Learning and perceptual similarity among cuticular hydrocarbons in ants

Nick Bos a,1,⇑, Stephanie Dreier a,⇑, Charlotte G. Jørgensen a,b, John Nielsen c, Fernando J. Guerrieri a,d, Patrizia d’Ettorre a,e

⇑These authors contributed equally to this study.

aCentre for Social Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark
bDepartment of Medicinal Chemistry, University of Copenhagen, Jagtvej 162, 2100 Copenhagen, Denmark
cDepartment of Life Sciences, Bioorganic Chemistry, University of Copenhagen, Thorvaldensvej 40, DK-1871 Frederiksberg, Denmark
dMax Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Jena, Germany
eLaboratory of Experimental and Comparative Ethology (LEEC), 13, University of Paris, France

ARTICLE INFO

Article history:
Received 25 July 2011
Received in revised form 24 October 2011
Accepted 25 October 2011
Available online xxxx

Keywords:
Ants
Camponotus
Conditioning
Generalisation
Learning
Nestmate recognition

ABSTRACT

Nestmate recognition in ants is based on perceived differences in a multi-component blend of hydrocarbons that are present on the insect cuticle. Although supplementation experiments have shown that some classes of hydrocarbons, such as methyl branched alkanes and alkenes, have a salient role in nestmate recognition, there was basically no information available on how ants detect and perceive these molecules. We used a new conditioning procedure to investigate whether individual carpenter ants could associate a given hydrocarbon (linear or methyl-branched alkane) to sugar reward. We then studied perceptual similarity between a hydrocarbon previously associated with sugar and a novel hydrocarbon. Ants learnt all hydrocarbon-reward associations rapidly and with the same efficiency, regardless of the structure of the molecules. Ants could discriminate among a large number of pairs of hydrocarbons, but also generalised. Generalisation depended both on the structure of the molecule and the animal’s experience. For linear alkanes, generalisation was observed when the novel molecule was smaller than the conditioned one. Generalisation between pairs of methyl-alkanes was high, while generalisation between hydrocarbons that differed in the presence or absence of a methyl group was low, suggesting that chain length and functional group might be coded independently by the ant olfactory system. Understanding variations in perception of recognition cues in ants is necessary for the general understanding of the mechanisms involved in social recognition processes based on chemical cues.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

The ability to discriminate among different stimuli is fundamental in many aspects of an animal’s life, from food location and predator avoidance to social behaviour and communication. Social insects are notable models for studying stimulus detection, perception, learning and memory because they combine behavioural plasticity and experimental accessibility. It has been long known that honeybees can learn relationships between different sensory cues (e.g. colour or odour) with a reward of sugar solution (Frisch, 1919). Since then, the honeybee has become a model organism for a plethora of cognitive studies analysing the functional properties of sensory systems (review in Menzel and Giurfa, 2006). In natural conditions, honeybees encounter floral odour blends consisting of tens to hundreds of components (Knudsen et al., 1993) and individual components of odorants can be categorised according to the selectivity of receptor neurons and behaviours related to these components. However, many other insects have also developed complex perceptual abilities (Akers and Getz, 1992, 1993). Recently, Reinhard et al. (2010) showed that when honeybees are conditioned to a complex blend of odours, they only learn specific key odorants. No correlation was found with molecular structure, but concentration appeared to have a positive effect on whether an odorant would become a key odorant. However, other studies suggested that the perceptual qualities of odours do correlate with specific molecular features. For example, changes in carbon chain length or structure, or changes in functional groups, affect generalisation from conditioned to test odorants (Laska et al., 1999; Guerrieri et al., 2005a). There is also evidence for a neuro-chemical modulation of perception, for instance, bees injected with octopamine agonists significantly increased their ability to discriminate nestmates from non-nestmates based on chemical cues (Robinson et al., 1999).

Nestmate recognition is a fundamental feature of insect societies and thus the identification of social recognition cues and the study of recognition mechanisms are essential for an integrated understanding of their advanced social organisation. Social recognition cues are encoded in the complex pattern of hydrocarbons present on the cuticle of social insects; these cuticular hydrocar-
bons generally range from about 20 to over 40 carbons in chain-length, with three principal structural classes: linear alkanes, methyl-branched alkanes and alkenes (review in e.g. Lenoir et al., 1999; Howard and Blomquist, 2005; Heffetz, 2007; Martin and Drijfhout, 2009; d’Ettorre and Lenoir, 2010). It has been suggested that certain hydrocarbon classes, such as methyl-alkanes and alkenes, have evolved a signal function while other classes, such as alkanes, serve little role in nestmate recognition (cf. in wasps: Dani et al., 2001; in honeybees: Dani et al., 2005; in ants: Akino et al., 2004; Lucas et al., 2005; Martin et al., 2008a; Guerriero et al., 2009; but see Greene and Gordon, 2007). Indeed, honeybees tested with the classical ‘proboscis extension response’ conditioning paradigm appear to learn certain alkenes better than alkanes when confronted with a discriminatory task (Châline et al., 2005).

Ants, like honeybees, can learn to solve different tasks (e.g. Wehner, 2003), and particularly Camponotus ants can be trained in controlled laboratory conditions to investigate learning and odour discrimination. This has been done with free walking ants (Dupuy et al., 2006); and with harnessed ants (Guerriero and d’Ettorre, 2010). Dupuy et al. (2006) studied free walking workers of two different Camponotus species that were presented with two volatile substances. One substance was positively reinforced with sugar solution, while the other was negatively reinforced with quinine solution. Individual ants were shown to learn single odours efficiently. The substances tested were volatile odour compounds either present in flowers or in honeybee pheromones (see Balderrama et al., 2002).

Chemical communication is especially important in nestmate recognition and our understanding of what part of the multi-component chemical cues of the ant’s cuticle might contain information about species, nestmate, task or fertility recognition is increasing (e.g. Greene and Gordon, 2003, 2007; d’Ettorre et al., 2004; Heffetz, 2007; d’Ettorre and Lenoir, 2010; Holman et al., 2010). In contrast, we know very little about how ants detect and perceive such information, and so far, no study has systematically investigated perceptual similarity among cuticular hydrocarbons in ants. Meskali et al. (1995) have supplemented the cuticle of Camponotus vagus workers with (Z)-9-tricosene, an alkene naturally absent in this species. This change in the composition of cuticular hydrocarbons was followed by a significant increase in antennation level and threats expressed by non-treated individuals towards treated individuals. In C. vagus, it has also been established that the proportions of certain hydrocarbons vary among worker subcastes suggesting that cuticular hydrocarbons serve as cues allowing discrimination between subcastes (Bonavita-Cougourdan et al., 1993).

Fine-tuned discrimination of cuticular hydrocarbons is expected to increase the accuracy of nestmate and within-colony recognition. Conversely, ants need to categorise cuticular profiles by taking inter-individual variation into account. This individual variation requires generalisation between cuticular profiles that are similar but not identical to decrease errors in discrimination between nestmates and non-nestmates (van Zweden and d’Ettorre, 2010). Indeed, Argentine ants show similar levels of aggression toward nestmate cuticular profiles supplemented with hydrocarbons with the same branch position but differing in chain length, suggesting that they are perceived as similar (van Wilgenburg et al., 2010).

Here we studied in individual Camponotus aethiops ants: (i) associative learning and perception of single hydrocarbons usually found on the cuticle of ants; (ii) the relationship between structure of hydrocarbons and perceptual similarity (generalisation). Using an original experimental procedure, we conditioned individual foragers of C. aethiops to associate a given single hydrocarbon with sugar solution (reward), and subsequently tested the conditioned ants for generalisation between the conditioned hydrocarbon and a novel hydrocarbon. We used an array of eight hydrocarbons combining different carbon chain lengths and presence/absence of a methyl group.

2. Materials and methods

2.1. Study organism

Nine queenright colonies of the carpenter ant C. aethiops (Latr.) were collected in spring 2006 in the Apennines, Italy (Moraduccio, 44°10’32.75”N, 11°29’3.08”E) and brought to our laboratory in Copenhagen. Each colony was housed in a plastic box (27×17×9.5 cm) with a plaster floor, which was connected to an equally sized plastic box serving as a foraging arena. The colonies were kept at 22±2°C under a 12:12 LD photoperiod. Each foraging arena contained a vertical wooden stick (platform) on which ants could be collected and put back (cf. Dupuy et al., 2006; Fig. 1A and B). Ants were fed with honey water and mealworms (Tenebrio molitor, Linn.). However, in order to increase ant motivation for sugar rewards, each colony was deprived of honey 10 days before the start of the experiments. All experiments were performed with individually marked workers. Each test ant was marked with a dot of enamel paint on its thorax after the first training trial. Test ants were typically foragers, i.e. medium-size workers present in the foraging arena.

2.2. Experimental setup

We trained individual ants to associate a sugar solution reward (30% w/w) with a hydrocarbon (S+), and later tested them for discrimination between the trained stimulus (S+) and a novel hydrocarbon (N) in the absence of a reward. Each hydrocarbon was diluted in pentane (Sigma–Aldrich, HPLC grade, >99.9% purity) to a final concentration of 10 µg/ml.

Training was conducted in Petri dishes (100 mm diameter× 15 mm high), and a clean Petri dish was used for each trial. The bottom of each Petri dish was covered with filter paper and the side walls were coated with Fluon® to prevent the ants from escaping. One of the quadrants contained a glass cover slide (18×18 mm) treated with a hydrocarbon (S+), by applying 20 µl of hydrocarbon solution on the edges (making sure that the solution did not touch the filter paper). This solution contained 0.2 µg of hydrocarbon, which is within the natural range found on the cuticle of ants of similar size (Holman et al., 2010). The treated cover slide was baited with a 1 µl droplet of sugar solution placed on its centre. In the opposite quadrant, a similar cover slide held a 1 µl droplet of water, but no hydrocarbon (Fig. 1C). The location of the cover slides was pseudo-randomised. The slides were never positioned in the same location for more than two consecutive trials to prevent the ants from using any directional preferences for locating the sugar solution.

2.3. Training procedure

At the beginning of each experiment, an ant was gently taken from the platform placed within the foraging arena and transferred to the Petri dish using a piece of filter paper the ant could climb on. The ant was randomly released in one of the two empty quadrants and allowed to search for the reward (Fig. 1C). When the sugar solution was found, the ant was allowed to ingest it. The time needed to find the sugar reward was recorded. Subsequently, the ant was gently recaptured with the help of the filter paper and released on the platform in its colony, so that it could perform trophallaxis with nestmates. Once its intake was unloaded, a motivated forager typically came back to the platform within 3–4 min, and was at that point picked up for the next trial. The ant was always left for a minimum of 1 min in its colony. Each ant performed six consecutive training trials, in order to build an association between a given hydrocarbon (S+) and the reward.

2.4. Test phase

After the training phase, two choice tests were conducted. The surface of the Petri dish was divided by a light pencil line into four quadrants of equal size. The test Petri dish gave the ant a choice between two cover slides, one treated with the trained stimulus (S+) and the other one with a novel hydrocarbon (N). This time however, no reward was present on either slide (Fig. 1D). The cover slides were placed in opposite quadrants and given that the hydrocarbons have low volatility the ants had to approach the cover slide to perceive the stimulus. The ant was allowed to walk for 2 min, during which its location was recorded. The time the ant spent in each of the four quadrants of the Petri dish was recorded using the software EthoLog v. 2.2.5 (Ottoni, 2000). Afterwards, a droplet of sucrose solution was provided on the coverslip containing S+. The ant was transferred back in its colony, and after a minimum of 1 min, a second choice test was conducted. Any association between a hydrocarbon and reward would be evinced by the ant remaining more time in the quadrants containing the hydrocarbons than in the empty quadrants. Perceptual dissimilarity (low generalisation) between hydrocarbons presented in the test would be evinced by the ants spending more time in the S+ quadrant than in the N quadrant.

A control test was conducted for each hydrocarbon, where a naïve ant (without training) had a choice between a slide with a hydrocarbon, and a slide with pentane. No preference for any quadrant was expected in this control test, indicating no spontaneous preference for a given hydrocarbon.

A total of eight hydrocarbons, including five linear alkanes and three mono-methyl alkanes were used (Supplementary Table 1S), some of which are present on the cuticle of *C. aethiops* (van Zweden et al., 2009). We chose pairs of hydrocarbons differing in structural similarity as S+ and N stimuli. For example, when conditioned with a linear hydrocarbon, the ant was presented in the test with another linear hydrocarbon of different chain-length, or with a branched hydrocarbon having a methyl group in a certain position (carbon 3 or carbon 11). All hydrocarbons used as S+ were also used as N for another, fully independent, set of ants. A total of 24 independent pairs of hydrocarbons were tested. For each pair of hydrocarbons, 10 replicates were performed representing 10 ants conditioned and tested individually, for a total of 240 ants from nine colonies. For the control tests (naïve ants), 10 ants were used for each hydrocarbon, for a total of 80 ants tested.

2.5. Data analysis and statistics

During the training trials we measured the time required by each ant to find the reward. The performance over the six trials was analysed using a Friedman’s ANOVA, followed by post hoc comparison to assess differences between trial 1 and trial 6 (Wilcoxon test for matched pairs). During the choice test we measured...
the time that each ant spent in the four quadrants. The time was subsequently normalised, and differences between the percentage of time spent in quadrant S+ and the percentage of time spent in quadrant N were analysed using Wilcoxon test for matched pairs, and effect sizes were calculated using G*Power 3.1.3.

A preference index (PI) was calculated and defined as $(t_{S+} - t_{N}) / (t_{S+} + t_{N})$. This PI varies between $-1$ and $+1$, with $0$ meaning that there was no preference between S+ and N (complete generalisation), positive values meaning preference for S+ and negative values preference for N.

Comparison between PI from hydrocarbon A to B and PI from B to A (between symmetric pairs) was done using Mann–Whitney U tests. In order to establish whether there was a defined pattern for asymmetric generalisation, the analysis between difference in carbon chain length ($S+ - N$) and preference index was performed separately for pairs containing linear alkanes and pairs containing at least one methyl-alkane using Spearman rank correlations.

2.6. Synthetic hydrocarbons

The linear alkanes were purchased from Sigma–Aldrich (Steinheim, Germany), and the mono-methyl-alkanes were all synthesised as described in the Supplementary material.
3. Results

3.1. Training: Building the association between a hydrocarbon and sucrose reward

The time needed by the ant to find the sugar reward diminished significantly over the six trials for all hydrocarbons tested (Friedman's ANOVA, $\chi^2 = 11.74-39.30$, df = 5, $n = 20-30$, $P < 0.05$ in all cases). Indeed, trials 1 and 6 were always significantly different (Fig. 2; eight hydrocarbons tested, Wilcoxon tests for matched pairs, $Z = 2.30–4.28$, $n = 20–30$, $P < 0.05$ in all cases).

3.2. Choice test: Specific response to the hydrocarbon associated to sucrose

In 23 out of 24 cases, the ants spent significantly more time in the quadrants containing hydrocarbons than in the empty quadrants (Wilcoxon test for matched pairs, $Z=1.89–2.80$, $n=10$, $P<0.05$). The exception ($n$-C$_{19}$/n-C$_{28}$) showed an almost significant trend (Wilcoxon test for matched pairs, $Z = 1.89$, $n = 10$, $P < 0.06$). So, we performed the subsequent analyses on the time spent by the ants in the two quadrants containing hydrocarbons.

Ants spent significantly more time in quadrant S+ than in quadrant N in 12 out of 24 cases (Wilcoxon test for matched pairs, $Z = 1.99–2.80$, $n = 10$ for each pair of hydrocarbons, $P < 0.05$ in 12 cases, see Fig. 3). High preference for S+ means that the hydrocarbons are perceived as dissimilar and thus there is low generalisation between hydrocarbons.

The preference of the ants in the second choice test did not differ from the first one (Wilcoxon test for matched pairs, $Z = 0.05–1.78$, $n = 10$, $P > 0.05$) in all cases except for one ($n$-C$_{28}$/n-C$_{22}$), in which the ants had an even stronger preference for $n$-C$_{28}$ in the second test than in the first.

By contrast, in the control tests, for each pair of hydrocarbons, naive ants spent an equal amount of time in both quadrants (Wilcoxon test for matched pairs, $Z = 0.36–1.69$, $n = 10$ for each pair of hydrocarbons, $P > 0.05$ in all cases except for one ($n$-C$_{28}$/n-C$_{22}$), in which the ants had an even stronger preference for $n$-C$_{28}$ in the second test than in the first).

Fig. 3. Choice test. Relative amount of time spent in each quadrant during the first choice test for each pair of hydrocarbons. Dark gray bars denote S+ quadrant; light grey bars denote N quadrant. The first hydrocarbon represents S+, while the second represents N in each pair (e.g. in the first line n-C$_{19}$ is S+ and n-C$_{21}$ is N). Stars denote significance level *$P<0.05$, **$P<0.01$. Effect sizes are given as Cohen's d.

pair of hydrocarbons, $P > 0.05$), suggesting that there is no spontaneous preference (or avoidance) for any specific hydrocarbon.

3.3. Asymmetry in generalisation

In some cases generalisation among stimuli depended on the animal’s experience, i.e. ants conditioned to a stimulus $A$ generalised to a stimulus $B$, whilst ant conditioned to stimulus $B$ did not generalise to stimulus $A$ (cf. Guerrieri et al., 2005a,b). When hydrocarbon pairs included only linear alkanes, the preference index (PI) from hydrocarbon $A$ to $B$ was significantly different from the PI from $B$ to $A$ in five out of seven cases (Fig. 4A, Mann–Whitney U test, $Z = 1.97–2.34$, $n = 10$, $P < 0.05$). When tested hydrocarbon pairs contained at least one methyl-alkane, none of the PI were significantly different (Fig. 5A; Mann–Whitney U test, $Z = 0.60–1.59$, $n = 10$, $P > 0.05$).

Among linear alkanes, there was a significant relationship between difference in carbon chain length ($S^+$ minus $N$) and preference index ($P < 0.05$, Spearman’s correlation). Black bars and black dots show the cases where $S^+$ is the longer hydrocarbon of the pair tested; white bars and white dots show the cases where $S^+$ is the shorter hydrocarbon.

Fig. 4. Perceptual similarity of linear alkanes. (A) The preference index for quadrant $S^+$ over $N$ for each alkane pair tested. Values around 0 mean that there was no preference between $S^+$ and $N$; positive values mean that there was a preference for $S^+$. Exact $P$-values and $Z$-test values for independent samples are shown. (B) Among linear alkanes there was a significant relationship between difference in carbon chain length ($S^+$ minus $N$) and preference index ($P < 0.05$, Spearman’s correlation). Black bars and black dots show the cases where $S^+$ is the longer hydrocarbon of the pair tested; white bars and white dots show the cases where $S^+$ is the shorter hydrocarbon.

(Fig. 4B; Spearman’s correlation, $r_s = -0.58, P < 0.05$), whilst among pairs containing at least one methyl-alkane no such relationship was found ($P > 0.05$, Spearman’s correlation), suggesting that they are perceived as similar.

### 4. Discussion

We developed an experimental protocol to study whether ants can associate single hydrocarbons to another stimulus. The first important result of our study is that individual *C. aethiops* workers can associate long-chain hydrocarbons to a sugar reward, supporting the results of Bos et al. (2010), where ants learned to associate a complete chemical profile to a sucrose reward. The fact that all eight hydrocarbons (five linear alkanes and three methyl-alkanes) were efficiently learnt by the ants suggests that both classes of substances can be detected and may have a potential role in chemical communication and recognition. In ants, the evolution of similar learning performances for hydrocarbons of different classes can be understood in terms of the relative importance of chemical cues for the identification of several categories of individuals. For example, in *C. vagus*, cuticular hydrocarbons are thought to be important to discriminate both nestmates from non-nestmates (Bonavita-Cougourdan et al., 1987) and functional subcastes among nestmates (Bonavita-Cougourdan et al., 1993). Similarly, in *Camponotus fellah*, cuticular hydrocarbons are involved in nestmate recognition (e.g. Boulay and Lenoir, 2001), while some hydrocarbons have a possible role as fertility signals and are abundant on the queen’s cuticle and on queen-laid eggs in *C. floridanus* (Endler et al., 2006).

The second important result of our study is that ants can discriminate most of the hydrocarbons learnt, but their discrimination performance is dependent on the structure of the hydrocarbon molecule and the previous experience of the individual. In general, for linear alkanes ants usually searched for sugar close to the conditioned hydrocarbon (S+) when discrimination was from lower to higher chain length molecules (e.g. when $n$-$C_{19}$ was S+ and $n$-$C_{28}$ was N). In the reciprocal situation, ants had no preference for either of two hydrocarbons presented in the choice test, showing that they were generalising from higher to lower chain length molecules. This suggests both that the significance of the stimulus is dependent on the animal’s experience acquired during conditioning and that odour detection and perception might follow an inclusion criterion: If molecules pre-
sented in the tests are similar in structure to those presented as conditioned stimuli and carry a shorter chain, these molecules are perceived as similar to the conditioned stimulus (cf. Guerrieri et al., 2009). Ants would then use an inclusion rule that produced the observed asymmetry in generalisation. However, when the difference in chain length is too large (for example n-C_{20}/n-C_{22}) the ants did not generalise. Asymmetry in generalisation has previously been found in honeybee (e.g. Guerrieri et al., 2005a; Sandoz et al., 2001); for instance, generalisation responses of bees conditioned to the alarm compound were much higher than those of bees conditioned to the floral odour. Such asymmetry was further linked to different biological significance of these compounds for bees (Sandoz et al., 2001). Asymmetry in generalisation has been hypothesised to be due to experience-dependent differences in salency of odours or innate odour preferences. However, it could also be due to glomerular activation patterns and/or lateral inhibition by local interneurons in the antennal lobes, the first integration centres of olfactory stimuli in the insect brain (cf. Guerrieri et al., 2005a).

Finally, in the present study, we found that ants that learnt to associate food to a methyl-branched hydrocarbon (+S) also searched for food around the novel methyl-branched hydrocarbon (N), meaning there was high generalisation among this class of molecules. Also, ants perceived hydrocarbons that differed among each other by the presence or absence of one methyl group (i.e. linear versus methyl-alkanes) as dissimilar, especially when there was also a difference in chain-length (e.g. when n-C_{20} was S+ and 11-meC_{19} was N, and vice versa), and thus expressed low generalisation. This confirms the importance of carbon chain length as a key feature for generalisation among hydrocarbons, and suggests that chain length and functional group might be coded independently by the ant olfactory system. Chain length of hydrocarbons differs greatly on the cuticle of ants (e.g. van Zweden et al., 2009), thus distinguishing between them might be important.

We start elucidating which molecular features are significant for perceptual similarity in ants (see also van Wilgenburg et al., 2010). The substances we assayed in the present study have long carbon chains, low volatility and are typically present on the ants’ cuticle. However, cuticular hydrocarbon profiles are blends of many molecules in different ratios. When the ratios of certain hydrocarbons in the profile are highly correlated with each other, they might be interpreted as a single variable (Martin et al., 2008b), thus simplifying an apparent complex profile. Our results suggest the possibility that ants might indeed perceive multiple compounds as a single variable, through generalisation. Generalisation might also blur the line between different chemical profiles, as differences present in the profile, apparent to us by use of GC-MS, might not be perceived as different by the ant. To fully understand how generalisation affects nestmate recognition, and possibly further improve multivariate analysis of chemical data in this species, a comprehensive study using hydrocarbons of all different classes, present on the cuticle of the species is needed. Recent work suggests that olfactory generalisation allows honeybees to adjust their sensitivity to differences in concentrations of chemical stimuli according to the animal’s experience (Wright et al., 2008). Honeybees generalised between two concentrations of the same stimulus when both were paired with sucrose reward. Conversely, generalisation was low when one concentration was paired with sucrose reward and the other one was paired with salt solution (Wright et al., 2008). Flowers, the main food sources for honeybees are recognised by their perfumes. These perfumes are complex odors blends composed by similar (but not always equal) proportions of volatile substances. Therefore, generalising among relative concentrations allows bees to precisely locate exploitable food sources. Further studies should address whether ants are capable of generalising among complex cuticular hydrocarbon blends, since this might be of great importance in the context of nestmate recognition.

Acknowledgements

We are grateful to Bernhard Seifert and Alain Lenoir for confirming the identity of ant specimens. Many thanks to Martin Giurfa for discussion on pheromone perception and Gösta Nachman for help with statistical analysis. We thank all the members of the Centre for Social Evolution, University of Copenhagen, for a stimulating working environment. This work was supported by the EU-Marie Curie Excellence Grant, EXT-CT-2004-014202 to PD. FJG was supported as a post-doctoral fellow by the Danish National Research Foundation.

Appendix A. Supplementary data


References


