Relationship between C/N ratio of their habitat in scarabaeoid larvae and decomposition abilities against decayed wood and/or humus

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Xylophagous scrabaeoid beetles are ecologically known to be major woody decomposer in the forest ecosystem of warm-temperate zone. Lucanid larvae belonging to the superfamily Scarabaeoidea inhabit and utilize mainly rotten wood and/or humus. They also show a food preference against the types (brown-, white-, and soft-rot) and stages of decay at a genus level, taxonomic rank. Although the food preference is thought to be related to the kinds and the activity of enzyme, it has not been clarified how they have digestive ability for food. In this study, therefore, I tried to reveal the relationship between C/N ratio (used as an indicator of organic matter decomposition) in their habitat and digestive abilities against major polysaccharides consisting of woody- (CM-cellulose and β-1,4-xylan) and fungal cell wall (β-1,3-glucan) in larval midgut, main digestive and absorption site of some species of Lucanidae and *Trypoxylus dichotomus* distributing in Japan.

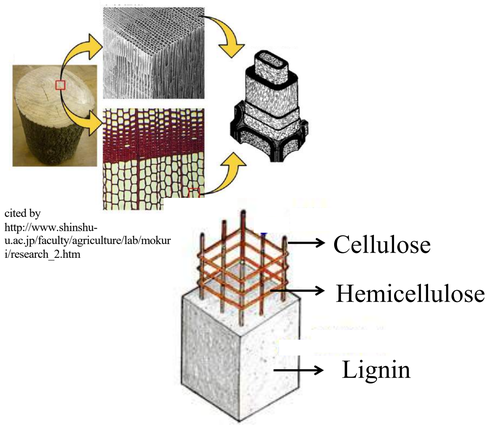
From the results, the correlations were observed between C/N ratio and glycanase and glycosidase against CM-cellulose and between C/N ratio and those against β-1,3-glucan. Although correlation was not observed between C/N ratio and glycanase (β-1,4-xylanase) against β-1,4-xylan, a tendency was indicated that β-1,4-xylanase activities are high in the larvae that inhabit the habitat under the condition of an earlier stage of decay. Since the enzyme induces and produces generally the inducer (substrate), the correlations observed in this study are thought to be related to substrate (woody- and fungal biomass) contents in their preferred habitat.

Background

○Wood

The major components:

- Cellulose (polysaccharide)
- Hemicellulose (polysaccharide)
- Lignin (aromatic compound)



cited by http://www.shinshu-u.ac.jp/faculty/agriculture/lab/mokuri/research_2.htm

○Major biological wood decomposition

●Wood-rotten fungi

Decomposition method in each wood rotten fungi

	Cellulose	Hemicellulose	Lignin
Brown rotten fungi	◎	◎	×
White rotten fungi	○	○	○
Soft rotten fungi	○	○	(○)

Decomposition rate (Takahashi, 1986)
◎:Rapid、○:normal、×:cannot almost decompose

● Xylophagous insects

e.g., Longhorn beetle, termites, and beetles

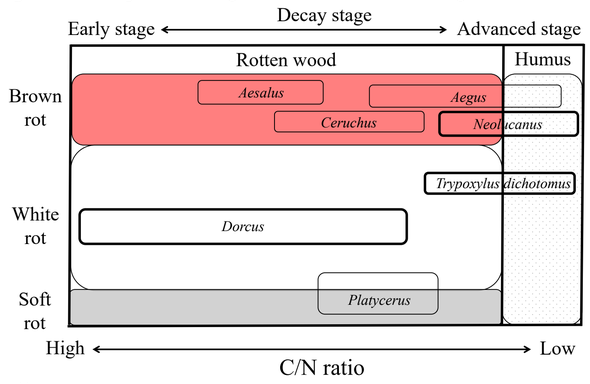
●Termites and cockroach

Major woody decomposer in tropical zone

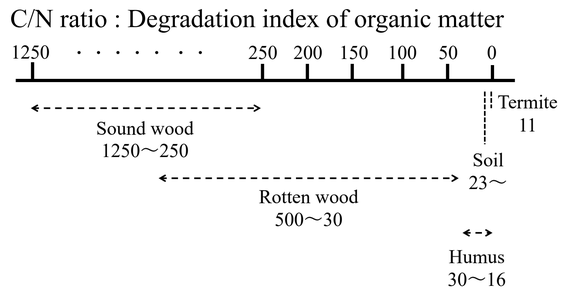
provided by Ms. Haruka Osaki(Lab. Ecological Science, Graduate School of Science, Kyushu University)

●Xylophagous beetles

Major woody decomposer in warm-temperate zone



C/N ratio : Degradation index of organic matter

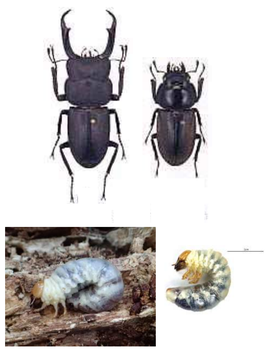


Objectives

Elucidation the relationship between C/N ratio of their preferred habitat and digestive enzymatic activities against the food digestion

Samples and Methods

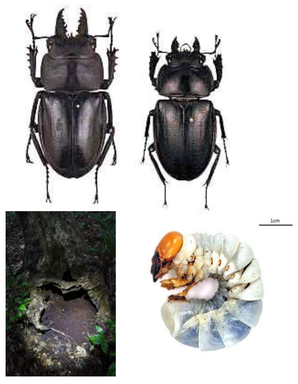
○**Samples**



Dorcus rectus rectus
3rd instar larvae

Informations

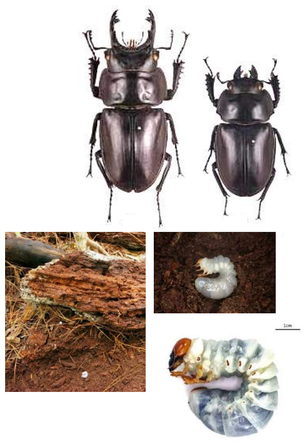
- Food preference: white rotten wood
- Collecting condition: white rotten wood
- Collecting place: Ito campus, Kyushu university
- Individual numbers: $n = 6$



Neolucanus protogenetivus
3rd instar larva

Informations

- Food preference: humus originated from brown rotten wood
- Collecting condition: white rotten wood
- Collecting place: Amamiohshima-island, Kagoshima prefecture
- Individual numbers: $n = 1$



N. insulicola insulicola
3rd instar larvae

Informations

- Food preference: humus originated from brown rotten wood
- Collecting condition: brown rotten wood under condition of an advanced stage of decay (in the individuals collected in Nosoko) and humus (that in collected in Yarabu)
- Collecting place: Nosoko ($n = 1$) and Yarabu ($n = 3$), Ishigakijima-island, Okinawa prefecture

Trypoxylus dichotomus
3rd instar larvae

Informations

- Food preference humus and/or white rotten wood under condition of an advanced stage of decay
- Collecting condition: white rotten wood under condition of an advanced stage of decay
- Collecting place: Ito campus, Kyushu university
- Individual numbers: $n = 6$

○**Methods**

○The measurement of C/N ratio

Grinding and drying of larval habitat (rotten wood or humus) ⇨ The measurements of carbon and nitrogen concentrations by SUMIGRAPH

○The substrates used in glycanase and glycosidase activities

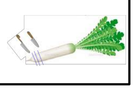
Polysaccharide focused	Substrates used in this study and the enzyme that acts on the substrate	
	Glycanase	Glycosidase
Cellulose (Woody cell wall)	CM-cellulose CM-cellulase	PNP-β-D-glucoside β-glucosidase
β-1,4-xylan (Woody cell wall)	β-1,4-xylan β-1,4-xylanase	PNP-β-D-xyloside β-xylosidase
β-1,3-glucan (Fungal cell wall)	β-1,4-glucan β-1,4-glucanase	PNP-β-D-glucoside β-glucosidase

The kinds of polysaccharide-degrading enzymes

○Glycanase (end-type): releasing various the decomposition products by acting on sugar chain of the inside of polysaccharide

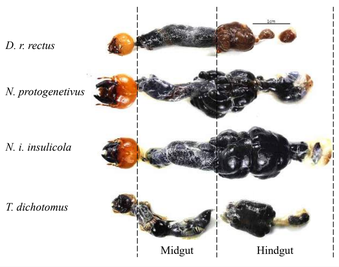
○Glycanase (exo-type): releasing monosaccharide from the disaccharide at the non-reducing end of the crystalline region

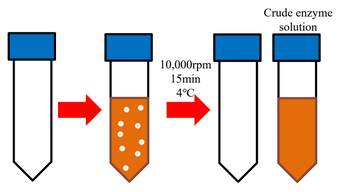
○Glycosidase: releasing monosaccharide from the non-reducing end of oligosaccharides and disaccharides etc

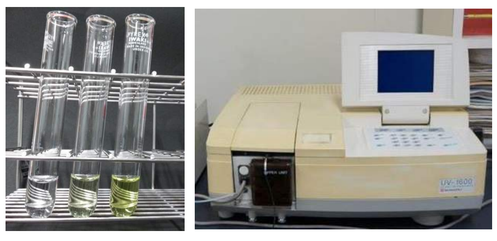


○The measurement of glycanase and glycosidase activities

Larval anatomy ⇨ Extraction of crude enzyme solution ⇨ Enzyme reaction and the measurement (Anzai et al., 1991)





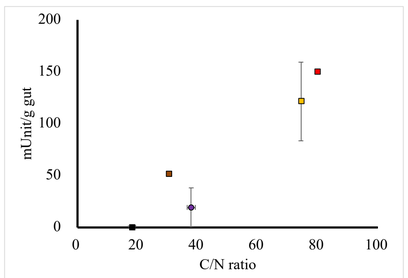


Results and Discussion

Since midgut is a main site of digestion and absorption in insects, I focused on digestive enzymatic activities of larval midgut.

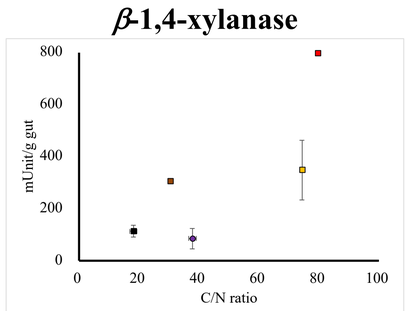
○**The relationship between C/N ratio and glycanase activities in midgut**

CM-cellulase



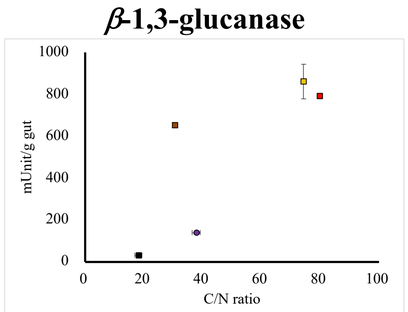
There is the correlation between C/N ratio and CM-cellulase activity ($r = 0.661$, $p < 0.01$).

β-1,4-xylanase



There is no the correlation between C/N ratio and β-1,4-xylanase activity ($r = 0.413$, $p = 0.099 > 0.05$).

β-1,3-glucanase

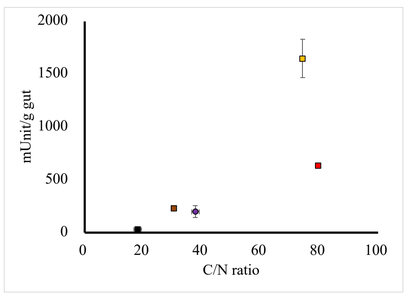


There is the correlation between C/N ratio and β-1,3-glucanase activity ($r = 0.847$, $p < 0.001$).

⇨ The correlations were observed in between C/N ratio and CM-cellulase and between C/N ratio and β-1,3-glucanase activities, respectively.

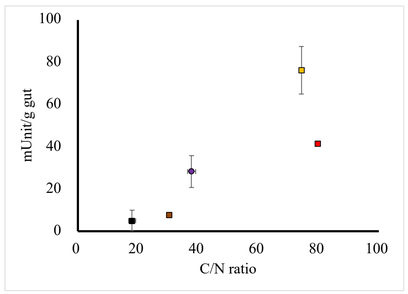
○**The relationship between C/N ratio and Glycosidase activities in midgut**

β-glucosidase



There is the correlation between C/N ratio and β-glucosidase activity ($r = 0.856$, $p < 0.001$).

β-xylosidase



There is the correlation between C/N ratio and β-xylosidase activity ($r = 0.818$, $p < 0.001$).

- Dorcus rectus
- Neolucanus insulicola insulicola (Mt. Yarabu)
- N. i. insulicola (Nosoko)
- N. protogenetivus
- Trypoxylus dichotomus

⇨ The correlations were observed in between C/N ratio and β-glucosidase and between C/N ratio and β-xylosidase activities, respectively.

○**From these results,**

⇨ It was suggested that the digestive abilities against CM-cellulose (non-crystal cellulose) and β-1,3-glucan may be related to the decay stage (C/N ratio) of larval preferred habitat.

This study can be a basic research for sustainable biodiversity conservation of forest ecosystem in warm-temperate zone.

Future issues

Since the above relationship may be related to the amount of substrate in the food, I would like to clarify the woody and fungal biomass in the future.

Acknowledgement

I greatly appreciate Prof. Kunio Araya (Kyushu University) for giving valuable advices and the samples of the larvae o f *N. protogenetivus* and *N. i. insulicola,* Prof. Hirosi Anzai (Nihon Unoversity) for giving experimental technique, and Ms. Haruka Osaki (Kyushu University) for providing the photos of cockroach.

The larvae of the genus *Neolucanus* used in this study were collected by the collecting permissions of Ministry of the Environment, Setouchi town, Amami city and Ishigaki city, Okinawa Prefecture.