Learning influences host choice in tsetse

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A learning capacity for feeding is described in many insect species including vectors of diseases, but has never been reported in tsetse flies (Diptera, Glossinidae), the cyclic vectors of human (sleeping sickness) and animal trypanosomoses in Africa. Repeated feeding on the same host species by a disease vector is likely to increase the within-species disease-transmission risk, but to decrease it between species.

An experiment with cattle and reptiles in a stable provides evidence that the species of host selected for the second blood meal in tsetse flies depends on the host encountered for the first blood meal when the between-meal interval is 2 days. This preference disappears when the between-meal interval is extended to 3 days. The energetic advantages of this acquired preference and its importance in trypanosomoses epidemiology are discussed.

Keywords: tsetse flies; trypanosomosis; feeding preference; learning

1. INTRODUCTION

In insects, an acquired feeding preference can overcome variations in the quality and distribution of food resources, as described for honeybees (Apis mellifera), other hymenoptera and vectors of diseases, with important epidemiological implications for the latter (Thorpe & Jones 1937; Lewis & Tuminson 1988; Bicker & Hähnlein 1994). For example, mosquitoes tend to return to the same villages, houses, host species and oviposition sites (McCall & Kelly 2002). The biological vectors of animal and human trypanosomoses in West Africa are tsetse flies of the palpalis group (Genus Glossina, subgenus Nemorhina) living in riparian vegetation (Challier & Gouteux 1980; Bouyer et al. 2005b, 2006), that feed on hosts including reptiles, ruminants and humans (Weitz 1963). Within the same tsetse species, between-population differences are observed in the hosts fed on, depending on host availability (de La Rocque et al. 2005). Preliminary experiments suggested that tsetse flies might possess a learning capacity (Bouyer et al. 2005a); this paper uniquely demonstrates that a tsetse vector’s first host encounter can strongly influence host choice on subsequent feeding cycles, with the magnitude of this effect being diminished by starvation.

2. MATERIAL AND METHODS

On-station experiments were made in a stable (10.4×4.0×2.0 m high), isolated by mosquito netting at CIRDES, Burkina Faso, with cohorts (125 flies per cohort) of teneral male Glossina palpalis gambiens (referred to hereafter as ‘flies’) drawn from a laboratory population (size approximately 100,000). Flies were marked the day after emergence (with acrylic paint on the pronotum) with different colours according to the cohort and rotations between cohorts. On day 2, separate cohorts were exposed to their first host (no choice situation) consisting of either caged (mesh size 2.5×5.0 cm) reptiles (R) or a tethered bovid (C) placed in the stable before the flies (see table 1 for details of the experimental design): two monitor lizards, Varanus niloticus coded V.n. 1 (190 cm long, 3 kg) and V.n. 2 (100 cm long, 2 kg); a crocodile, Crocodilus niloticus coded C.n. (115 cm long, 15 kg); a 5-year-old 134 kg female Bos Taurus coded B.t., a 4-year-old 155 kg female Bos indicus coded B.i. 1, local breed; and finally a 6-year-old 336 kg male B. indicus coded B.i. 2, local breed. In four trials, two monitor lizards were put in the same cage with two other lizards to provide a total host bait mass closer to that of one cow. Flies were left for 2 h (between 8 and 12.00 a.m.) and captured before host removal (two persons with protective clothing for 30 min). Engorged flies (average 89 per cohort) were released 2 days later (seven repetitions, minimal between-meal lag (BML) observed in nature) or 3 days later (two repetitions) with a similar number of teneral flies (no feeding history, eight repetitions) into the same stable but now holding both hosts (R+C, choice situation). Replete flies (average 63 per cohort) were caught for dissection to determine the origin of the blood in the crop. From well-established knowledge of tsetse physiology, the blood meal is evacuated from the crop and anterior midgut within 24 h, thus excluding confusion of blood types between hosts of two successive meals. Thin smears were made of the blood meals and stained (10% Giemsa) for host species determination by microscopy examination of erythrocytes: oval and nucleated for reptiles, round and anucleated for cattle.

The analysed response of flies was the proportion π that fed on cattle (choice situation). Our aim was to test the hypothesis that previous experience of feeding on reptiles is sufficient to cause a tsetse fly to feed on the same species on a second feed. The explanatory variables were (i) first host species (bovid, reptile or none for teneral flies) and (ii) the BML (2 or 3 days). Categories were (i) starvation, (ii) first meal on a bovid and BML of 2 days, (iii) first meal on reptile and BML of 2 days, (iv) first meal on a bovid and BML of 3 days, and (v) first meal on reptile and BML of 3 days. Preliminary analyses showed that the response was greater than the expected binomial variation. To account for this, a beta-binomial logistic regression model was applied (Griffiths 1973): a within-batch correlation coefficient ρ was estimated in addition to the coefficients of the fixed effects. Wald tests were used to test linear combinations of the coefficients under various null hypotheses. Statistical analyses were made with R software (R Foundation for Statistical Computing, Vienna; http://www.R-project.org) and the R package aod (v. 1.1-10; http://cran.r-project.org).

Probabilities π were fitted for each category of the explanatory variable. To facilitate their interpretation, relative risks (RRs) were computed as follows: RRπ,ij = κ̂i/κ̂j, where κ̂i was the fitted probability of a fly choosing a bovid or a reptile for its second blood meal, given its feeding history, and κ̂j(R=π) was the fitted probability to be compared with. Model coefficients were regarded as the realisation of a multivariate Gaussian distribution, with the coefficients as the mean, and their variance–covariance matrix as the variance. Ten thousand samples drawn from this distribution were used to compute the corresponding RRs. The empirical 0.025 and 0.975 quantiles of the simulated values were used to compute their 95% confidence intervals.

3. RESULTS

The engorgement rates of teneral flies were similar on reptiles (0.63, s.d. 0.16) and bovids (0.70, s.d. 0.15), and did not increase when both were presented together (0.62, s.d. 0.16). The engorgement rates of already fed flies on both hosts were slightly higher (0.73, s.d. 0.18), but did not change with the origin of the first host nor the BML (p > 0.05).
The estimated within-batch correlation coefficient was $f = 0.06$ ($z = 2.85$, $P(z) = 0.002$), thus confirming the need to use a statistical model accounting for this correlation. Teneral flies preferred to feed on cattle: $p = 0.85$ (0.79; 0.92) (95% confidence interval), a preference also observed for flies previously fed on cattle at a BML of 2 days ($p = 0.94$ (0.89; 0.99)). Flies previously fed on reptiles preferred reptiles ($p = 0.44$ (0.34; 0.55)). The fitted probabilities for the latter were significantly different from the former (d.f. = 1, 20; $P = 0.03$ for cattle and $P = 2 \times 10^{-8}$ for reptiles). Observed data and fitted probabilities are shown (figure 1). When BML was 3 days, the preference for cattle was similar independent of the first meal source and not significantly different from teneral flies (first meal = reptile: d.f. = 1, 20; $P = 0.74$; first meal = cattle, d.f. = 1, 17, $P = 0.33$); model outputs were compatible with a common probability for teneral flies and flies previously fed on cattle or reptiles (d.f. = 2, 20; $P = 0.59$).

RRs are displayed in table 2 for a BML of 2 days (none were different from 1 when the BML was 3 days). For example, for tsetse that had taken their first meal from cattle 2 days earlier, the RR of feeding on cattle versus reptile for their second meal was 15.94, whereas for tsetse that had taken their first meal from cattle 2 days earlier, the RR of feeding on cattle versus reptile for their second meal was 2.1 in comparison with tsetse that had taken their first meal from reptile(s). The value of each RR was compatible with the assumption that the feeding history influences the choice of host for the second blood meal in the expected direction: under these experimental conditions, flies with a first blood meal on cattle preferred cattle over reptiles for their second blood meal while those feeding first on reptiles preferred reptiles for their second blood meal. All RR values but 2 (second column, fourth line in table 2a and 2b).
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