VOLATILES THAT SOUND BAD: SONIFICATION OF DEFENSIVE CHEMICAL SIGNALS FROM INSECTS AGAINST INSECTS

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ABSTRACT

Defensive chemicals such as volatiles are essential for many insects against the attack of predatory insects, but in the research domain of chemical ecology there remains a need to better understand how intrinsic physicochemical constants of volatiles determine the intra- and interspecific diversification of such compounds produced by prey insects, knowing that many predatory insects primarily rely on chemical cues during foraging. To apprehend and explore the diversity of emitted chemicals as related to the receiver's perception, here we aim to transform chemical into acoustic signals by a process of sonification, because odours and sounds are similarly perceived in their spatiotemporal dynamics. Since insects often emit a complex mixture of repellents, we prototyped a sonification software to process physicochemical parameters of individual molecules, prior mixing these sonified data by following the chemical profile of specific insect defensive secretions. In a proof of concept, the repellence of insectivorous ants towards single chemicals was compared with the repulsive response of humans towards the auditorily translated signals. Expected outreaches of our ongoing project called 'SonifChem' are, among others, to explore the repulsive and even the attractive bioactivities of chemicals emanating from any (biological) source.

1. INTRODUCTION

Insects occupy a central position in the research area of chemical ecology. They store from their food, recycle and/or autogenously produce bioactive chemicals playing essential intra- and interspecific roles [1]. Under the nearly permanent risk of being killed by predators, they developed during evolution defence strategies that often involve defensive allomones such as volatiles [1], [2]. Volatiles have a repellent effect in that they keep an organism from the chemical source in the absence of contact [3], thus they hinder a predator's approach. However, volatility in its own right is a disadvantage because evaporating volatiles are lost and they must be produced again (regardless of the real risk incurred). We may expect from such benefits and costs that defensive volatiles are an adaptive trait and, as such, an integral part of the defence strategy that also includes the morphology, physiology, and behaviour of the insect, and that is shaped by evolutionary processes.

The bioactivity of chemicals is tested in a bioassay, which is the testing of a (chemical) stimulus on a living organism or part of its sensory system. Studies based on quantitative structure-activity relationship (QSAR) indicate a correlation between detection and pungency thresholds of (single) volatiles acting on humans [4]-[6], but the field of QSAR of complex mixtures remains under development mainly because descriptors used so far are applicable only to binary mixtures [7]. In the chemical ecology of volatiles emitted by insects, moreover, a majority of research on the evolution of chemically-based defensive strategies focus on the visual signalling of the defence rather than on its chemistry [8], [9]. For plant-feeding insects that sequester deleterious plant metabolites for their own defence, the diversity of defensive chemicals of the insect can reflect the one of plant secondary metabolites [10]. For autogenously-produced chemicals the question remains, however, which factors determine their intra- and interspecific diversification.

2. OBJECTIVES

Here, we launched a research exemplarily based on phytophagous insects in which there is evidence that the biosynthesis of defensive allomones is at least partly unrelated to plant chemistry. But the novelty of our approach lies in the perception of allomones. We aim to offer researchers a new experimental paradigm to perceive and explore predator-prey interactions by approximating the real (insect) world. Our approach complements classical bioassays of testing volatiles on a potential predator in that the repellence of volatiles is modelled through a non-chemical sensory canal and by which this bioactivity can be experienced by, and tested on, humans (see also section 4).

Our objective is to transform chemical into acoustic signals by a process of 'sonification' that is the use of non-speech audio to convey information or percept data [11], in order to predict the repellence of volatiles. This technique of rendering sound in response to data and interactions through an auditory display is used today in various domains such as seismology, climatology, neurophysiology, medical diagnostics, sport, etc. [12]. Thus sonification is used (besides in art and entertainment) in warning, monitoring, and/or to explore data [11]. It has been applied on chemicals such as DNA and proteins [13], but, as far as we know, never in order to scientifically study evaporating volatiles, a fortiori those emitted and perceived by insects. Yet emitted volatiles reach the olfactory system quite similarly to emitted air vibrations that reach the auditory system. The two types of stimuli share features in the perception of their spatiotemporal dynamics, and this may explain that at least in perfumery analogies are often made between odours and (musical) sounds. The novelty of our approach may be explained by the relatively new applications found for the sonification process itself.

3. STUDIED MATERIAL

The insects studied here belong to the sawfly subfamily Nematinae (Hymenoptera, Tenthredinidae). Their larvae feed on plants, generally living freely on leaves, so that they are easily preyed upon by insectivorous predators which belong to two major groups, birds and insects such as ants, wasps and true bugs. A characteristic of nematine larvae is the presence of ventro-abdominal eversible glands [14]. Under disturbance, the larva can turn the glands inside-out, and the secretion then starts to evaporate, which can be perceived even by humans [15].

The chemistry of glandular secretions is known for nematine species from approximately ten genera ([16] and literature therein). It is often composed of a complex mixture of volatiles and over 100 chemicals are identified so far, belonging to the structural classes of the aliphatics, aromatics, and terpenes. They function as an antipredator defence, and comparative bioassays suggest that the defence is aimed primarily against predatory insects [14], [17]. Volatiles, detected or not in the glandular secretion, have also been tested as pure compounds for their repellence against ants, and they are from inactive to sometimes highly active [17].

4. STATE OF THE ART

Insects defensive volatiles can be multifunctional by including chemical precursors, solvents, and/or wetting agents of the active compounds, and their occurrence can reflect phylogenetic relationships (*e.g.*, [15], [16], [18]).

But, it remains difficult to evaluate how intrinsic physicochemical parameters of (individual) defensive volatiles influence the overall bioactivity of a mixture on antagonistic insects, and thus whether this source of variation also determines the diversity of allomones. It is intriguing that closely related nematine species can have a differing chemical profile of their volatile secretion, and same volatiles can occur in rather unrelated species (see *e.g.* [16], [19] *versus* [20]). Chemicals from host plants sometimes explain their presence in nematines, but such a dependency is not likely for some major compounds such as the monoterpenes dolichodial and citral, and the aromatic compound benzaldehyde [15], [16], [19].

The case of nematines highlights a general pattern of the state of the art in chemical ecology when considering defensive allomones. Chemical analyses allow the (sometimes long) listing of allomones from which the specific repellent bioactivity may be known, but it is often nearly impossible to reconstruct a chemical profile by mixing the chemicals since these can be commercially unavailable, etc. Using a native secretion in bioassays poses other practical problems such as (seasonal and/or geographical) unavailability of species, and an undetermined absolute amount of the secretion collected.

Thus, we cannot conveniently explore the bioactivity of a specific chemical profile at once, let alone their qualitative and quantitative interspecific variations, although this diversity is most probably driven to some extent by predator-prey interactions. One way to circumvent these difficulties would be, as a first approximation, to use our own sense of olfaction, but humans have a limited ability to discriminate and identify more than a few constituents in a mixture [21], and some odours can repel insects while attract humans. All this prompted us to search for another modelling system, as described in section 2.

5. COMPILATION OF DATA

Here, we conceived and designed a methodological prototype for the sonification of single volatiles. In parallel we compared the response of ants to volatiles with the one of humans to the sonified chemicals, and we will broaden this comparative approach by using mixtures of volatiles, emitted by nematine larvae and other insects. For each of the 109 molecules known from the secretion of over 20 Nematinae species belonging to the taxa *Nematus*, *Craesus*, *Hoplocampa*, and Cladiini [16], [19], [22], [23], we compiled physicochemical constants (see Table 1) from standard works (mainly [24]). The database also included 22 volatiles tested singly on ants (see later) and among which 9 occur in the nematine species, the others being related compounds plus several solvents.

6. SONIFICATION METHODOLOGY

The data stored in a MySQL table were transformed to MIDI controls using Max/MSP, the data being linearly scaled to fit MIDI norms (Fig. 1). So far, this 'parameter mapping' (reviewed by [25]) has been performed by setting a positive or negative correlation between molecule and sound parameters. Correlations were set positive between the molecular weight and the sound duration, and between the possible occurrence of chemical functional groups (plus other characteristics) and several sound effects, whereas the correlation was set negative between the number of carbon atoms and the sound frequency (Table 1). Although molecular weight and number of carbons are typically related parameters for organic compounds, we wished larger molecules to be perceived as sounding lower and lasting longer, and vice versa.

To produce sound sequences the synthesizer Massive vers. 1.3.0 (Native Instruments GmbH, Berlin, Germany) was chosen for its versatility, the richness of its soundbank, and the possibility of fine-tuning sounds through several effects/parameters (Table 1). Three preset sounds (out of over 1,300 available) were empirically selected, slightly modified, and saved as new NMSV files along with their assignment to the volatile structural classes to which the molecules belong: 'Cloud' to aliphatics, 'Diagrammatic' to aromatics, and 'Cliff' to terpenes. Our selection criteria were to avoid sounds that would be too percussive, evocative, complex, short, etc., because such sounds would not withstand the process of parameter mapping, and because we searched for one basic sound rendering a general pattern for one chemical class.

The gathered sound sequences lasted 7 to 24 sec including an almost silent tailing. They were routed to Max/MSP by the virtual audio bus Soundflower to be recorded individually as AIF files, declipped with iZotope RX™ 2, and then stored in a Molecule Sound Library (Fig. 1). We also built a Species Sound Library where these files were mixed, that is, set at one of four possible loudness levels by following the published relative concentration of volatiles in nematine secretions (Fig. 1).

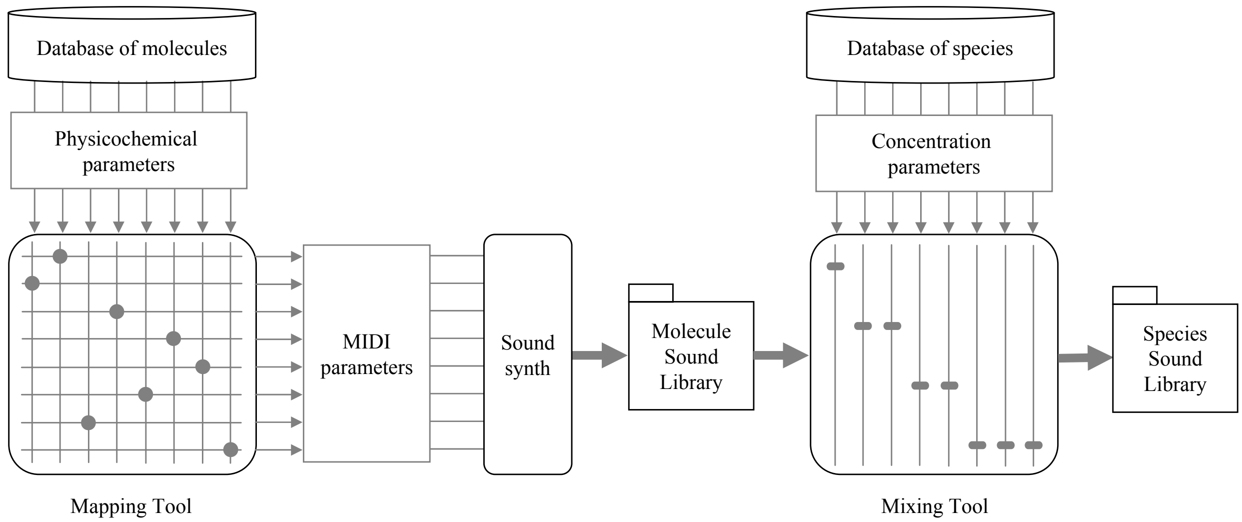

Figure 1: Schematic overview of the sonification steps. Physicochemical data from insect defensive volatiles are transformed into a Molecule Sound Library, before building a Species Sound Library. For further explanations, see text.

Table 1: Physicochemical parameters of volatiles used in this study, and their assignment to sound parameters during the process of sonification

Physicochemical parameter	**Data from molecules [range]**	**Range used**	**SOUND EFFECT (type): Parameter**	**MIDI range**
Molecular weight	[32.04 – 424.74]	0 → 425	Duration, in sec	1 → 10
Number of carbon atoms	[1 – 29]	1 → 29	Note, pitch	108 → 33
	[1 – 29]	1 → 29	EQ: Frequency	127 → 0
Functional groups, etc.	Aldehyde-group(s) [0 – 2]	0 → 2	FEEDBACK: Amp	0 → 127
	Acid-group [0 – 1]	0 → 1	NOISE (Metal): Amp	0 → 127
	Alcohol-group [0 – 1]	0 → 1	EQ: Boost	0 → 64
	Ketone [0 – 1]	0 → 1	MODULATION OSC (Filter FM): FM of Filter	0 → 127
	Ester [0 – 1]	0 → 1	INSERT 1 (Clip): Dry/Wet	0 → 127
	(Remaining) double bond(s) [0 – 3]	0 → 3	FILTER 2 (Highpass 4): Resonance	80 → 127

Taking into account several physicochemical parameters, the sonification as depicted in Figure 1 processes the range of values from the studied volatile molecules, so as to 'translate' and scale these values into a set and range of MIDI sound parameters.

7. BIOASSAYS

All 122 molecules were incorporated while setting up our sonification system that resulted in the two sound libraries. But so far, we only used from the first library those 22 files corresponding to molecules tested on ants.

Their 'auditory repulsive effect' was tested on 35 volunteers (mean $\pm$ SD age: 25 $\pm$ 4 years; 6 females, 29 males) who were not aware about the true chemoecological purpose of the experiment, as we told them about our wish to test the psychological effect of sounds in a multimedia context. Each volunteer was tested singly, in standardized conditions, staying in front of a computer coupled to two nearby placed loudspeakers. After receiving a short explanation about the experimental procedure, the volunteer could start the test by clicking the keyboard's space bar. This launched a first sound that possibly caused the person to walk backwards because the sound could be disliked for whatever reason (*i.e.*, loudness, frequency, etc.). Thus, we had asked the volunteer to walk backwards until reaching no more, no less a comfortable distance. The distance was recorded (precision: 25 cm) before she/he went back to the computer, to launch the second audio file, etc. The order of hearing the successive sequences was randomized among the individuals tested. The auditory repulsiveness of a given sonified molecule equals the mean ratio (in %) of the distance travelled by a person upon hearing this molecule to the maximum distance travelled by this person (and that reached from 1.5 to 7.5 m in our experimental conditions).

To quantify the repellence of a volatile on ants, workers of *Crematogaster scutellaris* (Olivier) (Formicidae) were feeding, in the laboratory, on honey water surrounding a small round podium placed in a Petri dish containing 40 ants. The number of feeding ants was counted at t = 0 ($F_{t=0}$) and, concurrently, a filter paper impregnated with 0.25 µl volatile was placed on the podium. The value 'F' was recorded again at t = 0.5 min and, once a min, from 1 to 5 min. This bioassay replicated 2 to 4 times per compound is detailed elsewhere [17]. For each volatile, the repellence was equal to 100 % minus the lowest mean ratio (in %) of an F-value, whenever obtained between t = 0.5 and 5 min, to $F_{t=0}$.

8. RESULTS AND ASSESSMENT

Our preliminary test results reveal a correlation (considered significant at $P < 0.050$) between the chemical repellence of volatiles against ants and the auditory repulsiveness of the sonified volatiles against humans (r_S = 0.4558, t = 2.29, P = 0.033, two-tailed, Spearman rank-order correlation, n = 22 volatiles; Fig. 2). However, this figure shows that the volatiles, generally, are underestimated in their auditory repulsiveness compared to their (chemical) repellence, which is especially the case for terpenes.

The two bioassays, on humans and on ants, were designed in a way to be comparable in testing a repellent effect. The ants were attracted by the food but repelled by the volatiles, and we counted those individuals actually tolerating the volatile vapours. Similar conflicting behaviours were indirectly obtained with the volunteers. These were asked to launch the sounds, thus staying at start close to the sound source. Then, they were asked upon hearing the sound to stay just at a comfortable distance. Without this request very sensitive people would systematically walk far away, so to say, to stay on the safe side. At the other extreme, some volunteers never walked away very far. The auditory repulsiveness was calculated, however, to offset inter-individual discrepancies. Finally, in our dual testing approach neither the bioassay on ants, nor the one on humans explicitly addresses the question about why volatiles are repellent, which is our ongoing research topic.

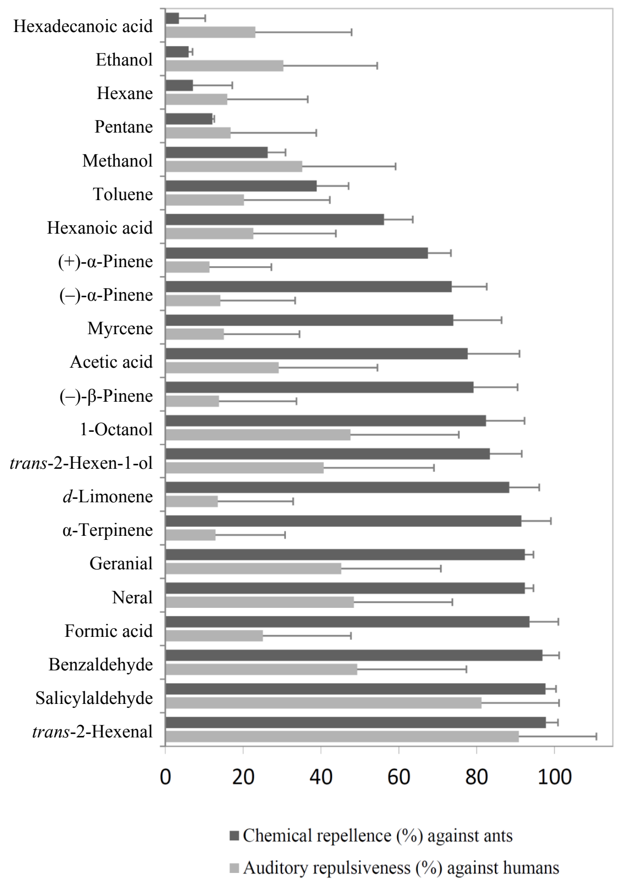

Figure 2: Preliminary results of testing on ants the repellence of single volatiles *versus* on humans the 'auditory repulsiveness' of the sonified molecules. Values are given as percentages $\pm$ SD. The two isomers of citral, geranial and neral, were tested in a racemic mixture on ants, whereas separately on humans, so that a same value is mentioned twice for the ants response to this volatile.

Though the aforementioned, statistically significant outcome is promising, the following experimental weaknesses shall be considered in the future. First, only one ant species was used, while other predatory insects may react differently to volatiles. This is one reason why we will apply our sonification device on other predator-prey systems. Second, the volunteers were not selected, on any socio-cultural criteria. But they were confronted to repulsive sounds (not much more affective ones such as music), which should render their response more comparable to ant responses than if attractive stimuli would have been evaluated on both organisms. Third, the number of used volatiles is rather low. Instead of integrating more volatiles, our wish with the current Molecule Sound Library is to adapt the parameter mapping, to assign other preset sounds to the chemical classes, to optimize the procedure of testing humans, and/or to use another synthesizer, then observing the effect of such modifications on the correlation between ant's and human's responses. Fourth, a major advance will be also to compare published results from bioassays where live nematine larvae and ants are interacting (*e.g.*, [14]) with those from the Species Sound Library to be tested on volunteers.

9. CONCLUSIONS

Our intention is to use the present methodology SonifChem on chemical profiles, knowing that the software prototyped so far requires several adjustments. In the pilot study based on an identical, chemical dataset we found that the chemical repellence against ants is correlated with the auditorily translated repulsiveness against humans, which suggests that this bioactivity can predict that one. Thus by avoiding problems related to chemically-unstable volatile mixtures, and without the necessity of performing (time-consuming) bioassays, sometimes on rare or otherwise unavailable insect species, researchers literally by themselves will be able, nevertheless, to estimate the bioactivity of defensive allomones.

10. PROSPECTS

Our ongoing work shall allow a derived but essentially realistic perception and monitoring of a chemical bouquet, it may be expanded by exploring the repulsive, and perhaps the attractive, bioactivity of chemicals emanating from any (biological) source, and by which it may complement existing devices in olfaction research [26], [27]. This research underlies applications used in various economic areas such as the development of Electronic Noses. Several companies deliver services, to monitor environmental odour nuisances. Such companies are contacted by others that carry out industrial and agricultural activities, by associations of residents, etc., and they may be interested in SonifChem.

In contrast to the avoidance and management of smelly odours, an outreach of our research could be the development of a derived software to be used in the fragrance/perfume industry. The software would then contribute in neuroscience topics dealing with olfactory modelling and the hedonic processing of odours (*e.g.*, [28], [29]).

For the society more generally, it will be possible to display SonifChem through an exhibition in schools, museums, and other educational institutions. Thus, (young) people will have a 'dynamic feeling' of insect-originating odours playing important ecological roles in the insect life.

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