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The energetic grooming costs imposed by a parasitic mite (*Spinturnix myoti*) upon its bat host (*Myotis myotis*)

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Parasites often exert severe negative effects upon their host's fitness. Natural selection has therefore prompted the evolution of anti-parasite mechanisms such as grooming. Grooming is efficient at reducing parasitic loads in both birds and mammals, but the energetic costs it entails have not been properly quantified. We measured both the energetic metabolism and behaviour of greater mouse-eared bats submitted to three different parasite loads (no, 20 and 40 mites) during whole daily cycles. Mites greatly affected their time and energy budgets. They caused increased grooming activity, reduced the overall time devoted to resting and provoked a dramatic shortening of resting bout duration. Correspondingly, the bats' overall metabolism (oxygen consumption) increased drastically with parasite intensity and, during the course of experiments, the bats lost more weight when infested with 40 rather than 20 or no parasites. The short-term energetic constraints induced by anti-parasite grooming are probably associated with long-term detrimental effects such as a decrease in survival and overall reproductive value.

Keywords: Chiroptera; energetics; grooming; metabolism; time and energy budget

1. INTRODUCTION

Several recent studies have demonstrated that parasites may exert severe negative effects on their hosts' fitness. For example, in his review on birds, Møller (1997) showed that ectoparasites may retard the timing of reproduction and reduce the survival and growth of young, eventually affecting overall reproductive success and individual reproductive value. Not surprisingly, hosts have thus evolved sophisticated physiological, immunological and behavioural defences against parasites (Combes 1991). Yet, although most recent studies have clearly made a link between parasitism and various hosts' life-history traits, few have measured the actual energetic costs induced by parasitism (Schall *et al.* (1982), Oppliger *et al.* (1996) in reptiles; Munger & Karasov (1989) in mammals; Booth *et al.* (1993), Møller *et al.* (1994) with regard to birds). Among the array of hosts' anti-parasitic behaviours are mate selection, avoidance of infected social groups or sites, nest sanitation and grooming (Hart 1997). Although the latter is efficient at reducing parasite loads in both birds (Clayton 1991; Hart 1997) and mammals (Mooring *et al.* 1996) (notice that there exists no study of the efficiency of anti-parasite grooming in free-ranging bats), the extra energetic costs it entails have to our knowledge never been properly investigated. This is particularly surprising as such a time-consuming activity fundamentally affects a species' daily time budget, modifying the fine balance of energy allocation that trades off various vital activities such as feeding, resting, sleeping and reproduction and, ultimately, it alters life-history strategies (Stearns 1992).

Here we present the results of a laboratory study that measured both the metabolism (oxygen consumption) and behaviour (time budget) of wild-captured greater mouse-eared bats (*Myotis myotis*) that were experimentally submitted to various levels of infestation by the haematophagous ectoparasite *Spinturnix myoti* (Acari, Dermanyssoidea).

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2. MATERIAL AND METHODS

(a) *Study organisms*

Fourteen subadult, non-reproductive greater mouse-eared bats (*M. myotis*) were captured with mist-nets during the summer of 1999 at the entrance to two nursery roosts in the upper Rhône valley (Valais, Swiss Alps). The bats were kept in a large, semi-outdoor enclosure at Lausanne University (under licenses from the Nature Conservation and Veterinary Services of the canton of Valais) and fed *ad libitum* on a mixed diet consisting of mealworms (*Tenebrio molitor*) and crickets (*Acheta domesticus*).

The mites used in the experiments were collected from the same colonies as the bats and were kept on our captive bats. *Spinturnix myoti* (Mesostigmata, Spinturnicidae) is a specific haematophagous ectoparasite of *M. myotis* (and its sibling species *Myotis blythii*). It occurs exclusively on wing and tail membranes and completes its entire life cycle on its bat host (Rudnick 1960). The development of eggs, pre-larvae and first nymphal stages takes place in the genital tract of the female mite. The female mite later gives birth to male and female deuteronymphs, which already have an adult appearance (Evans 1968).

(b) *Experimental conditions*

The behaviour and oxygen consumption of the bats were monitored in parallel during experimental runs. Three experiments were carried out on every individual bat, with each run corresponding to a given parasite load (intensities of no, 20 or 40 mites) according to a randomized succession. All parasites present on a bat were either removed prior to the experimental runs (intensity 0) or bats were infested with 20 or 40 parasites each and parasites remaining on the bats at the end of runs were counted. These parasite intensities are close to natural conditions

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as observations made on 166 greater mouse-eared bats gave an average load of 12.8 parasites per bat (s.d. = 10.8 and range = 0–56 mites). Two bats could be studied simultaneously; they were placed in separate respirometric boxes (see § 2c, below), which did not allow contact (either visual, olfactory or acoustic) with each other. All experiments took place in an almost totally dark and silent environment. Each run lasted (mean ± s.d.) 24.1 ± 0.6 h (range = 23–28 h), during which time the bats had no access to food or drinking water; this enabled us to monitor their time and energy budgets during a nearly complete daily cycle. The first hour after the onset of an experiment was excluded from the analysis as it often corresponds to a period of stress due to entry into the metabolic chamber. Moreover, in order to standardize the duration of each run among individuals, the analysis was restricted to 22 h of recording (60 − 1380 min from the onset of the experiment). Individual body mass was measured both prior to entering the respirometric box and just after the end of an experiment. Since the runs sometimes lasted more than 24 h, experiment duration was added to the analyses on weight losses as a covariate, assuming linear decreases in body mass during runs; due to technical problems, four out of 42 experiments could not be used in this covariance analysis.

(c) *Energetics*

Oxygen consumption ($\dot{V}O_2$) was measured using an open-air flow respirometer. Animals were placed in a metabolic chamber (10 cm × 16 cm × 10 cm = 1.6 l air volume) during the trials, which was submersed in a thermoregulated water bath at 35 ± 0.1 °C, i.e. well within the bats' thermoneutral zone (Hock 1951). Outside air was dried over silica gel and pushed through the two metabolic chambers at a flow rate of *ca.* 10.65 l h^{-1}. The flow rate was controlled and measured continuously by a calibrated mass flow controller (model 5850E, Brooks Instruments, Veenendaal, The Netherlands) that was connected to a control and read out equipment (model 5878, Brooks Instruments). The effluent air was sequentially passed through a column of KOH (in order to fix the expired CO_2) and a silica gel column. Finally, oxygen concentration was measured using an oxygen analyser (Gas purity analyser Xentra 4100, Servomex, Esslingen, Switzerland). The oxygen analyser was calibrated monthly using pure nitrogen gas (⩾95%) and pure oxygen gas (⩾95%).

Oxygen concentration was recorded on paper by a potentiometric recorder (recorder 320, Scientific Instruments, Basel, Switzerland) and digitized (using BATSCAN software, A. Hirzel 2000). This allowed us to obtain the instantaneous and total oxygen concentrations precisely. Finally, oxygen consumption was calculated according to Depocas & Hart (1957) as $\dot{V}O_2 = V_2 \times (F_1O_2 - F_2O_2)/(1 - F_1O_2)$, where V_2 is the flow rate measured after the bats were removed from the metabolic chamber and F_1O_2 and F_2O_2 are the oxygen concentrations prior to the bats' entrance to the metabolic chamber and after their exit, respectively. The mean oxygen consumption per hour was calculated from these data.

(d) *Time budget analysis*

The bats' behaviour was monitored using an infrared video camera (Canon Ci-20 PR, Tokyo, Japan) that was connected to a time-lapse video tape recorder (Panasonic AG 6720, Osaka, Japan). The time-lapse option enabled us to record a 24 h run consecutively using a normal 180 min video cassette. Analysis of the behavioural data was performed by the same person (M.S.G). Four types of behaviour were distinguished and directly coded from video playbacks on a monitor screen (time-event recorder software, O. Simonin 1999): (i) resting (corresponding in fact to resting and sleeping, which could not be distinguished), (ii) movement (any slight change in position within the metabolic chamber which lasted < 1 min), (iii) exploration (periods of > 1 min with the bat moving around within the chamber), and (iv) grooming (using teeth or feet for scratching). All of the activities exhibited by the bats could be classified under these four categories. The proportion of time devoted to each activity as well as the absolute durations of activity bouts were calculated.

(e) *Statistical analysis*

Data analyses were performed using the program S-PLUS 2000 (MathSoft Inc. 1988–1999, Seattle, WA, USA). Percentages were square-root arcsine transformed prior to running the statistical analyses. Every variable was then tested for normality (Kolmogorov–Smirnov one-sample test) and heteroscedasticity (one-way ANOVA). No variable deviated from normality and heteroscedasticity, except for the duration of grooming and resting bouts; as normal distributions could not be achieved by transformation of these two variables, we replaced their actual values by their respective ranking values (thereby achieving normal distributions) and performed parametric statistical tests on the ranked data. ANOVAs or ANCOVAs (with parasite load and individual bats as factors since each bat was submitted to the three treatments) were applied in all cases. Moreover, pairwise comparisons were performed using *post hoc* Tukey procedures in order to assess which levels of parasite load yielded results that differed significantly from each other.

3. RESULTS

(a) *Parasite number*

The numbers of parasites present on the bats did not change during the course of an experiment except in three trials out of 42 ($n = 17$ mites instead of 20, $n = 39$ mites instead of 40 and $n = 42$ mites instead of 40 due to two births).

(b) *Energetics*

(i) *Mean oxygen consumption (VO_2)*

Oxygen consumption was significantly greater at higher parasite intensities (figure 1): the values (mean ± s.e.m.) obtained were 48.5 ± 1.3 ml O_2 h^{-1} when the bats were infested with 40 parasites, 44.4 ± 1.4 ml O_2 h^{-1} when the bats were infested with 20 parasites and 40.1 ± 1.2 ml O_2 h^{-1} when the bats were not infested with parasites (ANOVA, $r^2 = 0.910$) (parasite load, $F = 50.3$, d.f. = 2,26 and $p < 0.001$ and individual, $F = 12.51$, d.f. = 13,26 and $p < 0.001$). This corresponded to average increases in oxygen consumption of 11.0% and 21.3% at 20 and 40 parasites, respectively, when compared with the reference value (100%) represented by no parasite.

(ii) *Body mass losses*

The bats lost more weight during an experiment at higher parasite intensities (ANCOVA, $r^2 = 0.693$) (parasite load, $F = 5.23$, d.f. = 2,33 and $p = 0.011$ and run duration as a covariable, $F = 64.04$, d.f. = 1,33 and $p < 0.001$) (note that individual had no effect, $F = 0.74$, d.f. = 13,20 and $p = 0.71$). However, significant differences only occurred between no and 40 parasites (*post hoc* Tukey test). Corrected for experiment duration, these body mass

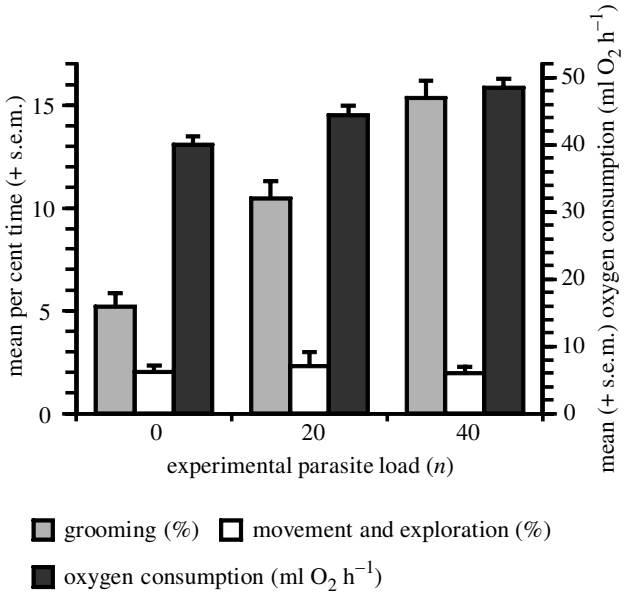

Figure 1. Mean (+ s.e.m.) percentages of time spent in grooming, movement and exploration (pooled together) and mean oxygen consumption (ml O_2 h^{-1}) for 14 *M. myotis* that were experimentally submitted to three parasite loads (no, 20 and 40 mites).

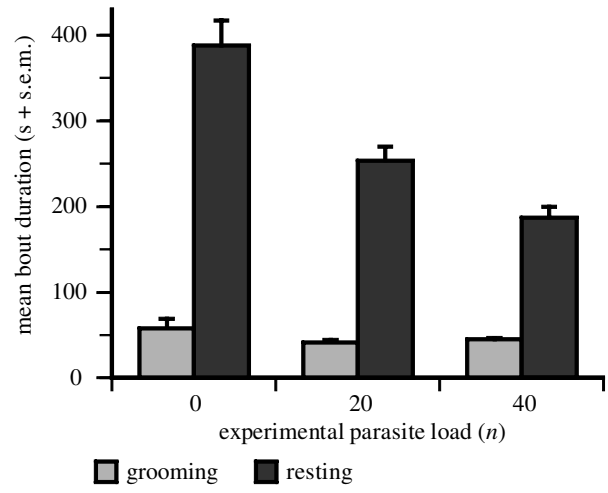

Figure 2. Mean (+ s.e.m.) durations of grooming and resting bouts for 14 *M. myotis* that were experimentally submitted to three parasite loads (no, 20 and 40 mites).

losses corresponded to (mean ± s.d. with s.d. showing among individual variation) 3.8 ± 0.4 g day (intensity $n = 40$ parasites), 3.4 ± 0.4 g day (intensity $n = 20$ parasites) and 3.3 ± 0.5 g day (intensity $n = 0$ parasites).

(c) *Time budget*

(i) *Time allocated to the various activities*

The number of parasites significantly influenced the time budget variables. Parasite load affected the time devoted to grooming (ANOVA, $r^2 = 0.849$) (parasite load, $F = 28.39$, d.f. = 2,26 and $p < 0.001$ and individual, $F = 2.25$, d.f. = 13,26 and $p = 0.038$) (figure 1). Grooming (mean ± s.e.m.) was more pronounced at 40 parasites (15.4 ± 0.8%) than 20 parasites (10.5 ± 0.9%) and also when the bats were infested with 20 rather than no parasites (5.2 ± 0.6%) (*post hoc* Tukey test). Accordingly, the time spent resting decreased with the number of parasites (ANOVA, $r^2 = 0.800$) (parasite load, $F = 42.03$, d.f. = 2,26 and $p < 0.001$ and individual, $F = 1.52$, d.f. = 13,26 and $p = 0.18$). In contrast, parasite intensity affected neither movement (ANOVA, $r^2 = 0.007$) ($F = 0.14$, d.f. = 2,39 and $p = 0.87$) nor exploration (ANOVA, $r^2 = 0.016$) ($F = 0.32$, d.f. = 2,39 and $p = 0.73$); these two behaviours played a tiny part in the overall activities of the bats (figure 1).

(ii) *Mean duration of grooming and resting bouts*

The number of parasites only influenced the overall time devoted to grooming (see § 3c(i)), but not its mean bout duration (figure 2) (ANOVA, $r^2 = 0.376$) (parasite load, $F = 0.723$, d.f. = 2,26 and $p = 0.49$ and individual, $F = 1.09$, d.f. = 13,26 and $p = 0.41$). Accordingly, there was a strong negative relationship between the number of parasites and the duration of resting bouts (figure 2) (ANOVA, $r^2 = 0.659$) (parasite load, $F = 21.57$, d.f. = 2,26 and $p < 0.001$ and individual, $F = 0.55$, d.f. = 13,26 and $p = 0.868$). Significant differences occurred between no, 20 and 40 parasites and also between 20 and 40 parasites (Tukey test). Thus, parasites primarily affected the number of grooming bouts and the progressive shortening of resting bouts with increasing parasite loads stems from numerous interruptions due to more frequent grooming phases.

4. DISCUSSION

This study reveals that parasitic mites may dramatically affect the time and energy budgets of their bat hosts. On average, bats that were experimentally infested with 20 and 40 parasites invested two and three times more time in grooming activity, respectively, than when they harboured no parasite (figure 1).

This suggests that grooming in mouse-eared bats is controlled by a stimulus-driven model (Mooring *et al.* 2000), i.e. that the level of grooming is not totally preprogrammed but is at least partly induced by a stimulus such as cutaneous irritation, which is emphasized by the tissue immune system (Hart 1997). The tremendous rise in grooming activity is clearly associated with extra energetic costs. The bats' energy expenditures corresponded to a rise of *ca.* 0.5% in overall metabolism (+ 11% at 20 mites and + 21% at 40 mites) for each additional mite (from no parasites upwards), which in turn caused a significantly greater drop in the body mass of the bats during the course of experiments at higher parasite intensities.

Consistent with the present results, several studies have established a positive relationship between parasite load and grooming activity (Møller 1991; Mooring 1995; Eckstein & Hart 2000). Moreover, relatively similar proportions of time spent grooming (range 2.2–24% in Burnett & August (1981), Winchell & Kunz (1996), McLean & Speakman (1997), Fleming *et al.* (1998) and Shen & Lee (2000) versus 5.2, 10.5 and 15.4% for no, 20 and 40 parasites, respectively, in this study) have been observed in free-ranging bats, which suggests that our measurements on captive bats are representative of natural conditions.

Despite the large amount of grooming observed, the number of parasites present on a bat remained constant during the experimental runs. Hence, anti-parasite

grooming may firstly serve to disturb sucking parasites, but may remain inefficient at reducing their loads or damaging them physically. However, it should be noted that the tiny room available in the respirometric chamber might have hampered grooming movements other than scratching, for instance wing stretching, which are frequently observed in the wild. In addition, a live mite that was eventually removed from a bat could have recovered its host without difficulty in such a small box. Moreover, within colonial clusters in nature, grooming may effectively enable bats to reduce their individual parasite loads as it could readily cause the transfer of mites from one host to another. Investment in anti-parasite grooming may therefore represent a decisive selective advantage for a given bat.

Mouse-eared bats compensated for the extra time invested in grooming by drastically diminishing the time they devoted to resting, in particular through a reduction in the length of their resting bouts. Although resting and sleeping could not be distinguished from our video sequences, one may assume that both the quantity and quality of their sleep was affected by intense parasitism as has been demonstrated in great tits (Christe *et al*. 1996). A shortening of the duration of sleeping bouts in vertebrates causes an alteration in the sequence of sleeping phases (Meddis 1975; Toates 1980), with corresponding long-term negative effects upon the organism, such as a decrease in survival and overall reproductive value.

Under natural conditions, the consequences of parasites on roosting bats' energetic strategies may be more acute than suggested by our data. Indeed, in the wild, animals often face a limited food supply whereas our captive bats were fed *ad libitum*. In addition, the energetically detrimental effects imposed by parasites upon young must be much greater even than suggested by this study since the majority of newborns harbour several dozen if not several hundreds of mites each (Christe *et al*. 2000). Finally, other costs imposed by parasites, which were not considered in the present study, surely also influence bats' energetic decisions. First, mounting an immune response may cause mid-term, extra energetic costs (Demas *et al*. 1997), which could not be assessed here. Second, haematophagous mites probably play a chief role in the transmission of blood parasites as well. In this respect, our estimations may actually represent the minimum energetic impacts of parasitic mites upon their roosting bat hosts.

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