0.324	0.455	-0.71	0.493
		rank (mid versus high)	-0.028	0.481	-0.06	0.955
[ <i>M<sub>9</sub></i> ] variation in PC2	<i>N</i> = 16	(intercept)	-0.067	0.273	-0.25	0.809

The PCA revealed that two retained components explained 81% of the variance in the data. PC1 (47%) represented a gradient along which subjects displayed variable tendencies towards hygienic behaviour (figure 1*a*). Each hygiene proxy loaded positively onto PC1 (H1: 0.48; H2: 0.66; H3: 0.86), and a generalized linear model showed that PC1 correlated negatively with geohelminth infection (figure 1*c* and table 2). Other predictors could not explain significant variation in infection intensity (table 2). Finally, variation along PC1 was unrelated to age and dominance rank, but females scored significantly higher than males (table 2), indicating greater hygienic tendencies.

#### 4. Discussion

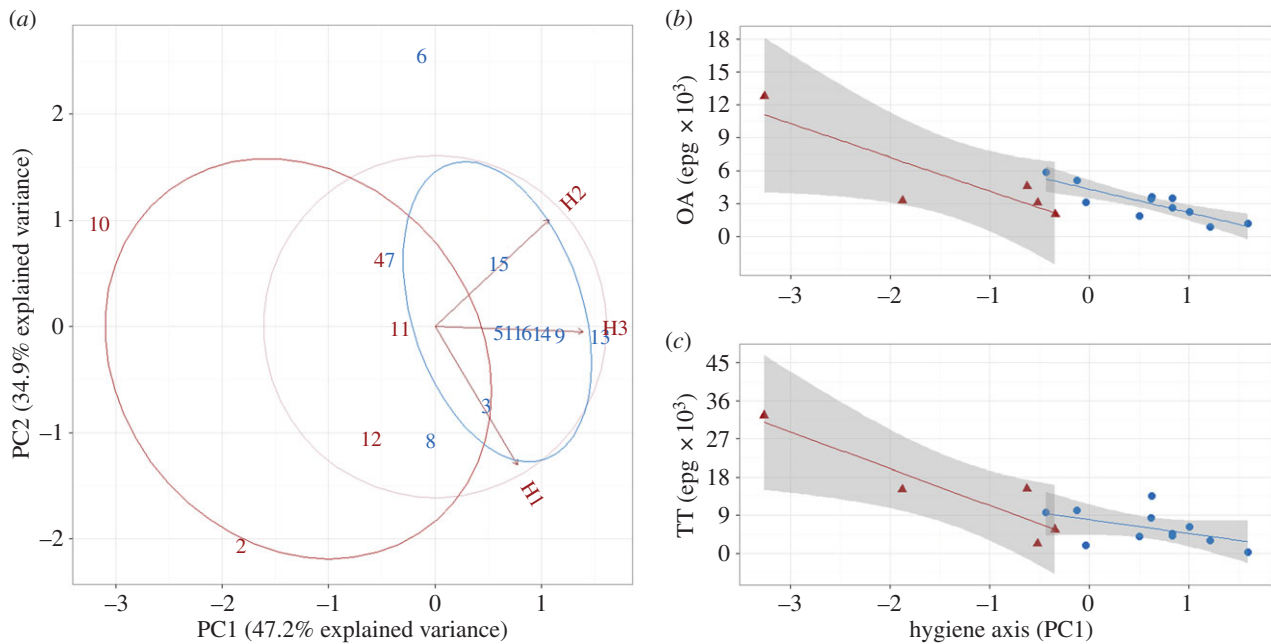
The negative correlation between hygienic tendencies (PC1) and geohelminth infection supports the infection-avoidance hypothesis as a plausible ultimate explanation. These results have implications for our understanding of how behavioural traits, here foraging decisions, might shape an animal's health and more deeply, how hygiene might have evolved. Soil-transmitted helminths are among the most common parasites of primates, and TT is one of the most important in humans where it is endemic [17]. Despite their ubiquity, little is known about their cumulative effects on health and fitness in non-human primates, though such effects should increase with intensity of infection [18]. We cannot assess whether the infections observed in this study were of clinical significance, although parasite-reduction experiments in this population, mainly targeting OA, are showing that treated females maintain higher body masses and reproductive rates than do controls (AJJ MacIntosh, C Sarabian, J Duboscq, E Thomas, V Romano, A Kaneko, M Okamoto, T Suzumura 2015, unpublished data). While most geohelminths are not directly transmissible via fresh faeces [16], general preferences for avoidance of faeces or faecally contaminated substrates and food processing prior to consumption may limit infection risk by avoiding or removing a wide variety of infectious organisms

using the faecal-oral pathway, including geohelminths, parasitic protists and viral or bacterial pathogens.

It is also notable that female macaques scored higher in hygiene tendencies. Male-biased infection is common in mammals, including Japanese macaques [15], but it is difficult to distinguish the effects of exposure to infectious organisms from susceptibility once exposed [19]. Reduced risk sensitivity to potentially contaminated substrata may provide a behavioural mechanism of increased infection among males (figure 2).

Risk sensitivity may also explain why Koshima macaques avoided consuming wheat from real and replica faeces while consuming peanuts in all cases. This interpretation would suggest a trade-off between nutritional benefits and infection risk, as demonstrated previously in foraging herbivores [20]. Indeed, one grain of wheat represented a 16-fold reduction in calories from half a peanut. Furthermore, since macaques consumed wheat readily from plastic control substrates but not plastic replica faeces, visual cues may suffice in faeces recognition, as is also suspected in ruminants [9,11].

Similarly, our subjects appeared to display risk sensitivity in potato manipulation experiments, engaging more often in manipulation when given sand-covered potatoes. Captive great apes were also shown to wash apples more often when covered with sand [21], indicating general tendencies across primates. It was hypothesized that sweet potato washing in Koshima macaques might relate to taste preferences, since salt or brackish water is generally used [14]. However, this hypothesis is generally unlikely because we show that pre-washed potatoes were washed far less often. Removal of contaminants seems more plausible, as Koshima beach is heavily contaminated with macaque faeces and sand is conducive to nematode parasite transmission [22]. Yet, the alternative that sand's gritty texture deters ingestion cannot be excluded. Our observational data suggest that macaques do remove other contaminants like soil from food items, so examining the contaminant type and the nutritive value of food items should allow for better discrimination among competing hypotheses.



**Figure 2.** Hygiene tendencies and infection with faecally transmitted geohelminths. (a) Biplot of principal components showing individual Japanese macaques (numbers) with respect to the three hygiene proxies. Conventional 68% concentration ellipses are given for females (blue, rightmost ellipse) and males (red, leftmost ellipse). Purple ellipses reflect the data centroid (centremost ellipse). Arrows indicate the direction of increase for (H1) faeces avoidance, (H2) sweet potato manipulation and (H3) acorn manipulation. (b,c) Scatterplots of hygienic tendencies (PC1) versus geohelminth infection intensity expressed as faecal egg counts (eggs gram<sup>-1</sup> faeces, epg): OA and TT. Regression fits (lines) with 95% confidence intervals (shaded grey) are shown for females (blue circles) and males (red triangles). (Online version in colour.)

Ultimately, animals must defend themselves against pathogenic organisms, and this may be achieved in part through hygienic tendencies manifest as patterns of behaviour. The health benefits of reducing the burden of infection with parasites readily transmissible via faecal contamination should improve fitness, so such behavioural tendencies are expected throughout the animal kingdom.

**Ethics.** This study adhered to the Guidelines for the Care and Use of Nonhuman Primates set by the Primate Research Institute of Kyoto University. Permission to conduct the experiments was provided by the Wildlife Research Center of Kyoto University (no. 2013-2-10).

**Data accessibility.** All data used in this study are provided in the electronic supplementary material.

**Authors' contributions.** Both authors conceived of and designed the study, analysed the data and wrote the article. C.S. collected and processed all data. Both authors gave final approval for publication and agree to be held accountable for all aspects of this work.

**Competing interests.** We have no competing interests.

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