Plastic degradation by two wax moths, *Galleria mellonella* and *Achroia grisella*

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1 | Wax moth larvae can eat plastics

- Plastics are a major global issue[a]
- Current recycling/remediation methods are inefficient
- Some species of insects can eat plastics
- Wax moth larvae are capable of eating polyolefins (PE, PP, PS)[b] and other polymers

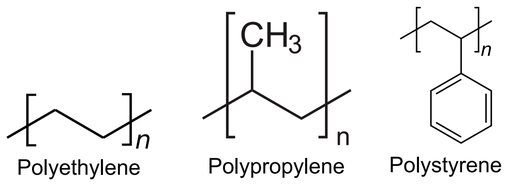


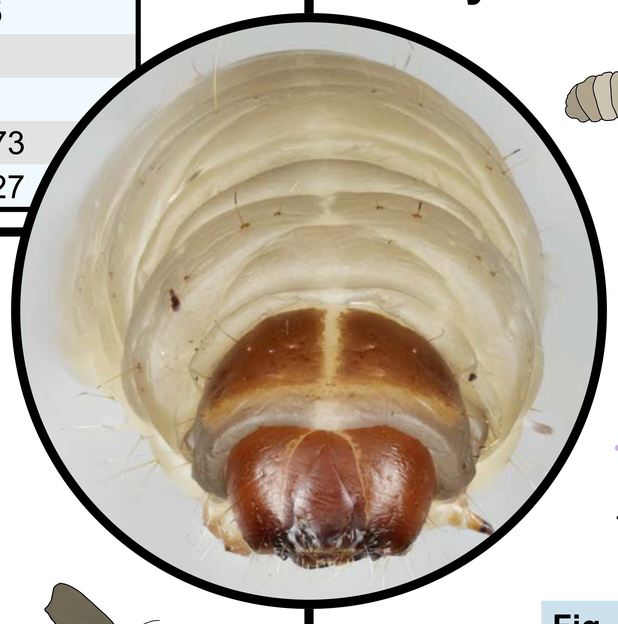
Fig. 1 A greater wax moth larva and the plastic substrates it is capable of eating.

2 | High-quality genomes for both species have been assembled

- RefSeq quality genomes of both wax moths have been assembled[c]
- Both are highly complete - 99.3% complete (lepidoptera_odb10 BUSCOs)
- Provides a reference for downstream analyses, biochemical basis of degradation

Table 1 Summary of assembly stats for both *G. mellonella* and *A. grisella* genomes.

Statistic	G. mellonella (GCF_026898425.1)	A. grisella (GCF_030625045.1)
Total size (Mb)	471.9	442.9
Contigs	221	525
N50 (Mb)	6.4	3.1
L50	25	43
Protein-coding genes	13,604	13,873
mRNAs	23,142	18,927



3 | Wax moth larvae prefer some plastics over others

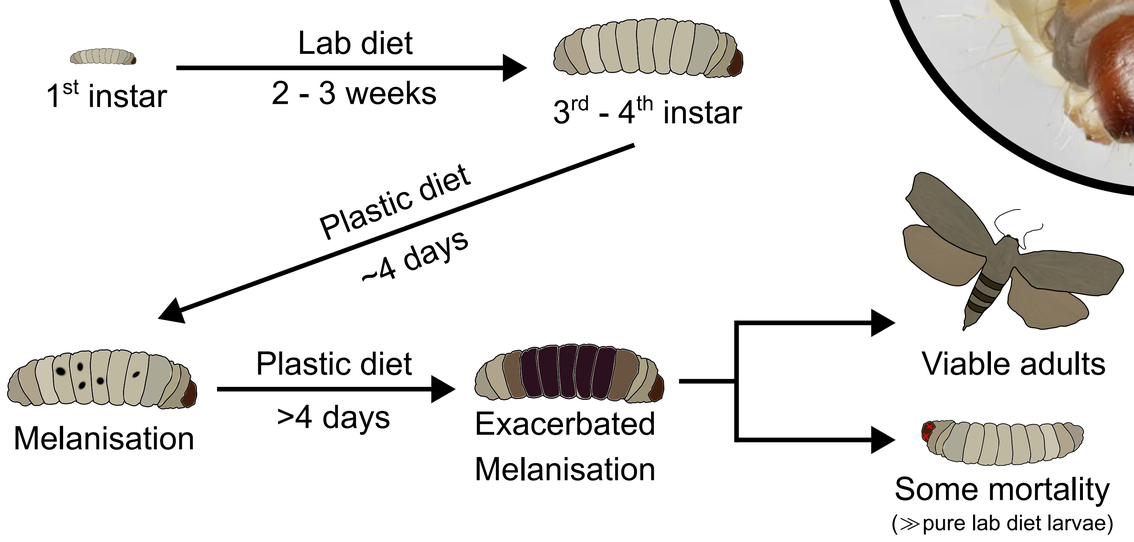
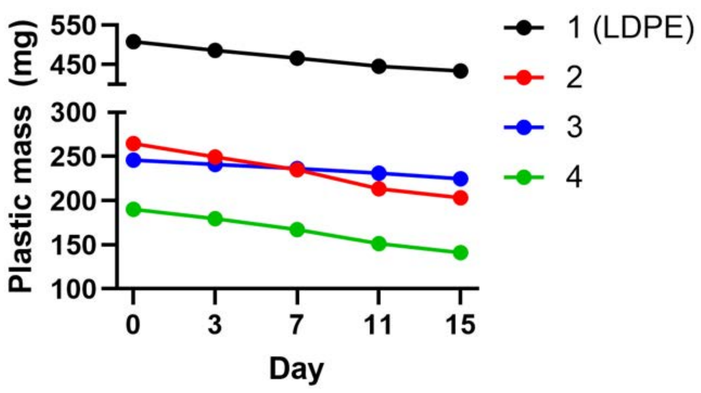


Fig. 2 Schematic diagram of the general method used for plastic-feeding experiments.

- Four-day plastic feeding periods in groups of eight after an initial period on lab diet
- Feeding plastic to larvae beyond 4th instar results in pupation
- Larvae preferred some plastics over others
- Due to size difference, *G. mellonella* larvae ate more than *A. grisella* larvae
- Cannibalism observed regardless of plastic



Plastic	Average mass loss* (mg/larva/day)
1 (LDPE)	0.641
2	0.519
3	0.178
4	0.411

*Not accounting for cannibalism

Fig. 3 Measured mass loss of plastics over a 15-day period by *G. mellonella* larval feeding (left) and calculated average mass loss per larva (right).

4 | Hexamerins in wax moths may be involved in degradation

- Large proteins called hexamerins are implicated in PE degradation[d]
- Copper co-factors contribute to activity?
- Originally non-enzymatic, typically involved in storage
- Degradation mechanism remains unknown
- Orthologs found in both species that share high level sequence identity

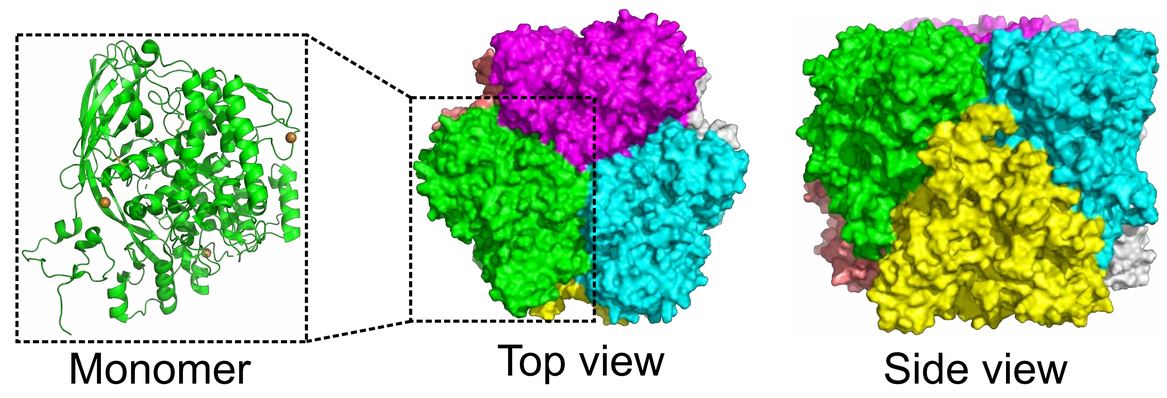


Fig. 4 Monomer structure and top + side views of a cryo-EM complex structure of GmCora, a hexamerin candidate PE-degrading protein originating from the greater wax moth identified by Bertocchini and co-workers. PDB: 8CAN[d].

5 | Transcriptomics reveals upregulated genes that may be active on plastics

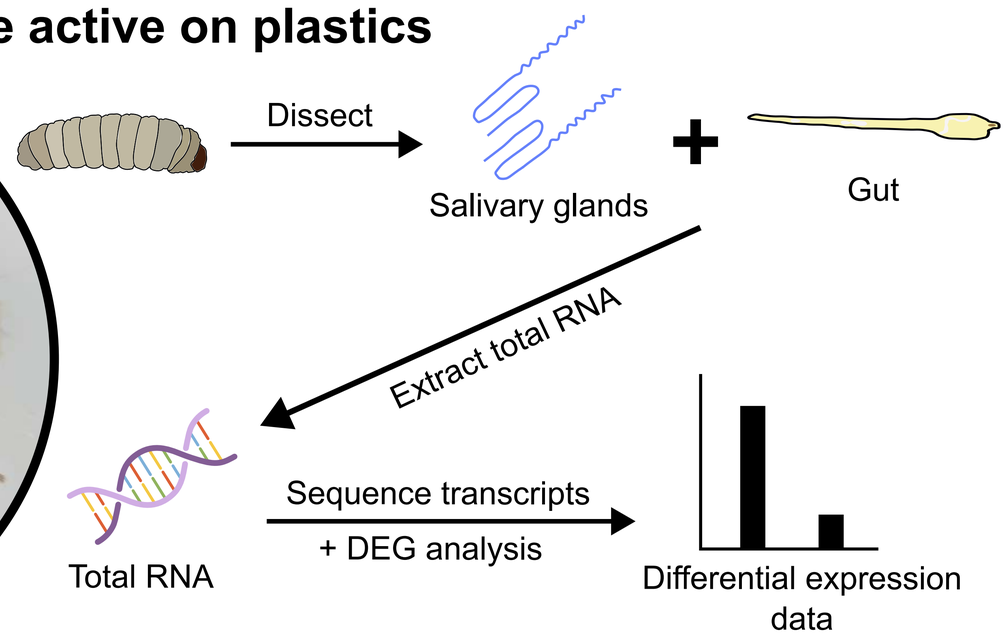


Fig. 5 Workflow for obtaining differential expression data in wax moth digestive tissue. DEG: Differentially expressed genes.

- RNA-Seq analysis of digestive tissue (salivary glands + gut) revealed several upregulated enzymes: peptidases/proteases, esterases/lipases, some CYP450s, acyltransferases
- Some enzymes exclusively expressed in gut or salivary glands

Table 2 Number of enzymatic GO terms identified in each digestive tissue of wax moth larvae. SP: Secretory signal peptide present; Up: Upregulated gene (+ve fold change).

	G. mellonella						*A. grisella*					
	Gut			Salivary glands			Gut			Salivary glands		
Enzyme class	All	SP	SP + Up	All	SP	SP + Up	All	SP	SP + Up	All	SP	SP + Up
Hydrolase		42	24				64	34	27			
Lyase							9					
	33			39			27					
				16								
Oxidoreductase	28			35			26			34		
							10					
	23			27						23		
				15								
	70			77						67	16	
Transferase				13								

6 | Future work

- RNA-Seq of digestive tissues from larvae fed plastic
- Proteomic analyses of digestive tissues
- Heterologous protein expression of candidate genes
- Catabolic pathway elucidation—appropriate techniques (GC-MS, IR, GPC, etc)?
- Expand to biopolymers

References
[a]Geyer *et al.*, *Sci. Adv.* **2017**, *3*, e1700782.
[b]1) Bombelli *et al.*, *Curr. Biol.* **2017**, *27(8)*, R292-3.
2) Sanluis-Verdes *et al.*, *Nat. Commun.* **2022**, *13*, 5568.
[b]3) Ruiz Barrionuevo *et al.*, *Environ. Sci. Pollut. Res. Int.* **2022**, *29(45)*, 68132-42.
[c]Young *et al.*, *G3* **2024**, *14(6)*, jkae070.
[d]Spínola-Amilibia *et al.*, *Sci. Adv.* **2023**, *9*, eadi6813.

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