Using insect sniffing devices for detection

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Emerging information about the ability of insects to detect and associatively learn has revealed that they could be used within chemical detection systems. Such systems have been developed around free-moving insects, such as honey bees. Alternatively, behavioral changes of contained insects can be interpreted by sampling air pumped over their olfactory organs. These organisms are highly sensitive, flexible, portable and cheap to reproduce, and it is easy to condition them to detect target odorants. However, insect-sensing systems are not widely studied or accepted as proven biological sensors. Further studies are needed to examine additional insect species and to develop better methods of using their olfactory system for detecting odorants of interest.

Introduction

The first studies to use arthropod and arachnid organisms as detectors were conducted by the United States Army in 1963 [1]. These studies attempted to use innate behavioral responses of certain invertebrates in order to detect the presence of hidden enemy personnel. Invertebrates such as ticks, mosquitoes, lice and cone-nosed bugs initiate a behavioral response to stimuli (e.g. changes in CO_2 or lactic acid concentration) that indicate the proximity of hosts (e.g. enemy personnel). These stimuli and predictable responses are pre-wired in the target organism and are necessary for their survival. Therefore, they exhibit behaviors that are explicit for locating and tracking hosts, such as detection of the presence of a hidden person. The initial results of the United States Army study were somewhat unspectacular; however, a subsequently tested arthropod-based detector reliably detected human presence when it was left stationary, and the response from this insect 'sentinel' was used to detect intrusion.

Insect learning has been studied extensively in relation to deciphering and predicting foraging behavior. However, it was not examined under the premise of developing volatile-sensing systems until the mid 1990s, when the United States Department of Defense (USDOD) initiated a substantial program to use live organisms for chemical detection. Some of the insects investigated include the parasitic wasp *Microplitis croceipes* C., the hawkmoth, *Manduca sexta* L., and the honey bee *Apis melifera* L. These studies aimed to determine the ability of these insects to learn specific odors related to explosives and toxins. This could potentially provide a method of alert when these odors were encountered in the environment. In addition, detection devices using honey bees and parasitic wasps were being developed during the time this research program was initiated. Since the USDOD program ended in 2006, research and development of insect-sniffing systems has continued in a limited number of laboratories around the world. Most notable are studies with honey bees at Inscentinel Ltd (http://www.inscentinel.com/), Montana State University and University of Montana [2,3] and Los Alamos Laboratories [4], as well as with parasitic wasps at the University of Georgia [5–7].

Associative learning in insects

Our knowledge of the ability of insects to learn and remember originated with Nobel Prize winning scientist Karl von Frisch and his work with the honey bee *A. melifera* [8]. Although these animals usually learn in their natural environment, they can also be trained to learn and remember odors through Pavlovian conditioning. A previously unrecognized volatile chemical compound (i.e. the conditioned stimulus) can be associated with food or another resource (i.e. the unconditioned stimulus) through a conditioning regime that is a form of associative learning (Box 1). Consequently, the animal learns to recognize an odor as an opportunity to locate food and will exhibit a behavioral response (i.e. the conditioned response) when the odor is detected. Controlling the physiological state of the animal further enhances this conditioning. A stronger link between the food and the odor is made if the animal is starved before being conditioned [9].

In some insect species, there is the potential to also condition the animals to other resources. The parasitic wasp *M. croceipes* has been conditioned to link an odor with its larval host [10]. In this case, the conditioned stimulus is an odor, but the unconditioned stimulus is the host (i.e. moth larvae) feces (also known as frass), and the conditioned response is depositing an egg in the host (ovipositing). Wasps conditioned to associate one odor (odor 1) to food can also be conditioned to associate another odor (odor 2) to its host. Subsequently, when the wasp is exposed to one of the odors, it will exhibit a resource-dependent behavior that can indicate which odor it is detecting. In the case of *M. croceipes*, this discovery indicates that a wasp exhibits food-searching behavior only to the odor linked with food. If it exhibits host-searching

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Box 1. Associative learning by Pavlovian conditioning – using the parasitic wasp *M. croceipes* as an example

Pavlovian conditioning, also known as classical conditioning, is a form of associative learning. Initially, an animal, such as a parasitic wasp, will exhibit an unconditioned response (UR) to an unconditioned stimulus (US). In this case, food is the unconditioned stimulus and food-searching is the unconditioned response. (Figure Ia) Parasitic wasps search for a potential food source in response to food odors. Smell and taste sensors in the antennae help to guide the insect to the food resource. The wasp will not perform the unconditioned response to a stimulus that is not associated with food. (Figure Ib) The conditioned stimulus is an odor that is not related to the wasp's natural foraging environment. Pavlovian conditioning is accomplished by presenting the odor (i.e. the conditioned stimulus [CS]) and then the food (i.e. the unconditioned stimulus [US]) to the wasp for 10 s and then providing a 1–2 min break and repeating this regime 2–4 more times. (Figure Ic) The wasp will then associate the odor with food and, when presented with the odor alone, exhibit the food-searching behavior (i.e. the conditioned response). The searching behavior is the same as it was before conditioning, but now it is a response to the odor (i.e. the conditioned stimulus), not to food. (Figure Id) The conditioning procedure will vary according to the animal being conditioned.

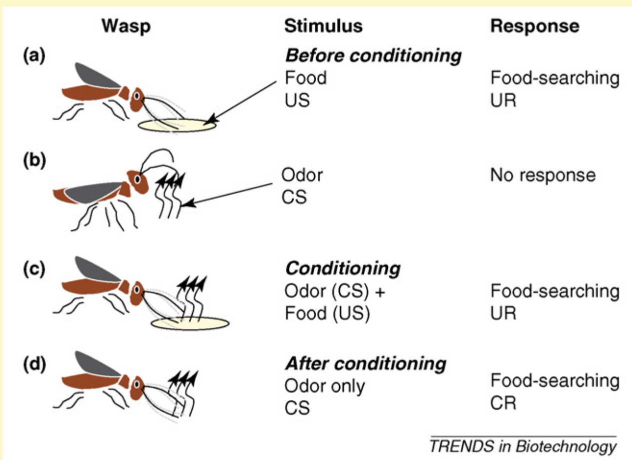

Figure I. Associative learning by Pavlovian conditioning – using the parasitic wasp *M. croceipes* as an example.

behavior, it has detected odor 2, which was associatively linked with host. Researchers have conditioned these wasps to detect odors associated with food toxins [11], explosives [12] and plant odors [5].

Several studies have also examined factors that affect insect learning and memory, such as molecular structure of the odor [13], blocking and overshadowing effects [14], and the ability to detect salient odors in a binary blend [15] or in an odor complex [16]. It is extremely important to understand how conditioning to individual compounds leads to generalized behavior in response to similar compounds and how conditioning to complex mixtures affects recognition and inhibition of individual components of the odor blend. These studies are primarily focused on understanding the relationships between the bioprocesses that make it possible for insects to navigate their environment using olfaction. However, they are also useful in the development of insect-sniffing sensors.

Learning and memory are also dependent on the particular insect being studied. Different insect species can live for years or days, and memory retention and learning limits are different for each species. Conditioned *M. croceipis* live for ~2–3 weeks as adults, and they can remember odors for at least 48 h without food [17]. When provided with food, the wasp will associatively learn the current odors it is encountering while feeding, and its association with other odors is diminished. By contrast, the adult honey bee lives for ~1–4 months and can retain learned odors for several days or, with multiple conditioning trials of the target odor, for up to its whole life [18,19].

Methods of using insects as 'sniffer' systems

Insects can be used as either free-moving or restrained organisms to detect chemicals. Free-moving insects are allowed to move towards odor sources without being constrained in a device and are tracked during flight or after arrival to the odor source. Tracking methods enable the locations of the organisms to be known. Electronic and video interrogation in an enclosed environment is used to monitor the behavioral or reflexive responses of restrained organisms.

Free-moving detectors

Insects conditioned to detect specific chemicals or mixtures will typically orient and move towards the odor source when they are within its range. In some instances, the insects might come across the target odor during general foraging and interrogate a specific location. In such a scenario, there must be a method for detecting the trajectory or location of the target animal as it moves towards and forages at the chemical source. Honey bees have been conditioned to associate volatile compounds from land mines with food and are tracked using 'light detection and ranging' (LIDAR) measurements [3]. LIDAR is a technique that measures the properties of scattered light to determine the distance to the object that scatters the light. Further studies enhanced the LIDAR detection of free-flying honey bees by measuring the back-scattered return signal caused by the wing-beat modulation [2,20]. Honey bees then are tracked using light scattered by their unique wing-beat frequency as they search for food; however, owing to their associative learning experience, they locate land mines instead of food resources within their foraging range. Theoretically, the beat frequency of any insect could be tracked using this LIDAR method. Other methods of tracking have been used for general entomological studies of insect migration. Two of the most promising methods to track conditioned insects once they have located the source of the odorant are harmonic radar and radio telemetry. Harmonic radar involves the placement of a transponder on the insect and is a promising tracking technique because the transponder consists of an antennae and electronics that use the incoming radar signal as an energy source. The transponder then re-emits a signal that is a harmonic of the incoming signal. Consequently, it does not require a battery on the insect, thus reducing the weight being carried. However, it is difficult to track insect movement in some environments that can disrupt the transponder signal, such as areas with heavy vegetation. Radio telemetry requires that a radio transmitter is mounted on the insect, which is then tracked with a receiver. Currently, harmonic radar detection is limited to insect species that are large enough to carry the transponder, such as honey

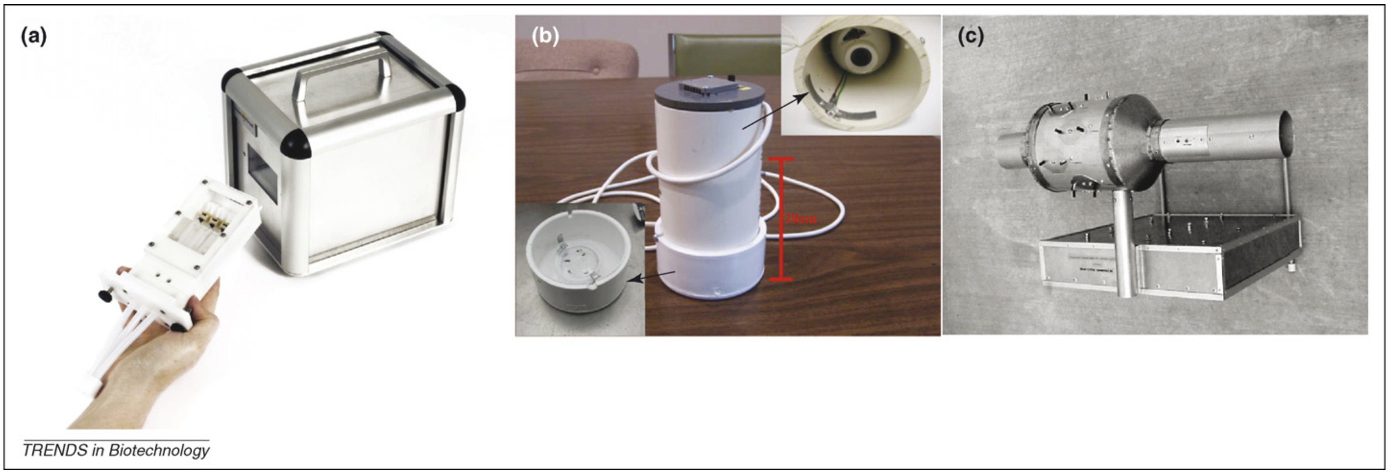

Figure 1. Bee, wasp and hawkmoth chemical detectors. **(a)** A device using three honey bees that are held in place, with their proboscises allowed to extend. An optical device is used to measure the extension of the proboscis as a chemical they were trained to detect passes over their antennae. Photograph courtesy of Rothamsted Research. **(b)** Wasp Hound chemical detection device. The Wasp Hound is 20 cm in length and 7.5 cm in diameter. A cartridge contains five conditioned wasps used for the detection process. A web camera is located inside the top of the Wasp Hound and captures insect behavior. Photograph courtesy of G.C. Rains. **(c)** A device to detect chemicals using the Hawkmoth contains ten moths, with electrodes used to measure changes in voltage. Five moths are trained to detect a target odor, and the other five moths are used as a control to account for false-positive readings. Voltage change is initiated by movement of the feeding muscles of the moth. Hawkmoth detector reproduced with permission from [22], © 2004 IEEE.

bees, bumble bees and moths [21]. The usability of other available methods (e.g. Doppler radar and fluorescent dyes) for the tracking of free-moving insects is limited by a lack of specificity or low resolution when a small number of insects are followed.

Restrained organisms

The behavioral response of insects to a conditioned stimulus can also be observed in insects that are restrained within a detection device. In the UK, the commercial enterprise Inscentinel Ltd uses a detection device in which three honey bees are held in cassettes, with the head of each bee protruding so that the proboscis is easily observed (Figure 1a) (http://www.inscentinel.com/). Air samples are brought into the device and passed over the bee antennae, and a vision system measures the proboscis extension response. This response is essentially an extension of the bee's tongue and can be measured visually or electronically. This device has reportedly been successful in detecting chemicals associated with security, medical diagnoses and food safety (http://www.inscentinel.com/).

Our laboratory has focused on the parasitic wasp *M. croceipes*. We have demonstrated that this species can be easily conditioned. It is extremely flexible in its ability to sense different compounds, with sensitivities of 10^{-7} mol/liter for some odors [5]. In one study, *M. croceipes* also distinguished different isomers of six-carbon alcohols on the basis of the position of the alcoholic group, and they were also able to distinguish between 1-hexanol and 1-hexanal [13].

We have also developed a portable device to present air samples to conditioned wasps and to interpret the food-searching behavior of five conditioned wasps held in a cartridge (Figure 1b) [6]. Video footage of the wasp behavior is recorded remotely in a laptop computer. A user-developed software program analyzes the video of wasps to determine when they have detected a chemical that they were conditioned to recognize [7]. A result can be determined within 20–30 s after the wasps are exposed to the air sample. We dubbed this device the 'Wasp Hound®' to illustrate its connection conditioned animals used to sense chemicals. With the Wasp Hound, we were able to detect the presence of 3-octanone in some corn samples used as feed for livestock at parts-per-million levels [6].

A different device has been developed to hold 10 noctuid hawkmoths. With this device, the feeding response of each moth is measured by electromyography [22]. Moths were trained to detect cyclohexanone through classical conditioning, and a voltmeter is used to detect spikes in the signal from the feeding muscles of the moth. Five moths were conditioned to the target chemical, and five were used as an unconditioned control to determine if responses from the conditioned moths were false positives. The device showed promise for the detection of chemicals associated with explosives, but its size (17 kg and 0.25 m^3) renders it less portable (Figure 1c). Miniaturization of the electronic components could result in a smaller sensor.

Why use insects?

There are several advantages of using insects as sensing devices, in both the restrained and the unrestrained approaches. Insects can be conditioned with impressive speed for a specific chemical-detection task. Moths and wasps can be conditioned in minutes and then used in purpose-built detectors, whereas free-flying honey bees are able to detect explosives only after two days of conditioning. This efficient conditioning regime allows for these sensors to be produced on demand as needs arise.

Moreover, insects are very sensitive and have been demonstrated to detect chemicals within a complex chemical background, which can be difficult for electronic noses (i.e. sensor) currently in production. When the detection limits of *M. croceipes* were compared with that of an electronic nose, wasps were found to be almost 10-fold more sensitive to several chemicals [5]. To our knowledge, there are no comparative studies between insects and canines. Such studies would be of great interest because they could potentially increase the level of acceptance for insect sniffers in many applications. Given that insects are viewed as being more expendable, they might be a preferred sensing method in scenarios (e.g. toxic or physically unstable environments) that are deemed too dangerous to use highly trained canines. In addition, the demand for canines is high, and insect detectors could fill some of this demand in the future.

A further advantage of using insects is their low cost. Maintaining a wasp or honey bee colony can cost 'pennies' per insect, and no special skills to train the insect to target odors is required. The main investment costs are the hardware and software that are required to interface with the insects; however, these can be minimal if off-the-shelf components are used. Furthermore, once the equipment has been purchased, backups are only needed in case of malfunction or damage.

There are numerous potential application areas in which insect-sniffing systems could be used. Some public programs involved with anti-terrorism, land mines and national security already investigate the use of insects for the detection of explosives [4]. The sensing of chemicals associated with food safety, agriculture, and forensic investigations has also been investigated in our own research programs [5,6,11,12].

It appears that restrained insects are more easily adaptable than are free-moving insects to indoor applications, in which a specific sample needs to be detected and no searching for samples is required. Generally speaking, a restrained detection system is not suitable to search for samples, although the potential for this application will be discussed below. By contrast, free-flying insects obviously are best suited to foraging in natural environments, because parameters present in artificial environments, such as lighting, windows and colors, can disrupt their foraging acuity.

Potential pitfalls

The use of any biological organism raises the potential for variability in sensitivity and response to target compounds, which can reduce the reliability of detection. Genetic differences can furthermore attribute to variations. Each individual insect has a given probability that its olfactory detection and/or behavioral response is genetically different to another insect, leading to variability in response to similar stimuli. Conditioning variability can also cause differences in the strength of the associative conditioning. Conditioning relies on exposure to the unconditioned stimulus, followed by the conditioned stimulus for a specified period of conditioning. These training regimes are commonly repeated in three to six sessions, with a set time-interval between conditioning. Variation in any of these important conditioning parameters, such as length of conditioning, number of conditioning sessions, and interval between the condition assays, affects the conditioned response to the conditioned stimulus, and hence the response of the insect to the stimulus [17].

The physiological status of the insect also has a major impact on the variability in response. Conditioning to food is usually accomplished when the insect has been starved for a set period of time, thereby improving the link to the conditioned stimulus. In parasitic wasps, the strength of the conditioned response is different if the wasp has been conditioned after not having been fed for 24 h or for 48 h [17]. It has been found that the longer the starvation period, the better the resulting associative learning. Variable environmental conditions in which the conditioning and testing is carried out can also contribute to a reduction in detection robustness. Further research is needed to determine the effect of changes in humidity, temperature, time of day, lighting and other parameters on associative learning. Furthermore, background odors mixed with the target odor also have an effect on the level of sensitivity and whether the insect is able to detect the conditioned stimulus. In addition, temperature and humidity affect insect physiology as well as the characteristics of the odor.

Although many basic tenets of learning, memory and perception are known and applicable across orders of arthropods, any new species that might be used as a chemical sensor must undergo behavioral studies to determine physiological limits, innate behavioral responses, basic memory and learning factors associated with the type of sniffing system to be developed. Standardized procedures to evaluate the sensory capabilities across different insects would also be useful when comparing the odor detection abilities of different insect species. Current conditioning and testing protocols are unique to each laboratory in which they are conducted.

Future perspectives

Carefully controlling current conditioning and testing protocols can address most if not all of the physiological and environmental issues that arise in insect-sniffer systems. However, if this technology is to advance beyond an interesting and novel approach to chemical detection, studies that might help in bringing the advantages of insect sensing to mainstream applications must be examined. For example, canines that detect arson or cadaver are often placed in dangerous conditions, either through exposure to toxic chemicals or unstable structures. Development of alternative sensing systems using insects would be advantageous because the loss of an insect sensor would be less expensive than losing a trained canine.

Mathematical algorithms for behavior modeling

Mathematical models could enable us to better understand and interpret the insect response to chemical stimuli; and if credible models could be developed, we might develop indices that correlate strongly to the concentration levels of chemical stimuli. Several studies have focused on quantitatively modeling insect behavior using 'forward models', which predict future insect movements based on the current status of the chosen variables and control signals. Forward models are used in scientific studies to explain motor control, context-dependent action, and cognition [23]. These models are based on the current state and control parameters that predict the future state of a system. By contrast, neural network models, which adapt their mathematical and parameter structures based on the patterns of input and output data of the system, can be more effective in capturing the associative learning of odors by the insects [24–26]. A locomotor activity portrait of the fruit fly, *Drosophila melanogaster* M., was con-

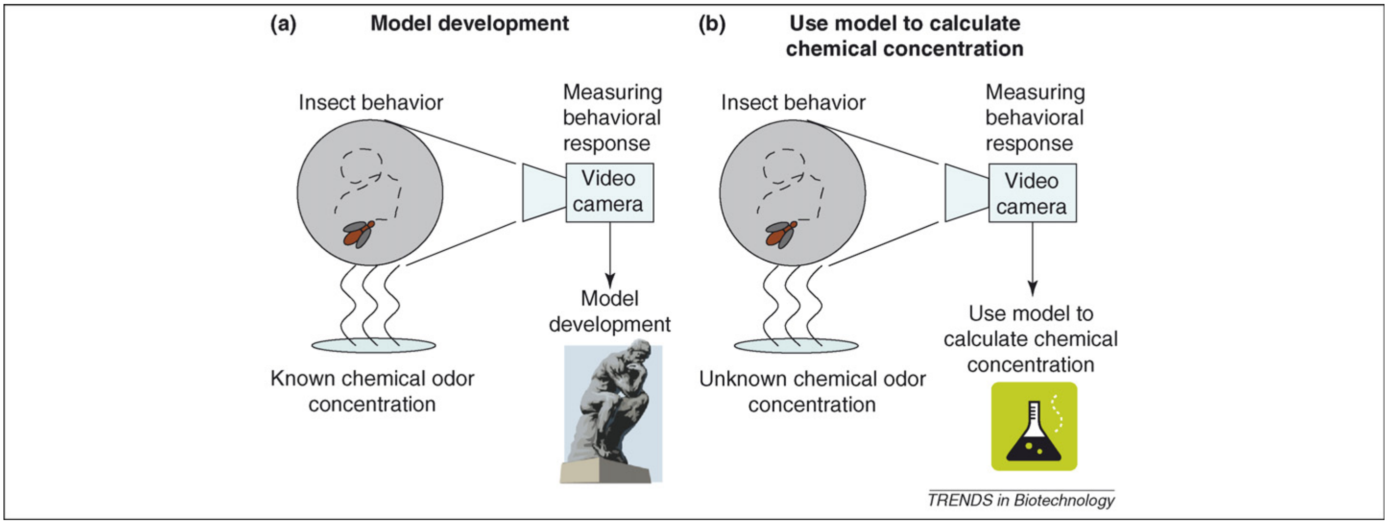

Figure 2. Development and use of behavioral algorithms with conditioned insects. **(a)** After an insect is conditioned to a chemical odor at a known concentration, the behavior of that insect to a different concentration is then measured by an optical system. Several insects are conditioned and tested over a range of chemical odor concentrations to develop mathematical algorithms of behaviors that are able to predict chemical concentration. **(b)** Once algorithms have been developed over a sufficiently robust array of conditions, such as a range of chemical odor concentrations and varying levels of potential background odors, they can be used to predict the characteristics of the chemical stimuli by tracking the behavior of the insect and feeding such behavior into the behavioral algorithms.

structed by a video-tracking device similar to that in the Wasp Hound, but without the odor stimulation. This device recorded multiple parameters, such as the total distance moved, the number and duration of episodes of activity and inactivity, and the mean walking speed [27]. The locomotive variables such as the radial distance moved by the insect and rotational angle around the center can be measured from the video images of behavioral responses after conditioning to a specific chemical odor; and these variables can then be modeled as stochastic (i.e. random) processes or stochastic neural networks. Once a credible model has been developed, the odor concentrations can then be predicted directly from the video images for unknown levels of chemical stimuli (Figure 2). Given that such a model would be able to determine concentration levels, a detector could be developed that guides a user towards the source of the chemical stimuli; for example, along the path that has the steepest concentration level gradient. Such a detector could also be placed on a robotic device that would use the model outputs based on insect behavior as feedback to move towards the source. Once a model is experimentally calibrated to specific odors, the sensors could also be used to detect concentration levels of odors that are correlated to toxin levels in foods, such as aflatoxin in peanuts, corn and milk.

Electro–bio interface

Quantitative and qualitative detection of chemical stimuli would be improved if signals from within the nervous system of the insect could be used. Behaviors are the result of signals that originate in the insect 'brain' as it interprets signals from the antennal lobe. Consequently, monitoring neuronal signals within the insect would provide a more direct and accurate measurement of the response to chemical stimuli. It has been shown that conditioning modulates the spatial and temporal changes in the antennal lobe and that these changes are positively correlated with behavioral responses in *M. sexta* moths [28]. As electronics continue to miniaturize and nanotechnology develops, mobile interfacing with odorant receptors, brain neurons, and other parts of the insect physiology opens up the potential to measure the response to stimuli without artificially inhibiting the insect response. Furthermore, it enables us to control insect movement, thereby creating a biological robot capable of sensing odors and moving to sampling locations that are difficult to breech with canines or other sensors. Such a robot was developed using insect behavior of a moth to model the control algorithms that tracks an odor plume [29]. In addition, a handheld insect antennal array of four insect antennae was developed to track odor plumes to their source. This device was guided by changes in auditory pitch interpreted by an operator moving the device over the ground [30].

Genetic selection

Using multiple insects as detectors in two current insect-sniffing devices reduces the effect of naturally occurring genetic variability (http://www.inscentinel.com/) [6]. Free-flying insect detection systems rely on a large number of organisms converging on a target location. Therefore, if some insects are genetically 'slow learners', or are not well conditioned, others in the arena still respond. There have not been any published attempts to increase levels of detection sensitivity through selective breeding. However, it stands to reason that those insects that respond the best to conditioning as measured by parameters associated with the conditioned response (e.g. length of time responding to a conditioned stimulus) can be selected for breeding the next generation of sniffer insects. Insect genetic lines can be selected for their ability to pick out an odor in difficult backgrounds (selectivity), or for their ability to detect odors in low concentrations (sensitivity), and furthermore for their memory and learning capabilities. By continually eliminating poor performers, certain behavioral traits in the genetic lines can then be enhanced and used to generate exceptional detection sensors.

Insects for specialized applications

There are millions of species of insects, and many of these might already be tuned through evolutionary selection to detect chemicals that would fit a specific desired application. For example, fungi-eating beetles should in theory be easily conditioned and sensitized to pathogenic fungi that grow on food. The hide beetle, *Dermestes maculatus* D., which colonizes corpses, is an attractive candidate for investigations and scientific studies to find clandestine gravesites without the need for associative conditioning. Female hide beetles are naturally attracted to animal remains in an advanced stage of decomposition, and thus exhibit specific behaviors when they encounter chemicals produced by decayed bodies. Blowflies are another group of insects that are naturally associated with decomposing human remains. Currently, it is suspected that they are generalists in terms of what they will colonize; however, this tenet might not be true. When developing from egg to adult on a specific carcass species, the adult blowflies might be attracted to those odors encountered during their development. Their colonization of human remains could potentially pass the chemical attractiveness to their maggot offspring and then to the adult flies, which as a consequence would seek out human remains for colonization. Another potentially very useful example includes the early detection of forest fires. The jewel beetle *Melanophila acuminate* D. is able to detect smoldering wood from a distance of several miles, owing to infrared sensory organs on its antennae and thorax, and this could be exploited for the detection of forest fires before they erupt [31,32].

Conclusions

Insect-sniffers are potentially useful tools for the quantitative and qualitative analysis of odors in medical, forensic, and food safety applications. Harnessing the sensitivity of insects has been accomplished through the development of chemical detection devices such as the Wasp Hound, which monitors the behavioral response of classically conditioned parasitic wasps to air samples. Changes in the behavior of the wasps indicate a detection of the chemical that they were conditioned to detect. Honey bees have also been conditioned to detect chemicals and respond both in a contained system, such as that used by Inscentinel, Inc., and in a free-flying system tracked by LIDAR. Detection of explosives has been proven with each of these systems, and other applications have also been demonstrated, such as the detection of aflatoxin in corn samples by the Wasp Hound. Other potential applications include fire detection, medical diagnoses, aflatoxin detection in peanuts, grains and milk, and detection of illegal drugs, arson and cadavers. Future detection systems might be developed around other insect species that have innate responses to chemical targets, or that have been selectively reared to have enhanced response to chemicals of interest. In addition, mathematical modeling of behavior might lead to methods that are able to identify the proximity or the concentration of a chemical. Further developments comprising the selection of higher-performing genetic lines within an insect species, such as the parasitic wasp or honey bee, could enhance reliability and sensitivity to specific odors. Therefore, further scientific and engineering research is needed to enhance the utility of insect-sniffer detection systems and, furthermore, to increase the level of acceptance from a larger community of research and commercial enterprises. Once this technology has proven its ability to perform in a real-world commercial application, there should be a coincidental increase in interest to develop insect sensors for other attractive applications.

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