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Energy costs of blood digestion in a host-specific haematophagous parasite

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Summary

Fleas consume and digest blood from their hosts. We hypothesized that the energy costs of digestion of blood by fleas is dependent on the host species. To test this hypothesis, we studied CO_2 emission, a measure of energy expenditure, during digestion of a blood meal taken by *Parapulex chephrenis* from a preferred (*Acomys cahirinus*) and a non-preferred (*Gerbillus dasyurus*) host. We predicted that the energy cost of digestion would be lower for *A. cahirinus* blood than that for *G. dasyurus*. Male and female fleas consumed similar amounts of blood per unit body mass, independent of host species. Our prediction was supported in that fleas expended significantly more energy digesting blood of *G. dasyurus* than blood of *A. cahirinus*. We also found CO_2 emission rates of fed fleas were higher than those of unfed fleas and differed significantly among stages of blood digestion when a flea fed on *G. dasyurus* but not when it fed on *A. cahirinus*. When fed on *G. dasyurus*, fleas spent less energy during earlier than later stages of digestion.

Key words: CO_2 emission, digestion, flea, host specificity, metabolic cost, *Acomys cahirinus*, *Gerbillus dasyurus*.

Introduction

Net nutritive and energetic value of food consumed by an animal depends on a variety of factors including ecological, chemical, morphological and/or physiological constraints of the forager (Lee and Houston, 1993; Brown et al., 1994; Kam et al., 1997; Piersma and Drent, 2003; Johnston et al., 2005). An interplay of cost/benefit evaluations concerning each of these factors and their interactions results in a foraging decision. One of the most important factors affecting a foraging decision is the energetic cost of food processing. Indeed, the cost of food processing in terms of time and/or energetic cost of digestion has been incorporated into many cost/benefit foraging analyses as an important variable (Kersten and Visser, 1996; van Gils et al., 2003; Piersma et al., 2003). Of particular importance is the energetic cost of digestion, as different types of food entail different energy costs.

Cost/benefit analyses for foraging decisions that take into account the energetic outlay for digestion have been done extensively for various vertebrates such as reptiles (Segor and Nagy, 1994), birds (Piersma et al., 2003; van Gils, 2005) and mammals (Williams et al., 2004). However, this is not the case for invertebrates, where only few studies have been made (Beiras and Camacho, 1994; Yang and Joern, 1994). Furthermore, energetic components of digestive physiology have been examined only rarely in parasitic organisms, although parasites form a large proportion of the diversity of life. Parasitism is suggested to be more common than all other feeding strategies (Sukhdeo and Bansemir, 1996) and parasites supposedly make the same decisions that every animal has to make regarding resource acquisition.

Different aspects of digestive physiology of parasites, especially haematophagous arthropods such as mosquitoes (Pascoa et al., 2002; Briegel, 2003), fleas (Richards and Richards, 1968; Vatschenok et al., 1976) and ticks (Coons et al., 1986; Rechav and Fielden, 1995), have been reported. In particular, different ectoparasitic arthropods have been shown to increase their metabolic rate during blood digestion (Fielden et al., 1999, 2004; Gray and Bradley, 2003). However, the energetic costs of digestion of a food resource (e.g. blood) extracted by a parasite from different hosts have never been studied. These costs would include secretion of enzymes, the metabolism of the blood components, the excretion of the toxic by-products and the heat increment of feeding (see Clements, 1992 for a review).

Differential energy costs of digestion of food obtained by a parasite from different hosts may have important ecological

and evolutionary implications. A lower energy cost for resource processing would allow a parasite to allocate more energy for other competing requirements of an organism (host location, mating, oviposition). This would supposedly increase fitness reward stemming from selection of an appropriate host and, thus, can shape the coevolutionary process between hosts and parasites. At the evolutionary level, differences in the energy cost of digestion of a resource extracted by a parasite from one host species rather than from another host species may reflect specific adaptations of a parasite to exploit successfully a particular host species. Specific adaptations to this host species were favoured by natural selection due to differential fitness rewards between hosts. Thus, energy expended by a parasite to process food obtained from a host may be an indicator of evolutionary success of a parasite in the exploitation of a host species.

We examined energy expenditure, as determined by measurement of CO_2 emission, of the flea *Parapulex chephrenis* Rothschild after feeding on blood of different hosts. Fleas (Siphonaptera) are holometabolous, blood-sucking, ectoparasitic insects that exploit higher vertebrates, being most abundant and diverse on small to medium-sized mammal species (Marshall, 1981). The larvae of fleas are not usually parasitic and feed on organic debris and materials found in the nest of the host. After emergence from the pupa and cocoon, adult fleas must locate a suitable host to complete their life cycle. Fleas vary in the degree of association with their host species from being highly host-specific to being host-opportunistic (Marshall, 1981).

P. chephrenis is a highly host-specific parasite associated with the Egyptian spiny mouse *Acomys cahirinus* Wagner (Krasnov et al., 1997). *A. cahirinus* is a specialized rock-dweller that co-exists in rocky habitats with the gerbil *Gerbillus dasyurus* Wagner, from which *P. chephrenis* is absent. When *A. cahirinus* was recorded occasionally in other habitats, it was also parasitized by *P. chephrenis*. Moreover, *P. chephrenis* was able to discriminate between different host species, selecting *A. cahirinus* over *G. dasyurus* in host-choice experiments (Krasnov et al., 2002a). In the laboratory experiments, *P. chephrenis* was shown to be able to survive and reproduce on *G. dasyurus* (Krasnov et al., 2002a). However, the egg production of *P. chephrenis* fed on this host was almost six times less than when it fed on *A. cahirinus* (Krasnov et al., 2002a).

Physical and chemical properties of blood are important characteristics of a host to which a host-specific ectoparasite is expected to be adapted (Marshall, 1981). However, studies of the effect of feeding ectoparasites on different host species are scarce and indirect (e.g. Prasad, 1969). Moreover, in most laboratory studies of the rate of blood digestion, ectoparasitic arthropods were fed on laboratory animals rather than on their natural hosts. For example, wild rodents were used as hosts in only eight of 27 studies on fleas while the others used mainly laboratory mice, rats, hamsters and guinea pigs (cited by Vatschenok, 1988).

We hypothesized that different host species exert different energetic costs on the digestion of blood by a flea. To test this hypothesis, we studied CO_2 emission during digestion of a single blood meal extracted by a host-specific *P. chephrenis* from a preferred (*A. cahirinus*) and non-preferred (*G. dasyurus*) host. We predicted that the energy costs of digestion would be lower for digesting blood from *A. cahirinus* than blood from *G. dasyurus*.

Materials and methods

Animals

Fleas and rodents were obtained from our laboratory colonies. The flea colony was started in 1999 from field-collected specimens of *A. cahirinus*. We used newly emerged fleas that did not feed prior to the experiments.

Progenitors of the rodent colonies were captured at the Ramon erosion cirque, Negev Highlands, Israel (30°35′ N, 34°45′ E). The rodents were maintained in glass cages (60×50×40 cm) at 25°C with a photoperiod of 12 h:12 h (L:D), using dried grass as bedding material. They were offered millet seeds and alfalfa (*Medicago* sp.) *ad libitum* and commercial cat chow or larvae of flour beetles once a week. No water was available to the rodents as alfalfa supplied enough for their needs. The infestation level of fleas on rodents in our colonies comprised approximately 75% of the natural level of flea infestation (6–8 fleas per rodent; see Krasnov et al., 1997). No adverse effects on body mass and food intake of infested rodents were observed. The experimental protocol met the requirements of the 1994 Law for the Prevention of Cruelty to Animals (Experiments on Animals) of the State of Israel by the Ben-Gurion University Committee for the Ethical Care and Use Animals in Experiments (License IL-19-04-2001). Details on maintenance of fleas and rodents were published elsewhere (Krasnov et al., 2002a, 2003).

Experimental design

Feeding of fleas on rodents was carried out at a room temperature of 25°C and relative humidity of 70%. We used *A. cahirinus* and *G. dasyurus*; 10 male adults of each species. Each rodent (*A. cahirinus* or *G. dasyurus*) was placed in a wire mesh (5×5 mm) tube (10 cm length and 2 cm diameter) that limited movement and did not allow self-grooming. To avoid the potential effect of acquired resistance (Willadsen, 1980), rodents were subjected to *P. chephrenis* parasitism 3–5 times (once daily) prior to experiments. Each tube with a rodent was placed in an individual white plastic pan, and 20 male or female fleas were placed on each rodent. Fleas were collected after 2 h. Groups of fleas (N=5–10) were weighed to the nearest 0.01 mg (Precisa Balance, model 290 SCS; Precisa Instruments AG, Dietikon, Switzerland) prior and after feeding and the difference was calculated as blood consumed. Fleas with high midgut engorgement (see below) only were weighed after feeding.

We assessed the level of flea midgut engorgement by examination of each flea under a light microscope (without

dissection) and by using the following classification: (a) low (less than 30% of midgut is filled with blood); (b) medium (30–70% of midgut is filled with blood); and (c) high (more than 70% of midgut is filled with blood). We selected fleas with high midgut engorgement, weighed them in groups (N=5) and placed them individually into 20 ml glass vials covered by a 5×5 cm nylon screen. Then, vials were placed in refrigerated incubators (FOC225E; Velp Scientifica srl, Milano, Italy) and maintained at 25°C and 92% relative humidity.

We measured CO_2 emission in newly emerged, unfed fleas as well as in fed fleas at different stages of blood digestion. Classification of the stages of blood digestion was modified from a classification of Ioff (1941) and has been used in our previous studies (Krasnov et al., 2002b). We distinguished the following stages: (1) early – midgut stretched and fully filled with light scarlet or dark red blood; (2*)* middle – the contour of the midgut is jagged and the content is dark brown or black; and (3) late – midgut contains only remnants of digested blood or is empty. The duration of each stage of blood digestion in *P. chephrenis* fed on *A. cahirinus* or *G. dasyurus* is reported elsewhere (Krasnov et al., 2003). In brief, the first, second and third stages of digestion of the first blood meal lasted 7–8, 9–10 and 14–15 h, respectively, when a flea fed on *A. cahirinus*, and 8–9, 12–13 and 10–11 h, respectively, on *G. dasyurus*. We measured CO_2 emission after 3, 12 or 24 h post-feeding. Prior to a measurement, we examined the midgut of each flea under light microscopy to verify the blood digestion status. Treatments, therefore, differed in host species (*A. cahirinus* or *G. dasyurus*), flea sex (male or female) and stage of blood digestion (1st, 2nd or 3rd). Each treatment (6–10 fleas) was replicated 10 times, totalling two hosts × two sexes × three stages of digestion × 10 replicates + 10 measurements of CO_2 emission of unfed fleas=130 experiments. Fleas were assigned to different treatments at random.

Respirometry

A flow-through respirometry system was used to measure CO_2 emission. Incurrent air was scrubbed of CO_2 and H_2O vapour by Drierite (700 ml volume) and Ascarite (25 ml volume) columns, respectively, and was pumped through a respirometer chamber made of tygon tubing (6.5 mm internal diameter, 3 ml volume) at a flow rate of 50 ml min^{-1}. Flow rate was controlled by a mass flow controller (model FC-260; Tylan, Rancho Dominquez, CA, USA). This dry, CO_2-free air constituted the baseline measurements for all flow-through measurements. Carbon dioxide content (p.p.m.) of air exiting the respirometer chamber, measured by a CO_2 analyzer (model 6262; LI-COR, Lincoln, NE, USA) in conjunction with data-acquisition software (Datacan V; Sable Systems, Henderson, NV, USA), was sampled every 2 s. Tygon tubing (3.3 mm internal diameter) was used to plumb the system. A stable temperature for the air inside the respirometer tubing (25°C) was regulated by placing the chamber and preceding 6 m of incurrent tygon tubing into a water bath (model 1013S; Fisher Scientific, Pittsburgh, PA, USA). Carbon dioxide emission for fleas was recorded for 1 h. Baseline measurements were made before and after each recording to determine zero CO_2 and to correct for instrument drift. Fleas were measured in groups (N=6–10) since CO_2 emission of individuals was very low and only slightly above baseline levels. Before being placed in the respirometer chamber, groups of fleas were weighed to the nearest 0.01 mg. Details of the protocol as well as details on the repeatability of the measurements can be found elsewhere (Fielden et al., 2004; Krasnov et al., 2004a).

Data analysis

The effect of host species and flea sex on the amount of blood consumed by a flea per individual and per unit body mass of unfed flea was analyzed using two-way analysis of covariance (ANCOVA; with body mass as a covariate because of sexual size dimorphism) and two-way analysis of variance (ANOVA), respectively.

All computerized CO_2 emission recordings were processed using the analysis package of Datacan V. Each recording was converted from p.p.m. to μl CO_2 h^{-1}. We analyzed both mass-specific (per unit body mass) and mass-independent metabolic rate of CO_2 emission. Distribution of all dependent variables did not deviate significantly from normal (Kolmogorov–Smirnov's tests; non-significant), so parametric statistics were applied. We analyzed mass-specific rates of CO_2 emission of fed fleas using three-way ANOVA with host species, flea sex and stage of blood digestion as independent variables. These variables were also used in ANCOVA of mass-independent rate of CO_2 emission with body mass as a covariate (Packard and Boardman, 1999). In addition, we analyzed mass-specific and mass-independent rate of CO_2 emission in newly emerged *versus* adult fleas using one-way ANOVAs and ANCOVAs (with body mass as a covariate), respectively, with flea feeding state (unfed and three stages of digestion) as an independent variable separately for male and female fleas fed on each host species. We used Tukey's HSD test for multiple comparisons. This test is highly conservative (Winer et al., 1991).

We also calculated mass-specific energy cost of a flea for digestion of 1 mg of blood of *A. cahirinus* or *G. dasyurus* during one hour of the first, second or third digestion stages. We refer to the energy expended to digest 1 mg of blood as specific dynamic effect (SDE), as suggested by Withers (1992). Withers (1992) defined SDE as reflecting '…the energetic requirements of many processes that occur as a consequence of food digestion, including mechanical processing, energy exchange through catabolic and anabolic biochemical pathways and amino acid deamination and nitrogen excretion' (p. 108). First, we calculated the mean volume of CO_2 emitted per hour per mg of body mass of newly emerged unfed fleas (no sexual difference in this parameter was found in *P. chephrenis*; see Results). Then, we used this value to calculate the difference in the mass-specific volume of emitted CO_2 between a digesting flea and that of an unfed flea for each respirometric replicate. Difference in body mass of fleas before and after feeding (reduced to 1 mg of body mass before feeding) was considered to be equal to the mass-specific

Table 1. *Mass-specific and mass-independent rates of CO_2 emission in newly emerged male (M) and female (F) unfed and fed* P. chephrenis *fleas at 25°C*

					Rate of CO_2 emission		
Status	Host	Sex	Stage of digestion	Body mass (mg)	Per flea (µl h^{-1})	Mass-specific (µl mg^{-1} h^{-1})	Mass-independent (µl h^{-1})
Unfed	–	M	–	0.11±0.01	0.07±0.01	0.7±0.04	0.17±0.07
	–	F	–	0.18±0.04	0.15±0.03	0.82±0.15	0.17±0.05
Fed	*Ac*	M	1	0.14±0.01	0.19±0.02	1.31±0.11	0.28±0.05
			2	0.13±0.01	0.14±0.01	1.12±0.09	0.25±0.03
			3	0.14±0.01	0.19±0.02	1.39±0.17	0.27±0.08
		F	1	0.24±0.04	0.31±0.03	1.29±0.13	0.27±0.04
			2	0.31±0.03	0.32±0.03	1.20±0.18	0.20±0.04
			3	0.26±0.04	0.50±0.04	1.98±0.20	0.44±0.04
	Gd	M	1	0.11±0.01	0.34±0.06	2.44±0.40	0.44±0.03
			2	0.18±0.01	0.50±0.02	2.60±0.048	0.56±0.07
			3	0.90±0.08	0.24±0.03	1.50±0.16	0.28±0.02
		F	1	0.27±0.05	1.43±0.31	4.83±0.63	1.34±0.12
			2	0.35±0.06	0.96±0.14	3.49±0.65	0.71±0.14
			3	0.21±0.05	0.43±0.04	2.24±0.29	0.43±0.07

Fed fleas were on either *Acomys cahirinus* (*Ac*) or *Gerbillus dasyurus* (*Gd*) as hosts.

amount of blood consumed. The quotient of mass-specific difference in the volume of emitted CO_2 between digesting and unfed fleas and mass-specific amount of consumed blood was considered as a mass-specific indicator of SDE (energy expended for digestion of 1 mg of blood) during the first, second or third digestion stage. To convert metabolic rate measured as the rate of CO_2 emission to an energy equivalent, we assumed a respiratory quotient (RQ) of 0.8. This was determined previously for unfed females of the tick *Amblyomma marmoreum* by Lighton et al. (1993) and seemed a reasonable assumption for haematophagous arthropods. An RQ of 0.8 gives an energy equivalent of 24.5 J ml^{-1} CO_2 (Schmidt-Nielsen, 1990). SDE of a whole flea was calculated in a similar way. These values were used to calculate the energy cost of blood digestion and were analyzed using three-way ANOVA and three-way ANCOVA (with initial body mass as a covariate), respectively, between host species and flea sexes and among digestion stages.

Results

Male and female *P. chephrenis* consumed similar amounts of blood per unit of unfed body mass, independent of host species (ANOVA, $F_{1,116}$=1.7 for the effect of sex and $F_{1,116}$=0.7 for the effect of host species, P>0.3 for both). The same was true for the amount of blood taken by an individual flea when controlling for the confounding effect of body mass (ANCOVA, $F_{1,115}$=2.1 for the effect of sex and $F_{1,115}$=0.6 for the effect of host species; P>0.1 for both). During 2 h of feeding, an individual flea consumed an average of 0.43±0.08 mg of *A. cahirinus* blood or 0.63±0.13 mg of *G. dasyurus* blood per mg of unfed body mass.

Mass-specific rate of CO_2 emission of fed fleas was higher than that of unfed fleas (ANOVA; males, $F_{1,38}$=13.9 if fed on *A. cahirinus* and $F_{1,38}$=12.2 if fed on *G. dasyurus*; females, $F_{1,38}$=7.2 if fed on *A. cahirinus* and $F_{1,38}$=16.4 if fed on *G. dasyurus*; P<0.005 for all; Table 1). The same was true for mass-independent rate of CO_2 emission (ANCOVA with body mass as a covariate; ANOVA; males, $F_{1,37}$=12.7 if fed on *A. cahirinus* and $F_{1,37}$=6.9 if fed on *G. dasyurus*; females, $F_{1,37}$=13.8 if fed on *A. cahirinus* and $F_{1,37}$=4.8 if fed on *G. dasyurus*; P<0.05 for all; Table 1).

Both mass-specific and mass-independent rates of CO_2 emission differed significantly among stages of blood digestion and were affected significantly by host species and flea sex, being, in general, higher (1) when digesting *G. dasyurus* than *A. cahirinus* blood and (2) in females than in males (Table 2). However, significance of the interaction terms indicated that differences in CO_2 emission among stages of digestion were manifested differently in male and female fleas fed on different hosts. Indeed, mass-specific rate of CO_2 emission did not differ between consecutive stages of blood digestion in both male and female fleas fed on *A. cahirinus* (Tukey's HSD tests; P>0.1 for all; Table 1). By contrast, male and female fleas fed on *G. dasyurus* demonstrated significantly higher mass-specific rates of CO_2 emission during the 1st and 2nd digestion stages than during the 3rd stage (Tukey's HSD tests; P<0.05; Table 1). The same was true for mass-independent rates of CO_2 emission (Table 1). In addition, mass-independent rates of CO_2 emission in females fed on *G. dasyurus* were significantly lower during the 2nd digestion stage than the 1st stage (Tukey's HSD tests; P<0.05; Table 1).

Mass-specific SDE of fleas showed a significant effect of host species ($F_{1,108}$=58.9; P<0.001); fleas expended

Table 2. *Summary of ANOVA and ANCOVA (with body mass of a fed flea as a covariate) of mass-specific and mass-independent rates of CO_2 emission, respectively, at different stages (digestion stage factor) of* A. cahirinus *or* G. dasyurus *(host factor) blood digestion by male and female (sex factor)* P. chephrenis

Analysis	Effect	d.f.	*F*
ANOVA	Host	1	56.5**
	Sex	1	15.9**
	Digestion stage	2	4.06*
	Host × sex	1	8.2**
	Host × digestion stage	2	11.6**
	Error	108	
ANCOVA	Host	1	22.8**
	Sex	1	7.2**
	Digestion stage	2	3.3*
	Host × sex	1	6.5**
	Host × digestion stage	2	6.3**
	Host × sex × digestion stage	2	3.4*
	Error	109	

Only significant interactions are shown: *P<0.05, **P<0.001.

significantly more energy digesting blood of *G. dasyurus* than blood of *A. cahirinus* (Fig. 1). The difference between digestion stages in SDE was marginally significant ($F_{2,108}$=2.9; P=0.06) whereas SDE was similar in males and females ($F_{1,108}$=0.7; P>0.3). The only significant interaction was between host species and digestion stage factors ($F_{2,108}$=3.6; P<0.03). This was manifested in lower SDE during the third stage of digestion than the second stage for males or the two first stages for females when fleas fed on *G. dasyurus* (Fig. 1).

The ANCOVA of SDE for a whole flea with body mass as a covariate demonstrated a similar trend. In both males and females, mass-independent SDE was affected by host species ($F_{1,107}$=22.5; P<0.001), being significantly higher for *G. dasyurus* blood than for *A. cahirinus* blood during the first and second (Tukey's HSD tests; P<0.05) stages but not the third stage of digestion (Tukey's HSD tests; P>0.05) (Fig. 2). This pattern also explains the significant effect of the digestion stage factor ($F_{2,107}$=3.2; P<0.05) as well as the significance of the interactions of host species with digestion stage and a three-way interaction term ($F_{1–2,107}$=3.5–6.4; P<0.05 for both). The effect of flea sex was marginally significant ($F_{1,107}$=3.0; P=0.056), whereas interaction between host species and sex was significant ($F_{1,107}$=6.5; P<0.01). This, together with significance of the three-way interaction, was manifested in a tendency of females to spend more energy than males during the first stage of digestion of *G. dasyurus* blood (Fig. 2).

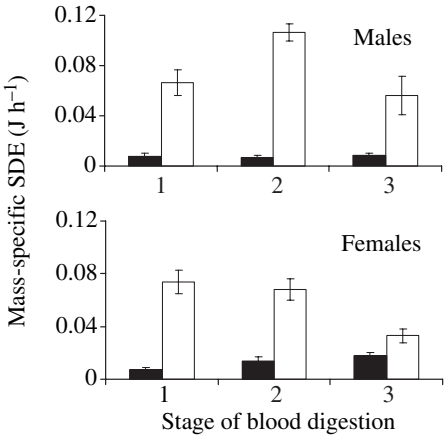

Fig. 1. Mean (± S.E.M.) mass-specific specific dynamic effect (SDE) in *P. chephrenis* digesting blood of *A. cahirinus* (filled columns) or *G. dasyurus* (open columns).

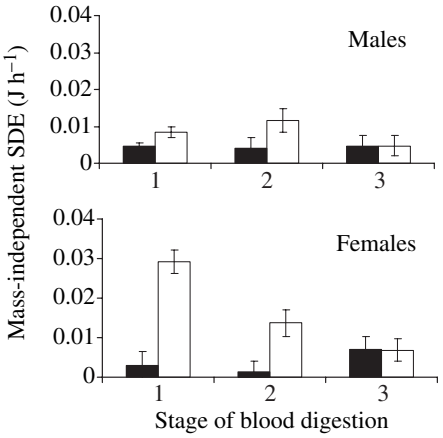

Fig. 2. Mass-independent (adjusted means ± S.E.M.) specific dynamic effect (SDE) in *P. chephrenis* digesting blood of *A. cahirinus* (filled columns) or *G. dasyurus* (open columns).

Discussion

Energy costs for blood digestion were lower when a flea fed on its preferred host rather than on a non-preferred (=strange) host and, therefore, our prediction was confirmed. In addition, this study demonstrated that (1) consecutive stages of blood digestion had different metabolic costs; (2) blood meals increased the metabolic rates of fleas as measured by CO_2 production; and (3) female fleas tended to spend more energy than males during the earlier stages of blood digestion from a non-preferred host.

Effect of host species

Besides the lower energy costs of *P. chephrenis* to digest blood of *A. cahirinus* than blood of *G. dasyurus,* it took *P. chephrenis* less time to digest the *A. cahirinus* blood (9.1±0.6 *versus* 15.4±0.9 h for the 2nd stage of digestion; Krasnov et al., 2003). This between-host difference in digestion time could be due to between-host variability in the resistance of blood cells (both red and white) to haemolytic activity of the flea digestive system (Vatschenok, 1988). Consequently, a blood meal from *A. cahirinus* would probably produce a higher fitness reward for *P. chephrenis* than a blood meal from *G. dasyurus*. Indeed, a previous study showed that female *P.*

chephrenis produced 4–6 times more eggs when fed on *A. cahirinus* than on *G. dasyurus* (Krasnov et al., 2002a). A reduction in egg production and viability for parasites fed on a non-preferred host has been reported for other species. As early as the beginning of the twentieth century, Goeldi (1905) recognized that mosquito egg production was affected by host blood. The rat fleas *Xenopsylla cheopis* and *Xenopsylla astia* failed to reproduce when they fed on humans (Seal and Bhattacharji, 1961), and fecundity and egg hatchability in *X. cheopis* were higher when the fleas fed on *Rattus rattus* than on *Bandicota bengalensis* (Prasad, 1969). Moreover, flea species that are commonly believed to be host opportunistic demonstrate differential reproduction output, dependent on host species. *Xenopsylla conformis* produced three times the number of eggs when exploiting the jird *Meriones crassus* than the gerbil *Gerbillus dasyurus* (Krasnov et al., 2004b).

Differential fitness reward of a haematophagous ectoparasite according to host species suggests that ectoparasites can adapt to feed on blood with host species-specific physiological and biochemical parameters (viscosity, protein, glucose, lipid content). However, little information is available on host blood characteristics that could account for preferences in haematophagous parasites. Host preferences shown by tsetse flies for certain mammals do not appear to be based on the nutritional value of the blood (Moloo et al., 1988), whereas some species of mosquitoes show a preference for human blood over mouse blood based on amino acid composition, specifically isoleucine content (Harrington et al., 2001).

The energy cost of digestion is not the only parameter that determines energetic efficiency of foraging and, ultimately, of host selection by a parasite. Other expenditures associated with foraging activities include searching for the host, feeding site selection for piercing the skin of the host and obtaining a blood meal, time required for a blood meal and surviving anti-parasitic grooming effort of a host. For example, hosts can differ in abundance and spatial distribution and, thus, in their availability for a parasite. The difference in feeding rate of a haematophagous parasite on different hosts could be a result of the morphology of the mouthparts that can penetrate the skin of some hosts but not others (Marshall, 1981). Previous studies on the patterns of flea foraging demonstrated that these costs are usually lower for a preferred host than for other hosts. For example, time from contact with the host to the beginning of feeding, which can be considered the latency of foraging decision, was less in *P. chephrenis* feeding on *A. cahirinus* than on *G. dasyurus* (Krasnov et al., 2003).

Digestion and metabolic rate

Higher CO_2 emission in fed *versus* unfed fleas supports our previous findings on *Xenopsylla ramesis* that fleas that had a blood meal had significantly higher metabolic rates than newly emerged unfed fleas (Fielden et al., 2004). This difference in energy expenditure is mainly a consequence of the energy costs of consuming, processing and digesting blood by the haematophagous arthropods. In addition, feeding may stimulate some physiological process that causes differential metabolic responses in starving fed and unfed haematophagous arthropods such as sperm or egg production (Gray and Bradley, 2003).

In fleas fed on *G. dasyurus*, energy costs generally decreased at the third stage of digestion. This decrease of energy expenditure may be associated with the absence of a digestive process *per se* at the third stage of digestion (Vatschenok and Solina, 1969). Indeed, the earlier stages of digestion in fleas include haemolysis and digestion of blood to haematin (the final product of blood digestion; Vatschenok, 1988), whereas the third stage seems to reflect mechanical (release of the undigested remnants and final products) rather than biochemical processes (Vatschenok and Solina, 1969; Brukhanova et al., 1978). The lack of differences in energy cost of different digestion stages in fleas fed on *A. cahirinus* can be related to overall low energy expenditure during this process and, thus, possible undetectability of these differences by our equipment.

Sexual difference

Female fleas invested more energy for digestion of blood of a non-preferred host than did males. In addition, time required for digestion of *G. dasyurus* blood has been found to be longer in females than in males (Krasnov et al., 2003, but see Vatschenok et al., 1976 for *X. cheopis*). This suggests a strong female-biased sexual difference in the total amount of energy spent for digestion of a blood meal from a non-preferred host.

This difference between sexes can be related to the unpredictability of host finding by a flea and to the association of flea reproduction with blood feeding. Adult fleas alternate periods on the host with periods in the burrow or nest (Ioff, 1941). Since a host may not always return in a regular or predictable manner to its nest or resting area, survival of fleas depends in part on their ability to use other host species. Indeed, female *P. chephrenis* can survive and reproduce when fed on *G. dasyurus*, although its fecundity decreases drastically when exploiting this host (Krasnov et al., 2002a). Furthermore, blood feeding in fleas triggers sperm transfer, mating behaviour, egg maturation and oviposition (Iqbal and Humphries, 1970; Hsu and Wu, 2001; Dean and Meola, 2002). Male fleas are able to mate after a single blood meal (Iqbal and Humphries, 1976), but females of most flea species have to feed repeatedly for their eggs to mature (Vatschenok, 1988). Consequently, the urgency of a blood meal is more critical for females than for males. As a result, females would benefit from investing energy in digesting blood from a strange host, especially given that the location and successful attack of a preferred host is not guaranteed. Another, not necessarily alternative, explanation of the sexual differences in the digestion of blood of a non-preferred host is related to the fact that, during digestion, fleas essentially transform host blood into eggs or sperm. Vitellogenesis or oogenesis may be more energy costly than spermatogenesis, especially when food with biochemical components that differ in some way from that of the specific host is consumed.

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