Entrapped sand as a plant defence: effects on herbivore performance and preference

ERIC F. LOPRESTI,[1] PATRICK GROF-TISZA,[1]
MORIA ROBINSON,[2] JESSIE GODFREY[3] and RICHARD
KARBAN[1] [1]Department of Entomology, University of California, Davis, California, U.S.A, [2]Department of Evolution and Ecology, University of California, Davis, California, U.S.A and [3]Department of Plant Sciences, University of California, Davis, California, U.S.A

Abstract. 1. Abrasive material in the diet of herbivorous organisms comes from a variety of sources, including crystalline silica or calcium in plant tissues, accidentally ingested soil while digging or grazing, and entrapped substrate on the surfaces of plants. A wide variety of plants entrap substrate, usually with glandular trichomes.

2. A previous study demonstrated that entrapped sand provided resistance to herbivory in the field. In this study, the following questions were addressed: how does entrapped sand on *Abronia latifolia* (Nyctaginaceae) leaves and stems affect preference and performance of a common herbivore, the large-bodied caterpillar *Hyles lineata* (Sphingidae); does this effect differ from those experienced by an internally feeding leaf miner?

3. Using a combination of experimental and observational approaches, it was found that sand comprised ~4–5% of ingested weight during normal feeding of *H. lineata* caterpillars. This entrapped sand caused extensive wear to their mandibles, they avoided sand-covered plants when given the choice, and the sand negatively impacted performance metrics, including pupal weight, development time, and growth rate. In contrast, a leaf-mining caterpillar did not have a preference for or against feeding on sandy plants.

4. These results are similar to studies on mandibular wear due to grasses, and herbivorous insects that feed on these two plant groups may have similar morphologies. It is hypothesised that increased wear potential may be a convergent solution to abrasive plants in both mammals (hypsodonty) and insects.

Key words. *Abronia*, insect herbivory, plant defence, plant–insect interactions, psammophory, sand armour.

Introduction

Herbivores of all sizes must cope with plant defences, whether physical or chemical, internal or external (LoPresti, 2016). Physical defences include deterrent or puncturing structures such as thorns or prickles as well as abrasive internal crystals (Farmer, 2014). Internal crystals promote costly wear on mouthparts and reduced digestive capabilities in insect herbivores (Massey & Hartley, 2009). Herbivorous animals unintentionally ingest soil, sand, and other inorganic substrates while feeding, possibly with similar consequences as ingestion of internal crystals. This

incidental ingestion may contribute to wear on mouthparts, a phenomenon extensively examined in comparative studies of mammalian dentition (Janis & Fortelius, 1988; Williams & Kay, 2001; Mendoza & Palmqvist 2008; Lucas *et al.,* 2014). While incidental soil ingestion in insects is less well studied, soil feeding in an Orthopteran causes mandibular wear (Kuřavová *et al.,* 2014); similarly, insects wear down their mandibles when feeding on tough plants (e.g. Wainhouse *et al.,* 1990; Massey & Hartley, 2009). Whether by mammals or insects, an investigation of sticky plants that entrap sand and dust with glandular trichomes found less herbivory on sand-covered plants compared with sand-removed treatments (LoPresti & Karban, 2016).

Sand or dust entrapment on plant surfaces occurs in many glandular-sticky plants. Sand-covered plants are commonly found worldwide in sandy or dusty environments, including

Correspondence: Eric F. LoPresti, Department of Entomology, University of California, 380 Briggs Hall, 1 Shields Avenue, Davis, CA, 95616 U.S.A. E-mail: lopresti.eric@gmail.com

coastal and desert dunes (see lists in Jürgens, 1996; Neinhuis *et al.,* 1996; LoPresti & Karban, 2016). Termed 'psammophory' by Jürgens (1996) and 'sand armour' by Farmer (2014), sand entrapment may provide the plant with a number of benefits, including resistance to herbivory, resistance to abrasion, increase in reflectance and lower leaf temperature (Neinhaus *et al.,* 1996; Jürgens, 1996). While only resistance to herbivory has been experimentally tested, these other benefits probably occur to varying degrees in those plants, of at least 34 families, which entrap substrate (LoPresti & Karban, 2016). These factors all probably drove the repeated evolution of psammophory in many phylogenetically disparate plants lineages (Jürgens, 1996; LoPresti & Karban, 2016).

LoPresti and Karban (2016) determined that the sand covering of *Abronia latifolia* (sand verbena: Nyctaginaceae) was not a crypsis defence; plants covered in stem-coloured sand received similar herbivory to plants covered in background-colored sand. Both of these treatments received far lower herbivory than did experimentally cleaned plants without sand, suggesting that sand is a physical defence for this species. While this study established an anti-herbivore function of sand entrapment under natural conditions, we lack a more mechanistic understanding of how it functions as a physical defence. In addition, as the previous study did not differentiate among chewing herbivores, it remains unclear whether sand is more effective against some herbivores than others. For instance, while external chewers encounter sand as a routine part of feeding, internally feeding leaf miners avoid extensive interaction with the plant surface. Given that these internally feeding herbivores are consumed (e.g. suffer predation) when the leaves in which they reside are consumed by herbivores (E. F. LoPresti, pers. obs.), it is possible that they use sandy plants as a refuge.

Given these limitations of previous field research, we used two *Abronia*-feeding caterpillars in the laboratory to answer several questions:

1 Do herbivores intentionally avoid sand?
2 How much sand do they ingest under natural feeding on psammophorous plants?
3 Does ingestion and processing of plant-entrapped sand negatively affect these herbivores?
4 Does preference against sandy leaves differ between functionally different *Abronia*-feeding caterpillars?

Materials and methods

Natural history

The genus *Abronia*, the sand verbenas, occur across much of western North America in sandy habitats, including coastal dunes and deserts. All species that the authors have examined (*fragrans, latifolia, maritima, nana, pogonantha, turbinata, umbellata,* and *villosa*) entrap sand to some degree using sticky exudates from glandular trichomes on stems, pedicels, flowers, buds and leaves, although location and density of these trichomes vary by species.

During field work, we have found three caterpillars on California *Abronia* species in occasional abundance: a hawkmoth

caterpillar, *Hyles lineata* (Lepidoptera: Sphingidae), and two leaf-mining caterpillars, *Lithariapteryx abroniaella* (Lepidoptera: Heliodinidae) and *Euphyia implicata* (Lepidoptera: Geometridae) (E. F. LoPresti, pers. obs.). *Hyles lineata* feeds on entire leaf laminae by chewing the leaf completely, beginning along the edge, in the process ingesting much of the sand stuck to leaf surfaces (E. F. LoPresti, pers. obs.). The leaf-mining species feed internally on mesophyll, leaving both epidermal layers (and all entrapped sand) intact. Both miners exit and re-enter leaves frequently, presumably when resources become scarce in one leaf and require relocation and mine initiation in another (pers. obs.). *Euphyia* occasionally feed externally on floral tissue, but on leaves they feed solely internally (pers. obs.).

Quantification of sand ingestion and mandibular wear

We quantified sand ingestion in both wild-caught and laboratory-reared caterpillars. We collected final-instar *H. lineata* on 17 and 18 March 2016 near Anza Borrego, California, from a large patch of roadside *Abronia villosa*. Caterpillars were immediately frozen for dissection. We also reared caterpillars to their final instar on sandy or non-sandy plants in the laboratory. Beginning on 21 March 2016, we randomly assigned 20 fourth-instar caterpillars (from the same population) to two treatment groups ($n = 10$ each). One group received 'clean' *Abronia latifolia* leaves, and the other sandy *A. latifolia* leaves, Leaves used for both treatments were collected from *A. latifolia* growing in patches of iceplant (*Carpobrotus edulis*). *Carpobrotus edulis* grows in thick mats, covering the sand surface nearly completely, and reduces the accumulation of sand on *A. latifolia* stems and foliage to extremely low levels (although no *A. latifolia* is completely sand-free). Caterpillars were reared in 163-ml plastic containers (Dart Industries, Mason, Michigan) until pupation. Plant material was refreshed daily and potting soil was provided to each as a pupation substrate when caterpillars reached the end of their final instar. To quantify sand ingestion, we sacrificed three individuals of each treatment for gut content analyses on 28 March. Gut contents were removed from the frozen collections (both field and laboratory), weighed and dried. In order to extract consumed sand, organic material was removed from dried gut contents using a combination of nitric acid (70%) and hydrogen peroxide (30%) in 2 ml Eppendorf tubes, a modification of a method developed for inductively coupled plasma-mass spectrometry analysis (Hansen *et al.,* 2013). Cleaned sand was then weighed.

To examine mandibular wear, when the remaining caterpillars pupated, the exuvial mandibles were collected. Mandibular micrographs were taken on these collected mandibles with a Hitachi TM3030 (Tokyo, Japan) tabletop scanning microscope after we removed the mandibles, rinsed them with 100% ethanol, dried, and mounted them.

Choice tests

Live third- and fourth-instar *H. lineata* were collected from the same site at the same time as described in the previous section. *Lithariapteryx abroniaella* caterpillars were collected on 23 and 24 March 2016 and on 12 April 2016 on *A. latifolia* at the UC Davis Bodega Marine Reserve. All mines in one patch

(~2 × 5 m) were collected and returned to the laboratory for processing; we gently removed caterpillars from any mines that were inhabited and used them for choice tests.

Both *H. lineata* and *L. abroniaella* were placed in Petri dishes and given a choice of either a 'sandy' or 'nonsandy' *A. latifolia* leaf of the same size. For each leaf pair, one leaf was randomly selected (by coin flip) to be dipped in sand, then gently shaken. The presentation of treatments was right/left switched each time to account for any potential bias. For *H. lineata*, we recorded the preferences of 46 caterpillars after 30 min. Choice scores were: preference for non-sandy leaf (leaf damage on non-sandy treatment only), preference for sandy leaf (leaf damage on sandy treatment only), no preference (both eaten equally), or no choice (no herbivory).

We tested the preference of *L. abroniaella* in the same fashion. We placed 48 *L. abroniaella* in petri dishes overnight and recorded in which leaf (or none) they had created a mine the next day. No caterpillar created multiple mines, though several pupated rather than forming a new mine.

Effects of mandibular wear and sand ingestion on herbivore performance

To quantify the effects of sand ingestion on caterpillar performance, we performed a second feeding experiment where we switched diets partway through an instar (as mandibles are replaced at each moult). In this experiment, final-instar *H. lineata* caterpillars were assigned to the same treatments (sandy/non-sandy *A. latifolia*), but half of the individuals of each treatment were switched to the opposite treatment partway through development (at 2 g of body mass). This created four treatments (n = initial, survived and used in analyses): first sandy, then sandy (S-S, n = 21, 16); sandy, non-sandy (S-NS, n = 21, 16); non-sandy, sandy (NS-S, n = 22, 15); and non-sandy, non-sandy (NS-NS, n = 20, 19). We hypothesised that if mandibular wear was a factor, those caterpillars fed sandy plants earlier in development would perform worse than those initially fed on non-sandy plants. For this experiment, we used early-instar individuals collected near Minden, Nevada, on 27 May and 8 June. We applied treatments on the first day that caterpillars moulted into their final instar (23 May to 18 June 2016), assigning successive individuals to the next treatment in a randomly chosen order (NS-S, S-S, S-NS, NS-NS) to assure interspersion of treatments across time. We weighed each caterpillar daily, at the same time refreshing food, which was provided *ad libitum*. When a caterpillar exceeded 2 g, we began the second diet treatment (whether a switch or continuation) with that caterpillar. As in the prior experiment, potting soil was placed in each container prior to pupation in order to allow construction of a pupal chamber. Once pupae were hardened, they were weighed and sexed. Individuals that contracted a virus and did not complete development (n = 18) were excluded from analysis (i.e. the 18 that did not survive in the above sample sizes).

Statistics

All statistics were performed in R version 3.1.3. We tested the null model that choice was independent of treatment for both choice tests using the exact binomial test. We used ANOVA followed by Tukey's honestly significant difference comparison of treatment means to test if the factorial treatments affected pupal weight and larval period. Sex was a significant predictor of pupal weight but not pupal period and thus was used as a covariate for pupal weight only. Similarly, linear models were used to test whether timing and order of diet treatment affected cumulative weight gain for each day on the first and second diet. Cumulative weight gain ('growth') was calculated by subtracting each caterpillar's initial weight from the daily recorded value. We log-transformed caterpillar growth to meet the assumptions of normality.

Results

Sand ingestion by caterpillars

Sand was conspicuous in the guts of field-collected and laboratory-reared *H. lineata* feeding on sandy *Abronia* leaves (Fig. 1a,b). Field-collected *H. lineata* from *A. villosa* had 4.22% sand ($\pm$ 0.54 SE, n = 10) of total wet gut contents, while those raised in the laboratory on sandy *A. latifolia* averaged 4.94 $\pm$ 0.39% (n = 3). In contrast, individuals raised on non-sandy *A. latifolia* had only 0.39 $\pm$ 0.09% (n = 3) sand in their guts. (*Abronia villosa* was not available locally to test the caterpillars on in the laboratory.)

Leaf miner life history

Of the leaves with mines of *L. abroniaella*, 28 of the 73 on 23 and 24 March and 20 of the 75 on 12 April were occupied by living caterpillars. Previous observations of single stems suggested that each caterpillar created several mines during its development (LoPresti & Karban, 2016). We do not know whether this 32% occupancy figure represents an average of three mines per caterpillar over its life span or whether the average caterpillar which reaches pupation makes more, but some fraction of empty mines are probably from caterpillars killed by predation or parasitism.

Choice tests

Hyles lineata caterpillars overwhelmingly chose to feed on non-sandy leaves (31/36, 86%), a highly significant preference (binomial test, two-tailed, P < 0.0001). This supports the hypothesis that sand on leaf surfaces is a deterrent to feeding.

Lithariapteryx abroniaella created new mines with roughly equal frequency in sandy and non-sandy leaves (10:9, respectively, binomial test, two-tailed, P > 0.99). Therefore, we have no evidence to support the hypothesis that this internal feeder prefers physically defended leaves; however, sand on the leaf's exterior did not deter creation of new mines either.

Mandibular wear

Mandibular wear of *H. lineata* caterpillars fed the sandy *A. latifolia* treatment was extensive, far more than in the control
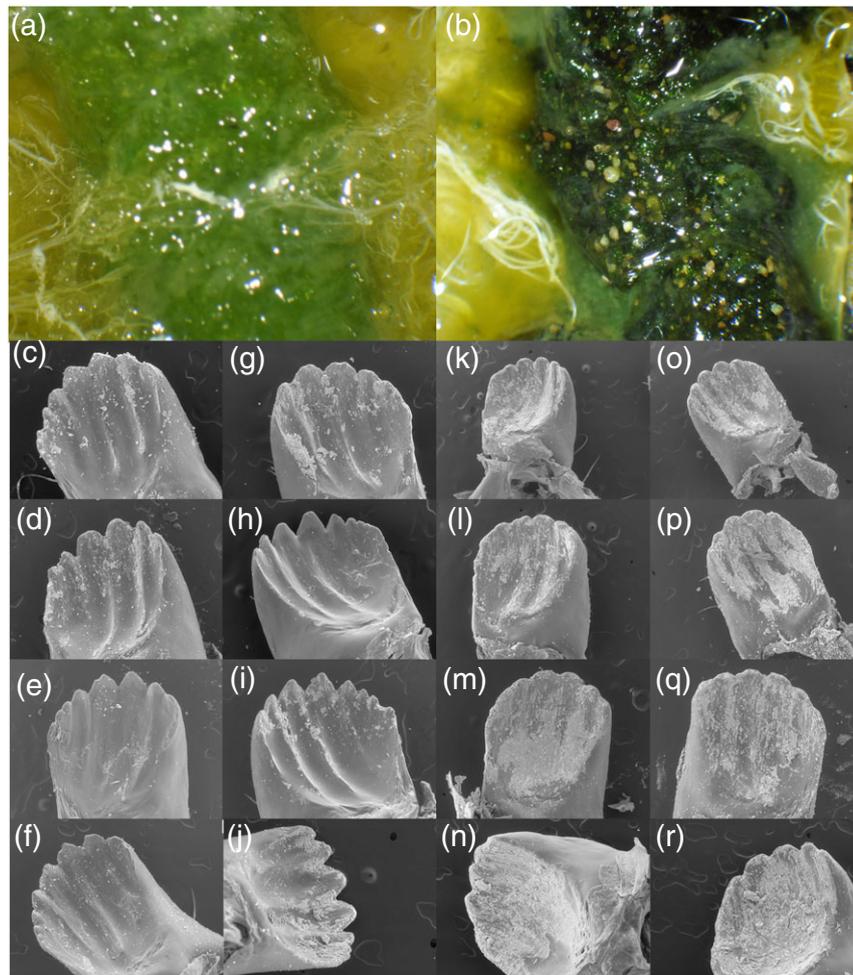
Fig. 1. Comparison of the gut contents of final-instar *Hyles lineata* caterpillars fed on non-sandy (a) and sandy (b) *Abronia latifolia*. Comparison of mandibles (taken from final-instar shed skin upon pupation) of caterpillars fed on non-sandy (left mandibles, c–f; right mandibles, g–j) and sandy (left mandibles, k–n; right mandibles, o–r) leaves. Note the pointy teeth in the non-sandy-fed individuals and the worn to nearly flat teeth in the sandy-fed individuals. [Colour figure can be viewed at wileyonlinelibrary.com].

group (which did ingest some sand; no field-collected *A. latifolia* is completely sand-free). Wear was obvious and extensive on mandibles of caterpillars from the sandy treatment; the mandibular teeth were originally triangular but they became worn and almost flat. While other studies (e.g. Whitehill *et al.,* 2016) have quantified mandibular wear as a ratio of distance between 'tooth' peaks to height of the 'tooth' peaks, we had few samples ($n = 4$/treatment) and damage was obvious and extensive. Locating the tooth peaks would have been very subjective on several sandy treatment individuals. Therefore, all mandibles that were photographed are presented in Figure 1.

Effects on correlates of fitness

Initial period. Weights of caterpillars ($n = 68$) for the first period (up to 2 g) were reduced in the sandy treatment compared with controls (Fig. 2a). Both initial weight (coefficient = 1.2 ± 0.1, $t = 8.4$, $P < 0.0001$) and sand treatment (coefficient

= -0.2 ± 0.1, $t = 2.6$, $P = 0.01$) were significant predictors of weight 24 h after the initiation of the experiment (day 1). After day 1, many passed 2 g and moved to the later treatment, so analyses were not performed.

Weights of the caterpillars ($n = 67$) in the later treatment (Fig. 2b,c) were predicted significantly by initial weight in days 1 and 2 post-switch (Fig. 2d). Both initial and later treatments were significant predictors by day 3. In days 4 and 5 only initial treatment was a significant predictor (Fig. 2d). (Initial and later treatments were always additive; an interactive model did not fit any better on any day.) The increasing significance of the initial treatments in later days is consistent with mandibular wear causing the largest effect later in life when the caterpillar was processing increasing volumes of plant matter.

Overall performance metrics. Larval period was shortest in caterpillars fed non-sandy plants second (Fig. 4: Table 1). This result was driven by the fact that the second treatment lasted
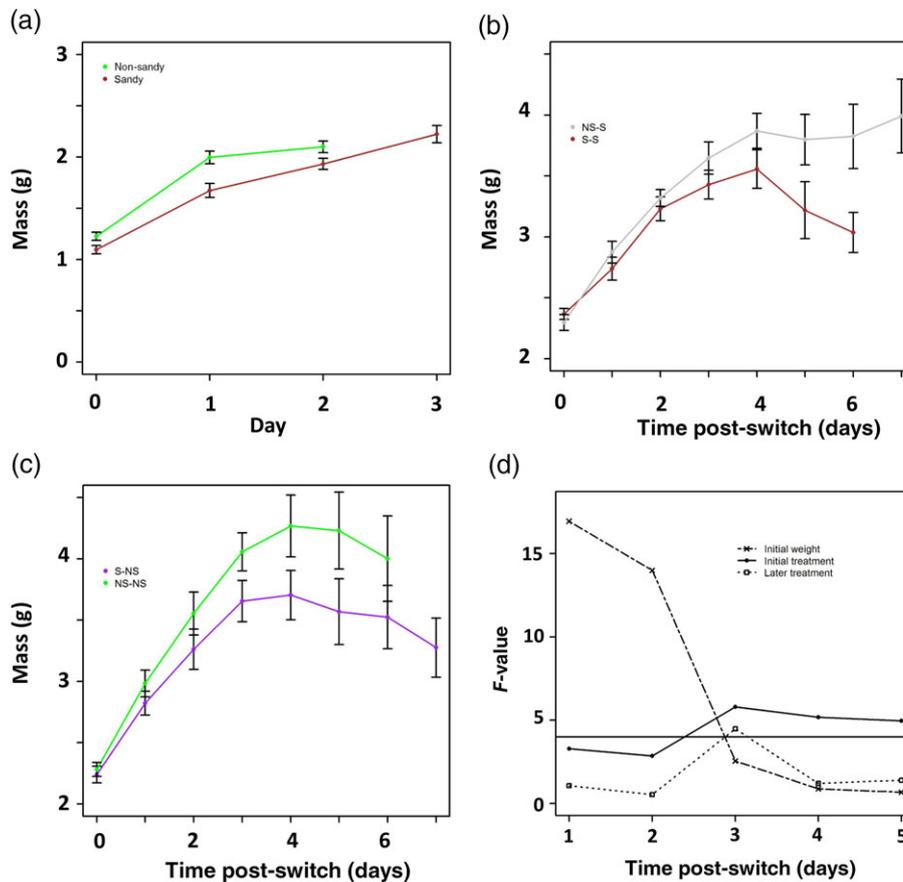
Fig. 2. Growth rates. (a) Weights of caterpillars in the initial treatments [all caterpillars feeding on either sandy (S) or non-sandy (NS) *Abronia latifolia*]. Note that all caterpillars are only present on day 1 ($n = 68$). (b) A comparison of caterpillars fed S plant matter in the later treatment (NS-S, $n = 15$; S-S, $n = 18$). (c) A comparison of caterpillars fed NS plant matter in the later treatment (S-NS, $n = 16$; NS-NS, $n = 18$). (d) *F*-values for the coefficients of initial weight, initial treatment, and later treatment, by day after diet switch. The solid horizontal line denotes significance level. These are coefficients of the full model; each best-fitting model included only the significant variables each day. [Colour figure can be viewed at wileyonlinelibrary.com].

far longer than the first [first treatment, 2.2 ± 1.2 days; second treatment, $4.6 + 1.9$ days (SD reported)].

Pupal weights were significantly lower for caterpillars fed on sandy material (S-S) than on those fed on non-sandy material (NS : NS) (Fig. 3). In the overall analysis, both initial treatment and sex were significant predictors of pupal weight (Table 1). The later treatment and the interaction term were both marginally significant ($P = 0.07, 0.10$, respectively). As the first treatment lasted far less time than the second, and compensatory growth could have occurred, initial treatment driving pupal weight suggests a long-lasting effect of the initial treatment.

Discussion

We found field and laboratory evidence for high rates of sand ingestion, and a cost to this ingestion, in the large-bodied, externally feeding caterpillar, *H. lineata*. In contrast, a leaf-mining species did not have any aversion to sand. This demonstrates that sand entrapment is not a universally effective defence against all guilds of herbivores.

This under-appreciated plant defence is phylogenetically widespread; over 200 species across the plant phylogeny entrap sand in glandular trichomes (LoPresti & Karban, 2016). More species probably pick up abrasive particles incidentally through the action of rain or wind-blown dust (Janis & Fortelius, 1988). These results suggest that sand entrapment could be an additional benefit of stickier or denser trichomes, contributing to any other selective advantage of these physical defences.

Any herbivore that feeds externally on these plants probably ingests some quantity of sand, with possible deleterious effects. *Hyles lineata* caterpillars fed on sandy plant material had reduced pupal weight and growth rate, and lengthened larval period compared with individuals fed on non-sandy plant material. These results probably stem from several mechanisms working simultaneously: mandibular wear decreasing processing efficiency, the occupation of gut volume by non-nutritive matter, and a reduction in nutritional absorption due to gut abrasion (e.g. Massey & Hartley, 2009). While all of these mechanisms could produce the observed results, the significant effect of the initial treatment on later growth rates shows that food processing later in the instar is affected by diet early

Table 1. Analysis of variance (ANOVA) and pairwise Tukey's honestly significant difference test results for the effects of timing (first diet for caterpillars < 2 g versus second diet for caterpillars > 2 g) and order of diet treatment (diet 1 × diet 2) on pupal weight and duration of the larval period.
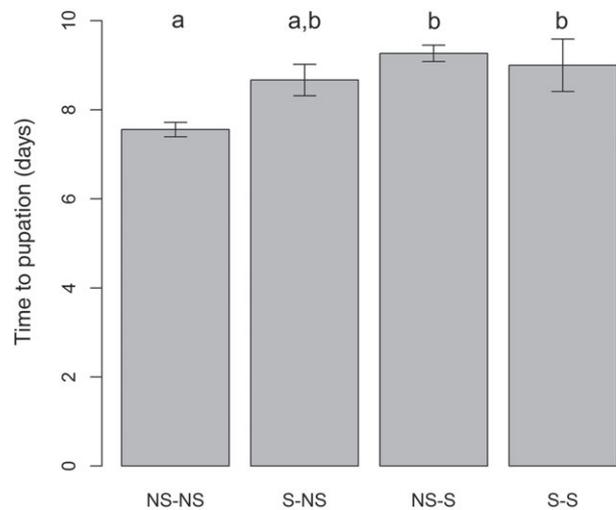
	Source	d.f.	SS	*F*-value	*P*-value	Pairwise test	*P*-value
Pupal weight	Sex	2	2.56	4.09	0.02	S : NS-NS : NS	0.05
	First diet	1	1.72	5.23	0.02	NS : S-NS : NS	0.08
	Second diet	1	1.1	3.51	0.07	S : S-NS : NS	0.03
	First × second diet	1	0.89	2.85	0.10	NS : S-S : NS	0.99
	Error	52	16.25			S : S-S : NS	0.99
						S : S-NS : S	0.99
Time to pupation (days)	First diet	1	4.38	1.63	0.21	S : NS-NS : NS	0.22
	Second diet	1	17.10	6.36	0.01	NS : S-NS : NS	0.02
	First × second diet	1	7.77	2.89	0.09	S : S-NS : NS	0.05
	Error	62				NS : S-S : NS	0.75
						S : S-S : NS	0.94
						S : S-NS : S	0.97

S, sandy; NS, non-sandy; in treatments, the first noted is the initial treatment and the second noted is the final treatment.
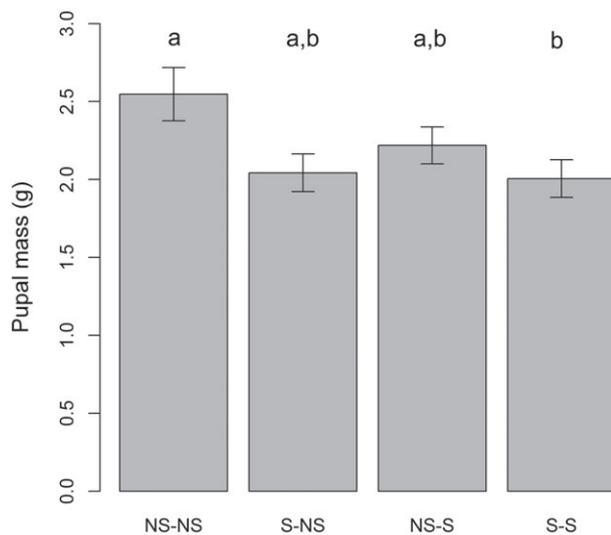
Fig. 3. Pupal masses of caterpillars fed on non-sandy (NS) and sandy (S) plant material. The first noted is the initial treatment (from the beginning of the final instar up to 2 g), and the second noted is the final treatment (from 2 g until pupation). $n = 18, 14, 13, 14$ for NS-NS, S-NS, NS-S, and S-S, respectively. Letters indicate significant differences between treatments.

Fig. 4. Total larval periods for caterpillars in each of the four treatment combinations. NS, non-sandy; S, sandy. This is time to pupation (days), not time to burial. (After the cessation of larval feeding, *Hyles lineata* spends 1–3 days constructing a pupal chamber before pupating.) $n = 18, 14, 13, 14$, for NS-NS, S-NS, NS-S, and S-S, respectively.

in the same instar. This is consistent with our observations of heavy mandibular wear occurring from feeding on sand-covered plants (Fig. 1k–r), although permanent damage to the gut could produce this effect as well.

This work contributes a new dimension to the study of abrasive particles used in plant anti-herbivore defence. Previous work has focused on plant-produced abrasive materials, which takes many forms. Conifers form hard stone cells in the bark, which increase mandibular wear and slow development of weevils (Wainhouse *et al.,* 1990, Whitehill *et al.,* 2016; J.G.A. Whitehill, pers. comm.). Other plants build putatively defensive phytoliths, small silica crystals in vacuoles or cell walls, or calcium-rich crystals found in the same tissues (Farmer, 2014). Grasses are best known for these defences, which slow development of

herbivores and reduce their fitness (e.g. Massey & Hartley, 2009), although some caterpillars respond plastically to these abrasive particles by increasing head and mandible size during moults (Bernays, 1986).

Our results are similar to those found by Massey and Hartley (2009) when caterpillars were fed diets of high- and low-silica grass diets. They found both short-term and long-term effects of silica in the diet. Compared with controls, growth rate, nitrogen absorption and feeding efficiency were lower in caterpillars fed on higher-silica grass species and then switched to lower silica species, suggestive of mandibular wear as a mechanism. They also performed an experiment where they raised caterpillars on high- and low-silica diets and then, at their final moult, raised them all on a low-silica diet. They found a negative effect of early exposure to silica, which demonstrates a mechanism independent of mandibular wear operating as well (as mandibles

are moulted). They suggested that this result was due to digestive tract abrasion, although a plastic effect reducing feeding efficiency could have contributed as well (i.e. Bernays, 1986). This plastic response at moult was not operating in our experiment, as we performed our experiment during a single instar. Our work complements these prior studies by demonstrating that this effect can occur with silica that was not produced by the plant. Psammophory is more phylogenetically widespread than stone cells in conifer bark and, while phytoliths and various calcium crystals are found commonly and broadly among plants, they are in high concentrations in some families only (Farmer, 2014). The main difference between these internal crystals and entrapped sand is location in or on the plant, a distinction that is often important and overlooked in plant defence (LoPresti, 2016). In addition, grain size of entrapped sand is likely to be far larger than within-tissue crystals, a difference that could have large functional effects on mechanisms of these defences. [This variable depends on the soils present in the environment; even clay stuck to plant surfaces could be a deterrent (Lev-Yadun, 2007).]

Herbivores adapt to the physical and chemical challenges of their host plants in quite remarkable ways. Therefore we may reasonably expect that herbivores that feed upon psammophorous plants will have behaviours (e.g. *H. lineata*, a generalist feeder, overwhelmingly chooses to feed on non-sandy leaves) or physical traits to minimise sand ingestion or the consequences of that ingestion. These traits may be especially pronounced in specialists. In a similar situation, a suite of distantly related rodents that feed on salt-encrusted *Atriplex* (Chenopodiaceae) leaves have evolved similar dental morphologies used to scrape the salt covering off before ingestion (Mares *et al.,* 1997). We observed *H. lineata* feeding on sandy *Abronia* spp. to see if any action by the mandibles or labrum might be pushing sand off the leaf surface before mastication. We did not observe any obvious scraping in this generalist feeder, although alternative behavioural avoidance mechanisms could be exhibited by other herbivores feeding on these plants and warrant further investigation. These behaviours could include cleaning leaves before ingestion, feeding more heavily after rains dislodge substrate, scraping the epidermal layers off and feeding on mesophyll, among many others.

Lithiapteryx and other miners are able to avoid any ingestion of sand during their internal feeding, although they may ingest some during the initial entry into a leaf (as they form several mines throughout their larval stages). This minimal interaction with sand on the leaf surface is not a large deterrent, as they made mines equally frequently in sandy and non-sandy leaves when given the choice. In extensive surveys of *Abronia* species, we (EL, PG-T, RK) found gastropods, four specialist leaf-mining caterpillars, and *Hyles* feeding on leaf tissue; in addition, damage indicative of herbivory by mice and deer was observed. How slugs and snails deal with sand-coatings and whether wear on the radula is functionally significant is an interesting question, as radula teeth are replaced continuously (Runham, 1962). Despite this possible mechanism for coping with wear, gastropods do avoid plants with siliceous outer layers (Grime *et al.,* 1968).

Many herbivorous mammals have teeth with high crowns, allowing much wear (hypsodont dentition), or grow them constantly (hypseledonty). The latter strategy is impossible for insects as the exoskeleton cannot grow once it has hardened. These dental traits are probably necessary for coping with large quantities of abrasive plant material, either because of internal abrasiveness or because 'the accumulation of grit on surface structures is a more typical feature of plant food items than of animals' (Janis & Fortelius, 1988). Comparative work suggests that incidental consumption of sand or soil (whether on the plants ingested, or non-entrapped soil ingested while feeding), not phytoliths, is probably the most important predictor of hypsodonty in extant mammals (Damuth & Janis, 2011, 2014). Whether insects have mandibular morphology similar to 'hypsodonty' in mammals remains an open question. We know that larger head sizes and larger mandibles occur in grass-feeding insects, both among species (reviewed in Hocheli, 1996) and as a plastic response to diet (Bernays, 1986). We do not know whether grass-feeding insects can tolerate greater wear, which would be a remarkable convergence to mammalian hypsodonty. Bernays *et al.* (1991) found that caterpillars and grasshoppers that feed on grass or tough leaves had shorter and broader mandibles than do relatives that feed on less abrasive food. However, a comparison of the durability (e.g. thickness of the cuticle of the cutting or grinding surface) was not conducted. Bernays *et al.*'s list of closely related species differing in feeding ecology (table 3 in that paper) would be a good starting point for investigations of this sort. (Mastication strategies differ between insect taxa; see Bernays & Janzen, 1988 for a detailed comparison of Sphingidae and Saturniidae in this regard.)

Most *Abronia* species grow in sandy areas and acquire their sand from the action of wind, which either moves the sand particles onto the plant or moves the sticky prostrate stems and leaves over the sand where they pick up particles. In these environments, the amount of sand caught by a given plant, or even leaf, is a function of abiotic factors (e.g. aspect, prevailing wind direction, cohesiveness of the substrate) and biotic factors (e.g. presence of mat-forming plants, structure of an individual plant). While LoPresti and Karban (2016) demonstrated that sand removal in the field increased herbivory, and we show here a mechanism by which it reduces herbivore performance, the response of herbivores to natural variation in sand entrapment has not been quantified. Large and/or mobile herbivores (such as late-instar *H. lineata*) could sample multiple plants and choose less well-defended plants or tissues (that field-collected caterpillars had large quantities of sand in their guts suggests they may not be choosy in the field). Female moths could oviposit preferentially, assuming they had some way of assessing the sandiness – or future sandiness – of a plant.

These results open a number of new lines of inquiry from the perspective of plant and herbivore trait function and evolution. For example, how do specialist, large-bodied herbivores cope with this physical defence? From the perspective of plant trait evolution, does sand entrapment join the suite of anti-herbivore traits thought to facilitate plant success in harsh abiotic environments, where tissue regeneration is especially costly? Further study of the plant traits that mediate sand entrapment, the shared traits of herbivores feeding on these plants, and the evolutionary history of both groups would improve our understanding of sand entrapment and its ecological and evolutionary implications.

In conclusion, our study demonstrated that entrapped sand on plant surfaces has a negative impact on the performance metrics of an externally feeding caterpillar. This probably underlies the strong preference against feeding on sandy plants in the choice tests and the previous field study (LoPresti & Karban, 2016). Mandibular wear was pronounced and extensive in the mandibles of caterpillars feeding on sandy plants, and probably underlies some of the reduction in performance. This phenomenon of sand-entrapping defence has long been suggested (as far back as Beal, 1878!), but never investigated mechanistically. These results open a number of new lines of inquiry, including how specialist herbivores cope with this physical defence and whether this or other abrasive foods select for hypsodonty in insect mandibles.

Acknowledgements

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12483

File S1. Pupal weights and periods for all which constructed pupal chambers (several died before pupation).

File S2. Caterpillar weights up to the diet switch at 2 grams.

File S3. Caterpillar weights after the diet switch at 2 grams.

References

Beal, W.J. (1878) Hairs and glandular hairs of plants, their forms and uses. *The American Naturalist*, **12**, 271–282.

Bernays, E.A. (1986) Diet-induced head allometry among foliage-chewing insects and its importance for graminivores. *Science*, **231**, 495–497.

Bernays, E.A. & Janzen, D.H. (1988) Saturniid and sphingid caterpillars: two ways to eat leaves. *Ecology*, **69**, 1153–1160.

Bernays, E.A., Jarzembowski, E.A. & Malcolm, S.B. (1991) Evolution of insect morphology in relation to plants [and discussion]. *Philosophical Transactions Biological Sciences*, **333**, 257–264.

Damuth, J. & Janis, C.M. (2011) On the relationship between hypsodonty and feeding ecology in ungulate mammals, and its utility in palaeoecology. *Biological Reviews*, **86**, 733–758.

Damuth, J. & Janis, C.M. (2014) A comparison of observed molar wear rates in extant herbivorous mammals. *Annales Zoologici Fennici*, **51**, 188–200.

Farmer, E.E. (2014) *Leaf Defence*. Oxford University Press, Oxford, U.K.

Grime, J.P., MacPherson-Stewart, S. & Dearman, R. (1968) An investigation of leaf palatability using the snail *Cepaea nemoralis* L. *Journal of Ecology*, **56**, 405–420.

Hansen, T.H., de Bang, T.C., Laursen, K.H., Pedas, P., Husted, S. & Schjoerring, J.K. (2013) Multielement plant tissue analysis using ICP spectrometry. *Plant Mineral Nutrients: Methods and Protocols, Methods in Molecular Biology* (ed. by F. J. M. Maathuis). Springer, Berlin, Germany.

Janis, C.M. & Fortelius, M. (1988) On the means whereby mammals achieve increased functional durability of their dentitions, with special reference to limiting factors. *Biological Reviews*, **63**, 197–230.

Jürgens, N. (1996) Psammophorous plants and other adaptations to desert ecosystems with high incidence of sandstorms. *Feddes Repertorium*, **107**, 345–359.

Kuřavová, K., Hajduková, L. & Kočárek, P. (2014) Age-related mandible abrasion in the groundhopper *Tetrix tenuicornis* (Tetrigidae, Orthoptera). *Arthropod Structure & Development*, **43**, 187–192.

Lev-Yadun, S. (2007) Defensive functions of white coloration in coastal and dune plants. *Israel Journal of Plant Sciences*, **54**, 317–325.

LoPresti, E.F. (2016) Chemicals on plant surfaces as a heretofore unrecognized, but ecologically informative, class for investigations into plant defence. *Biological Reviews*, **91**, 1102–1117.

LoPresti, E.F. & Karban, R. (2016) Chewing sandpaper: grit, plant apparency, and plant defense in sand-entrapping plants. *Ecology*, **97**, 826–833.

Lucas, P.W., Casteren, A.V., Al-fadhalah, K., Almusallam, A.S., Henry, A.G., Michael, S. *et al.* (2014) The role of dust, grit and phytoliths in tooth wear. *Annales Zoologici Fennici*, **2450**, 143–152.

Mares, M.A., Ojeda, R.A., Borghi, C.E., Giannoni, S.M., Diaz, B., Braun, J.K. *et al.* (1997) How rodents overcome halophytic plant defenses. *Bioscience*, **47**, 699–704.

Massey, F. & Hartley, S. (2009) Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. *Journal of Animal Ecology*, **78**, 281–291.

Mendoza, M. & Palmqvist, P. (2008) Hypsodonty in ungulates: an adaptation for grass consumption or for foraging in open habitat? *Journal of Zoology*, **274**, 134–142.

Neinhuis, C., Muller-Doblies, U. & Muller-Doblies, D. (1996) Psammophora and other sand-coated plants from Southern Africa. *Feddes Repertorium*, **107**, 549–555.

Runham, N. (1962) Rate of replacement of the Molluscan radula. *Nature*, **194**, 992–993.

Wainhouse, D., Cross, D.J. & Howell, R.S. (1990) The role of lignin as a defence against the spruce bark beetle *Dendroctonus micans*: effect on larvae and adults. *Oecologia*, **85**, 257–265.

Whitehill, J.G.A., Henderson, H., Strong, W., Jaquish, B. & Bohlmann, J. (2016) Function of Sitka spruce stone cells as a physical defense against white pine weevil. *Plant, Cell & Environment*, **39**, 2545–2556.

Williams, S.H. & Kay, R.F. (2001) A comparative test of adaptive explanations for Hypsodonty in ungulates and rodents. *Journal of Mammalian Evolution*, **8**, 207–229.