

FAECAL FIRING IN A SKIPPER CATERPILLAR IS PRESSURE-DRIVEN

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Accepted 9 October 1997; published on WWW 9 December 1997

Summary

Many leaf-rolling caterpillars have a rigid anal comb attached to the lower surface of the anal plate (or shield) situated above the anus. This comb is widely assumed to be a lever used to 'flick' away frass pellets. An alternative mechanism to explain pellet discharge is proposed on the basis of observations on the caterpillar of the skipper *Calpododes ethlius*. The model proposes that the underside of the anal plate serves as a blood-pressure-driven surface for the ejection of faecal pellets. Rather than acting as a lever, the anal comb serves as a latch to prevent the premature distortion of the lower wall of the anal plate until the anal haemocoel compartment is fully pressurized. The anal comb is swung into position during pellet extrusion by retractor muscles attached at its base and held in place by

a catch formed by a blood-swollen torus of everted rectal wall. As the caterpillar raises the blood pressure in its anal compartment by contracting its anal prolegs, the comb eventually slips over the toral catch. This causes the underside of the anal plate to move rapidly backwards as the blood pressure is released, projecting the pellet resting against it through the air. Simulation suggests that a local blood pressure of at least 10 kPa (75 mmHg) would be required to accelerate the lower surface of the anal plate outwards at a rate fast enough to discharge a 10 mg pellet at an observed mean velocity of 1.3 m s⁻¹.

Key words: caterpillar, faecal pellet, blood pressure, anal comb, defecation, skipper, *Calpododes ethlius*.

Introduction

The frass-expelling behaviour of leaf-rolling caterpillars in the family HesperIIDae (the skippers) is well documented in the early literature (Edwards, 1885; Scudder, 1889). Helen Selina King (1880) stated that the larva of *Calpododes* (*Hesperia*) *ethlius* (the Brazilian skipper) feeds from its tubular case and 'forcibly ejects all excrement from the upper free end'. To expel a faecal pellet, this caterpillar extends its abdomen beyond the confines of the leaf roll, tips the abdomen up and fires the pellet over distances many times its body length. As frass odours may attract insect predators and parasitoids (Takabayashi and Takahashi, 1989; Mattiacci and Dicke, 1995), the survival of a leaf roller may depend on the dispersal of evidence of its feeding activity (Stamp and Casey, 1993), although this remains to be substantiated.

The ability of certain caterpillars to project pellets some distance away from the feeding area is attributed to the presence of a cuticular comb situated directly above the anus (Gerasimov, 1952). This anal comb, described as being able to 'flick' or 'flip' the pellet away (Stehr, 1987; Scoble, 1992), is a sclerotized prong or fork situated on the ventral face of the anal plate (Peterson, 1948). The prevailing idea that the anal comb is a simple mechanical lever capable of flicking pellets away from the anus is probably invalid. The most notable contradictions are an apparent absence of protractor muscles required to power the

backward flicking motion of the anal comb and a pattern of mechanical wear on the comb inconsistent with the lever model. Furthermore, the lever model fails to explain why the abdomen tip remains pressurized with blood after the pellet has left the rectum but before it is fired off, or why the anal prolegs contract during defecation. We propose that the skipper caterpillar uses haemostatic pressure to eject its faecal pellets. In this model, the anal comb acts as a mechanical latch holding back the lower surface of the anal plate until it is finally driven outwards by a temporary elevation in haemolymph pressure in the recently described 'tokus' haemocoel compartment in the terminal abdominal segment (Locke, 1997). Blood flow between this tokus compartment and the general haemolymph is normally closed off by valves (Locke, 1997) during defecation (S. Caveney and H. McLean, in preparation). Further evidence for the pressure-driven latch/catch model comes from the observation that trimming the anal comb causes the anal pressure plate to expand backwards before the comb is extended and the pellet to fall off rather than be projected through the air.

Materials and methods

Caterpillars

Larvae of the Brazilian skipper, *Calpododes ethlius* Stoll (Lepidoptera, HesperIIDae), were collected from a greenhouse

colony maintained by M. Locke for 25 years. The caterpillars, reared on the leaves of *Canna* lilies, are assumed to have retained their field behaviour under greenhouse conditions. The life cycle lasts approximately 5 weeks at 25 °C. Early to mid fifth-instar caterpillars, approximately 50 mm in body length and weighing 1.0–1.3 g were used. Actively feeding larvae were removed from the plants and placed within rolled-up 50 mm×50 mm squares of *Canna* leaf inside Petri dishes 90 mm in diameter, and allowed to continue to feed and to drop several pellets before they were used. The defecation cycle is approximately 20 min long at room temperature.

Video microscopy

The firing velocities and trajectory angles of pellets fired off by the caterpillars were determined from video sequences recorded at a shutter speed of 1/2000 s using a Canon L1 Hi8 camcorder equipped with a Canon AF-Macro CL80–120 mm zoom lens. For close-up observations, caterpillars were videotaped with a colour video camera (Hitachi KP-C550CCD) mounted on a trinocular head of a Wild MZ8 stereodissecting microscope using a Volpi Intralux 5000-1 fibre optics ring illuminator. Descriptions in this paper are based on a frame-by-frame (1/60 s) analysis of the video-taped sequences. The near-transparent cuticle of this caterpillar allows the disposition of its internal organs, including the gut and its food contents, to be observed under natural conditions. Gut movements associated with defecation, and the flow of haemolymph (coloured yellow or green depending on caterpillar age), are clearly visible. Both are important features in understanding the mechanism of faecal pellet discharge. The frame-by-frame movements of the tip of the transparent anal comb, and of the cuticle of the lower surface of the anal plate adjacent to the comb, were followed by marking the structures with a vividly coloured model paint (Testors blaze orange enamel).

Scanning electron microscopy

Digitized images of sputter gold-coated tissue were taken with a Quartz PCI version 3 image-capturing system linked to a Hitachi S-570 scanning electron microscope.

Pellet volume and density

A typical faecal pellet has a round-ended barrel shape. Pellet volume was estimated using a representative pellet radius calculated from the geometric mean of three dimensions, its length and its two orthogonal diameters. The estimated pellet volume divided by its mass should be constant provided that the volume estimates are accurate. The slope of the regression line fitted to pellet mass (range 3–20 mg) *versus* pellet volume data was 1.057 ($r^2=0.8015$, $P<0.001$, $N=118$) (S. Caveney, H. McLean and D. Surry, unpublished data), suggesting that an average pellet has the density of water.

Modelling

The model used is based on the simple physics of a load (the faecal pellet) being accelerated from rest by a constant force

applied by the release of internally generated pressures (through the mechanism of the anal plate) acting over a very short distance. The release velocity v of a known mass $V\rho$ (where V is pellet velocity and ρ is density) leaving the plate is then described by the equation:

$$v = \sqrt{2pA\delta K/\rho V}, \quad (1)$$

where p is the pressure (the blood pressure) acting on an effective area A perpendicular to the direction of motion as it travels a distance δ along the line of motion before discharging the load, and K is the efficiency of energy transfer to the load. Determination of a range of experimental values of v allows the blood pressure to be approximated as:

$$p = \frac{1}{2}\rho Vv^2/A\delta K, \quad (2)$$

given that the dimensions and travel distance of the plate can be estimated from the video footage, and the volume and density of the pellet can be determined. This also assumes, for simplicity, that the pressure is constant during the short time the pellet is accelerating. The effective pressure applied to the pellet is related to the internal blood pressure through K . K has a value of 1 where energy transfer is 100% efficient (i.e. complete elastic resilience in the structures involved). Values of $K<1$ account for the energy losses arising from the distortions and friction associated with load and pressure plate movement that reduce the discharge velocity of the load and imply that the blood pressure is greater (by $1/K$) than the effective driving pressure. The above simplifications are justified in the context of the uncertainties in the other variables involved. The definition of A as the component of the contact area perpendicular to the motion reduces A significantly below the true contact area, since the actual contact area is at an acute angle to the direction of motion. The actual contact areas that apply the internal blood pressure to the pellet are not perpendicular to the direction of motion. Instead, the release of the pressurized anal plate squeezes the pellet against two further contact areas at the base of the anal prolegs. The downward force of the anal plate induces opposing forces from these contact areas, so that the opposing forces largely cancel out, but leave a net longitudinal force that fires the pellet away. The pressures required are then increased by $(1/\sin\alpha)$ compared with those that would be needed if the same contact area were perpendicular to the direction of motion, where α is the angle between the pressure plate face and the line along which the pellet is fired ($\alpha=90^\circ$ if the pellet had no secondary contact points).

Results

Feeding habits

To construct a feeding chamber in the *Canna* leaf, a fifth-instar caterpillar methodically folds a section of the upper margin of the leaf under the leaf blade, holding it in place by spinning a series of multistranded silk fibres that secure the marginal region of the leaf fold to points on the leaf's undersurface. Tension on the developing leaf fold is exerted by the silk fibres as they mature and shorten. As the fold develops,

the first-formed fibres become slack and are tightened by the caterpillar adding extra silk strands to them. The caterpillar is much shorter (50 mm) than the rolled-back sections of the leaf (range, 90–150 mm in length). Consequently, unless feeding, it is completely hidden from view. When feeding, it may feed on either end of the leaf roll and over time reduce the leaf roll's length to that of its body, or it may partly emerge from its chamber to consume adjacent leaf surface to as far back as the leaf midrib.

There is little pattern, however, to the orientation of the abdomen tip at the moment the pellet is expelled. Some pellets are shot out with a wide trajectory, others are simply fired vertically downwards. The caterpillar can be upright or inverted in its chamber at the time it extends its abdomen to eject a pellet. As a consequence, the distribution of faecal pellets on the soil beneath the food plant tends to be random.

Anatomy of the abdomen tip

Externally, the terminal abdominal segment (segment 10) of a caterpillar consists a pair of anal prolegs with associated pleural (basal) regions, an anal plate (the supra-anal lobe) and a region of cuticle around the anus (Scoble, 1992). The anal plate is stiffened dorsally into a shield-like sclerite, whereas the ventral surface is flexible (Fig. 1). Internally, this segment constitutes a separate and distinct 'tokus compartment' (Locke, 1997) of the caterpillar haemocoel. Haemolymph in the tokus compartment bathes the epithelium of the posterior rectum and several groups of intrinsic and extrinsic muscles of the rectum and the anal prolegs (Henson, 1937; Eaton, 1982). The tokus compartment is delimited anteriorly by the rectal (cryptonephric) complex (Ramsay, 1976). The posterior wall of the rectal complex forms a diaphragm-like barrier to the flow of haemolymph between the tokus compartment and the general haemocoel (Locke, 1997). Valves in this diaphragm are thought to permit blood to flow periodically between the tokus compartment and the main haemocoel (Locke, 1997). None of the other abdominal segments is apparently partitioned by intersegmental diaphragms. The terminal segment (as well as segment 9) in caterpillars lacks a pair of spiracles. Consequently, the tokus is aerated by four tracheae that radiate from the enlarged spiracles on abdominal segment 8. One pair of tracheae enters the anal plate, and a single trachea enters each proleg (Locke, 1997). The valves are located where the dorsal pair of tracheae penetrate through the tokus diaphragm (Locke, 1997). In skipper caterpillars, the tokus also contains retractor muscles that insert at the base of the anal comb and control its position (described below).

The anal comb

The anal comb is a flattened and ribbed structure attached to the underside of the anal plate (Fig. 1A). The two walls of the comb are strongly ribbed and uneven in length (Fig. 1A,C). The ventral wall, attached to the base of the anal plate, is longer (1.2–1.4 mm) than the dorsal wall (0.6 mm), attached closer to the tip of the plate (Fig. 1C). The proximal half of the ventral wall forms part of the surface cuticle of the anal lobe, whereas

its distal half and the dorsal wall of the comb form a hollow structure suspended from the anal plate. The projecting sides of the comb are formed of unsculptured pliable cuticle. The ribbed walls flatten out at the vertex of the comb, where they are fashioned into a set of approximately 20 short peg-like teeth. The teeth at the centre of the comb may fuse and consequently appear wider (Fig. 1B). The teeth have a slight upward curvature (or backward curvature, depending on the comb orientation). The comb teeth of newly ecdysed caterpillars are smooth and rounded (Fig. 1B) but they have a worn appearance in feeding caterpillars (Fig. 1D). Tooth wear is particularly pronounced on the convex (ventral) face of the teeth, where layers of cuticle are seen to have flaked away (Fig. 1D). The significance of this comb wear, which appears to be use-related, is described in the Discussion.

Two sets of retractor (flexor) muscles attach to the base of the anterior wall of the comb. A main set of muscle fibres extends forward to the posterior edge of the dorsal border between segments 9 and 10 (marked by a white band of epidermal pigment, see Fig. 2B) and a smaller set fans out to scattered points on the anal shield. These muscles appear to be the homologues of dorsolateral muscles DL1 and DL3 of segment 10 in the *Manduca sexta* caterpillar, which serve to retract the lower wall of the anal plate and expand the anal orifice (Eaton, 1982, 1988). The anal comb has no extrinsic protractor (extensor) muscles. At rest, the comb is held vertically and forms a curtain over the anus.

The pellet ejection surface

Two further regions of surface cuticle play a role in pellet ejection. The first, consisting of a small central region of the underside of the anal plate together with the dorsal surface of the anal comb, serves as the primary ejection surface (Fig. 1C, asterisk). This smooth region of cuticle is surrounded by a crescent-shaped ring of 20–25 tactile bristles inserted in prominent sockets (Fig. 1C). These mechanosensitive bristles, which may be distinguished from the pointed bristles on the anal segment and prolegs by their clubbed and grooved ends (Fig. 1E), are in a position to detect when the faecal pellet touches the underside of the anal plate (Fig. 1C). A secondary surface involved in pellet firing is the pliable cuticle flanking the ventrolateral margins of the anus (termed the paraprocts by Scoble, 1992). This cuticle, covered by an unusually dense pile of fine hairs less than 50 µm in length, appears to serve as a 'trampoline' surface that absorbs some of the kinetic energy of the pellet during its discharge and determines the trajectory of the pellet (see Fig. 6A).

Events leading to pellet discharge

Pellet discharge occurs in five stages, each stage associated with a precise sequence of discrete events. They are described below and shown in Figs 2 and 3. The deduced positions of the anal comb during pellet discharge are shown in Fig. 4. The durations of the five stages were estimated from video-taped recordings of 10 different sequences obtained from six different caterpillars.

Preparatory events

A skipper caterpillar first signals its intent to drop a faecal pellet by briefly extending its anal comb past the posterior margin of the anal plate (Fig. 2C). The caterpillar then detaches its anal prolegs from the leaf surface and arcs its abdomen

upwards, by as much as 70° to the horizontal. At the same time, the anal plate starts to swell, although the total volume of blood in the tokus compartment remains unchanged at approximately $13.7 \pm 1.7 \mu\text{l}$ (mean \pm s.d.). The anal comb then retracts, and a prominent line of white pigment marking the

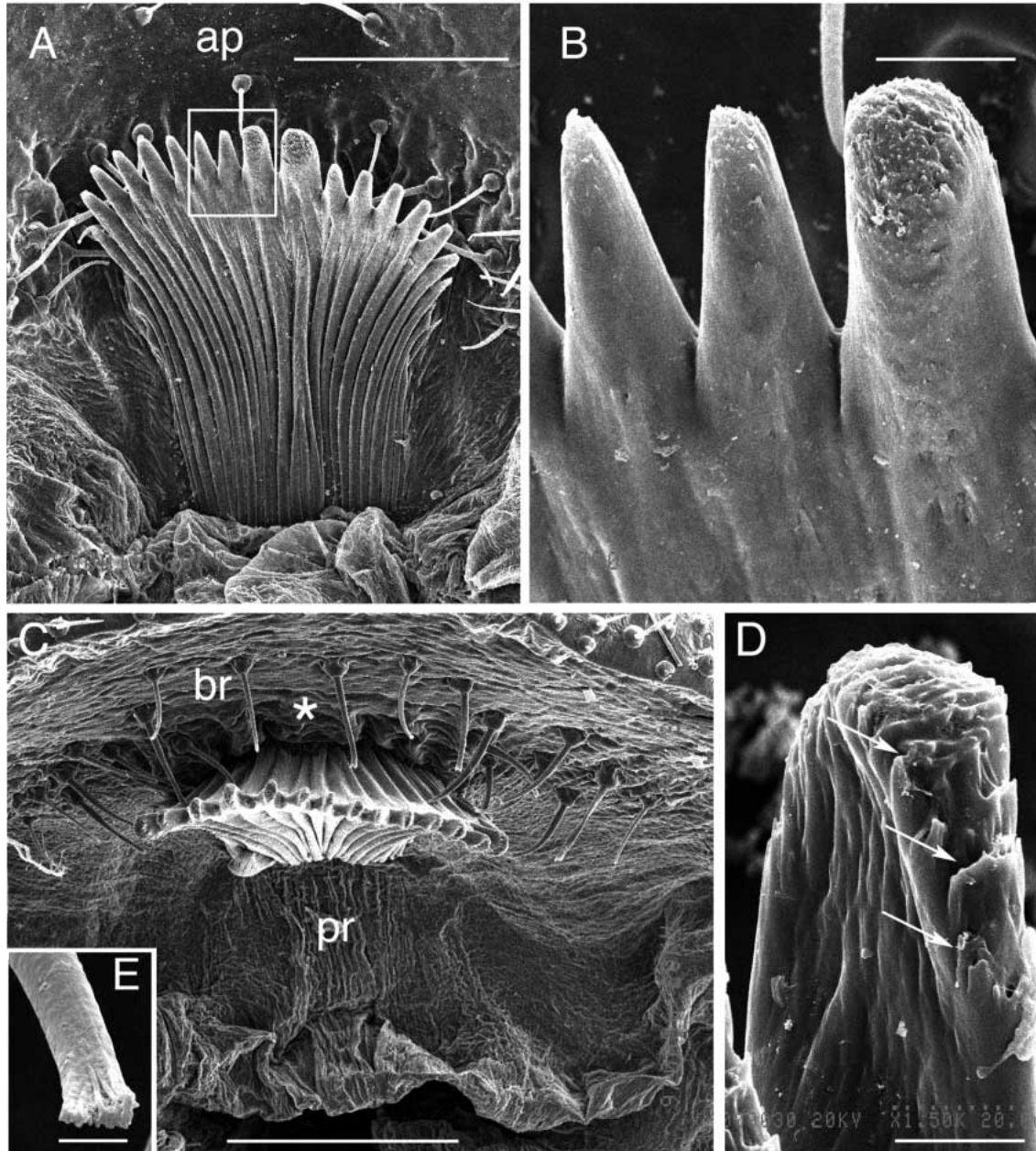


Fig. 1. Scanning electron micrographs of the tip of a skipper caterpillar's abdomen, showing the structures associated with pellet discharge. (A) Anal comb of a newly emerged caterpillar shown from its ventral aspect in position below the lower surface of the anal plate (ap). Scale bar, 0.5 mm. (B) Margin of the comb in a newly emerged caterpillar (area outlined in A) showing the smooth-walled teeth. Scale bar, 50 μm . (C) Rear-view of the circum-anal area of segment 10 shown immediately after pellet discharge. The anal comb projects outwards towards the viewer. Its short dorsal wall is attached to the wrinkled lower surface of the anal plate and its long ventral wall is attached to the dorsal lining of the posterior rectum (pr). The junction between the pliable lower surface and the rigid upper surface of the anal plate is seen at the upper corners of the micrograph. The proposed pressure plate area consists of a smooth region of anal plate cuticle (asterisk) and the dorsal wall of the anal comb suspended from it (see text for details). Sensory bristles (br) border the pressure plate, which appears here in its collapsed and pleated state. Scale bar, 0.5 mm. (D) Tip of a chipped and worn tooth as seen in anal combs of actively feeding caterpillars. Pieces of cuticle have flaked off from the comb surface (arrows) to give it a rough appearance. Scale bar, 2 μm . (E) Tip of a mechanosensitive bristle adjacent to the pressure plate with its characteristic sculpted blunt end. Scale bar, 20 μm .

border between segments 9 and 10 becomes more scalloped in appearance (Fig. 2C) as segment 9 shortens and remains contracted until after the pellet is discharged (compare Fig. 2B with Fig. 2C–G). The anus remains closed throughout this preparatory phase. The time lapsed in preparation for defecation varied from 0.5 to 3.5 s (mean 1.8 ± 1.2 s) and largely depended on how long the abdomen tip waved about after the prolegs lifted off the leaf surface.

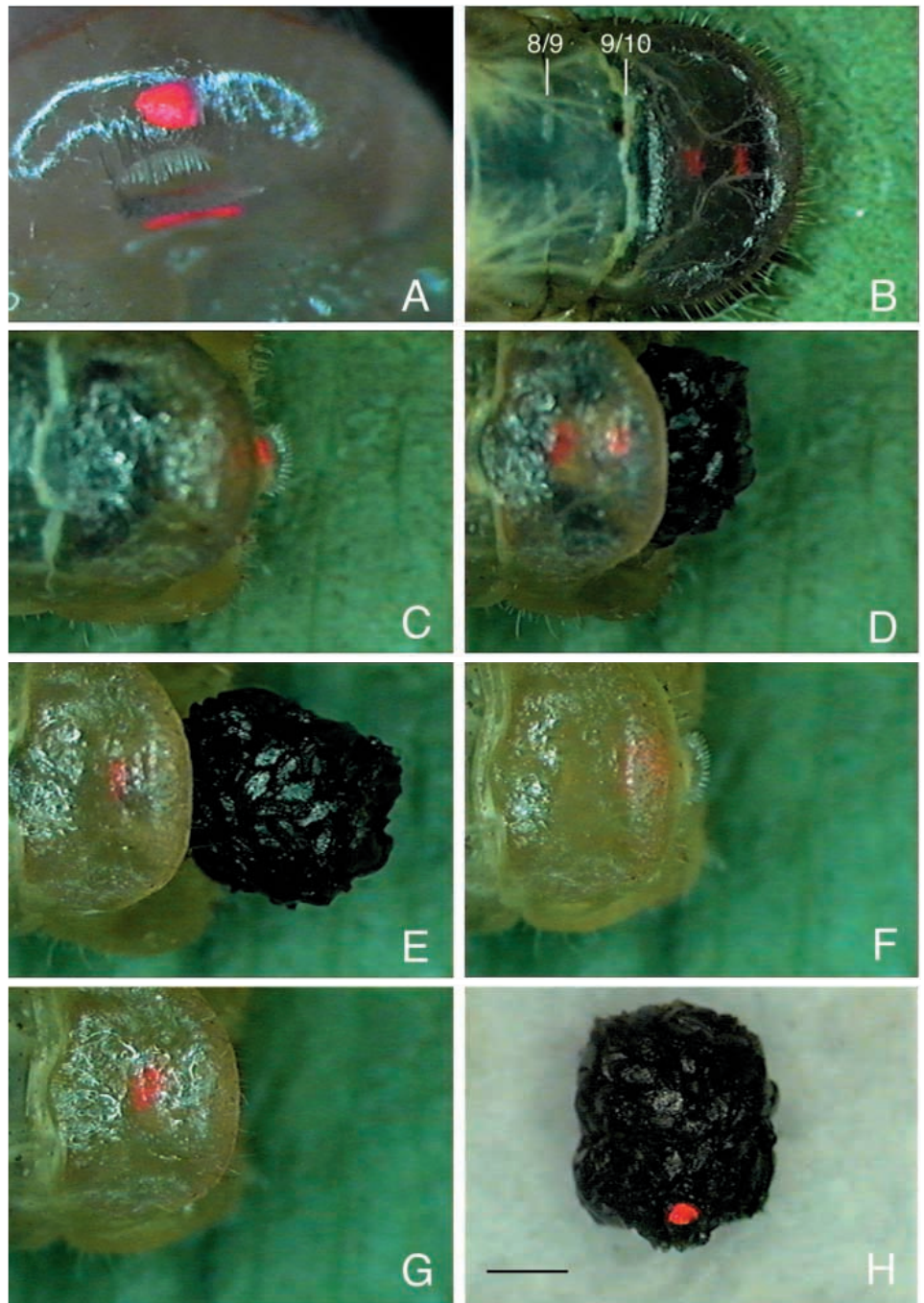
Extrusion events

The rectal muscles involved in expelling a faecal pellet in the *Manduca sexta* caterpillar are described in Eaton (1982,

1988). In *Calpodex ethlius*, the pellet emerges after the epiproct (a cuticular area flanking, and including, the ventral side of the anal comb) and the paraprocts (two cuticular areas lateral to the anus) pull back and dilate the triangular-shaped anal orifice (Fig. 3A).

The separation of the area surrounding the anus is brought about by the contraction of muscles attached directly to the anal region. The rectum now shortens to expel the pellet through the expanded anus (Figs 2D, 3B). As the rounded leading face of the pellet starts to emerge, it pushes out a ring-shaped collar (torus) formed by four circumferentially arranged and everted sections of the lining of the posterior rectum (Fig. 3A,B). The

Fig. 2. The movement of the anal comb during pellet discharge was tracked on video tape by marking the base of the comb with red paint. (A) Rear view of abdomen tip showing marker paint spots at the anterior (ventral) and posterior (dorsal) border of the anal comb, which in this image projects towards the viewer (centre). Images B–H (1 mm scale bar shown in H) are all taken from a single discharge sequence from the animal in A. (B) Abdomen tip viewed from above, with the paint spots revealing the comb to be in its resting position. The white band of pigment marking the segment 9/10 border is indicated, as is the thinner and broken white line marking the segment 8/9 border. (C) Comb extension prior to pellet extrusion. The paint spot marking the dorsal edge of the comb is visible but the ventral spot is obscured by the margin of the anal plate. (D) The comb in its retracted position during pellet extrusion. The anal plate is elevated and both paint spots are visible. (E) The comb now flexes downwards, causing the pellet to tilt upwards and to come into contact with the central area of the lower surface of the anal plate. Viewed from above, the two paint spots appear to converge as the comb flexes downwards. The anterior spot disappears behind the pellet just before pellet discharge, as shown here. (F) Expanded lower surface of anal plate with the comb extended immediately following pellet discharge. The posterior paint spot has disappeared by being transferred to the pellet. (G) Comb retracting back into the resting position with only the paint spot marking its anterior margin now present. (H) The fired faecal pellet with the transferred paint spot showing where the approximate centre of the pressure plate exerts force on the pellet during its discharge.



outer margin of this torus is formed from the bristle-free paraprocts folding back on themselves. Although initially a flattened collar around the pellet, the primary torus soon swells with blood to form a fleshy cushion before the pellet is halfway out of the rectum (Fig. 3B). The leading edge of a secondary torus, formed from the everted lining of a more anterior region of the posterior rectum, now emerges between the primary torus and the pellet (Fig. 3B). This secondary torus, too, first emerges as a flattened collar around the pellet. Both the pellet and the secondary torus continue to slip outwards beyond the primary torus, which begins to retract. At its maximum extension, the secondary torus covers the trailing third of the pellet's surface

(Figs 3C, 4B). The primary torus now begins to collapse and fold back into the rectum as it slips off the trailing surface of the pellet (Fig. 3C). Whereas the dorsal rim of the primary torus never extends beyond the dentate tip of the anal comb during pellet extrusion, the secondary torus stretches over the pellet surface to a point well beyond the tip of the comb (although at this time the anal plate is lifted out of the way of the emerging pellet and the anal comb is flattened against the plate's lower surface (Figs 2D, 3C).

The secondary torus now begins to swell and slip back, holding the pellet temporarily in place on its swollen pedicel (possibly as in the action of a suction cup) (Figs 3D, 4C). As it retracts, the swollen everted rectal wall pushes on the comb tip and wedges it between the primary and secondary tori (Figs 2D, 4B), a process aided by the comb retractor muscles flexing the comb at this time (Fig. 4B). Pellet extrusion typically takes 1 s, the time ranging between 0.9 and 1.2 s (mean 1.0 ± 0.1 s, $N=8$). In two cases examined, the movement of the pellet stalled when half-extruded, which delayed the completion of pellet extrusion by approximately 1 s.

Positioning events

The rectum continues to pull back into the body and moves the pellet closer towards the tip of the abdomen. The pellet tips up and comes to rest against the lower surface of the anal plate (Figs 2E, 3E), touching and bending the blunt-ended bristles of the anal plate (Fig. 1C). Pellet tilting is apparently due to the anal comb, wedged between the two tori, arcing downwards as the everted rectum continues to pull back into the body. By this time, the primary torus has slipped back into the rectum and disappeared from view (Figs 3E, 4C). The comb now lies in a near-vertical position with its tip separated from the faecal pellet by the upper margin of the secondary torus (Figs 2E, 4D). The anal plate now starts to arch downwards, and the anal prolegs start to contract. This causes the inner faces of these three structures to make contact with the back surface of the pellet (Figs 3E, 4D). The plantae of the anal prolegs now retract and their crochets arches fold up (Fig. 3E). The rising blood pressure in the tokus compartment forces the flexible ventrolateral areas of the underside of the anal plate to swell and extend beyond the posterior margin of its rigid dorsal surface (Figs 3E, 4D). The central area of the underside of the plate, however, remains compressed into a concave shape by the fully flexed anal comb. The comb is held in place by the upper margin of the swollen secondary torus (Fig. 4D). The positioning phase of pellet movement has a rather constant duration of approximately 1 s (range 0.5–1.1 s; mean 0.8 ± 0.2 s).

Discharge events

The first sign that pellet discharge is imminent is a slight outward movement of the pellet relative to the abdomen tip. The central surface of the anal plate and the comb then suddenly shoot backwards and the pellet is projected through the air (Fig. 4E). This occurs within 0.5 ± 0.1 s of the pellet being tilted into position (Figs 2E, 4D). The abrupt release of the anal comb acts as a trigger allowing the central region of the anal plate to

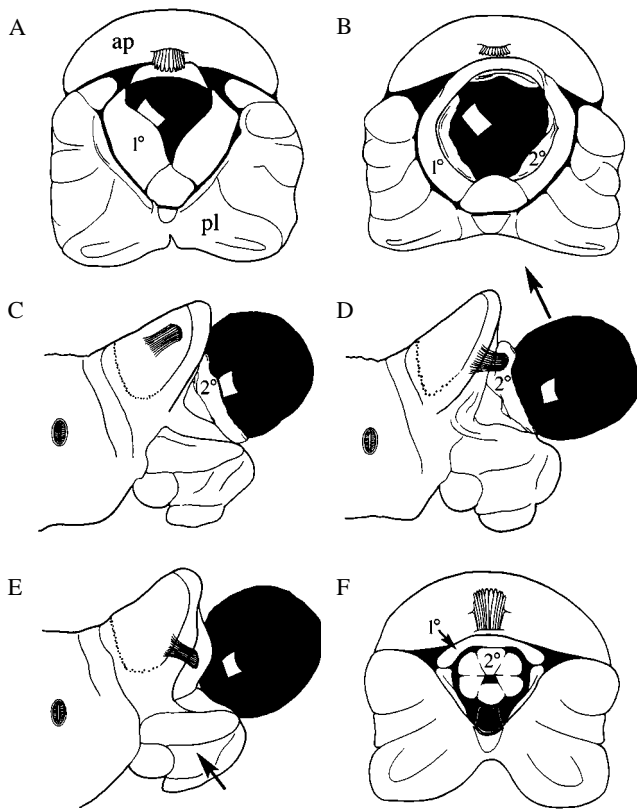
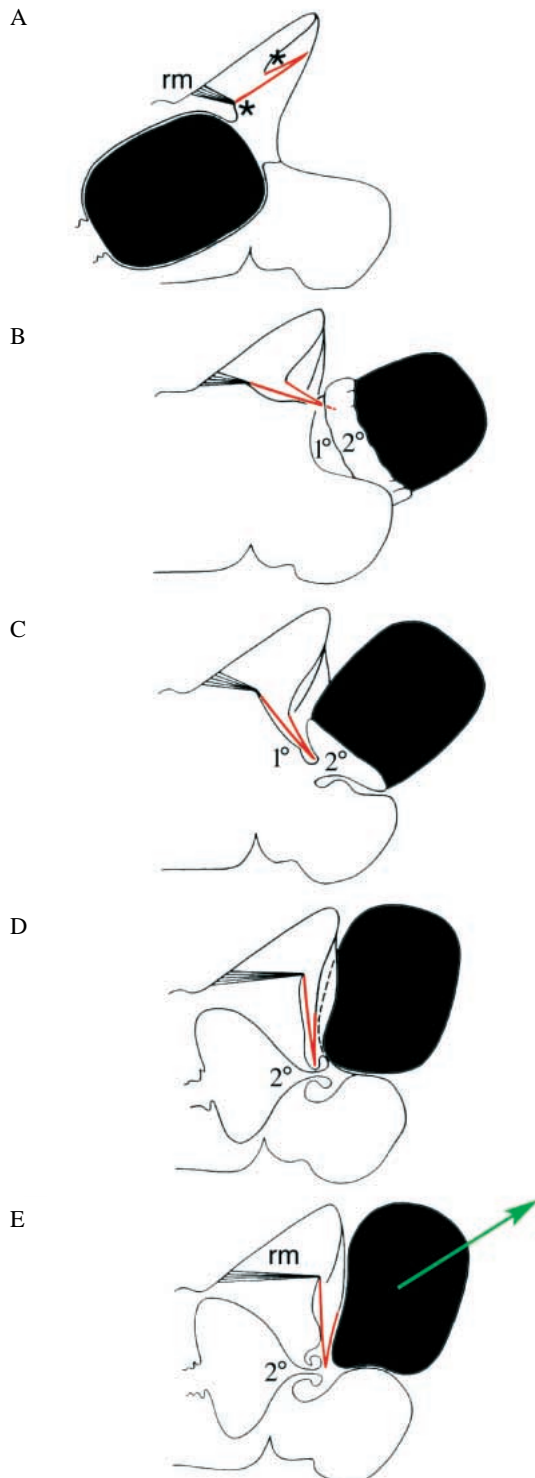


Fig. 3. Movements of the abdominal tip during pellet discharge seen in rear view (A,B,F) or side view (C,D,E). (A) Early stage of pellet extrusion revealing the four-part structure of the primary torus (1°), with the anal comb lying over its dorsal rim. A single food chunk (white area) marks the progression of the pellet. (B) Slightly later stage showing both primary and secondary (2°) tori wrapped around the emerging pellet. (C) Pellet extrusion at a fairly advanced stage. The comb is retracted and the primary torus has slipped back into the rectum as the secondary torus starts to grow. (D) The pellet, now seated on the secondary torus, is pulled towards the tip of the abdomen by the anal comb flexing downwards and pushing on the pellet through the torus. (E) As the comb continues to flex, it continues to pull the secondary torus towards the abdomen tip and the pellet tilts upwards. The prolegs retract and the lateroventral walls of the anal plate and the paraprocts bulge outwards. The arrow shows the direction of contraction of the anal prolegs. (F) Post-firing state showing the anal comb briefly extended and the secondary torus as a six-lobed pad immediately before it retracts into the rectum.

fill with blood, which changes instantly from a concave to convex shape as it moves backwards a short distance (Fig. 4E). The comb is also swollen with blood at this time (Fig. 4E) and this may help maintain its rigidity. By painting spots on the anal plate just posterior to the dorsal base of the comb (Fig. 2A, upper paint mark), we determined by frame-by-frame analysis that the distance of travel was between 0.8 and 1.2 mm, and that it was completed within 17 ms (1/60 s). A more precise



estimate, based on pellet velocities, suggests that plate travel is over within 2 ms (see below). These paint marks occasionally transferred to the pellet on its discharge and provided direct proof that the moving pressure plate strikes the pellet along the pellet's lateral wall, rather than on its end (Figs 2H, 4E).

The explosive nature of this movement, in which released blood pressure is translated into kinetic energy, projects the pellet through the air from its resting position against the anal plate. The mechanism that trips the flexed anal comb and initiates pellet discharge is not obvious. It could be due to one or a combination of events such as (i) the release of stored elasticity in the hyper-stretched retractor muscles or their contraction (these muscles are temporarily rotated around their comb insertion point and may act as weak comb extensors); (ii) the dorsal rim of the primary torus, which is swollen at the moment of discharge, driving the anal comb outwards over the secondary torus; (iii) the overall pressure in the anal compartment rising to a level beyond the ability of the secondary torus to retain the comb tip; and/or (iv) the gut continuing to pull on the secondary torus. All or several of these events could trip the anal comb and release the pressurized wall of the anal plate, causing it to travel backwards and to propel the pellet away. The anal prolegs continue to retract after the pellet has been fired. Indeed, for a brief instant after the pellet has gone, the rate of contraction of the anal prolegs actually accelerates, presumably because the internal pressure resisting the force exerted by the plantar muscles is momentarily relieved by the movement of blood into the pressure plate of the anal lobe. Altogether, the four stages described above take between 2.5 and 5.6 s (mean 3.9 ± 1.0 s in the six larvae examined). This interval represents between 0.2 and 0.5 % of the 20 min long defecation cycle in this caterpillar.

Recovery events

The dorsal rim of the primary torus and the reduced secondary torus (Fig. 3F) are immediately retracted into the

Fig. 4. The sequence of positions of the pressure plate and anal comb during preparation for pellet discharge. These schematic diagrams are an interpretation of comb movement made by video-taping the comb after painting its base at the two spots marked (asterisks) in A and observing the comb through the semi-transparent cuticle of the anal plate as shown in Fig. 2. (A) Comb in a resting position just before the faecal pellet is voided. rm, retractor muscle. (B) After the pellet is half-extruded from the rectum, the comb is extended over the primary torus (1°) and hooked beneath the dorsal margin of the secondary torus (2°). The comb is lowered into this position by the contraction of its retractor muscles. (C) As it arches forwards, the anal comb remains tightly wedged between the primary and secondary tori. The comb is presumably pulled by the contraction of extrinsic muscles attached to the hindgut. (D) The anal comb in the fully flexed state. The comb acts as a 'latch' that trips when it is forced backwards over a 'catch' formed by the secondary torus. (E) Position of the pressure plate and anal comb at the moment of pellet discharge. The forces acting on the pressure plate are predicted to distort the pellet's shape by pushing it against a cushion formed by the base of the anal prolegs as it is fired off along the trajectory shown by the arrow (see text for details).

Table 1. *Observed pellet firing data and selected values used in modelling*

	<i>N</i>	Mean \pm S.D. (range)	Model values
Pellet parameters			
Mass, ρV (mg)	118	11.05 \pm 3.87 (2.6–19.2)	10
Volume, V (mm ³)	118	11.75 \pm 4.17 (3.0–22)	–
Estimated density, ρ		0.93 \dagger	1
Parameters of initial pellet flight			
Larvae firing right-side up			
Trajectory angle (degrees)	21	11.0 \pm 17.5 (–21 – +41)	
Velocity, v (m s ^{–1})	21	1.30 \pm 0.32 (0.91–1.66)	
Pellet mass (mg)	21	10.1 \pm 3.4 (6.0–16.7)	
Larvae firing upside-down			
Trajectory angle (degrees)	10	28.0 \pm 15.9 (11–40)	
Velocity, v (m s ^{–1})	10	1.19 \pm 0.22 (0.87–1.57)	
Pellet mass (mg)	10	10.6 \pm 3.6 (6.6–18.5)	
Angle between upper surface of anal plate and firing angle, β (degrees)	15	11 \pm 5.3 (4–22)	
Pressure plate parameters			
Observed contact area (mm ²)	5	2*	–
Effective contact area, A (mm ²)		–	1.41
Observed displacement (mm)	5	1 (0.8–1.3)	–
Effective displacement, δ (mm)		–	0.71
Angle between direction plate moves and pellet trajectory, α (degrees)		–	45

*Value based on the maximum apparent area of contact seen when a pellet is swung into position before its discharge. As the pellet and the contact area are presumed to deform when the pellet is fired off, the actual area of contact may be greater than the observed contact area.

\dagger Regression fit, $r^2 = 0.8034$, $P > 0.001$.

rectum, and the triangular-shaped anus closes. The volume of the anal plate begins to drop as the prolegs expand to regain contact with the leaf surface. The extended anal comb (Fig. 2F) retracts and flexes downwards to cover the anus (Fig. 2G).

Analysis of pellet motion

The discharge velocity of a pellet was estimated from video sequences of its position after being fired off by the caterpillar. Interlacing of the video images allows two sequential images to appear together at the two ends of a flight trajectory lasting 1/60 s (Fig. 5A).

Trajectory and velocity

A sample set of initial pellet trajectories is shown in Fig. 5B. It is evident that the position of the caterpillar at the moment of pellet discharge (upright or upside-down) had some effect on the firing velocity or on a pellet's trajectory angle (Fig. 5B; data summarized in Table 1). The pellets were seen to rotate in flight about a nearly horizontal axis perpendicular to the trajectory (although a few misshapen pellets spun about the horizontal axis along the trajectory). The direction of rotation (indicated in Fig. 5B) implies that the pellet rests on, or at least strikes against, the paraprocts when the lower surface of the

anal plate drives the pellet away. In the absence of this contact, the pellet would have rotated in the opposite direction, since it is struck below its centre of gravity (Figs 2H, 4E).

Tilt of the anal plate in relation to the initial trajectory of the pellet

When a faecal pellet is fired off, its trajectory line is not in line with the direction in which the anal plate moves (Fig. 4E). Whereas the centre of the pressure plate swings outwards at an estimated angle of 50–60° relative to the upper surface of the anal plate, the pellet trajectory subtends a far more acute angle (β) to the plate, on average 11° (measured range 4–22°, $N=15$). This, too, implies that the pellet is momentarily squeezed between the plate and the paraproct cushion on which it rests before it is ejected. For modelling purposes, we estimated the angle (α) between the direction in which the pressure plate moves and the pellet trajectory to be 45° (Table 1).

Modelling the mechanism of pellet release and estimates of blood pressure involved

The model described in the Materials and methods section proposes a release mechanism in which released pressure in the anal compartment moves the pressure plate in a direction

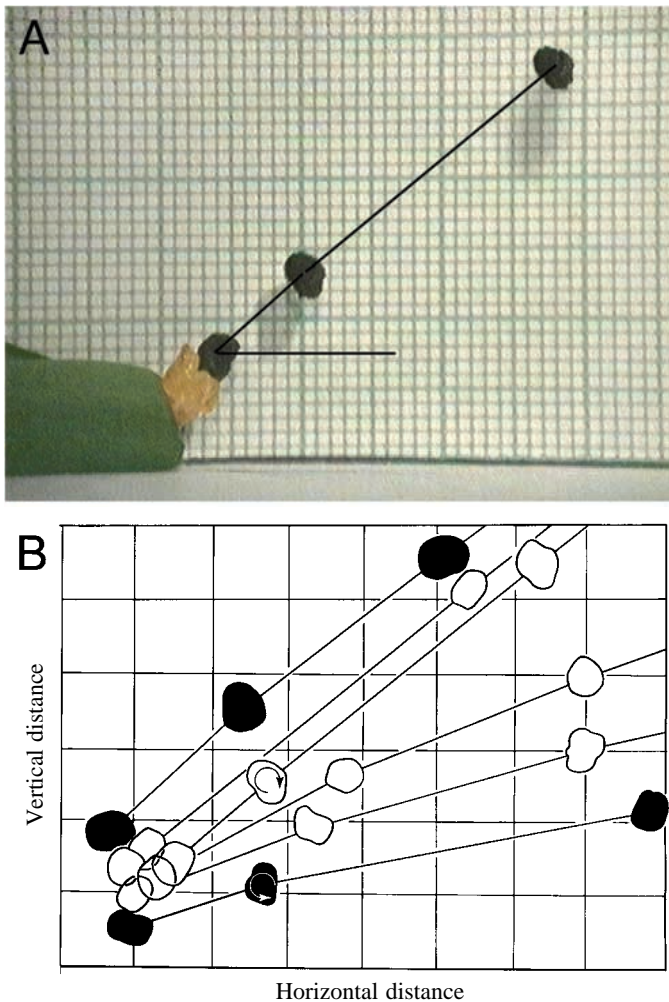


Fig. 5. Pellet dynamics. (A) Composite video image of the trajectory of a pellet made by superimposing its position in three successive $1/60$ s video frames. The tip of the abdomen of the caterpillar is seen projecting from the leaf-roll and elevated by approximately 45° to the horizontal. The pellet had a mass of 12.0 mg and an initial velocity of greater than 1.4 m s $^{-1}$ and tumbled in the direction indicated by the arrows in B. (B) A sample set of six pellet trajectories, showing their positions in space at the instant of discharge (lower left) and then along their flight trajectories during the following two intervals of $1/60$ s. The trajectory angle and discharge velocity of a pellet are not affected by the caterpillar being either right-side up (white pellets) or upside down (black pellets) in its leaf roll (see Table 1 for details). The background is a 5 mm \times 5 mm grid.

different from the initial trajectory of the pellet. The model considers the pellet to be squeezed between the lower wall of the anal plate and the paraprost cushions while it is being fired off (Fig. 4E). This feature is consistent with the observed direction in which the pellet tumbles during flight, as a pellet hit below its centre of gravity by the anal pressure plate alone would rotate through the air in a direction counter to that seen (Figs 4E, 5B). Compression against the paraprost cushion apparently reverses the rotary motion of the pellet.

Applying the mean values listed in Table 1 to equation 2 with

$K=1$, the model provides an estimate of the effective driving pressure. The blood pressure in the anal compartment at the moment of faecal pellet discharge will be greater than this by $1/K$, as discussed below. Taking an average value for v of 1.3 m s $^{-1}$, an observed pressure plate movement of 1 mm, an estimated total contact area of 2 mm 2 , an angle between the pressure plate area and its displacement relative to the trajectory of $\alpha=45^\circ$ (leading to $A=2$ mm $^2\times\sin 45^\circ=1.41$ mm 2 and $\delta=1$ mm $\times\sin 45^\circ=0.71$ mm) and a typical pellet mass ρV of 10 mg, the effective driving pressure involved can be estimated at approximately 8.45 kPa or approximately 63 mmHg, somewhat more than the human pulse pressure. Applying pressure values of this order to pellets of recorded differing masses (Fig. 6B), the model predicts the initial discharge velocities reasonably well in both magnitude and general trend. High-end pressures are estimated to be in the 10 kPa range (upper curve in Fig. 6B).

Acceleration forces

The initial acceleration of a pellet results from the application of considerable force, possibly in excess of 300 times its own weight. A pellet moves from a stationary state to a velocity of perhaps 1 m s $^{-1}$ in approximately 2 ms, assuming that the anal plate accomplishes the energy transfer over a distance of approximately 1 mm in the direction of pellet motion at constant acceleration. This motion implies a constant acceleration of $50g$ (i.e. 50 times the acceleration due to gravity). A $50g$ acceleration of a pellet weighing 10 mg is the same as saying that the force on the pellet is equal to 50 times its mass (or 0.5 g, approximately a quarter of the caterpillar's mass). This is a conservative estimate since pellet speeds of up to 1.7 m s $^{-1}$ were observed. The implied acceleration, other parameters being equal, varies with the square of the speed attained, suggesting accelerations of $300g$ or more. At these large values of acceleration, it is almost certain that the pellet will experience considerable distortion during the short period over which the forces are applied (Fig. 4E). In fact, the actual details of the pellet firing over the 2 ms or less of acceleration probably involve significant compression of the rear of the pellet around the contact areas, followed by a rebound of shape which combines with the applied pressure to accelerate the pellet. Thus, it is recognized that the proposed model using observed contact areas and constant pressures is highly oversimplified.

Pellet elasticity

Implicit in this model is the notion that the faecal pellet, and possibly the surfaces that temporarily exert force on it, are to some extent elastic. This resilience allows the momentarily compressed pellet to revert to its original shape after being fired off, rather than becoming permanently deformed. A pellet unable to recoil elastically would be crushed when squeezed between the anal plate and the proleg cushions (Fig. 6A), and the distance it could be fired by haemostatic pressure in the anal plate would be diminished. An exploratory attempt was made to gain a rough idea of the efficiency of energy transfer from the pressure-driven plate to its pellet load by dropping fresh pellets from a height of 100 mm or 200 mm onto either a

A

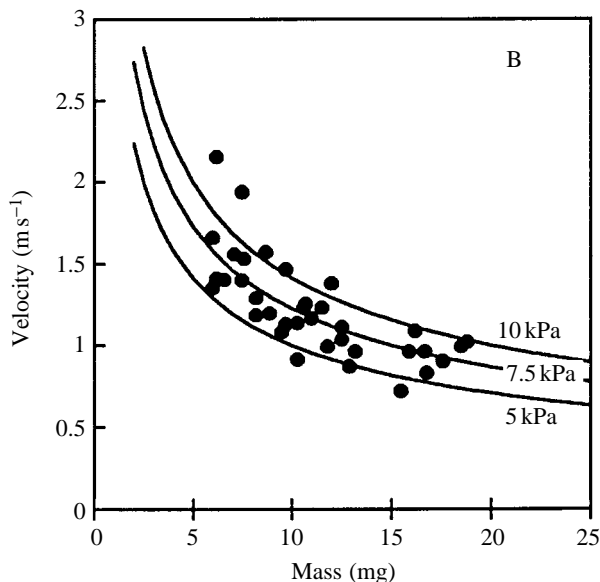
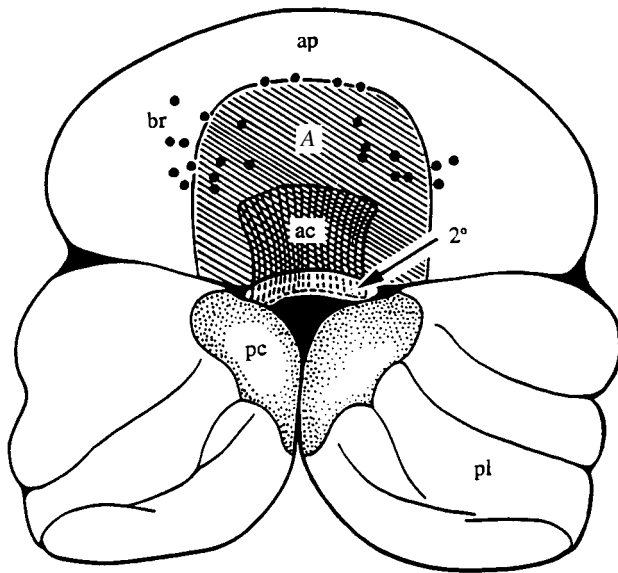


Fig. 6. A model of pellet discharge. (A) Diagram of the rear view of the caterpillar showing the predicted firing plate (area A, diagonal hatching) on the lower surface of the anal plate and the two paraproct cushions (pc, stippled areas) at the base of the anal prolegs (pl). The pellet is squeezed against the paraproct cushions during its discharge. The anal comb (ac) forms the lower part of the firing plate and is shown here with its tip hooked behind the secondary torus (2°). Sensory bristles (br) are shown as filled circles. (B) Blood pressures predicted to be involved in pellet discharge, based on observed pellet mass and initial velocity. The theoretical velocity curves bracketing the bulk of the experimental data points were derived from equation 1 using the model parameters listed in Table 1. The model curves shown predict that the blood pressure in the anal compartment rises to between 5 and 10 kPa (38–75 mmHg) at the moment of pellet firing, assuming a high efficiency (K) of energy transfer between the plate and the pellet ($K=1$). As K is reduced, the estimated pressure increases proportionally.

metal (aluminium) or a thin (1 mm) rubber surface under the force of gravity, recognizing that neither correctly simulates the anal plate/pellet interaction. The terminal speeds attained from these heights are 1.4 and 2.0 m s^{-1} respectively, neglecting air drag, which reduces these speeds only slightly. As the pellets are semi-cylindrical in shape and often bounced off sideways when they landed on their edges, only the heights of pellets that bounced vertically or near-vertically were recorded. Dropped from 100 mm, pellets bounced $11.3 \pm 2.2 \text{ mm}$ off the metal surface ($N=28$, highest bounce recorded, 17 mm) and $11.4 \pm 2.1 \text{ mm}$ off the rubber surface ($N=38$, highest bounce recorded, 15 mm), demonstrating a maximum coefficient of restitution (energy after collision/energy before collision) of 0.17 with little dependence on the interacting material. From 200 mm, the pellets bounced to $21.9 \pm 3.1 \text{ mm}$ ($N=26$, highest bounce, 28 mm) off metal and to $23.6 \pm 3.2 \text{ mm}$ ($N=25$, highest bounce, 30 mm) off the rubber surface, demonstrating a maximum coefficient of restitution of 0.15, again with little sensitivity to the interacting material or to the speed of impact. These experiments, which suggest more than 80% loss of energy in the interaction, probably exaggerate the energy loss compared with the anal plate/pellet interaction. Considering that the pellet is normally compressed and moulded into shape within the rectum, and then pulled against the pressure plate immediately before firing, the effective elasticity in the pellet (i.e. at its points of contact with the plate) and the elasticity of the proleg membranes themselves probably lead to a more efficient energy transfer. A high-end estimate for K of 0.5 (50% energy efficiency) might be appropriate in interpreting the pressure predictions shown in Fig. 6B, thus indicating actual blood pressures a factor of two greater than the effective driving pressure shown. The details of the brief instants of firing would be an interesting area for future research.

Effects of drag and gravity

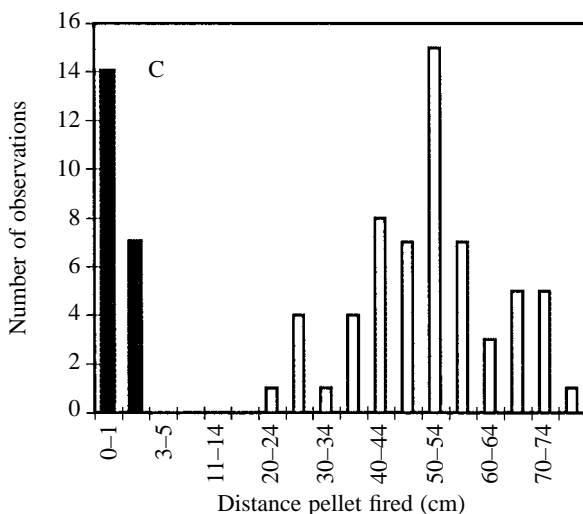
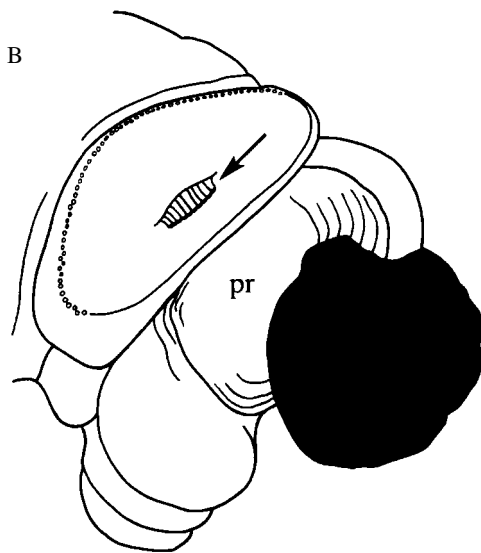
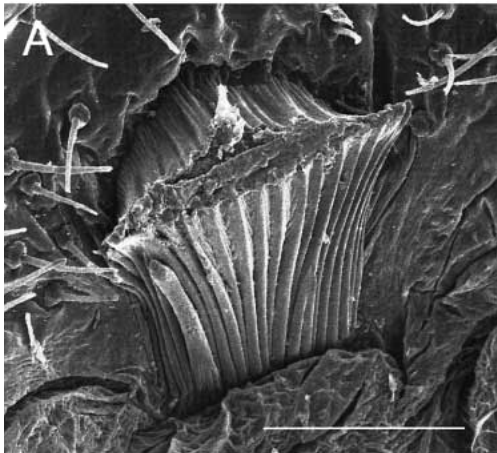
Flight speeds and angles were estimated directly from the video images and were not corrected for the effects of either air drag or gravity on the flight trajectory. Analytical estimates indicated that air drag introduces errors of only approximately 1%. The effects of gravity on the trajectory are somewhat larger, implying that both speeds and trajectory angles are underestimated by as much as 5%.

Removal of the tip of the anal comb

We tested whether the ability of a skipper caterpillar to discharge its faecal pellets is impaired when the tip of its anal comb is cut off (Fig. 7). The region of the anal comb removed was restricted to its dentate margin and common shaft (Fig. 7A). Although surgery induced slight to moderate bleeding from the trimmed comb, the operation did not prevent or delay defecation. The number of pellets voided by comb-trimmed caterpillars left to feed and recover overnight (between 31 and 44 pellets in 15 h, $N=5$) was similar to the number voided by a companion caterpillar with an intact comb (36 pellets). A few hours after the operation, a local

phenoloxidase reaction in the scar tissue and damaged comb cuticle gave the wound a black colour, which was useful in following the movement of the truncated comb during pellet expulsion.

Video sequences of defecation in caterpillars with clipped combs showed that the anal plate and comb went through



typical preparatory movements (mean $t=1.8\pm 0.3$ s, where t is time), and that the primary and secondary tori developed normally as the pellet was extruded but then continued to grow in size (Fig. 7B) (mean $t=1.9\pm 0.5$ s). The pellet could not be retracted properly nor tilted up to its normal prefiring position and failed to be discharged to any distance (Fig. 7C). A truncated comb was too short to hook securely behind the secondary torus and moved outwards over the top of the pellet as the blood pressure in the tokus compartment rose. Instead of being fired off, the pellet rested against the abdomen tip and was slowly pushed off by the anal lobe and retracted prolegs (mean $t=1.11\pm 0.62$ s) and fell to the ground. The anal comb took approximately 50 ms (range 2–5 video frames, i.e. 33–83 ms) to complete its outward movement compared with less than 17 ms (1 video frame; predicted actual time 2 ms, see above) in normal animals.

Discussion

The mechanism behind pellet ejection may be described in general terms as one in which hydrostatic pressure drives a movable plate to which a load-anchoring lever is attached. The rod-shaped lever acts as a latch holding the plate stationary until sufficient pressure has developed to trip the latch. The pressure stored behind the plate then drives the plate rapidly outwards, firing the load away after compressing it between the pressure plate and a secondary plate. This description of the forces involved in pellet discharge is analogous to the way in which a solid object – such as a tiddlywink – placed on a passive resilient surface may be shot off by pressing on it. Neither of the forces acting on the pellet is in line with its initial trajectory.

Highlights of the model

This model of a pressure-driven mechanism for faecal pellet firing in skipper caterpillars is summarized below.

Pellet discharge is powered by the abrupt release of blood pressure in the anal compartment of the haemocoel

During pellet extrusion, the blood pressure in the anal compartment rises, largely through the activity of retractor muscles in the anal prolegs. A similar process may occur in other caterpillars as they drop faecal pellets. The particular role of the anal prolegs in pressurizing the anal compartment might explain why their musculature – in particular that of the plantae

Fig. 7. Caterpillars with trimmed anal combs are unable to disperse their faecal pellets by firing them through the air. (A) Truncated comb seen by scanning electron microscopy 24 h after the comb-trimming operation. The wound is tightly sealed by this time. Scale bar, 0.5 mm. (B) After the anal comb has been trimmed, rectal eversion is unrestrained and the tori swell excessively during defecation (pr, posterior rectum). (C) Pellet travel distance. The pellets of normal animals (open columns) are fired through the air and roll some distance on landing (data from six animals). The pellets of comb-trimmed animals (filled columns) fell away from the abdomen and rolled only a short distance (data from five comb-trimmed caterpillars).

– differs from that of the prolegs on abdominal segments 3–6. In the anal prolegs, the basal muscles are reduced and the muscles of the plantae enlarged. Unlike in the other prolegs, the latter include both dorsal and ventral fibres (Snodgrass, 1935).

A pressurized anal plate fires off the faecal pellet

Haemostatic pressure in the anal compartment is predicted to be at least as high as 10 kPa (75 mmHg) at the moment the pellet is discharged. The pressure plate comprises the dorsal face of the anal comb and a central area of the lower surface of the anal plate. Whereas this part of the anal plate is free of setae, the band of mechanoreceptive setae around its margin presumably allow the caterpillar to sense when the pellet is in an optimal firing position.

The anal comb is a latch preventing the premature release of blood pressure

Pellet discharge is apparently not powered primarily by comb protractor (extensor) muscles. The anal comb does not have powerful protractor muscles to swing the comb backwards to ‘flick’ the pellet away, nor does there appear to be an appropriate fulcrum on which the comb might pivot. However, the ribs on the anal comb are consistent with a structure that can bear large lateral loads without bending, such as might be required to hold the anal plate in place. That the anal comb acts as a latch is revealed by experiments in which the comb was trimmed. Comb-trimmed caterpillars were unable to project pellets through the air for two reasons. First, a shortened comb is unable to restrain the outward movement of the secondary torus and the pellet resting on it. Normally, the anal comb makes solid contact with the everted rectal wall between the primary and secondary tori. As it flexes downwards, the comb pulls the secondary torus towards the base of the anal plate and tilts the pellet upwards. A truncated anal comb, in contrast, is able to make at most slight contact with the everted rectal lining. In this instance, as the anal plate was lowered and the shortened comb thrust downwards, the comb was unable to hold the rectal wall in place. Consequently, the secondary torus became more swollen and the pellet extruded further backwards out of the anus and could not therefore be fully tilted and raised to its normal firing position against the lower wall of the anal plate. Second, a shortened anal comb is not able to prevent the lower wall of the anal plate from expanding prematurely. Normally, the pellet fires off when the anal comb disengages from the secondary torus and, together with the lower face of the anal lobe, the comb moves rapidly backwards. Because the truncated comb fails to engage fully the everted rectal wall between the primary and secondary tori, the blood pressure in the anal compartment causes the volume of the anal plate to increase gradually and its lower surface to bulge out before anal comb extension. Although a truncated anal comb is able to move backwards quite rapidly (presumably driven by blood pressure and probably retractor muscle relaxation and stored elasticity in the cuticle) in the absence of the release of

restraining force of the secondary torus, it usually failed to make contact with the pellet during this stroke. This was due both to comb length and to the mis-positioning of the pellet. In instances where the comb grazed the upper surface of the pellet during its backstroke, little projectile force was evident.

The everted rectal lining acts as a catch controlling the release of the anal comb and the pressurized anal plate

The comb is held in place by a catch formed by the dorsal rim of a blood-swollen secondary rectal torus. The comb is released by being pushed over the catch formed by this torus when the rectal lining is being retracted back into the body, a time when the pellet comes to rest against the lower surface of the anal plate. The role of the catch is to hold the rectal lining and the extruded faecal pellet in place until sufficient blood pressure has built up in the anal plate. The friction associated with the rapid backward extension of the anal comb during pellet discharge accounts for the wear pattern on the teeth. It is unlikely that comb wear occurs during pellet retraction prior to discharge, as at this time the walls of the comb are held firmly sandwiched between the swollen primary and secondary tori. When the comb flexes, initially through contraction of its retractor muscles, then through the combined action of the rectum pulling forwards and the anal plate moving downwards, the teeth remain locked against the everted rectal cuticle. The cuticular intima of both primary and secondary tori are reported to be highly pliable and smooth and lack the cuticular pits found on the intima of the anterior rectum and elsewhere along the gastric tract (Byers and Bond, 1971). This might aid the smooth backward slippage of the comb while it is still in contact with the rectal cuticle.

Haemostatic mechanisms in the Lepidoptera

Haemocoelic pressure in most insects examined, including lepidopterans, is near-atmospheric (Wasserthal, 1996). Unlike in arthropods such as spiders (Stewart and Martin, 1974), blood pressure is not regarded as being of importance to insect locomotion (Jones, 1977). The typical lepidopteran pupa has a steady subatmospheric blood pressure between –200 and –600 Pa (reviewed in Wasserthal, 1996). In adult moths, however, local haemostatic forces are involved in proboscis extension (Banzinger, 1971) and in wing expansion following pupal–adult eclosion, when a maximum blood pressure of approximately 7 kPa (51 mmHg) was recorded (Moreau, 1974). In *Bombyx mori*, the larval blood pressure rises slightly at ecdysis, from 400–470 Pa (3–3.5 mmHg) in an immobile caterpillar to 1.3–2.0 kPa (10–15 mmHg). Local changes in blood pressure may be responsible for the eversion of a variety of integumentary structures in caterpillars, such as the protrusion of osmeteria in the Papilionidae (the swallowtails) and the extension of stemapodiform appendages (modified anal prolegs; Scoble, 1992) in the Notodontidae (for instance, the pussmoth *Cerura vinula*). We have not attempted to measure pressure fluctuations in the anal haemocoel compartment in actively feeding and defecating caterpillars. Nevertheless, the blood pressure we predict to be attained in the anal

compartment of a skipper caterpillar when it 'forcibly ejects' its faecal pellet (King, 1880) is physiologically attainable, being similar to that developed during wing expansion in the adult moth (Moreau, 1974).

Are there more fundamental reasons why the anal haemocoel compartment in caterpillars is pressurized during defecation?

Most caterpillars do not have a defecation sequence in which they extrude, retract, tilt and fire off pellets of frass. Instead, they merely extrude the frass and allow it to fall away (Eaton, 1988). Nevertheless, the anal haemocoel compartment in such caterpillars becomes pressurized during defecation. It seems plausible to suggest that the temporary rise in blood pressure in the tokus compartment serves a basic physiological role, subsequently adapted to a role as a pellet-firing mechanism in a few unrelated families within the Lepidoptera. New insight into the role of the tokus haemocoel in caterpillars comes from the recent work of Locke (1997). He proposes that special thin-walled tracheae present in the tokus haemocoel compartment as well as in a pair of tracheal tufts in abdominal segment 8 serve to supply oxygen to anoxic haemocytes that periodically cluster on them. The lung-like role of these 'aerating tracheae' (as described by Locke, 1997) may be facilitated through the transient pressure-driven flow of haemolymph from the tokus compartment to the main haemocoel, which may flush adherent and oxygenated haemocytes back into the general circulation as well as into the tissues of the rectal complex (S. Caveney and H. McLean, in preparation). In a feeding caterpillar, this normally occurs while the rectum is changing shape during defecation.

The periodic elevation of blood pressure in the tokus compartment represents a significant but previously overlooked feature in caterpillar physiology. It may have originally evolved in caterpillars to assist in non-projectile defecation or as part of a respiratory device to circulate oxygenated haemolymph. These transient elevations in blood pressure then became adapted to power a projectile mechanism to discharge frass pellets. Concomitant with this adaptation would have been the development of the anal comb, which appears to have arisen independently in several families of Lepidoptera (Gerasimov, 1952).

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (OGP 6797) to S.C., Harry Leung, Rod Hallam and Kim MacDonald provided technical assistance. Ian Craig assisted in the computer artwork and Alan Noon provided video equipment.

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