PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Evolution of Cellulose Digestion in Insects [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1991 **333**, 281-288
doi: 10.1098/rstb.1991.0078

The evolution of cellulose digestion in insects

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SUMMARY

Despite the abundance and diversity of species that include living or dead plant tissue in their diets, the ability to digest cellulose is rare in insects and is restricted to a small number of orders and families. In this paper it is argued that cellulolytic capacity is uncommon in insects simply because it is a trait that is rarely advantageous to possess. Although there is a growing body of evidence for the occurrence of symbiont-independent cellulose digestion in cockroaches and in higher termites from the subfamily Nasutitermitinae, cellulose digestion in insects is usually mediated by microorganisms. It is proposed that non-cellulolytic omnivorous scavengers and detritivores may be preadapted to evolve symbiont-mediated cellulolytic mechanisms because of the prevalence of mutualistic associations between such species and the microorganisms that normally reside in their hindguts. A scenario is proposed for the evolution of symbiont-mediated cellulolytic capacity in roaches and lower termites. Finally, it is suggested that biochemical studies of insect cellulases might provide crucial insights that would greatly advance our understanding of the evolution of cellulose digestion in insects.

1. INTRODUCTION

Considering the number and diversity of insects that thrive on diets consisting largely of plant tissue, it is striking how few are able to exploit the nutritive value of cellulose, which is doubtless the most abundant form of non-fossilized carbon on earth. Cellulose digestion is a complex process, involving a suite of enzymes with diverse modes of action. Although the production of a complete cellulolytic system is common among bacteria and fungi, it is unusual among animals. Cellulose digestion in animals is most often mediated by symbiotic cellulolytic microorganisms. Thus, a discussion of the evolution of cellulolytic capacity in insects requires that two major questions be addressed: (i) Why is cellulose digestion so rare, and (ii) why is symbiont-dependent cellulose digestion more common than symbiont-independent cellulose digestion? In this paper I first discuss some general aspects of the biochemistry of cellulose digestion. I then discuss the occurrence and mechanisms of cellulose digestion in insects, and review evidence for symbiont-independent cellulolysis. Finally, I conclude by posing tentative answers to the two questions posed above, and by suggesting future directions for research in this area.

2. THE ENZYMOLOGY OF CELLULOSE DIGESTION

All cellulolytic systems examined to date consist of a mixture of enzymes with different activities and specificities (Coughlan & Ljungdahl 1988). Cellulose digestion has been most thoroughly studied in white rot fungi, where the cellulase system consists of a mixture of three classes of soluble extracellular enzymes: (i) endo-β-1,4-glucanases, which randomly cleave β-1,4-glucosidic bonds along a cellulose chain; (ii) exo-β-1,4-glucanases, which cleave cellobiose units from the non-reducing end of a cellulose chain; and (iii) β-1,4-glucosidases, which hydrolyse cellobiose and water soluble cellodextrins (oligosaccharides) to glucose. Endoglucanases generally exhibit very low activity toward crystalline cellulose, but are quite active toward amorphous cellulose and water-soluble derivatives of cellulose, such as carboxymethylcellulose (CMC), whereas exoglucanases are more active toward crystalline cellulose and less active against CMC (Knowles *et al.* 1988). Endo- and exocellulases work synergistically in bringing about the hydrolysis of crystalline lignocellulose, and effective cellulolysis requires the presence of both classes of enzymes.

The digestibility of native cellulose is highly dependent upon its crystallinity and its association with other structural polymers, especially lignin (Wood & Saddler 1988). The cellulolytic systems of some organisms incorporate processes that effect the removal of lignin and the disruption of crystalline structure (amorphogenesis). For example, oxidative processes that cleave cellulose chains and destroy the orderly arrangement of cellulose molecules are believed to act as amorphogenizing systems in some brown rot fungi. In insects, the grinding action of the mandibles and the highly alkaline conditions that prevail in the midguts of some species might also serve to reduce the crystallinity of ingested cellulose.

The characterization of insect cellulases has been complicated by the presence of many enzymes in the gut fluids of cellulolytic species that are not insect enzymes at all, but rather are enzymes produced by cellulolytic microbial symbionts. Many insects, including species that are not able to digest cellulose, secrete endoglucanases and β-glucosidases analogous to those produced by white rot fungi (Martin 1983, 1987). These species lack the ability to digest cellulose because of the failure to produce exoglucanases or to have effective amorphogenizing systems that convert cellulose into a form that can be attacked by endoglucanases. The production by insects of enzymes active against crystalline cellulose, has only recently been shown (§5).

3. THE OCCURRENCE OF CELLULOSE DIGESTION IN INSECTS

Cellulose digestion has been shown in 78 species of insects from 20 families representing eight orders (table 1). Taxa in which cellulolytic capacity is common include the thysanuran family Lepismatidae (silverfish and firebrats), which has been elevated to ordinal status and renamed Zygentoma by Kristensen (1981), the Isoptera (termites), and the three coleopteran families Anobiidae (furniture beetles and death watch beetles), Buprestidae (metallic wood borers), and Cerambycidae (long-horned beetles). Cellulolytic capacity is also probably common in the orthopteran superfamily Blattoidea (roaches), the coleopteran family Scarabaeidae (scarab beetles), the dipteran family Tipulidae (crane flies), and the hymenopteran family Siricidae (woodwasps).

Termites are the most efficient cellulose-digesters, with assimilation efficiencies often approaching 99%. The xylophagous larvae of siricid woodwasps and anobiid, buprestid and cerambycid beetles are somewhat less efficient, with assimilation efficiencies in the range 12–68%. Cellulose digestion also occurs in a number of omnivorous species, such as silverfish, firebrats, and roaches, where efficiencies range from 40–90%. Cellulose digestion is rare in detritus feeders, and digestive efficiency is usually low-to-moderate, ranging from 11–50%. Cellulose digestion in foliage feeders is very rare.

Table 1. *The occurrence of cellulose digestion in insects*

The number in parentheses is the number of species in that family in which cellulolytic capacity has been shown. Species names can be found in Martin (1983, 1987)[a].

order family	order family
Thysanura	Coleoptera
Lepismatidae (5)	Scarabaeidae (2)
Orthoptera	Buprestidae (6)
Gryllidae (1)	Anobiidae (5)
Cryptocercidae (1)	Coccinellidae (1)
Blattidae (1)	Cerambycidae (29)
Blaberidae (1)	Curculionidae (1)
Isoptera	Trichoptera
Mastotermitidae (1)	Limnephilidae (1)
Kalotermitidae (2)	Diptera
Hodotermitidae (1)	Tipulidae (2)
Rhinotermitidae (6)	Hymenoptera
Termitidae (8)	Siricidae (3)
Plecoptera	
Pteronarcyidae (1)	

[a] Studies not referenced in Martin (1987): Lindsay (1940); Modder (1964); Zinkler (1983); Griffiths & Cheshire (1987); Zinkler & Götze (1987); Chararas & Noirot (1988); Hogan *et al.* (1988*a*, *b*); Kukor *et al.* (1988); Rouland *et al.* (1988); Scrivener *et al.* (1989).

4. THE MECHANISMS OF CELLULOSE DIGESTION IN INSECTS

Four mechanisms have been proposed to account for cellulose digestion in insects: (i) exploitation of the cellulolytic capacity of protozoan symbionts residing in the hindgut; (ii) exploitation of the cellulolytic capacity of bacteria residing in the hindgut; (iii) reliance upon fungal cellulases, originating in the food, that remain active in the gut following ingestion; and (iv) secretion by the insect of a complete cellulase system. The multiplicity of mechanism implies that cellulolytic capacity has evolved independently in different groups, which explains the irregular distribution of the trait among highly divergent taxa.

The first mechanism, the exploitation of protozoan symbionts in the gut, was the first to be demonstrated. The dependence of the lower termites *Reticulitermes flavipes* (Rhinotermitidae) and *Kalotermes flavicollis* (Kalotermitidae) and the wood roach, *Cryptocercus punctulatus* (Cryptocercidae), on hindgut protozoa for cellulose digestion has been recognized since the classic investigations of Cleveland (1924). The symbionts are anaerobic protozoa from unique genera of flagellates restricted to the guts of the lower termites and wood roaches.

The second mechanism, the exploitation of cellulolytic bacterial symbionts in the gut, has been assumed to explain the ability of many higher termites (Termitidae) to digest cellulose, but convincing evidence in support of this assumption is still lacking (Breznak 1982; O'Brien & Slaytor 1982). Hindgut bacteria have been shown to contribute to cellulose digestion in the American cockroach, *Periplaneta americana* (Blattidae) (Bignell 1977; Cruden & Markovetz 1979) and the rhinocerus beetle, *Oryctes nasicornis* (Scarabaeidae) (Bayon 1981), and seem likely to participate in the larvae of crane flies (Tipulidae) (Sinsabaugh *et al.* 1985; Griffiths & Cheshire 1987).

The third mechanism, the use of ingested fungal cellulases, was discovered in my laboratory. Ingested fungal enzymes are essential participants in cellulose digestion in the fungus-growing termite *Macrotermes natalensis* (Termitidae: Macrotermitinae) (Martin & Martin 1978), larvae of the siricid woodwasp *Sirex cyaneus* (Siricidae) (Kukor & Martin 1983), and the larvae of five species of cerambycid beetles (Kukor & Martin 1986; Kukor *et al.* 1988). This mechanism has also been shown in other species of fungus-growing termites (Abo-Khatwa 1978; Rouland *et al.* 1988), and probably accounts for the limited cellulolytic capacity of stonefly nymphs and caddisfly larvae (Sinsabaugh *et al.* 1985).

The fourth mechanism, the production by the insect of all of the enzymes necessary for cellulose digestion, remains controversial, and is discussed in detail in the next section.

5. THE EVIDENCE FOR SYMBIONT-INDEPENDENT DIGESTION OF CELLULOSE IN INSECTS

Symbiont-independent cellulose digestion has been proposed to occur in cerambycid beetle larvae, in silverfish and firebrats, and in one roach and several termite species. In this section, I conclude that only in the cases of the roach and termite species is the evidence for symbiont-independent cellulose digestion strong.

(*a*) *Cerambycid beetle larvae*

The ability of the wood-boring larvae of cerambycid beetles to digest structural polysaccharides has been the subject of investigations dating back to the early 1900s. Cellulolytic enzymes have been detected in the gut fluids of many species. Influenced by the investigations of Cleveland (1924) on the role of hindgut protozoa in cellulose digestion in lower termites, early investigators attributed cellulose digestion in wood-feeding beetles to symbiotic yeasts housed in evaginations of the anterior-most segment of the midgut (Buchner 1928). During the 1930s, however, it was conclusively demonstrated that these yeasts do not contribute to cellulose digestion (Mansour & Mansour-Bek 1934), whereupon it was concluded that the beetles must produce their own cellulolytic enzymes. No evidence in support of that conclusion was presented, however, and other possibilities were not considered. It is now known that ingested fungal enzymes bring about cellulose digestion in some cerambycid species, and it is possible that hindgut bacteria may be involved in others, but there still has been no convincing evidence presented for the occurrence of symbiont-independent cellulose digestion in any anobiid, buprestid or cerambycid species. Cellulose digestion in wood-boring beetles deserves further study.

(*b*) *Silverfish and firebrats*

Cellulolytic capacity has been shown in five species in the thysanuran family Lepismatidae, but evidence concerning the agents of cellulose digestion is conflicting. Lindsay (1940) claimed that cellulose digestion in *Ctenolepisma longicaudata* occurs in the crop, mediated by aerobic bacteria. Lasker & Giese (1956), on the other hand, concluded that cellulose digestion in *C. lineata* occurs in the midgut, mediated by cellulases secreted by the silverfish. More recently, Zinkler & Götze (1987) have asserted that cellulose digestion in *Thermobia domestica* occurs in the crop, mediated by insect enzymes secreted in the midgut and refluxed forward into the crop.

Despite the unresolved confusion in the literature, the study of Lasker & Giese (1956) is regularly cited as a definitive demonstration that the silverfish *C. lineata* secretes a cellulase system capable of digesting native cellulose. In that study, the authors produced aposymbiotic (symbiont-free) animals by subjecting eggs to surface sterilization and raising the neonates on a diet of sterile rolled oats. They observed that the activity of the midgut fluid toward insoluble cellulose and the ability of the silverfish to degrade [^{14}C]-labelled cellulose to [^{14}C]-labelled carbon dioxide were undiminished in the aposymbiotic animals when compared with animals that had their normal complement of gut microbes, and conclude that cellulose digestion is brought about by enzymes produced by the insects.

Despite the impeccable design of this experiment, several methodological ambiguities, not evident in 1956, preclude so definitive a conclusion. First, there is no indication that the purity of the labelled cellulose was checked before feeding it to the silverfish. Commercial labelled cellulose is notorious for the presence of labelled non-cellulosic impurities. Indeed, it is common to find that as much as 10 % of the counts in some preparations can be extracted with water and an additional 15–20 % can be extracted with base. Thus, the possibility cannot be ruled out that an impurity, not cellulose, was the source of the labelled carbon dioxide in the respired gasses collected from the aposymbiotic nymphs. Second, in assaying cellulolytic activity in the gut fluid, the substrate was 'regenerated cellulose' prepared according to Trager (1932). The preparation of 'regenerated cellulose' results in significant loss of crystallinity, making it susceptible to degradation by endoglucanases alone. Consequently, finding undiminished activity toward 'regenerated cellulose' in the midguts of the aposymbiotic animals does not establish that they still possess a complete cellulolytic system or that they are still able to digest native cellulose. Of course, these criticisms do not necessarily mean that Lasker & Giese are wrong in their conclusion that symbiont-independent cellulose digestion occurs in *C. lineata*, but only that the evidence in favour of that conclusion is not compelling. This important study deserves replication. The order Thysanura (or Zygentoma) includes the most primitive known insects. A knowledge of the mechanism by which these insects digest cellulose is a critical piece of information in any analysis of the evolution of cellulolytic capacity in insects.

(*c*) *Termites and roaches*

Studies of the higher termites *Trinervitermes trinervoides* (Nasutitermitinae) (Potts & Hewitt 1973, 1974), *Nasutitermes walkeri* and *N. exitiosus* (Nasutitermitinae) (Schulz *et al.* 1986; Hogan *et al.* 1988*a*) and the Australian wood-eating roach *Panesthia cribrata* (Blaberidae) (Scrivener *et al.* 1989) provide the strongest evidence to date for symbiont-independent cellulose digestion. The first indication that these species might not be dependent upon microbial symbionts for cellulose digestion was the demonstration that enzymes with activity toward crystalline cellulose are secreted by the midgut epithelium. The discovery of such enzymes does not, of course, show that they are the sole agents of cellulose digestion *in vivo*. Cellulose digestion could still be primarily a symbiont-mediated process, with insect-derived enzymes serving only in a subsidiary role. Indeed, the production of cellulases with detectable activity toward crystalline cellulose has been shown in three additional termite species (*Mastotermes darwiniensis*, *Coptotermes lacteus*, *Macrotermes mülleri*) in which cellulose digestion most likely does involve microbial symbionts. In *M. darwiniensis* (Mastotermitidae) (Veivers *et al.* 1982) and *C. lacteus* (Rhinotermitidae) (Hogan *et al.* 1988*b*), both of which are lower termites, hindgut protozoa are the most likely major agents of cellulolysis, and in *M. mülleri* (Termitidae: Macrotermitinae) (Routland *et al.* 1988), cellulose digestion is mediated by a mixture of termite enzymes and ingested fungal enzymes.

A demonstration of symbiont-independent cellulose digestion requires that evidence be obtained which shows a lack of participation by gut protozoa and bacteria. The involvement of symbiotic hindgut protozoa in cellulose digestion in the termites *N. walkeri* and *N. exitiosus* and the roach *P. cribrata* was easily ruled out. Protozoa do not occur in the hindguts of higher termites, and occur only in small numbers in the roach. Furthermore, elimination of protozoa from the roaches' hindguts had no effect on gut cellulase levels. As evidence against the participation of hindgut bacteria in cellulose digestion in *N. exitiosus*, Hogan *et al.* (1988*a*) cite their inability to detect soluble cellulases in the hindgut and the failure of treatments designed to solubilize membrane-bound bacterial cellulases (e.g. sonication and incubation with lysozyme) to increase the level of hindgut cellulase. As evidence against the importance of bacterial cellulolysis in *P. cribrata*, Scrivener *et al.* (1989) cite the failure of ingested tetracycline (which reduces the total counts of hindgut bacteria by 85%) to reduce the viability of the roaches on a cellulose diet or to reduce gut cellulase activity. These are very important studies, and my only reluctance in accepting the conclusion of the authors that cellulose digestion in these species is symbiont-independent is my concern that the evidence, strong though it certainly is, is not quite compelling. Is it not still possible that cellulose digestion is mediated by fastidious anaerobes that are not affected by tetracycline and whose cellulosomes are not dislodged from the cell membrane by using the techniques employed? Perhaps an electron microscopic study of the type that revealed the mode of action of bacteria during the degradation of plant tissue in the rumen (Dinsdale *et al.* 1978) would provide the definitive evidence necessary to preclude the involvement of hindgut bacteria in the degradation of plant cell wall polysaccharides in these insects.

6. WHY IS CELLULOSE DIGESTION RARE IN INSECTS?

Cellulose digestion generates glucose, which is fermented to volatile fatty acids (VFAs) in some insects. Glucose and VFAs can be used to meet an organism's carbon or energy requirement. However, the dietary factors that usually limit growth and reproduction in insect herbivores are nitrogen or water, not carbon or energy (Slansky & Scriber 1985). Most herbivorous insects are able to meet their carbon requirements easily by digesting such ubiquitous plant constituents as sucrose and other oligosaccharides, starch, phospholipids, neutral lipids, and proteins (Applebaum 1985). The capacity to exploit the energy content of cellulose is unlikely to confer any particular benefit on an insect that can meet its carbon and energy requirements by exploiting more easily digested constituents of the diet.

It is possible that fitness-related processes other than growth and reproduction might be energy limited. For example, flight and stridulation have high energy requirements, and the intensity and duration of these activities can be limited by the rate at which metabolic energy is supplied. However, the process of cellulose digestion is too slow to support an activity requiring a rapid rate of energy production. Because they can be more rapidly mobilized and digested than cellulose, metabolic reserves, such as glycogen, trehalose, proline and fat, or ingested nutrients, such as nectar and honeydew, are the metabolic fuels of choice for the support of these activities.

Therefore, I propose a very simple answer to the question posed in the heading to this section. I suggest that cellulolytic capacity is uncommon in insects simply because it is a trait that is rarely advantageous to possess.

I propose further that even among cellulose-digesting species there are some that derive little or no benefit from this capacity. When fed labelled purified cellulose, nymphs of the stonefly *Pteronarcys proteus* (Plecoptera) and larvae of the caddisfly *Pycnopsyche luculenta* (Trichoptera) digest 11 and 12%, respectively, of the cellulose they ingest (Sinsabaugh *et al.* 1985). Stoneflies and caddisflies acquire their capacities to digest cellulose by the ingestion of microbial cellulases present in their normal diets of microbe-rich leaf litter. There is no evidence that the carbon derived from cellulose digestion makes a crucial contribution to the carbon budgets of these insects, or, indeed, that fitness is enhanced in any way by the acquisition of cellulolytic capacity. It is entirely possible that these species benefit from consuming a microbe-rich substrate, not because it provides a source of cellulolytic enzymes that enable them to assimilate a fraction of the carbon they ingest, but rather because it provides a rich source of nitrogen or micronutrients. For these species, cellulolytic capacity may be an inconsequential by-product of an association with microorganisms that is beneficial for some other reason. Non-adaptive microbe-mediated cellulose digestion in insects that are dependent upon microbial associates for other reasons provides a possible clue to the answer to the question posed in the heading to the next section.

7. WHY IS SYMBIONT-DEPENDENT CELLULOSE DIGESTION MORE COMMON THAN SYMBIONT-INDEPENDENT CELLULOSE DIGESTION? OR IS IT?

The prevalence of symbiont-dependent *vis-a-vis* symbiont-independent cellulose digestion could reflect either the ease with which symbiont-mediated processes evolve or the difficulty with which cellulase systems entirely of insect origin evolve. These two

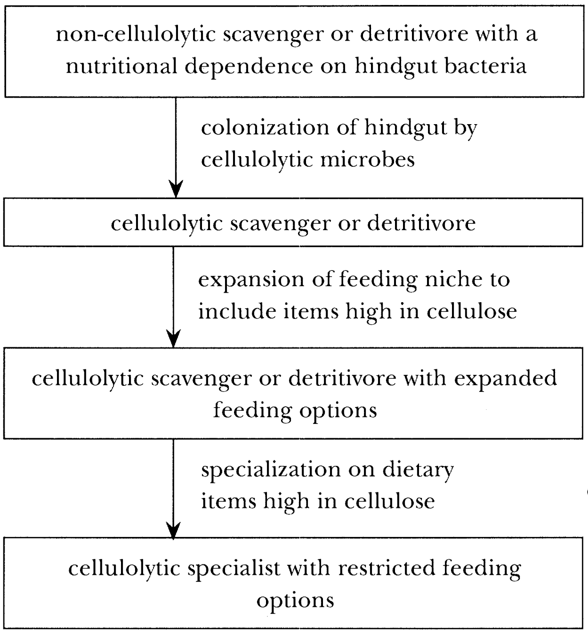

Figure 1. Scenario for the evolution of symbiont-mediated cellulose digestion in a scavenger or detritivore with a nutritional dependence on hindgut bacteria.

possibilities will be discussed separately. The possibility that the question is based upon an incorrect assumption will also be considered.

(*a*) *What circumstances favour the evolution of symbiont-mediated cellulose digestion?*

The hindguts of many omnivorous scavengers and detritivores, including roaches, crickets, scarab beetles and crane flies, provide conditions favourable for microbial growth and often harbour diverse and populous bacterial communities. Hindgut bacteria often make important contributions to their insect hosts. For example, in some species hindgut microbes supplement the nitrogen budget (by fixing nitrogen, conserving and upgrading uric acid nitrogen, or synthesizing essential amino acids) and provide critical micronutrients (vitamins and essential fatty acids) (Campbell 1989). The acquisition of cellulolytic capacity by an insect already dependent upon its hindgut bacteria for some other reason would only require that the hindgut become colonized by cellulolytic species. The resulting cellulolytic capacity might be entirely superfluous to the carbon budget of the host insect, but might also enable a change in the host's feeding niche to include dietary resources in which cellulose constituted the major carbon source. If a change in diet were followed by specialization on the new dietary resource, then cellulolytic capacity would no longer be superfluous, but would be indispensable.

This scenario (figure 1) can account for the pattern of cellulolytic capacity observed in roaches and lower termites, which are believed to have evolved from a roach-like ancestor. Most roaches are omnivorous scavengers. Their digestive enzymes include amylase, maltase, invertase, β-glucosidase, endocellulase, chitinase, esterase, lipase, and protease (Bignell 1981), enzymes that enable them to exploit the easily digested carbon sources commonly present in many types of food. Roaches also possess an abundant hindgut flora, which is believed to contribute significantly to nutritional status by degrading and conserving uric acid nitrogen, supplying critical micronutrients, and possibly scavenging carbon by fermenting otherwise unabsorbable sugars to VFAs. Hindgut bacteria are also believed to be responsible for the cellulolytic capacities of roaches in the family Blattidae. The significance of cellulose digestion to the carbon budget of roaches on their natural diets is not known.

By contrast, in wood roaches (Cryptocercidae) and lower termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Serritermitidae, Rhinotermitidae), all of which are wood feeders, cellulose digestion plays a crucial role in the carbon economy. The hindguts of the lower termites and wood roaches, like those of omnivorous cockroaches, contain a diverse bacterial community, but in addition also include large populations of cellulolytic protozoa, which are the major agents of cellulose digestion. Deprived of their protozoan symbionts, wood roaches and lower termites are unable to survive for long on a diet of wood or cellulose (Cleveland 1924; Cleveland *et al.* 1934; Orlova 1974; Mauldin *et al.* 1972; Mauldin 1977). In the termite *Zootermoposis angusticollis* (Hodotermitidae), more than half of the carbon requirement is met with carbon derived from symbiont-mediated digestion (Hungate 1938).

The roach-like Australian termite, *Mastotermes darwiniensis*, is the most primitive termite. This species has characteristics expected of a transitional form between an omnivorous roach, in which cellulolytic capacity augments digestive scope but is not essential, and a xylophagous lower termite, in which cellulolytic capacity is essential and has been made more efficient by the colonization of the hindgut by cellulolytic protozoa. These termites not only attack timber, but also sugar cane, vegetables, and flour. Feeding workers a diet in which the carbon source is starch rather than cellulose results in the elimination from the hindgut of the four large protozoan symbionts normally found there and the loss of cellulase activity (Veivers *et al.* 1983). However, the termites produce an amylase and a maltase, and are able to survive indefinitely on a starch-containing diet. Cellulolytic capacity, although adaptive, is not indispensable, as this species can utilize sugar- and starch-containing foods, in addition to wood.

In summary, insects with diverse bacterial communities in their hindguts are pre-adapted to evolve symbiont-mediated celluloytic processes. Thus, the prevalence of symbiont-dependent cellulose digestion may simply reflect the prevalence of mutualistic associations between omnivorous scavengers and detritivores and their hindgut bacteria.

(*b*) *What factors constrain the evolution of cellulolytic systems entirely of insect origin?*

It is premature to suggest even a tentative answer to this question. Implicit in the question is the assumption that cellulolyti systems of insect origin are very rare, or at least are much less common that symbiont-mediated systems. That assumption may require revision. At the present time strong cases have been made for symbiont-independent cellulose digestion in only one species of cockroach and a few species of higher termites in the subfamily Nasutitermitinae. It is possible, however, that further research will reveal that cellulolytic systems entirely of insect origin are actually very common in roaches and higher termites. It is also possible that the early claim of symbiont-independent cellulose digestion in the Thysanura will be validated, and it is even possible that further studies of cellulose digestion in wood-feeding anobiid, buprestid and cerambycid beetle larvae will uncover additional examples. If symbiont-independent cellulose digestion proves to be more common than is currently assumed, the more appropriate question may be 'How do the circumstances that favour the evolution of cellulolytic systems entirely of insect origin differ from those that favour the evolution of symbiont-dependent cellulase systems?'.

On the other hand, there remains a small but finite possibility that the claims of symbiont-independent cellulose digestion in the roach *Panesthia cribata* and the nasute termites will not hold up. It is still possible that cellulose digestion in these species will ultimately prove to be mediated by gut bacteria. Furthermore, the early claim of symbiont-independent cellulose digestion in the Thysanura may not be confirmed, and additional research on cellulose digestion in wood-feeding beetle larvae may not provide any additional cases. The possibility that no insect digests cellulose without some microbial contribution cannot be totally discounted, in which case the appropriate question would be 'What has precluded the evolution of cellulolytic systems entirely of insect origin?'.

We would be well advised to know what the real question is before we hazard an answer.

8. FUTURE DIRECTIONS

Recent success in cloning cellulase genes (Béguin *et al.* 1987) has resulted in significant progress in elucidating structure–activity relations in the endo- and exoglucanases of bacteria (Kilburn *et al.* 1989) and fungi (Tomme *et al.* 1989). A common feature of these microbial cellulases is that they consist of two functionally independent domains, a catalytic domain that does not bind to cellulose and a cellulose-binding domain that is not enzymically active. An understanding of the functional roles of these two domains has led to important insights into the nature of the synergism that occurs between the exo- and endo-cellulases of microorganisms. Similar studies of the structure–function relations of insect cellulases might provide valuable clues to the factors that favour or constrain the evolution of both symbiont-dependent and symbiont-independent cellulolytic systems.

The study of cellulose digestion in insects has stimulated important research in insect physiology, and has played a seminal role in shaping the field of symbiosis research. This area remains an inviting one for investigators with interests that range from biochemistry and molecular biology to ecology and evolution.

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Discussion

C. G. JONES (*Institute of Ecosystem Studies, Millbrook, New York, U.S.A.*). Is there any evidence that cellulose digestion enhances extraction or utilization of nitrogen?

M. M. MARTIN. I'm not aware of any evidence that cellulose digestion enhances extraction or utilization of nitrogen, but it is certainly a plausible suggestion. There is a little bit of protein associated with plant cell walls, which might be made available if the cellulose were digested. I don't have any information about the nutritional quality of that protein, although my recollection is that it isn't very good. However, cell wall lysis, chemical or mechanical, does not appear to be a prerequisite for the utilization of cell contents by insect herbivores. Apparently the cell walls are, or become, very porous under conditions that prevail in the guts of caterpillars, which neither digest cellulose nor grind up their food into small particles, so that cell contents leak out. This is work done by Dr Ray Barbehenn while he was a student of Professor Liz Bernays'. A paper describing it is currently under review.

E. A. BERNAYS (*Department of Entomology, University of Arizona, U.S.A.*). Professor Martin's story is certainly convincing. I wondered also about the pointlessness of digesting cellulose when it is nitrogen that is limiting. I thought perhaps that by digesting cellulose, nitrogen would be made available. Being an unbalanced suite of amino acids the symbionts would have the value of upgrading this unsuitable mixture to one that would actually be useful.

M. M.Martin. I would generalize Professor Bernay's point a little further. I suspect that supporting a diverse and metabolically active gut flora is often the most beneficial consequence of cellulose digestion, whatever the contribution of the gut flora to the insect host might happen to be. Upgrading nitrogen quality is certainly one way that feeding glucose to gut bacteria might benefit a cellulose-digesting insect. Others might be vitamin synthesis, assimilation of sulphate into sulfur-containing amino acids, nitrogen fixation, maintenance of reducing conditions, or metabolism of allelochemicals.