Applications of BNF and NWR Strategies in Insects

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Nitrogen is usually a restrictive nutrient that affects the growth and development of insects, especially of those living in low nitrogen nutrient niches. In response to the low nitrogen stress, insects have gradually developed symbiont-based stress response strategies—biological nitrogen fixation and nitrogenous waste recycling—to optimize dietary nitrogen intake. Based on the above two patterns, atmospheric nitrogen or nitrogenous waste (e.g., uric acid, urea) is converted into ammonia, which in turn is incorporated into the organism via the glutamine synthetase and glutamate synthase pathways.

Keywords: insect symbionts ; biological nitrogen fixation ; nitrogenous waste recycling ; GS/GOGAT cycle ; amino acid biosynthesis

1. Application Cases of BNF Strategy in Insects

A recent review paper showed that potential diazotrophs widely exist in Coleoptera, Diptera, Hemiptera, Blattaria, Hymenoptera, Lepidoptera, and Thysanoptera ^[1]. Nitrogen fixation has been extensively studied and convincingly demonstrated in cockroaches and termites. Besides this, BNF strategy can also be exploited by other insects for nitrogen provisioning, mainly including the longhorned beetle (e.g., *Anoplophora glabripennis, Prionoplus reticularis*), the weevil (e.g., *Conorhynchus palumbus*), the bark beetle (e.g., *Dendroctonus rhizophagus, D. valens*), the click beetle (e.g., *Agriotes obscurus, Selatosomus aeneus*), the stag beetle (e.g., *D. rectus*), and the bess beetle (e.g., *Odontotaenius disjunctus*) in Coleoptera; tephritid fruit flies (e.g., *Ceratitis capitata, Bactrocera tryoni*) in Diptera; the carmine cochineal (e.g., *Dactylopius coccus, D. opuntiae*) in Hemiptera; and leaf-cutter ants (e.g., *Acromyrmex echinatior, A. volcanus, A. octospinosus*) and sirex wood wasp (e.g., *Sirex noctilio*) in Hymenoptera.

BNF-related symbionts are not limited to the intestinal tract of insects. For instance, the carmine cochineal endosymbiont *Dactylopiibacterium* attaches to the surface of host ovary for vertical transmission, and expresses nitrogenase activity in host hemolymph and ovary ^[2]. Furthermore, BNF reaction occurred in vitro, such as in the immediate living environment of insects or insect-associated organisms, can also benefit to insect nitrogen budget. For example, the tunnels of some xylophagous insects, the nests of termite, or the fungus gardens cultivated by leaf-cutter ants fill with large quantities of frass and food residue, which act as preferred substrate for diazotrophs. Nitrogen nutrition in frass and food residue fixed by diazotrophs can be consumed by insects directly ^{[3][4][5]}.

2. Application Cases of NWR Strategy in Insects

Three alternative scenarios of symbiotic NWR, different in their patterns of amino acid synthesis using waste ammonia, may be functional in the symbiosis between insects and symbionts ^[6]: (A) Symbiont-mediated: Symbionts convert nitrogenous waste compounds of insects into EAAs for host assimilation and absorption; (B) Mediated by host cell–symbiont complex: Ammonia is firstly assimilated into non-essential amino acids (nEAAs, such as glutamine and glutamate) by GS/GOGAT in insect cells. nEAAs are used as ammonia donors to synthesize EAAs in symbionts; (C) Host cell-mediated: Ammonia is assimilated into glutamate by GS/GOGAT in insect cells. At the same time, intracellular symbionts synthesize carbon skeleton of EAAs and secrete them into insect cells. EAAs are assembled in insect cells catalyzed by transaminases. These NWR patterns could be employed by insects in Blattaria, Coleoptera, Diptera, Hemiptera, and Hymenoptera for nitrogen provisioning (Table S1).

2.1. Blattaria

Nitrogenous wastes in cockroaches (e.g., *Periplaneta americana*, *Blattella germanica*) are stored in their fat bodies in the form of uric acid. When dietary nitrogen is limited, endosymbiont *Blattabacterium cuenoti* inhabited in the fat bodies of cockroaches synergizes with the host to degrade uric acid and assimilate the degradation products into EAAs. In this process, cockroaches synthesize urate oxidase, allantoinase, and allantoicase, which are necessary for uric acid degradation, and supply nEAAs to *B. cuenoti*. *B. cuenoti* recycles nitrogen from urea and ammonia into glutamate under

the catalysis of urease and glutamate dehydrogenase, and then synthesizes all the EAAs, various vitamins, and other required compounds for the host insect [I][8][9]. During the evolution of cockroach to termite, *B. cuenoti* was gradually lost from the fat body, and its metabolic functions were replaced by the symbiotic flora in termite hindgut. Therefore, uric acid in termites (e.g., Reticulitermes flavipes) needs to be transferred to hindgut through malpighian tubules before it can be degraded by gut symbionts [10][11]. Mastotermes darwiniensis is the only lower termite that retains the endosymbiont B. cuenoti. However, compared with B. cuenoti in cockroaches, the genome of B. cuenoti in M. darwiniensis is sharp reduced, so it is speculated that uric acid degradation in M. darwiniensis should be co-mediated by B. cuenoti and hindgut symbionts [12][13]. Uricolytic strains, such as Clostridia, Enterobacteriaceae, also widely exist in the guts of Reticulitermes formosanus, Neotermes koshunensis, Glyptotermes speratus, Coptotermes fuscus, Cryptotermes domesticus, Hodotermopsis sjoestedti, O. formosanus, and Nasutitermes takasagoensis. It is estimated that an amount of uric acid nitrogen equivalent to 30% of the total nitrogen in an average termite colony may be recycled or redistributed annually through the action of gut uricolytic bacteria $\frac{[14]}{}$.

2.2. Coleoptera

The levels of uric acid in bark beetles (*D. rhizophagus*, *D. valens*) are significant different in whole eggs, larvae, and adults (male and female). Among them, the highest uric acid content is detected in female adults, whose guts contain various uricolytic bacteria, such as *Pseudomonas fluorescens*, *Serratia proteamaculans*, and *Rahnella aquatilis* ^[15]. Urea-hydrolyzing bacteria present in the egg surface and larval guts of *Anoplophora glabripennis* or the larval and adult guts of *Melolontha hippocastani* can incorporate nitrogen from ingested urea back into the insect tissues ^{[16][17]}.

2.3. Diptera

As mentioned above, adding urea or ammonia to artificial diets can significantly improve the female fecundity of olive fruit fly and the larval biomass of black soldier fly. In addition, the bacterium *Enterobacter agglomerans* isolated from the alimentary tract of the apple maggot fly, *Rhagoletis pomonella*, mediates purine (such as uric acid) degradation, and the degradation products were significantly attractive to *Anastrepha ludens* and *R. pomonella* ^{[18][19]}.

2.4. Hemiptera

Most plant sap-feeding hemipteran insects feed on diets with an extremely low or unbalanced nutrient content. Therefore, many plant sap-sucking insects (such as psyllids, whiteflies, mealybugs, aphids, cicadas, spittlebugs, and sharpshooters) rely on obligatory endosymbionts with much-reduced genome size to synthesize B vitamins, steroids, EAAs, and other nutrients [20][21][22][23]. The application of NWR strategy in hemipteran insects has been verified in shield bugs, brown planthoppers, cochineal insects, aphids, and others. Erwinia-like bacteria, vertically transmitted through eggs, are widely present in the midgut of stink bugs and they synthesize uricase, allantoinase, and allantoicase in the cecum of Parastrachia japonensis to catalyze the degradation of uric acid. Uric acid is recycled for EAAs syntheses with the aid of *Erwinia*-like bacteria, thereby leading to significant improvement of the survival rate of adults and nymphs $\frac{[24]}{2}$. Different from P. japonensis, Nilaparvata lugens on its own is capable of encoding partial uricolytic genes (e.g., uricase gene). Under the synergistic action of N. lugens and yeast-like symbionts in the fat body, uric acid is finally degraded and reutilized by insect-yeast association [25][26]. The genome of endosymbiont Dactylopiibacterium mainly located in the ovaries of the carmine cochineal insects (D. coccus, D. opuntiae) was sequenced, and the data showed that purine and [<u>2]</u> its genome In addition, species uric acid degrading genes were present in fungal (Rhodotorula, Cryptococcus, Trametes, Penicillium, Debaryomyces) associated with the carmine cochineal also exhibit urate oxidase activity in uric acid metabolism ^[27]. Acyrthosiphon pisum synergizes with the intracellular bacterium Buchnera through the host cell-mediated mode to assimilate ammonia from bacteriocyte metabolism into EAAs [<u>6][28]</u>

2.5. Hymenoptera

Current research on NWR strategies in Hymenoptera mainly focuses on Formicidae insects. *Camponotus compressus* showed specific gustatory preferences for urea ^[29]. The overall transcriptional activity of urease structural gene *ureC*, urease accessory gene *ureF*, glutamine synthetase encoding gene *glnA* and arginase family encoding gene *speB* in the obligate intracellular endosymbiont *Blochmannia floridanus* increase steadily with carpenter ants age, which promotes the conversion of urea in chemically defined diets to all but arginine of the EAAs to the genus *Camponotus* ^{[30][31]}. Gene content within the gut microbiomes of 17 *Cephalotes* species showed that nearly all core symbionts involve in the biosynthesis of EAAs and nEAAs, but only a subset encode pathways of NWR, such as symbionts in Burkholderiales, Rhizobiales, and Opitutales. Burkholderiales mediate the degradation of purine, xanthine, or uric acid into urea, while Rhizobiales and Opitutales catalyze the conversion of urea into ammonia ^[32]. Similarly, gene

function prediction in Bartonellaceae colonized in the midgut wall of *Dolichoderus* indicated that Bartonellaceae is capable of mediating nitrogen recycling and biosynthesis of several vitamins and all EAAs ^{[33][34]}.

It should be stressed here that some insects on its own is sufficient for nitrogenous waste compounds degradation. For example, *Aedes aegypti* can efficiently incorporate ammonia into amino acids via GS/GOGAT cycle to reduce the toxicity of high-concentration ammonium salts to cells ^[35]. Urate oxidase encoding gene is actively expressed in the specific developmental stages of *Drosophila* (*D. melanogaster*, *D. pseudoobscura*, and *D. virilis*), and their nitrogenous metabolites are eventually excreted in the form of allantoin, allantoic acid, urea or ammonia ^{[25][36]}. However, a recent genomic investigation showed that the bacteria of the family Acetobacteraceae isolated from the guts of *Drosophila* fruit flies also possess the genes responsible for uric acid degradation ^[37]. Similar to *D. melanogaster*, the blowfly *Lucilia sericata* express high levels of urate oxidase in the malpighian tubules that convert uric acid from the hemolymph into allantoin that is excreted ^{[36][38]}. The uric acid that accumulates in silkworm tissues is excreted as a nitrogen waste product, but the genes of *Bmwh3*, *BmABCG5*, and *Bm5'N* encoded by silkworm are involved in uric acid metabolism ^[39]. Interestingly, urease in fresh mulberry leaves can directly pass through the gut wall of silkworm larvae into the hemolymph without being digested. Urea in midgut or hemolymph catalyzed by urease to ammonia is utilized by silkworm larvae to synthesize protein ^[40].

References

- Bar-Shmuel, N.; Behar, A.; Segoli, M. What Do We Know About Biological Nitrogen Fixation in Insects? Evidence and Implications for the Insect and the Ecosystem. Insect Sci. 2020, 27, 392–403.
- de Leon, A.V.P.; Ormeno-Orrillo, E.; Ramirez-Puebla, S.T.; Rosenblueth, M.; Esposti, M.D.; Martinez-Romero, J.; Martinez-Romero, E. Candidatus Dactylopiibacterium Carminicum, a Nitrogen-Fixing Symbiont of Dactylopius Cochineal Insects (Hemiptera: Coccoidea: Dactylopiidae). Genome Biol. Evol. 2017, 9, 2237–2250.
- Pinto-Tomas, A.A.; Anderson, M.A.; Suen, G.; Stevenson, D.M.; Chu, F.S.T.; Cleland, W.W.; Weimer, P.J.; Currie, C.R. Symbiotic Nitrogen Fixation in the Fungus Gardens of Leaf-Cutter Ants. Science 2009, 326, 1120–1123.
- 4. Ulyshen, M.D. Insect-Mediated Nitrogen Dynamics in Decomposing Wood. Ecol. Entomol. 2015, 40, 97–112.
- Aylward, F.O.; Burnum, K.E.; Scott, J.J.; Suen, G.; Tringe, S.G.; Adams, S.M.; Barry, K.W.; Nicora, C.D.; Piehowski, P.D.; Purvine, S.O.; et al. Metagenomic and Metaproteomic Insights into Bacterial Communities in Leaf-Cutter Ant Fungus Gardens. ISME J. 2012, 6, 1688–1701.
- Macdonald, S.J.; Lin, G.G.; Russell, C.W.; Thomas, G.H.; Douglas, A.E. The Central Role of the Host Cell in Symbiotic Nitrogen Metabolism. Proc. R. Soc. B Biol. Sci. 2012, 279, 2965–2973.
- 7. Sabree, Z.L.; Kambhampati, S.; Moran, N.A. Nitrogen Recycling and Nutritional Provisioning by Blattabacterium, the Cockroach Endosymbiont. Proc. Natl. Acad. Sci. USA 2009, 106, 19521–19526.
- Patino-Navarrete, R.; Piulachs, M.D.; Belles, X.; Moya, A.; Latorre, A.; Pereto, J. The Cockroach Blattella Germanica Obtains Nitrogen from Uric Acid through a Metabolic Pathway Shared with Its Bacterial Endosymbiont. Biol. Lett. 2014, 10, 20140407.
- 9. Weihrauch, D.; O'Donnell, M.J. Links between Osmoregulation and Nitrogen-Excretion in Insects and Crustaceans. Integr. Comp. Biol. 2015, 55, 816–829.
- 10. Costa-Leonardo, A.M.; Laranjo, L.T.; Janei, V.; Haifig, I. The Fat Body of Termites: Functions and Stored Materials. J. Insect Physiol. 2013, 59, 577–587.
- 11. Scharf, M.E. Challenges and Physiological Implications of Wood Feeding in Termites. Curr. Opin. Insect Sci. 2020, 41, 79–85.
- Kinjo, Y.; Bourguignon, T.; Tong, K.J.; Kuwahara, H.; Lim, S.J.; Yoon, K.B.; Shigenobu, S.; Park, Y.C.; Nalepa, C.A.; Hongoh, Y.; et al. Parallel and Gradual Genome Erosion in the Blattabacterium Endosymbionts of Mastotermes Darwiniensis and Cryptocercus Wood Roaches. Genome. Biol. Evol. 2018, 10, 1622–1630.
- Sabree, Z.L.; Huang, C.Y.; Arakawa, G.; Tokuda, G.; Lo, N.; Watanabe, H.; Moran, N.A. Genome Shrinkage and Loss of Nutrient-Providing Potential in the Obligate Symbiont of the Primitive Termite Mastotermes Darwiniensis. Appl. Environ. Microb. 2012, 78, 204–210.
- Thong-On, A.; Suzuki, K.; Noda, S.; Inoue, J.; Kajiwara, S.; Ohkuma, M. Isolation and Characterization of Anaerobic Bacteria for Symbiotic Recycling of Uric Acid Nitrogen in the Gut of Various Termites. Microbes. Environ. 2012, 27, 186–192.
- 15. Morales-Jimenez, J.; de Leon, A.V.P.; Garcia-Dominguez, A.; Martinez-Romero, E.; Zuniga, G.; Hernandez-Rodriguez, C. Nitrogen-Fixing and Uricolytic Bacteria Associated with the Gut of Dendroctonus Rhizophagus and Dendroctonus

Valens (Curculionidae: Scolytinae). Microb. Ecol. 2013, 66, 200-210.

- 16. Ayayee, P.; Rosa, C.; Ferry, J.G.; Felton, G.; Saunders, M.; Hoover, K. Gut Microbes Contribute to Nitrogen Provisioning in a Wood-Feeding Cerambycid. Environ. Entomol. 2014, 43, 903–912.
- Alonso-Pernas, P.; Bartram, S.; Arias-Cordero, E.M.; Novoselov, A.L.; Halty-deLeon, L.; Shao, Y.Q.; Boland, W. In Vivo Isotopic Labeling of Symbiotic Bacteria Involved in Cellulose Degradation and Nitrogen Recycling within the Gut of the Forest Cockchafer (Melolontha Hippocastani). Front. Microbiol. 2018, 9, 124462.
- Lauzon, C.R.; Sjogren, R.E.; Prokopy, R.J. Enzymatic Capabilities of Bacteria Associated with Apple Maggot Flies: A Postulated Role in Attraction. J. Chem. Ecol. 2000, 26, 953–967.
- Robacker, D.C.; Lauzon, C.R. Purine Metabolizing Capability of Enterobacter Agglomerans Affects Volatiles Production and Attractiveness to Mexican Fruit Fly. J. Chem. Ecol. 2002, 28, 1549–1563.
- 20. Douglas, A.E. Microbial Brokers of Insect-Plant Interactions Revisited. J. Chem. Ecol. 2013, 39, 952–961.
- 21. Baumann, P. Biology of Bacteriocyte-Associated Endosymbionts of Plant Sap-Sucking Insects. Annu. Rev. Microbiol. 2005, 59, 155–189.
- 22. Douglas, A.E. How Multi- Partner Endosymbioses Function. Nat. Rev. Microbiol. 2016, 14, 731-743.
- 23. Ankrah, N.Y.D.; Chouaia, B.; Douglas, A.E. The Cost of Metabolic Interactions in Symbioses between Insects and Bacteria with Reduced Genomes. Mbio 2018, 9, e01433-18.
- 24. Kashima, T.; Nakamura, T.; Tojo, S. Uric Acid Recycling in the Shield Bug, Parastrachia Japonensis (Hemiptera: Parastrachiidae), During Diapause. J. Insect Physiol. 2006, 52, 816–825.
- Hongoh, Y.; Sasaki, T.; Ishikawa, H. Cloning, Sequence Analysis and Expression in Escherichia Coli of the Gene Encoding a Uricase from the Yeast-Like Symbiont of the Brown Planthopper, Nilaparvata Lugens. Insect Biochem. Mol. 2000, 30, 173–182.
- 26. Xue, J.; Zhou, X.; Zhang, C.X.; Yu, L.L.; Fan, H.W.; Wang, Z.; Xu, H.J.; Xi, Y.; Zhu, Z.R.; Zhou, W.W.; et al. Genomes of the Rice Pest Brown Planthopper and Its Endosymbionts Reveal Complex Complementary Contributions for Host Adaptation. Genome Biol. 2014, 15, 521.
- de Leon, A.V.P.; Sanchez-Flores, A.; Rosenblueth, M.; Martinez-Romero, E. Fungal Community Associated with Dactylopius (Hemiptera: Coccoidea: Dactylopiidae) and Its Role in Uric Acid Metabolism. Front. Microbiol. 2016, 7, 954.
- 28. Hansen, A.K.; Moran, N.A. Aphid Genome Expression Reveals Host-Symbiont Cooperation in the Production of Amino Acids. Proc. Natl. Acad. Sci. USA 2011, 108, 2849–2854.
- 29. Shetty, P.S. Gustatory Preferences of Ants (Camponotus-Compressus) for Urea and Sugars. Experientia 1982, 38, 259–260.
- Feldhaar, H.; Straka, J.; Krischke, M.; Berthold, K.; Stoll, S.; Mueller, M.J.; Gross, R. Nutritional Upgrading for Omnivorous Carpenter Ants by the Endosymbiont Blochmannia. BMC Biol. 2007, 5, 48.
- Zientz, E.; Beyaert, N.; Gross, R.; Feldhaar, H. Relevance of the Endosymbiosis of Blochmannia Floridanus and Carpenter Ants at Different Stages of the Life Cycle of the Host. Appl. Environ. Microb. 2006, 72, 6027–6033.
- 32. Hu, Y.; Sanders, J.G.; Lukasik, P.; D'Amelio, C.L.; Millar, J.S.; Vann, D.R.; Lan, Y.M.; Newton, J.A.; Schotanus, M.; Kronauer, D.J.C.; et al. Herbivorous Turtle Ants Obtain Essential Nutrients from a Conserved Nitrogen-Recycling Gut Microbiome. Nat. Commun. 2018, 9, 964.
- Bisch, G.; Neuvonen, M.M.; Pierce, N.E.; Russell, J.A.; Koga, R.; Sanders, J.G.; Lukasik, P.; Andersson, S.G.E. Genome Evolution of Bartonellaceae Symbionts of Ants at the Opposite Ends of the Trophic Scale. Genome. Biol. Evol. 2018, 10, 1687–1704.
- 34. Cook, S.C.; Davidson, D.W. Nutritional and Functional Biology of Exudate-Feeding Ants. Entomol. Exp. Appl. 2006, 118, 1–10.
- Scaraffia, P.Y.; Zhang, Q.F.; Thorson, K.; Wysocki, V.H.; Miesfeld, R.L. Differential Ammonia Metabolism in Aedes Aegypti Fat Body and Midgut Tissues. J. Insect Physiol. 2010, 56, 1040–1049.
- 36. Weihrauch, D.; O'Donnell, M.J. Mechanisms of Nitrogen Excretion in Insects. Curr. Opin. Insect Sci. 2021, 47, 25–30.
- Winans, N.J.; Walter, A.; Chouaia, B.; Chaston, J.M.; Douglas, A.E.; Newell, P.D. A Genomic Investigation of Ecological Differentiation between Free-Living and Drosophila-Associated Bacteria. Mol. Ecol. 2017, 26, 4536–4550.
- Baumann, A.; Skaljac, M.; Lehmann, R.; Vilcinskas, A.; Franta, Z. Urate Oxidase Produced by Lucilia Sericata Medical Maggots Is Localized in Malpighian Tubes and Facilitates Allantoin Production. Insect Biochem. Mol. 2017, 83, 44–53.
- 39. Tang, L.M.; Yang, D.H.; Wang, Y.H.; Yang, X.; Chen, K.; Luo, X.Y.; Xu, J.; Liu, Y.J.; Tang, Z.; Zhang, Q.Q.; et al. 5'-Nucleotidase Plays a Key Role in Uric Acid Metabolism of Bombyx Mori. Cells 2021, 10, 2243.

40. Hirayama, C.; Sugimura, M.; Saito, H.; Nakamura, M. Host Plant Urease in the Hemolymph of the Silkworm, Bombyx Mori. J. Insect Physiol. 2000, 46, 1415–1421.

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