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Patterns of Erythrocyte Digestion by Bloodsucking Insects: Constraints on Vector Competence

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ABSTRACT Two general patterns of erythrocyte digestion were observed in representative species from four insect orders. Ingested erythrocytes were hemolyzed rapidly, and blood meals remained liquefied within body lice, *Pediculus humanus* L. and the fleas *Ctenocephalides felis* (Bouché) and *Xenopyslla cheopis* (Rothschild). Peritrophic membrane was absent. In contrast, there was a lag time of 6–18 h before substantial degradation of erythrocytes within the blood meals of bed bugs, *Cimex lectularius* L.; the sand fly *Phlebotomus papatasi* Scopoli; and the mosquitoes *Anopheles stephensi* Liston and *Culex pipiens* L. Blood meals of sand flies and mosquitoes were clotted and surrounded by peritrophic membrane at 18–24 h after feeding. Clotting and peritrophic membrane were less pronounced in bed bugs. It is proposed that acquisition and maintenance of pathogen types (i.e., prokaryotic versus eukaryotic) within insects are constrained by the general pattern of bloodmeal processing.

KEY WORDS erythrocyte digestion, pathogen acquisition, coevolution

BIOLOGICAL TRANSMISSION of various types of pathogens tends to be associated with particular taxonomic groups of vector arthropods. For example, transmission of eukaryotic parasites occurs more frequently within Hemiptera and Diptera than Anoplura and Siphonaptera. The reverse is true for prokaryotic pathogens. There are many factors that contribute to the vectorial efficiency of an insect species (e.g., behavior and genetics). However, in each case, an insect first must successfully acquire and maintain the pathogen if the insect is to become a vector. With the notable exception of transovarial or venereal transmission, most insect vectors acquire pathogens orally via the blood meal. The pathogen must be compatible with the digestive physiology of the vector if continued development is to proceed. Thus it follows that the generalized pattern of bloodmeal "processing" exhibited by an insect taxon places constraints on the coevolution of pathogens within insects. To explore this concept, we examined the general patterns of erythrocyte digestion in representative species from several insect orders.

Materials and Methods

Seven insect species representing four orders were examined: adult *Pediculus humanus* L., late instar and adult *Cimex lectularius* L., newly emerged adult female *Ctenocephalides felis* (Bouché), adult female *Xenopyslla cheopis* (Rothschild), *Phlebotomus papatasi* Scopoli, nulliparous *Anopheles stephensi* Liston, and nulliparous *Culex pipiens* L.

Insects were blood fed on either our forearms or anesthetized rodents. Bloodmeal sizes were determined gravimetrically (engorged weight minus prefed weight). Blood meals were excised and diluted 1:200 in saline, and erythrocytes were counted on a hemocytometer. Engorged insects were processed at various intervals after feeding. Host erythrocyte density was measured immediately after feeding.

For comparative infectivity studies, groups of *C. lectularius, X. cheopis,* and *A. stephensi* were fed simultaneously on a single anesthetized mouse (CD-1 strain) infected with *Plasmodium berghei* Vincke & Lips (ANKA strain). Unfed insects were removed, and engorged insects were maintained at 21°C. At 18–24 h, 10–20 blood meals from each species were excised and examined for oocysts by two methods (Vaughan et al. 1991). Some blood meals were diluted in ≈20 μl saline, spotted onto glass slides, air dried, stained with Giemsa solution, and examined for ookinetes at 1000X-oil. Other blood meals were emptied into 30 μl of a 3% acetic acid solution that lysed the erythrocytes, leaving leucocytes and parasites intact. Samples were examined at 400X phase contrast with a hemacytometer to estimate absolute densities of ookinetes. Remaining insects were dissected 10 d later, and midguts were examined for oocysts.

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Table 1. Blood cell counts ($\times 10^{-3}$ per μl; mean ± SEM) in blood meals of various bloodsucking insects over time (26–28°C)

Time after feeding, h	*P. humanus*[a]	*C. felis*[b]	*X. cheopis*[c]	*C. lectularius*[d]	*P. papatasi*[e]	*An. stephensi*[f]	*Cu. pipiens*[g]
0	6,780 ± 665	4,577 ± 948	4,183 ± 991	—	—	8,746 ± 1,017	9,189 ± 772
2	1,331 ± 354	4,056 ± 993	487 ± 194	3,888 ± 866	8,562 ± 679	—	10,965 ± 1,113
4	25 ± 20	—	—	—	—	—	—
6	—	2,283 ± 526	22 ± 14	3,960 ± 468	9,268 ± 1,073	7,654 ± 1,127	8,910 ± 654
12	0 ± 0	—	—	—	7,617 ± 491	—	—
18	—	137 ± 97	0 ± 0	1,320 ± 563	—	3,533 ± 381	6,503 ± 755
24	—	38 ± 38	—	776 ± 505	3,838 ± 749	1,827 ± 433	6,367 ± 529
48	—	—	—	20 ± 2	641 ± 170	1 ± 1	708 ± 267

[a] Bloodmeal source; human with red blood cell count of $4,657 \pm 435 \times 10^{-3}$.
[b] Bloodmeal source; mouse with red blood cell count of $9,835 \pm 245 \times 10^{-3}$.
[c] Bloodmeal source; baby rat with red blood cell count of $5,470 \pm 150 \times 10^{-3}$.
[d] Bloodmeal source; human with red blood cell count of $4,487 \pm 459 \times 10^{-3}$.
[e] Bloodmeal source; hamster with red blood cell count of $7,450 \pm 276 \times 10^{-3}$.
[f] Bloodmeal source; mouse with red blood cell count of $8,252 \pm 527 \times 10^{-3}$.
[g] Bloodmeal source; mouse with red blood cell count of $7,745 \pm 265 \times 10^{-3}$.

Results and Discussion

There were two general patterns of erythrocyte digestion (Table 1). The first pattern, observed for lice and fleas, was characterized by rapid hemolysis and liquefication of the blood meal within 6 h after feeding. Formation of a peritrophic membrane was absent, and the blood meal remained liquid throughout the 48-h observation period. In the second pattern, observed for bed bugs, sand flies, and mosquitoes, there was a lag time of 6–18 h before substantial breakdown of erythrocytes began. A well-defined peritrophic membrane surrounded the blood meals of sand flies and mosquitoes at 18–24 h after feeding, and blood meals were clotted. Peritrophic membrane formation and bloodmeal clotting was much less pronounced in bed bugs.

The two patterns of erythrocyte digestion most likely reflect the different feeding strategies employed by these insects. Lice and fleas generally maintain a close association with the host animal, taking relatively small but frequent blood meals. Because the availability of blood is unlimited, erythrocytes are degraded quickly, most likely to expedite a rapid turnover of nutrients necessary for a constant output of eggs. In contrast, micropredators such as bugs and flies take large, relatively infrequent blood meals. For these insects, processing efficiency may take precedence over rapid cycling, the result being that only a small portion of the blood is actually digested at any one time. In bugs and many higher flies, most of the blood meal is stored in the expanded anterior midgut or diverticula while a smaller portion is digested in the posterior midgut. In nematoceran flies, excess water is removed (diuresis) and a peritrophic membrane is initiated usually before digestive enzymes peak (see Gooding [1972] for an excellent review on digestive processes). Such processes probably account for the lag time observed in erythrocyte digestion for bugs and flies.

We propose that these distinct patterns of erythrocyte digestion also delineate the general boundaries within which pathogens can survive and ultimately coevolve with insects. Specifically, rapid bloodmeal hemolysis and liquefication may tend to favor survival of prokaryotic organisms. Eukaryotic parasites such as protozoans and nematodes would likely be destroyed together with host erythrocytes if they remained in the gut lumen for more than a few hours. Bacteria and rickettsiae may better withstand the rapid liquefication of the blood meal because of their cell walls and tiny size. In contrast, delayed erythrocyte digestion may tend to favor survival of eukaryotic organisms by allowing sufficient time for requisite developmental events (e.g., differentiation, replication, or syngamy) to occur within the blood meal. Prokaryotes, particularly nonmotile forms, may become effectively "walled off" by clotting or peritrophic membrane, confining these organisms to the blood meal to be subsequently digested or expelled.

To test this hypothesis, we examined the initial developmental events (i.e., fertilization) of a rodent malarial parasite, *Plasmodium berghei*, within the blood meals of mosquitoes (*An. stephensi*), bed bugs (*C. lectularius*), and fleas (*X. cheopis*). As expected, gamete fertilization and ookinete formation were greatest in *An. stephensi* (Table 2). Fertilization was permissible but suboptimal within bed bugs. No ookinetes were detected in the blood meals of fleas. Smears of flea blood meals 18 h after feeding indicated that few if any intact cells remained, suggesting that the parasites were destroyed be-

Table 2. *Plasmodium berghei* ookinete densities within blood meals of three bloodsucking insects fed simultaneously on same gametocytemic mouse

Species	Blood meal size, μl	Geometric mean ookinete per μl
An. stephensi	0.9	309.0 (n = 13)
C. lectularius	4.4	47.2 (n = 7)
X. cheopis	0.3	0.0 (n = 10)

Table 3. Experimental infection of *X. cheopis* fleas allowed to feed for 18 h on a *Rickettsia typhi*-infected rat at various time interval after inoculation

D after inoculation	*Rickettsia typhi* PFU/ml host blood	*Rickettsia typhi* ingested per flea blood meal (theoretical)[a]	Resultant flea infection rate (%, $n = 20$)
0	<6	<0.004	0
3	<6	<0.004	0
5	<6	<0.004	30
7	170	0.1	100
9	8700	5.7	100
11	4300	2.8	100
13	660	0.4	100
15	330	0.2	80
21	<6	<0.004	10
28	<6	<0.004	0

[a] Calculation based on rickettsial body: PFU ratio of 2 and a flea blood meal size of 0.3 μl (Vaughan & Azad 1990).

fore the sexual stages could fertilize and penetrate the midgut. Oocysts developed only in *An. stephensi*.

The digestive pattern of fleas and lice, so destructive to malarial parasites, may in fact aid in the maintenance of prokaryotes. This was first demonstrated by Bacot (1915) when he compared the development of plague bacilli, *Yersinia* (=*Bacillus*) *pestis* (Lehmann and Neuman), in the flea *Nosopsyllus* (=*Ceratopsyllus*) *fasciatus* (Bosc) and the bedbug, *C. lectularius*. Bacot states that the "digestive processes of the flea result in a rapid destruction of both red blood cells and leucocytes, leaving the contents of its stomach very much in the condition of an artificial culture media" (789). In contrast, development of plague bacilli within bed bugs was much reduced "possibly due to the preservation of the structural character of the blood for many days after its ingestion into the crop" (791).

Likewise, our recent studies (Vaughan & Azad 1990) indicate that the acquisition of murine typhus rickettsiae (*Rickettsia typhi*) by *X. cheopis* fleas is extremely efficient, requiring only a few rickettsial organisms to result in flea infection (Table 3). Presumably, the rapid breakdown of erythrocytes facilitates release of internal components of the blood cells, including rickettsiae. Because no peritrophic membrane is formed, there are no obvious physical barriers preventing extracellular rickettsiae from coming into contact with their target cells, the midgut epithelium. In addition, peristalic contractions of the midgut (observed in both engorged fleas and lice) may act to circulate the liquefied blood and enhance the likelihood of rickettsial–epithelium contact.

There are substantial differences among insects with respect to the "processing" of blood meals. We believe that bloodmeal processing, especially erythrocyte digestion, helps to explain why biological transmission of certain categories of pathogens tends to be associated with certain categories of insects. In general, the relationship between vector efficiency and erythrocyte digestion may follow broad taxonomic lines, although many exceptions undoubtedly exist. A classic example is that of the protozoan, *Trypanosoma lewisi* Kent, developing within the midgut epithelium of the flea, *N. fasciatus* (Minchin & Thomson 1915). In this case, the trypanosome presumably escapes the harsh environment of the gut lumen by invading midgut epithelial cells very soon (≤6 h) after ingestion. Thus, the concept of erythrocyte digestion as a determinant of vectorial efficiency should not be restricted wholly to taxonomic relationships. Rather, we conclude that if a microbial species is to coevolve with an insect species (or group of species), it must first and foremost be able to develop within the physical and chemical constraints placed upon it by the insect's digestive physiology. Patterns of erythrocyte digestion help to define those constraints. Within these primary constraints, there then may exist secondary mechanisms (e.g., lectin-sugar interactions, see Pimenta et al. [1992]) that modulate or even dictate parasite–vector compatibility at the species level.

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