Original Article

WASHBOARDING: ARE MAN-MANAGED HONEYBEES PERFORMING A VESTIGIAL ACTIVITY FOSTERED BY CRYPTOGAMS?

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Abstract

A common view is that honeybees are mostly managed by beekeepers for commercial purposes or as a hobby, especially in Europe. This misconception is probably due to the lack of systematic studies on wild colonies of honeybees in Europe in comparison to other regions of the world. Since we are used to considering this species as "domesticated", we may be induced to disregard activities not distinctly linked with colony survival, reproduction, or productivity. Washboarding is one of them in which the entire colony stops resource collection activities; numerous individual bees assemble on the front side of the hive and perform repetitive movements back and forth. They are curiously synchronised but apparently without a scope. In this exploratory work, we carried out a literature review of available, mostly grey, literature. Assuming this behaviour may be linked to cavity-nesting and to tree trunks or rocks being rough surfaces hosting various cryptogams, we performed preliminary observations and manipulative experiments. From our survey, we depict that washboarding is frequently reported in grey literature/beekeepers' reports, but rarely mentioned in scientific literature. Beekeepers who responded to a designed questionnaire observed this behaviour in various ecological situations, with no trend emerging. Our preliminary experiment of placing cryptogams in front of managed hives resulted in honeybees removing lichens (foliose types) or covering with propolis, and all species were affected. Further research is needed to clarify if lichens are removed because of their chemical compounds, because of hosting potentially toxic microorganisms, or collected as resources.

Keywords: *Apis mellifera*, bee behaviour, beekeepers, citizen science, lichens, online survey

INTRODUCTION

Cavity-nesting in a tree or rock crevices is how the honeybee *Apis mellifera* naturally sets up a colony. This is well documented, even from the rock paintings of prehistoric peoples (Crane, 2001). Oleksa et al. (2013) first assessed free-living honeybees in tree cavities in Europe and they recorded the frequency of feral colonies of honeybee inhabiting rural avenues in Poland. Density of feral colonies was lower than that of managed populations. Certainly, most colonies are man-managed, even if free-living ones are commonly reported; however, wild colonies (which never inhabited a beehive) or feral ones (possibly swarmed from a nearby apiary and settled free) are poorly investigated.

Following Oleksa et al. (2013), other authors focused on wild/feral colonies, carrying out

systematic studies on free-living colonies. Kohl & Rutschmann (2018) surveyed a beech forest in Germany estimating an average colony density of 0.13 colonies per square kilometre. Requier & Leonhardt (2020, and citations herein) mentioned trees as primary nesting sites of wild colonies, and Kohl et al. (2022) found that colonies were not confined to the forest edges (as commonly expected) but were also recorded deep inside the forests. Other monitoring also found colonies in anthropogenic environments. Browne et al. (2020) detected the largest number of free-living colonies (almost 70%) settled in cavities in buildings, and Rutschmann et al. (2022) found colonies adopting electric power poles as nesting sites. Monitoring free-living colonies is often performed thanks to the collaboration of beekeepers or citizens (Browne et al., 2020; Bila Dubaić et al., 2021).

Life-history traits connected to free-living colonies may help to disentangle washboarding, an activity frequently recorded and reported by beekeepers. While performing this behaviour, individual bees can be observed with their heads down, scraping the substrate with their mandibles while rocking back and forth in an almost synchronous way. Inferences among scientist and beekeepers date back to the end of 19th century. Alfonsus (1932) nicely summed up all available hypotheses (Tab. 1), describing the behaviour in great details and as a mechanical cleaning. The behaviour is frequently observed on the front of the hive, seldomly also on the inner walls. Seeley &

Table 1.

Hypotheses on reasons behind washboarding behaviour, based on scientific reports in the literature

Available hypotheses explaining washboarding, found in the literature at the end of the 19th -beginning of the 20th century, as cited in Alfonsus (1932)		
Mentioned Author(s)	Year of report	Explanation for washboarding
Dönhoff	1860	playing instinct
Schmid & Kleine	1861	playing instinct
Mehring	1866	wax lining
Kleine	1866	scraping to remove fungi and lichens
Buttel-Reepen	1915	playing instinct
Haidak	1929	scraping
Vahan	1930	petting something
Betts	1931	propelling the content of the honey-sac and exuding other substances
Alfonsus	1932	mechanical cleaning
Available hypotheses explaining washboarding, found in the literature 21st - 22nd century, as cited in Taulmann (2017)		
Author(s)	Year of report	Explanation for washboarding
Gary	1992	cleaning the surface around the hive entrance, removing any loose particles, parasites, fungi, or other debris
Bohrer and Pettis	2006	
Garvey	2012	keeping active during scarcity of food sources
Taulmann	2017	applying an attractive scent to the surface around the entrance, to help foragers to orientate

Morse (1976) inferred that areas of smoothed bark, up to 30 cm around the entrance holes of natural nests in hollow trees, could be the result of washboarding to facilitate nest defence and traffic flow. Walton & Toth (2016) employed washboarding as a behaviour to estimate individual worker variability, finding a paired correlation with another behaviour, the application and manipulation of wax. The authors also confirmed washboarding as possibly related to nest maintenance tasks but underlined the purpose still to be a mystery. Despite the diversity of reasons hypothesized for this behaviour, washboarding still lacks solid evidence for any particular explanation. This could be linked to the fact that so far washboarding has mostly been observed on box hives, structures made by man to facilitate bee manipulation, favouring hypotheses linked with artificial conditions. The sole reports on washboarding in natural conditions were by Taulman (2017), who directly observed and videorecorded washboarding at two different natural forest hives. He hypothesised that the behaviour could be related to the application of tarsal pheromone, an oily substance which may be rubbed on the surface.

After surveying all hypotheses, we detected that no author mentioned washboarding as a vestigial behaviour, originally put in place in such natural surfaces as tree barks and rocky surfaces. Bees may act in response to potentially self-restoring cues, which elicit the inconsistently observed response. This hypothesis does not fit with the mechanical polishing or with the application of pheromones. If the aim is smoothing the texture, once polished the work is done, no need to repeat it; if the aim is the application of a scent, that should be constantly resumed. Among the possible cues for a preliminary investigation, we decided to address cryptogams, and lichens in particular. Lichens are found on any surface, including bark, rock crevices and man-made surfaces of almost all textures. Moreover, lichens release undetachable spores, propagules and Volatile Organic Compounds (VOCs), still poorly studied, which may be the cue to which honeybees respond.

Lichens produce a great variety of secondary metabolites, mostly depsides and depsidones. Numerous secondary metabolites growth regulators and lichen acids are produced by the symbiotic microorganisms (Pankratov et al., 2017). Lichen secondary metabolites represent from 0.1-5.0% up to 25% of thallus dry weight (Fahselt, 1994; Romagni et al., 2004) and are mainly deposited on the surface of the hyphae. Out of over one-thousand secondary metabolites known to occur in lichens, only approximately 7% have also been found in nonlichenized fungi or in higher plants (Hauck et al., 2009; Beckett & Minibayeva, 2013). Secondary compounds have various biological activities with antibiotic, antimycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects (*e.g.* Triggiani et al., 2009; Bačkorová et al., 2012; Munzi et al., 2014; Goga et al., 2017). While many animals feed on lichens, including primates (Wang et al., 2019), lepidoptera (Hesbacher et al., 1995; Karunaratne et al., 2008), orbit mites (Syed & Seaward, 1984) and terrestrial gastropods (Baur et al., 1992), bees have never been mentioned in relation to lichens, but the interaction could either be positively (employing some compounds in bee products) or negatively (removing lichens as a response to mechanical disturbance or toxic volatiles) correlated.

We ran an exploratory study to 1) investigate the frequency and distribution of the behaviour through data mining, internet search and a tailored-designed questionnaire circulated among researchers and beekeepers; 2) verify the presence of lichens on hives; 3) test the responses to transplants of bark strips covered with lichens placed on the hives. Because the test was a simplified preliminary approach to verify if any kind of reaction could be detected, some weaknesses should be expected. For example, we decided to allow beekeepers themselves to assess behavioural changes in their hives, and we did not run a simultaneous control, not being able to constantly register all hives at once. However, in the present

study a possible bees-lichens relationship is investigated for the first time and a citizen science approach is combined with experimental manipulations to validate the interest of stakeholders and the feasibility of further analyses.

MATERIAL AND METHODS

Data mining

Scientific literature. The little existing scientific literature related to washboarding mainly is concentrated on descriptive reports and hypotheses formulation while missing direct testing. The only exception is a poster presentation at an international conference, where data on worker age, time of day and surface texture were presented (Bohrer & Pettis, 2006). However, only the abstract is available for interpretation of the results.

Grey literature. Sporadic observations of washboarding frequently appear in grey literature, *i.e.* beekeepers' association newsletters and journals, discussion forums, technical reports, YouTube videos and social media groups. We carried out a random Google search of the term "washboarding" and even applied Boolean operators adding "behavior", "behaviour", "bees". The most common results were a few webpages, as beekeeper blogs and/or newsletter, and YouTube videos. We surveyed eleven blogs and ten videos (Tab. S1, Suppl. Mat.).

To directly involve stakeholders, we designed an electronic questionnaire (https://it.surveymonkey.com/r/MNKHL5R) with multiple answer choices integrating the kind of comments recorded on the different web channels but structured to identify trends. It was translated into English, Italian and Portuguese and circulated through beekeeper associations and researchers in the field of apiculture, via email, websites, social media and printed copies. In total, we received seventy filled-out questionnaires; during the analyses, we excluded those which had never observed the behaviour. Even if some responses may be biased or inaccurate, the importance relies in the inclusion of stakeholders' perception for future more-detailed studies on a topic of such a broad interest.

YouTube turned out to be a good source of videos (n=277) with washboarding bees, mostly uploaded by professional or amateur beekeepers. We ordered videos by the number of visualisations, restricting our analyses to those with more than 5000 visualizations. We then listed and summarised the hypotheses made by authors of videos or people commenting on them. We separately reported and commented on a few more videos (n=14; Tab. S2, Suppl. Mat.).

Field records and experimental elicitation of washboarding behaviour

Visual inspection of hives. In a relatively small area of northern Italy, we involved local beekeepers in direct recordings and observations at various field sites in a range of about 15 km^2 (45°13'59.96"N; 11°27'56.8"E, north-east of Italy; Fig. S1, Suppl. Mat.). Beekeepers allowed us to make a visual inspection of their apiaries to check for the presence of cryptogams or other biological material at macroscopic level (Fig. 1a). We surveyed each individual hive with the help of a magnifier (2x) focusing on the external surfaces (the front and side bars and the landing board) of ninety-six hives located in private gardens, open agricultural fields or under the shade of trees on the margin of urban areas.

Lichen stripes. On some of the apiaries, we experimentally induced a bee response by applying lichen stripes. We planned a very simplified experimental manipulation, aimed at validating if the approach of considering lichen in connection with bees could justify future supplementary studies. These stripes were artificially composed through the addition of random samples of bark slices hosting common foliose and crustose lichens (Fig. 1b-c). Bark portions had been previously collected from tree trunks or branches close to apiaries located in the countryside of Portugal (lichens labelled with D) and in a hilly area of Italy, (lichens labelled with A) in April 2018 and May 2019, respectively. Lichen specimens were identified in the laboratory, air-dried and stored at room

Fig. 1. Observations and experiments on hives: a) visual inspection of hive surfaces; b-c) lichen transplants composed of bark stripes glued on satin ribbons. b) D-type sample, collected in Portugal in 2018 and c) A-type sample, collected in Italy in 2019; d) placement of transplants. Photo record: Davide Romani.

temperature until use. Lichen nomenclature follows the online database of Italian lichens ITALIC (Nimis, 2016).

In July 2019, one-month old and one-year old bark slices were glued on satin ribbons, labelled individually, and placed (10 samples of each A and D) on the front bar and on the landing board of the hives. Beekeepers' activity, mainly carried out on top of the hive, did not influence the lichen stripes. Considering results from the data mining and the interest shown by beekeepers, we decided to involve them in the experiment. Volunteer beekeepers were therefore in charge of reporting direct observations of bee reactions to the stripes once they have been placed, through direct communications and video-records. The lichen stripes remained in place for two months, and in September 2019, we removed the stripes from the hives and brought them to the laboratory.

Laboratory analyses

The stripes were later retrieved and analysed under the microscope in the laboratory. We evaluated differences from photographic records taken before the placement in the field, recording any removal of parts or morphological and physiological changes in the lichens. We employed the Fv/Fm ratio, a parameter of the chlorophyll *a* fluorescence, as a lichen vitality index (Munzi et al., 2019) to check the health status of lichens after the experimental exposure. All measures were taken on *X. parietina*, the most common species among our samples, at room temperature using a Plant Efficiency Analyzer Handy PEA (Hansatech Instruments LTD, UK).

RESULTS

Data mining

From websites, we got a poor overview notwithstanding we analysed 129 posts. Washboarding was observed randomly in Europe as well as USA, even in the subtropics (Tobago Island), apparently at any time of the day (morning, noon), in different climatic conditions (sunny or rainy days), and with opposite hive orientations (East, West). Some washboarding was reported as a behaviour performed by given colonies, while some were never observed performing it. Many beekeepers never observed this behaviour during their activity (up to 53 years), and no trend of any kind apparently emerged. This urged us to better define response options in the questionnaire, trying to confirm or reject this absence of a trend.

Only twenty-one out of fifty-six respondents observed washboarding personally. However, among those who observed the behaviour, two out of three beekeepers observed it more frequently than once (Fig. 2). Most of them

(89%) did not notice any extra-material on the surface of the hives. Washboarding most frequently occurred on the front bar and the landing board (11 and 9 cases, respectively) and also a few times outside the hive on a wall and on a tree trunk (three cases each). Without having any clue on the relative distribution of hives in different habitats, we can at least confirm that washboarding was recorded both in hilly and flat environments, and mainly during sunny conditions. Seasonally, it was observed most frequently between March and July (58% of records). The information about the time of occurrence is not conclusive but could be useful to consider for future studies.

Hypotheses made by beekeepers replying to YouTube videos did not vary greatly, and repetitions were frequent. In Fig. 3, we summarised and grouped sixty-eight different hypothesis found among 266 posts. In many posts, people inferred that this behaviour was somehow connected to a period of scarce resources that left the honeybees with "free time" (n=26). This concept is certainly related to an anthropocentric view that washboarding was recreational (even imagining line dancing/gym/yoga classes taken by numerous individuals at once), if not even useless. "Sweep the porch" (n=13) was similarly a common expectation, in which the behaviour was interpreted as spending time in an activity of poor priority, possibly cleaning the outdoor surface in the absence of more pressing duties. Among the anthropocentric hypotheses, the most unrealistic was inferring that this role was a punishment for improper behaviour possibly carried out inside the hive. Other hypotheses

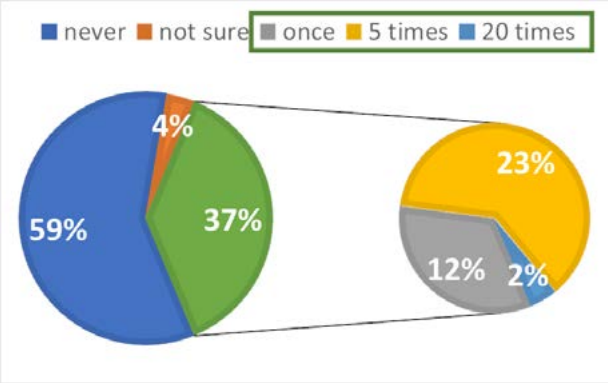

Fig. 2. Beekeepers responding to a questionnaire indicate the frequency of their observations of washboarding. On the right, details on the number of times that each respondent observed the washboarding behaviour.

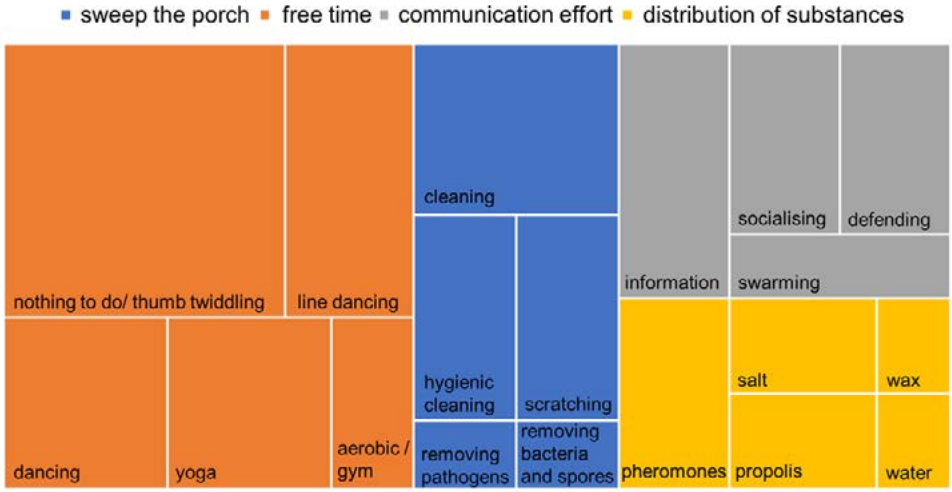

Fig. 3. Relative importance of possible explanations of washboarding, expressed as posts related to YouTube videos. They are grouped based on main themes.

showed a more scientific perspective. Some could be grouped as "communication effort" (n=12) and considered the possibility of washboarding being a way to transfer information among individuals and to increase defensive organisation or indicate a forthcoming swarming. It was also supposed to be a way to strengthen intraindividual relationships. Others referred to a careful "distribution of substances" (n=9): pheromones, salt, water, wax, propolis. About the latter, we found three more videos (lower visualizations than the ones we screened) in which a direct reference to a possible link between washboarding and propolis has been made (Tab. S2, videos nr. 8-10). Authors indicated they observed the same sequence of movements when bees were propolising and supposed washboarding could be mimicking propolis distribution.

Field records and experimental elicitation of washboarding behaviour

After careful visual inspection of ninety-six hives, none showed the presence of lichen colonization or other biological material. However, the possible presence of microscopic elements (e.g., spores or vegetative propagules) cannot be excluded.

All lichen species identified on the bark stripes belong to functional groups tolerant to medium-high nitrogen availability and medium-high solar radiation (Nimis, 2016). They were present in variable combinations on the bark slices (Tab. 2), and possibly with variable levels of

Table 2.

List of identified lichen species present on each bark stripe used during the field experiment

Lichens present (x) on bark stripes collected in Italy in May 2019										
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
Amandinea punctata		x						x	x	x
Caloplaca cerina						x				
Candelaria concolor	x	x	x	x	x	x	x	x	x	x
Hyperphyscia adglutinata	x	x	x	x	x	x	x	x	x	x
Lecania cyrtella			x		x		x			
Lecidella elaeochroma				x	x		x			
Phaeophyscia orbicularis	x		x		x	x		x		x
Physcia adscendens	x	x	x	x	x			x	x	x
Physcia aipolia										x
Physcia dubia						x				
Scoliciosporum sp.					x					
Xanthoria parietina	x	x	x	x	x	x	x	x	x	x
Lichens present (x) on bark stripes collected in Portugal in April 2018										
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Arthonia radiata		x								
Hyperphyscia adglutinata			x	x	x	x		x		
Lecanora chlarotera			x						x	
Lecidella elaeochroma		x								
Pertusaria sp.							x		x	
Physcia erumpens				x		x				x
Xanthoria parietina			x	x	x	x	x	x	x	x

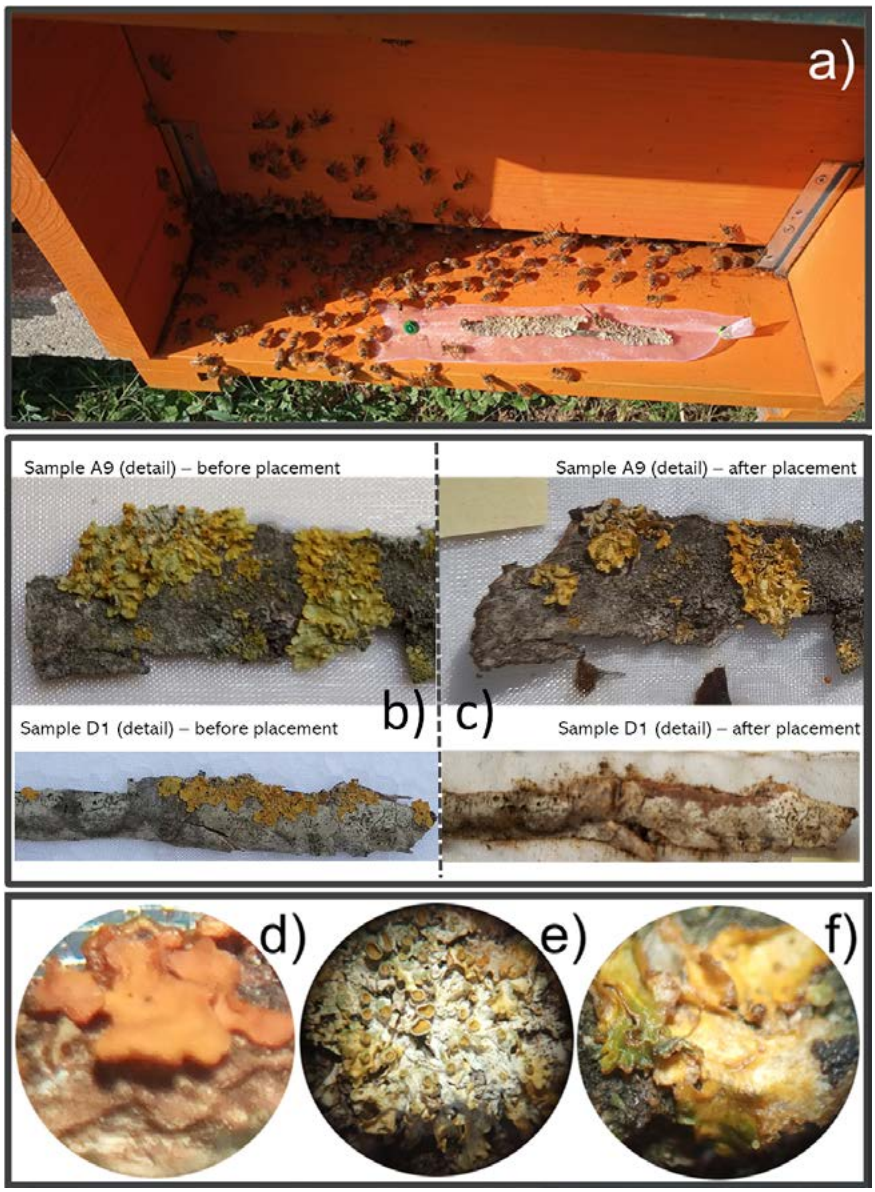

Fig. 4. Bee responses to lichen stripes transplant: a) washboarding behaviour; b) lichen stripe sample conditions before placing; c) same sample as in b), after two months of exposure in front of the hive: evidence of lichen removal; d-f) decoloration of those lichens present on the bark slice after two months of exposure.

vitality due to the two periods of drying treatments. The experimental placing of lichen transplants on the hives successfully elicited honeybee responses (Fig. 4a). We transplanted twenty lichen stripes, all in the morning. A beekeeper that could directly check five hives on the same afternoon, videorecorded washboarding bees on all of them (Fig. 4a; video S1). Other beekeepers reported to have observed washboarding after placing transplants. Since we could not remotely control all twenty hives, we cannot calculate the effective rate of response of colonies. However, honeybee response was assessed also through microscopic evaluation of the physical state of lichens. After two months of exposure on the hives, all lichens resulted in somehow being affected. Some thalli were mechanically removed, exposing the underlying bark (Fig. 4b-c). Foliose species, namely *X. parietina* and species belonging to the genus *Physcia*, were partially or totally removed from the substrate. Other thalli showed such morphological changes (Fig. 4d-f) as depigmentation or colour alteration. Very frequently, the surface of foliose and crustose lichens appeared covered with propolis. Out of all twenty samples, 77.2% had some lichens removed, 39.9% had discoloured lichens and 44.4% had traces of sprayed propolis. Physiological measurements, namely the Fv/Fm parameter, showed a decrease in the vitality of all samples, irrespective of location and time passed since the collection. However, they were not conclusive and did not allow us to distinguish between the effect of propolis, transplantation and exposure (Tab. S3) on thalli state.

Evidence of lichen-bee interactions out of videorecords

We can summarise the following:

- honeybees performed washboarding near nest entrance, on whatever surface (hives, occasional structures as a metal pot, tree trunks, rock cavities - Tab. S2, videos nr. 11-14);

- honeybees have been seldomly recorded while protruding their tongues directly on lichen thalli (Tab. S2, video nr. 14; photo records of beekeeper not shared), even distant from their hives;
- honeybees in hives with experimental manipulation started almost immediately washboarding behaviour directed towards the surface containing the lichen transplants (video S1).

DISCUSSION

The main aim of this study was to aggregate existing knowledge on washboarding. Descriptions originate from various sources, but this behaviour was rarely investigated scientifically. We found it first mentioned in 1860, and later washboarding was described and addressed only occasionally up to 2017. No experiments were performed, apart from those mentioned in the abstract of a conference. Washboarding, however, stimulate beekeepers' curiosity and hypotheses on reasons behind it. Many reports were found among beekeepers' blogs, associations' newsletters and videos uploaded to the YouTube platform. In agreement with our analyses (Fig. 3; Tab. S1 and S2), and with the responses to our questionnaire, the interest of this important group of stakeholders emerged and should be undertaken by the scientific community. As a preliminary step and hoping to promote interest, we performed an experimental manipulation of hives with the help of volunteer beekeepers. Our working hypothesis was that this behaviour, now performed on the clean surfaces of man-made hives, could be a vestigial trait linked to the presence of cryptogams on surfaces of natural nesting sites (tree trunks or rock crevices).

The absence of lichen colonization on the hives is not surprising, since hives are periodically subjected to maintenance and lichens are slow-growing organisms requiring years to reach relevant sizes. Washboarding has been observed most frequently outside but in some case on the inner surfaces of the hive. Although light conditions are not suitable for lichen colonization in hive interiors, we cannot exclude that lichen propagules (like spores and soredia) reach the inside walls. Most observations occurring on the outside surfaces match with the possibility of an organism, eliciting the behaviour, that needs light to grow. Moreover, we could also expect VOCs to penetrate the hives and elicit the behaviour: both lichens and/or microorganisms hosted by lichens could be responsible for their production.

The lichen stripes underwent a physical and chemical processing by honeybees. Foliose lichens were removed from the bark, possibly since they are anchored to the substrate through rhizines or hapteria (root-like structures). Occasional detachment and fragmentation of the thallus could have been caused by bees just walking on their surface. However, the total and precise removal of lichens from the substrate observed on some bark stripes is hardly compatible with accidental removal due to bees' passage or environmental conditions. When dead, lichens equally remain attached to the substrate for a long time in absence of a direct effect on the thalli, and no extreme meteorological events occurred during the transplant period. Crustose lichens did not undergo the same process, but their tight attachment to the substrate makes their mechanical removal impossible. Our experiment confirmed that washboarding causes lichens' manipulation in the proximity of beehives. However, what must be solved is whether lichens are removed for being unwanted guests or if they are collected as a nutrition source.

The two hypotheses also match with recent findings of lichens and their relationship with animals, insects included. Several papers recently investigated the relationship between invertebrates and lichens, used as hiding places, shelter or food (Karunaratne et al., 2008 and references therein). Numerous butterflies and moths showed different feeding habits and a certain specificity for lichen species (Pringle et al., 1994; Pöykkö, 2006). In the lichen association, the fungal symbiont is responsible for the production of a large range of metabolites, often unique to lichens. The presence of lichen compounds recorded in lichen-feeding

populations of butterflies (Karunaratne et al., 2002, 2008) suggested that invertebrates obtain protection against predators by assimilating lichen secondary compounds and using them as a chemical defence. Possibly, lichen substances may be included in the propolis, similar to many different substances assimilated from plants (Burdock, 1998; Anjum et al., 2019). Otherwise, such lichen compounds as usnic acid, vulpinic acid, and stictic acid, and lichen extracts were studied for toxicity and antifeedant activity against larvae of the polyphagous insect herbivore *Spodoptera littoralis* (Emmerich et al., 1993), adults of wheat weevil, *Sitophilus granarius* (Emsen et al., 2015) and other insects (Sachin et al., 2018). Toxicity may be the reason for our observations of propolis distributed on top of all lichen stripes which we had placed on hives.

To our knowledge no investigation linking lichens and bees exists. The above speculations are aimed at considering a newly emerging opportunity to investigate the disregarded relationship of bees and lichens and the intriguing washboarding behaviour.

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Supplementary Material

Link to the questionnaire used to collect information:

English: https://it.surveymonkey.com/r/MNKHL5R
Italian: https://it.surveymonkey.com/r/WZRRFLG
Portuguese: https://it.surveymonkey.com/r/FDSPRYH

Supplementary VIDEO S1: 14.JAS-66.2.Giovanetti-10.2478jas-2022-0016_video_S1.mp4

Original video recorded by the beekeeper Giorgio Casazza, on II July 2019,
the same day of stripes positioning.

Table S1.

Website list

	Website	Brief summary of content	Opinion/ info
1	https://beekeep.info/a-treatise-on-modern-honey-bee-management/the-basis-for-management/mysterious-behaviors-of-honey-bees/washboard-behavior/ Last accessed: 18 Jan. 2021	Site dedicated to informing beekeepers managed by The Apis Information Resource Center, developed by Dr. Malcolm T. Sanford, Professor Emeritus, University of Florida	intriguing behaviour; presentation of results of Bohrer, K., & Pettis, J.S.; hosting 2 videos and a link too a google page listing results for washboarding behaviour
2	https://honeybeesuite.com/washboarding-bees-arockin-and-alickin/ Last accessed: 18 Jan. 2021	Site dedicated to informing beekeepers and people managed by Sam Droege, USGS Patuxent Wildlife Research Center	Brief description in the blog, reporting results of Bohrer, K., & PettisJ.S.; hosting 1 video 22 replying posts
3	https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=7586 Last accessed: 18 Jan. 2021	Informative site of the Agriculture and Natural Resources, University of California Blog-Article by Kathy Keatley Garvey.	It would be interesting to find out; 14 replying posts
4	https://beekeepingforum.co.uk/showthread.php?t=33125 Last accessed: 18 Jan. 2021	UK Beekeeping Forum. Various posts.	reporting info of Bohrer, K., & Pettis, J.S.; 10 replying posts
5	https://www.beesource.com/forums/showthread.php?230659-Washboarding Last accessed: 18 Jan. 2021	Forum community dedicated to beekeeping, bee owners and enthusiasts. 6 discussions following a starter post.	reporting info of Bohrer, K., & Pettis, J.S.; 70 replying posts
6	http://iussi.confex.com/iussi/2006/techprogram/P2650.HTM Last accessed: 18 Jan. 2021	From the program of an International Conference (IUSSI 2006). Poster presentation related to washboarding behaviour by Bohrer, K., & Pettis, J.S.	The function of this behaviour remains to be elucidated, after experimental observations of worker age and surface texture.

7	http://www.myrmecos.net/2010/08/19/washboarding-bees/ Last accessed: 18 Jan. 2021	Informative website by Alex Wild. Posted video.	Description, no hypotheses; 13 replying posts
8	https://forum.honeyflow.com/t/theories-on-washboarding/9718 Last accessed: 18 Jan. 2021	Informative website by a private company. Posts following introduction of topic: Theories on Washboarding	Description, + hypotheses; 20 replying posts 1 video
9	http://www.keltronixinc.com/washboarding-bees/ Last accessed: 18 Jan. 2021	Informative website by a private company. Posts following introduction of topic: Washboarding Bees	Description, + videos, + hypotheses;
10	http://www.beeculture.com/ask-phil-november-2014/ Last accessed: 18 Jan. 2021	Bee Culture The Magazine of American Beekeeping. Article/post by Phil Craft, November 1, 2014	Responding to post
11	https://forum.canberrabees.com/t/bees-washboarding/200/2 Last accessed: 18 Jan. 2021	Site dedicated to informing beekeepers Posts following introduction of topic: Bees washboarding	Description, + videos; 2 replying posts

Table S2.

YouTube video list

	Link to Videos	Title / Author /date of post	Visualizations / comments
1	https://www.youtube.com/watch?v=lbwumXVTOz8	Bees Washboarding AlexWild73 20 Aug. 2010	45.313 / 24
2	https://www.youtube.com/watch?v=sxjc4tSKJFs&feature=emb_logo	Washboard Behavior Dr. James E. Tew 30 Aug. 2012	28.338 / 69
3	https://www.youtube.com/watch?v=tcJtJdke0AQ	Washboarding/Bees Don The Fat Bee Man 31 Aug. 2012	19.419 / 10
4	https://www.youtube.com/watch?v=0qncNbsh4_0&t=118s	Washboard Movement of Honey Bees (Example and Explanation) Honey Bee Honey 13 Nov. 2015	12.023 / 8
5	https://www.youtube.com/watch?reload=9&v=h6oaASApN-k4&feature=emb_logo	HONEY BEE Apis mellifera washboarding mystery solved? Dr. Tom Seeley is right. insidethehive.tv 18 Aug. 2019	9.871 / 37
6	https://www.youtube.com/watch?v=yUDBk9VTypc&t=149s	When Honey Bees Do The Washboarding Thing David Burns 4 Aug. 2016	8756 / 20
7	https://www.youtube.com/watch?v=DBZpa-6rs8A	Nectar Dearth, Wash Boarding Bees And Never Getting Poison Ivy Again Jason Chrisman 16 Aug. 2020	7.644 / 98
Link to Videos correlating washboarding to propolis distribution		**Title / Author /date of post**	**Visualizations / comments**
8	https://www.youtube.com/watch?v=tXWefJhw2rA&feature=youtu.be	Honeybee Propolis and Washboarding is there a connection? 18 bees 24 Aug. 2020	3.872 / 46

9	https://www.youtube.com/watch?v=y3gUqC694D4	Does bee washboarding on boxed hives mimic propolising in nature? 18 bees 10 Sept. 2020	2.774 / 27
10	https://www.youtube.com/watch?v=6NRjYNJEb6g	Bees Washboarding Jossamer Jedi 22 Nov. 2015	126 / 0
Link to other interesting Videos		**Title / Author /date of post**	**Visualizations / comments**
11	https://www.youtube.com/watch?v=EavTZt8ybDc	Wash boarding bees Justified Bee 7 May 2017	0/0
12	https://www.youtube.com/watch?v=1t8JyQTUmSs	Bees shimmering or washboarding John Haverson 17 Aug. 2016	282/0
13	https://www.youtube.com/watch?v=2WIeAd_ATko	Bee tree bees wash boarding today Hooterville Honey bees 22 Aug. 2020	22/1
14	https://www.youtube.com/watch?v=K6LJyVnqFYU&fbclid=IwAR2FsbjtCHHzZi16cOaXWijeQUnuWdhKhCuJOKRCQDqIsIZqZvc8tKs6JOY	Honeybees foraging on lichen? Very interesting honeybee behavior Swarmstead Bees & Gardening 22 Oct. 2020	362/13

Table S3.

Fv/Fm values (±SD; n=3) in *X. parietina* after exposure

Sample	Fv/Fm	Observations
D1	0.22±0.20	depigmentation, presence of propolis on the surface
D2	NA	absent
D3	0.22±0.39	depigmentation, presence of propolis on the surface
D4	0.16±0.17	depigmentation
D5	0.15±0.15	depigmentation
D6	0	depigmentation
D7	0.21±0.19	presence of propolis on the surface
D8	0.42±0.13	depigmentation
D9	0.42±0.37	depigmentation, presence of propolis on the surface
D10	0.15±0.13	depigmentation
A1	0.27±0.25	presence of propolis on the surface
A2	0.28±0.08	presence of propolis on the surface
A3	0.06±0.03	depigmentation
A4	0.16±0.13	presence of propolis on the surface
A5	0.39±0.06	presence of propolis on the surface
A6	0.41±0.05	no changes observed
A7	0.15±0.12	depigmentation
A8	0.32±0.29	depigmentation
A9	0.41±0.08	no changes observed
A10	NA	absent

Fig. S1. Study area, where apiaries under experimental manipulation were placed, 45°13′59.96″N; 11°27′56.8″E, north-east of Italy.

Maps credits: northern Italy at https://d-maps.com/carte.php?num_car=5880&lang=it; detail of the study area at https://www.google.it/maps (Immagini ©2022, TerraMetrics, Dati cartografici ©2022 Google)