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## Social and reproductive correlates of parasite ova emissions by baboons

In several species of rodents, the social and reproductive condition of a host individual has been shown to affect its susceptibility to intestinal parasites and the maturation rate of the parasites once established[1,2]. The relationship between social and reproductive condition of host individuals and most aspects of parasite development and transmission, including ova emissions, has not previously been examined for any non-human primate species. Information on parasite ova emissions by non-human primates is important for understanding the life-cycle of the parasites and for understanding the spread of parasites both within and between social groups and to individuals of other species, including humans and domestic stock[3].

In 1974, as part of an ongoing longitudinal study of free-living yellow baboons (*Papio cynocephalus*) in the Amboseli National Park, Kenya, we collected 100 faecal samples from the 29 adult and subadult members of a single well studied group[4]. Intestinal parasite ova were recovered from these samples by zinc sulphate flotation[5] and number of ova per g of faeces was analysed in relation to dominance rank and reproductive condition of host. Egg recovery and counting was done "blind"; samples were identified only by a sequence number and not by name or other information concerning the host. Two nematode genera, *Trichuris* and *Trichostrongylus* accounted for 89% ($N = 2,119$) of all parasite ova recovered in the samples. The distribution of number of ova per g of faeces for all samples combined was positively skewed and roughly Poisson in shape; thus, significance testing was carried out with the variance ratio[6].

Figure 1 shows that among adult and subadult males, high ranking individuals had higher egg emissions than did more subordinate individuals. All differences in mean egg counts between adjacent dominance rank classes were statistically significant ($P < 0.01$ for all comparisons); the Spearman rank correlation coefficient between mean egg count and dominance rank in adult and subadult males was 0.67 ($P < 0.05$). Subadult males generally had lower egg counts than did older, fully adult males, and ranks 13–16 were occupied exclusively by subadults. But, examination of the mean egg counts for ranks 1–12 alone, which eliminated the age-related low egg counts of subadults, still showed a strong correlation between mean egg output and dominance rank in adult males ($r_s = 0.56$, $P < 0.05$). Since adult male baboons change rank frequently[4], Fig. 1 is based only on samples collected when adult males were stable, or consistent, in their dominance relationships. Egg output by adult males during periods of inconsistency was usually lower than egg output before the period of inconsistency, but only a small number of such samples were obtained.

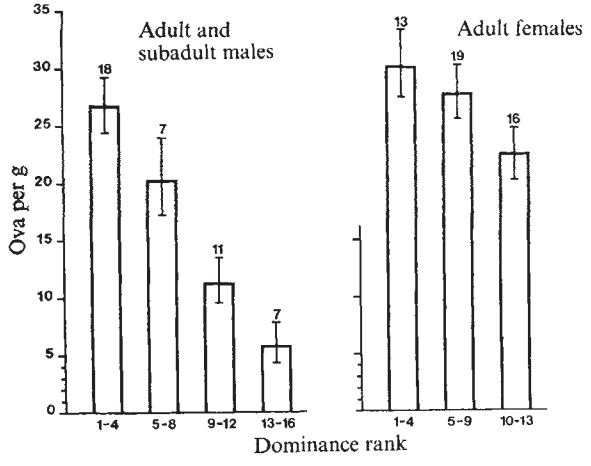


**Fig. 1** Parasite egg counts in relation to dominance rank. Shown are mean egg counts for each dominance rank class and 95% asymmetrical confidence intervals for the mean. Figure at top of each bar gives number of samples on which mean is based. For adult males, analysis included only samples collected during periods of rank stability. Subadult males are the same age as young adult females.

The mean egg count for adult females, 26.58 ova per g, was significantly higher than the mean egg count for adult males during periods of rank stability, 20.69 ova per g ($F_{max} = 1.28$, d.f. = 2,552, 1,490, $P < 0.01$), and this difference was even greater when subadult males and adult males inconsistent in their dominance relationships were included in the comparison. Parasite ova emissions among adult females, however, were not correlated with dominance rank ($r_s = 0.02$, $P > 0.05$), although middle ranking females did have significantly higher egg counts than did low ranking females ($F_{max} = 1.24$, d.f. = 1,054, 714, $P < 0.01$) (Fig. 1).

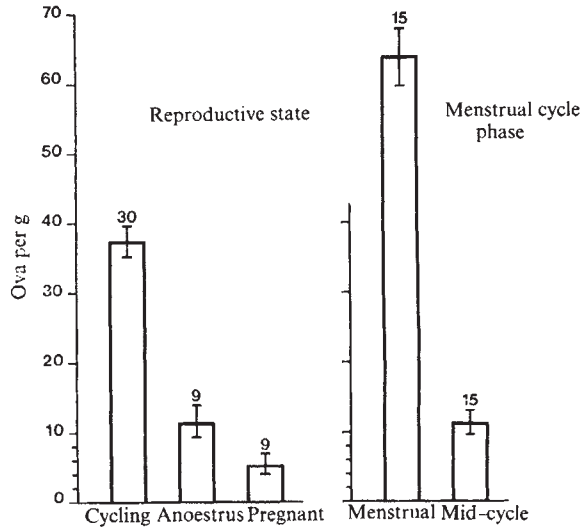


**Fig. 2** Parasite egg emissions in relation to female reproductive state and menstrual cycle phase. Conventions as in Fig. 1.

Adult female baboons rarely change dominance rank, but changes in their reproductive state or menstrual cycle phase occur frequently[4]. Sexually cycling females had significantly higher egg emissions than did anoestrous (lactating or otherwise non-cycling) females ($F_{max} = 3.26$, d.f. = 2,250, 206, $P < 0.01$); anoestrous females in turn had significantly higher egg emissions than did pregnant females ($F_{max} = 2.14$, d.f. = 206, 96, $P < 0.01$) (Fig. 2). Among sexually cycling females, mean egg counts were significantly higher during the one week immediately before and the one week immediately after the onset of menstruation compared with the remaining 3 weeks mid-cycle that include oestrus and ovulation ($F_{max} = 5.65$, d.f. = 1,912, 338, $P < 0.01$) (Fig. 2).

One simple but plausible explanation for the observed pattern of parasite ova emissions is that individuals differ in ingestion rate of infective and non-infective ova or in other aspects of food intake, gut passage time or faecal output. Alternatively, high ranking adult males and sexually cycling females may provide a generally more favourable internal environment for egg production by parasites than do individuals of other social and reproductive classes. Of course, the above correlations might also reflect actual differences in the burden of adult worms harboured by individuals of each social and reproductive class. Although unlikely, it is also possible that parasite ova emissions affect social and reproductive condition, for example, dominance rank, rather than the other way around as presumed by the above alternatives. Regardless of their precise

explanation, the relationships described in this note, if substantiated by more extensive studies, will be of interest and importance to many scientists concerned with primates, parasites or public health.

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# Inhibition of *Leishmania donovani* transformation by hamster spleen homogenates and active human lymphocytes

HAEMOFLAGELLATES are protozoan parasites responsible for many important diseases of man and domestic animals[1]. *Leishmania donovani*, the cause of visceral leishmaniasis or kala azar, is a widespread human parasitic haemoflagellate. Its life cycle involves man (and other mammals) and an insect vector (sandflies) with a different stage in each of the two host types. Transformation from one stage to the next includes morphological antigenic, physiological, and infectivity changes[2–5].

Transformation in these organisms is a fundamental developmental transition whose regulation is incompletely understood. In this report we present evidence that transformation from the mammalian (amastigote) stage of *L. donovani* to the insect and/or culture (promastigote) stage is inhibited by host hamster spleen homogenates and by a soluble factor(s) synthesised by activated human lymphocytes. In the absence of this factor transformation proceeds in an orderly series of steps (Fig. 1).

Synchrony of transformation by a population of amastigotes is rather poor; transformation to the promastigotes begins after 18 h with all cells capable of developmental transition (~ 60–80% of the cells undergo transformation) completing cytodifferentiation by about 60 h (Fig. 2). Transforming amastigotes do not incorporate $^{14}$C-thymidine[6] into DNA; however, promastigotes readily incorporate this substrate into DNA (~ 70% of recoverable $^{14}$C from labelled thymidine is found in DNA). This suggested that *de novo* DNA synthesis does not occur in transforming amastigotes.

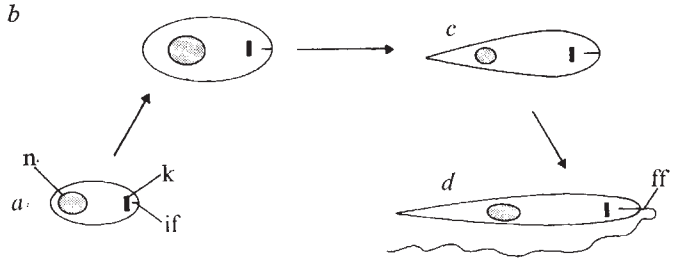


**Fig. 1** Morphological events during transformation of a *L. donovani* amastigote to the promastigote stage. *a*, Oval-shaped amastigote with nucleus (n), kinetoplast (k) and internal flagellum (if), 0–8 h; *b*, tenfold enlargement of the oval stage, ~ 8–16 h; *c*, elongation of the enlarged parasite, ~ 16–18 h; *d*, formation of free flagellum (ff) and further elongation to complete cytodifferentiation, ~ 18–20 h. Amastigotes were obtained as follows: male hamsters were killed with ether about 2 months after intraperitoneal inoculation with freshly isolated amastigotes (Malakal area Sudan strain, 1S) and the spleen excised in sterile conditions. The spleen was homogenised in 10 ml Tris (0.2 M)–sucrose (0.25 M) buffer, *p*H 7.8, with a Ten Broeck glass homogeniser. Homogenates were centrifuged at 150*g* for 10 min and the pellet discarded; the supernatant was centrifuged at 1,000*g* for 20 min and the resultant supernatant (first wash) discarded. The pellet was resuspended in 10 ml Tris–sucrose buffer and this procedure repeated two more times. After resuspending the final pellet (washed amastigotes) in a small amount of Tris–sucrose buffer, amastigotes were counted and $10^8$ cell aliquots incubated in HO–MEM[7] medium at 27 °C. Isolation procedures were carried out in the cold to retard spontaneous transformation, and sterile techniques were used to prevent bacterial contamination. The cell depicted was preincubated in glucose-free Locke's solution (see Fig. 2). About 10% of the preincubated cells started to transform after 8 h in transforming medium. Although stage *a* lasts longer for non-preincubated cells, the duration of time spent in the other stages is the same as that for preincubated cells.

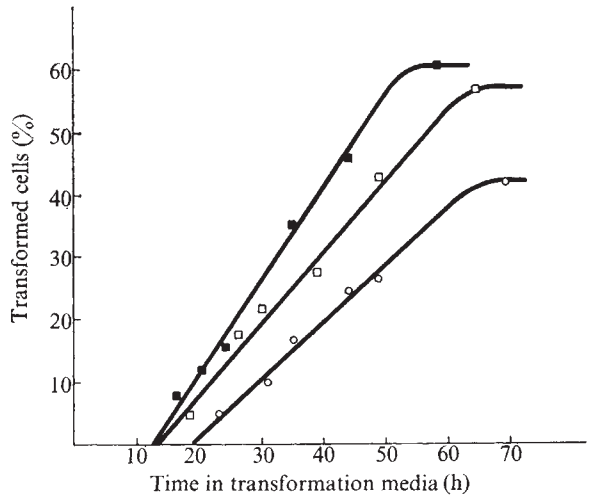


**Fig. 2** Transformation of *L. donovani* amastigotes to promastigotes: influence of preincubation in glucose-free Locke's solution on the times of initiation and completion of transformation by a population of washed amastigotes. ○, Control amastigotes transferred to transforming medium (incubated at 27 °C) immediately after isolation from hamster spleen; □, amastigotes preincubated for 8 h at 27 °C before transfer to transformation medium; ■, amastigotes preincubated for 8 h at 34 °C before transfer to incubation medium. Amastigotes (~ $10^8$ cells) were incubated in 25-ml Erlenmeyer flasks containing 5 ml HO–MEM (containing glucose). Samples were removed from flasks at various times and the percentage of transformed cells (stage *d* in Fig. 1) determined by microscopic observation. In all experiments the final samples were tested for bacterial contamination; the data from contaminated flasks were discarded.

We then incubated freshly isolated amastigotes in glucose-free Locke's solution (*p*H 7.7) for 8 h at 27 °C before transfer to transformation medium (HO–MEM)[7] to deplete possible contaminant compounds carried over during isolation that might inhibit cytodifferentiation. The cells transformed more synchronously and the times required for initiation and completion of transformation were markedly reduced (Fig. 2). Even shorter initiation and completion times for developmental transition of amastigote populations were found when cells were preincubated in glucose-free Locke's solution for 8 h at 34 °C before transfer to HO–MEM (Fig. 2).

To determine if a host factor(s) carried as a contaminant with amastigotes during their isolation delayed transformation, 2.5 ml of the first wash from infected spleen homogenates (see legend to Fig. 1) was added to 2.5 ml of