Correspondence

Polyethylene bio-degradation by caterpillars of the wax moth *Galleria mellonella*

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Plastics are synthetic polymers derived from fossil oil and largely resistant to biodegradation. Polyethylene (PE) and polypropylene (PP) represent ~92% of total plastic production. PE is largely utilized in packaging, representing ~40% of total demand for plastic products (www.plasticseurope.org) with over a trillion plastic bags used every year [1]. Plastic production has increased exponentially in the past 50 years (Figure S1A in Supplemental Information, published with this article online). In the 27 EU countries plus Norway and Switzerland up to 38% of plastic is discarded in landfills, with the rest utilized for recycling (26%) and energy recovery (36%) via combustion (www.plasticseurope.org), carrying a heavy environmental impact. Therefore, new solutions for plastic degradation are urgently needed. We report the fast biodegradation of PE by larvae of the wax moth *Galleria mellonella*, producing ethylene glycol.

PE comprises a linear backbone of carbon atoms (Figure S1B), which is resistant to degradation. Although PE is believed not to be susceptible to bio-degradation, a few attempts have been made, as PE is the most common packaging plastic. Slow (weeks/months) PE biodegradation has been observed, given appropriate conditions. For example, modest degradation of PE was observed after nitric acid treatment and incubation for 3 months in a liquid culture of the fungus *Penicillium simplicissimum* [2]. Slow PE degradation was also recorded after 4 to 7 months exposure to the bacterium *Nocardia asteroides* [3]. In both cases, fourier transform infrared spectroscopy

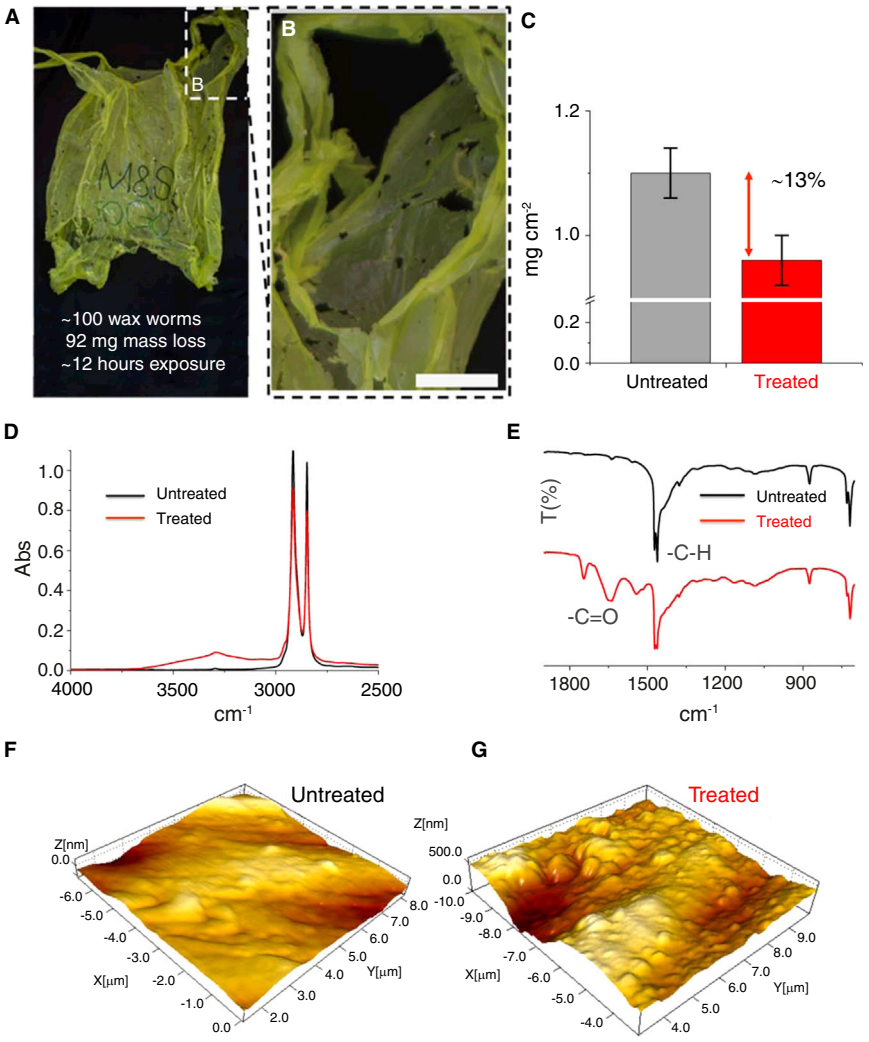

Figure 1. Polyethylene degradation by *Galleria mellonella*.
(A) Plastic bag after exposure to ~100 wax worms for 12 hours. (B) Magnification of the area indicated in A. (C) Gravimetric analysis of homogenate-treated versus untreated polyethylene (PE), showing a reduction (13%) of mass per unit of area in the former. (D,E) FTIR analysis of the homogenate-treated and control PE films. (F,G) Atomic Force Microscopy on homogenate-treated (G) and untreated (F) PE film (representative examples of 3 topographic maps each).

(FTIR) analysis of treated samples revealed formation of an absorbance peak around 3,300 cm^{-1}, a signature for ethylene glycol, confirming PE degradation. More recently, Yang *et al*. reported bacterial degradation of PE over several weeks [4]. However, no production of ethylene glycol from the biodegradation was described. The authors reported that PE biodegradation depended on the activity of microorganisms present in the gut of the larvae of the Indian mealmoth *Plodia interpunctella* (two bacterial strains, *Bacillus* sp. YP1 and *Enterobacter asburiae* YT1). Faster biodegradation (~0.13 mg cm^{-2} day^{-1}) of another plastic, poly(ethylene terephthalate) (PET) by a microbial consortium including a newly isolated bacterium, *Ideonella sakaiensis*, was described recently [5]. Although PET is a resistant material, one might expect its biodegradation to be easier than PE, as PET has a polyester backbone and can be hydrolysed. We report here the fast biodegradation of PE by the wax worm, the caterpillar larva of the wax moth *Galleria mellonella* of the snout moth (Pyralidae) family of Lepidoptera.

When a PE film was left in direct contact with wax worms, holes started to appear after 40 minutes, with an

estimated 2.2±1.2 holes per worm per hour (Table S1A). Figure 1A,B shows the result of leaving ~100 wax worms in contact with a commercial PE shopping bag for ~12 hours, which caused a mass loss of 92 mg. To exclude the possibility that mechanical action of the masticatory system was solely responsible for the observed PE breakdown, worm homogenate was smeared on and left in contact with PE films. Gravimetric analysis of the treated samples confirmed a significant mass loss of 13% PE over 14 hours of treatment (one-way ANOVA, $p = 0.029$) compared to the untreated samples (Figure S1C and Table S1B,C). This corresponds to an average degradation rate of 0.23 mg cm^{-2} h^{-1}, which is markedly higher than the rate of PET biodegradation by a microbial consortium recently reported [5].

To test if the PE polymer was chemically degraded by contact with the worm homogenate, we carried out FTIR analysis. When the FTIR probe was pointed on untreated samples, the spectroscopic results confirmed the identity of the PE film, with peaks at 2,921 and 2,852 cm^{-1} being the classical signatures of PE (Figure 1D, black line). However, when the probe was pointed on sample smeared with worm homogenate, an additional peak at ~3,350 cm^{-1} was seen (Figure 1D, red line). This FTIR peak corresponds to the one previously described as the ethylene glycol signature (also compare Figure 1E with Figure 4B in [4]) [3,6]. In addition, a peak at 1,700 cm^{-1} appeared in the treated sample, which is the classical signature of the carbonyl bond (Figure 1E, red line). The ethylene glycol signature was also seen when the probe was pointed close to holes in PE caused by intact worms, but not when the probe was pointed at a distance (Figure S1C–E).

The formation of products after treatment with wax worm extract was also characterised by high performance liquid chromatography coupled with mass spectrometry (HPLC–MS), covering a mass/charge (m/z) range from 100 to 600 (Figure S1F,G). Figure S1G shows the spectra for untreated PE (top, black) and the treated PE (bottom, red). In the samples treated with the wax worm extract three new peaks appeared at the lower end of the m/z region (110.0, 122.9 and 170.0). The chemical identity of these lighter fractions was not confirmed but their presence supports the hypothesis of PE degradation by the wax worm homogenate.

To analyse further the effect of wax worm homogenate on the PE surface, Atomic Force Microscopy (AFM) was performed (Figure 1F,G). After treatment with homogenate, we observed an obvious change in the topography of the PE surface (Figure 1G), corresponding to a significant (one-way ANOVA = 0.005) greater than 140% increase in surface roughness (Figure S1H and Table S1D). These results indicate that the physical contact of the wax worm homogenate with the PE surface modified the integrity of the polymer surface.

What allows the wax worm to degrade a chemical bond not generally susceptible to bio-degradation? The answer may lie in the ecology of the wax worm itself. They feed on beeswax, and their natural niche is the honeycomb; the moth lays its eggs inside the beehive, where the worms grow to their pupa stage, eating beeswax [7]. Beeswax is composed of a highly diverse mixture of lipid compounds, including alkanes, alkenes, fatty acids and esters [8]. The most frequent hydrocarbon bond is the CH_2–CH_2, as in PE (Figure S1B). Although the molecular details of wax biodegradation require further investigation, it seems likely that the C–C single bond of these aliphatic compounds is one of the targets of digestion. The appearance of holes when PE films are left in direct contact with wax worms, and the FTIR analysis of degraded PE, indicate chemical breakdown of the PE, including breakage of C–C bonds. It is not clear whether the hydrocarbon-digesting activity of *G. mellonella* derives from the organism itself, or from enzymatic activities of its intestinal flora [7], as with PE digestion by *Plodia interpunctella* [4]. Further investigation is also required to determine if related species have the capacity for PE degradation, and to analyse its molecular basis including the detailed nature of the products.

Nevertheless, given the fast rate of biodegradation reported here, these findings have potential for significant biotechnological applications.

SUPPLEMENTAL INFORMATION

Supplemental Information contains experimental procedures, one figure and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.02.060.

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