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Conditioning of *Apis florea* F. as a function of Duration of exposure to chemical vapours at R_c50 and R_c60 dilutions

Introduction

Various workers carried out adaptation experiments. As early as 1895, Zwaardamaker suggested that olfactory cross-adaptation could be used as a tool to detect odour similarities. The work of Adrian (1956), Ottoson (1956) and Stuvier (1958) indicates that two superimposed compounds were involved in adaptation. Lemagnen (1942–43) determined the olfactory threshold of tested odorants for mammals before and after 10 minutes exposure to the conditioning odorants. The difference between both threshold concentrations as a numerical expression of the degree of cross-adaptation was assumed to be indirectly related to the degrees of similarity between the primary information patterns. The tested compounds viz., terpineol, vanillin, safrole and tetralin were structurally and olfactorily almost totally unrelated. But the structures indicated that even two information patterns representing widely different odorant structure usually had certain structural detail in common. This was demonstrated more in his later work on cross adaptation with odorants belonging to bitter almond, camphor and musk type. After conditioning with benzonitrite, the odour of benzaldehyde was described as safrole like, while conditioning with a mixture of benzonitrite and safrole left an impression reminiscent of indole. The odour of benzaldehyde after conditioning with a mixture of safrole and indole was observed as benzene like. Similar reactions were found in a series of camphoraceous compounds. Engen (1963) studied cross adaptation effects in some mammals of a series of straight chain alcohols and was unable to detect a relationship between the magnitude of the effect and the chain length.

Waller *et al.* (1974) conditioned bees to the flower scents of lucerne clone 2490 and of sainfoin. Bees responded positively to ocimene, myrcene and limonene, but did not to linalool which seemed non-distinguishable by bees from the other three chemicals.

Material and methods

Two types of experiments, self- and cross conditioning were performed. The compounds were tested at R_c50 and R_c60 levels of chemical dilutions and at predetermined exposures of the bees to chemical vapours at steps of 30 min. 1, 2, 4 and 6 h (Gupta, 1982).

In self conditioning experiments, bees were first fed on 30 per cent sugar water (SW) solution for the period mentioned before transferring them to a chemical sugar solution (CS). In cross conditioning, bees were at first fed on one CS solution and then were allowed feeding on another, in both cases for the same interval of time. Reciprocal experiments were also carried out in each case in order to check authenticity of the first cross conditioning of bees as a function of time at R_c50 and R_c60 levels of concentrations.

The plan for obtaining the combination in each case is as follows.

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a) Combinations for self-conditioning experiments

Sugar → Linalool, Terpineol — Sugar constant

Sugar → D(+)-carvone, Linalool — Sugar constant

Sugar → Terpineol, D(+)-carvone — Sugar constant

b) Conditions for cross-conditioning reciprocal experiments

D(+)-carvone → Terpineol, Linalool — (D+)-carvone constant

Terpineol → D(+)-carvone, Linalool — Terpineol constant

Linalool → Terpineol, D(+)-carvone — Linalool constant

The data was statistically analysed by the method of CROW et al., 1960.

Results and discussion

It is known that each animal gets adapted to an environment in which it stays for a certain period of time. This seems true when bees were exposed to the chemical vapours. The sooner the bees become acclimated to the environment loaded with the chemical vapours, the less useful will be the compound in question. It is also presumed that the vapours of one compound may loose its repellent effect much earlier than a double or multiple load of chemical vapours to which bees may be exposed.

The data shown in tables 1 and 2 present the results on self- and cross conditioning experiments. In self-conditioning experiments, a group of 30 bees, fed on SW solution, kept on feeding more and more with the passage of time. The group of bees which were trans-

Table 1
Experiments on conditioning of bees to three promising chemicals by feeding sugar and sugar-chemical solution of R_c50 dilutions

Duration of exposure	Uptake by feeding (mg)							
	D(+)-Carvone constant							
	Self-conditioning Sugar				Cross-conditioning D(+)-Carvone			
	Linalool		Terpineol		Linalool		Terpineol	
	S	L	S	T	C	L	C	T
30 min	0.830	0.0015	0.0672	0.0039	0.2232	0.0026	0.2160	0.0046
1 h	0.1206	0.0032	0.1274	0.0046	0.4472	0.0038	0.4406	0.0072
2 h	0.2205	0.0047	0.2243	0.0048	0.5815	0.0038	0.5621	0.0107
4 h	0.4432	0.0043	0.4452	0.0055	0.5819	0.0035	0.5619	0.0109
6 h	0.6516	0.0044	0.5678	0.0052	0.5812	0.0038	0.5616	0.0106

Left hand figures show feeding on sugar solution (control) in self-conditioning experiment or on a single compound in cross-conditioning experiment. S/L indicates prefeeding of bees on sugar and post feeding on linalool. Similarly, C/L indicates prefeeding on D(+)-Carvone and post feeding on linalool and so on.

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Table 1 (contd.)

Uptake by feeding (mg)

Linalool constant

Duration of exposure	Self-conditioning Sugar				Cross-conditioning Linalool			
	D(+)-Carvone		Terpineol		D(+)-Carvone		Terpineol	
	S	C	S	T	L	C	L	T
30 min	0.0586	0.0033	0.0559	0.0049	0.1225	0.0045	0.1206	0.0043
1 h	0.1380	0.0042	0.1246	0.0073	0.2409	0.0077	0.2404	0.0077
2 h	0.2295	0.0048	0.2322	0.0105	0.4511	0.0121	0.4510	0.0147
4 h	0.5196	0.0051	0.4370	0.0106	0.4515	0.0124	0.4517	0.0105
6 h	0.5393	0.0048	0.5242	0.0109	0.4514	0.0124	0.4518	0.0148

Terpineol constant

Duration of exposure	Self-conditioning Sugar				Cross-conditioning Terpineol			
	Linalool		D(+)-Carvone		Linalool		D(+)-Carvone	
	S	L	S	C	T	L	T	C
30 min	0.550	0.0020	0.0517	0.0041	0.0809	0.0019	0.0810	0.0042
1 h	0.1279	0.0032	0.1264	0.0063	0.1630	0.0031	0.1665	0.0082
2 h	0.2369	0.0039	0.2464	0.0111	0.4650	0.0065	0.4682	0.0125
4 h	0.4493	0.0039	0.4311	0.0115	0.4630	0.0067	0.4550	0.0122
6 h	0.6031	0.0041	0.6033	0.0112	0.4641	0.0066	0.4582	0.0121

ferred to CS solution also behaved in the same manner up to 2 h after which the feeding was almost constant. By comparison, the difference between feeding from 30 min to 2 h on CS solution was relatively less, so much so that it declined by 48:1 up to 2 h when linalool was fed. This kind of behaviour was met with feeding of CS solution irrespective of the compound employed. This behaviour was true for two concentrations (R_c50 and R_c60) of the compounds experimented with. The only difference was that the feeding of CS solution at R_c60 was appreciably low, compared with R_c50. This suggests that the sensory organs of the bees get adapted to a single load of vapours of a compound within 2 h of exposure. This indicates also that increase in concentrations has a definite effect but not beyond a certain concentration.

Table 2
Experiments on conditioning of bees to three promising chemicals by feeding sugar and sugar-chemical of R_c60 dilutions

Uptake by feeding (mg)

D(+)-Carvone constant

Duration of exposure	Self-conditioning Sugar				Cross-conditioning D(+)-Carvone			
	Linalool		Terpineol		Linalool		Terpineol	
	S	L	S	T	S	L	C	T
30 min	0.0576	0.0011	0.580	0.0011	0.1244	0.0013	0.1233	0.0011
1 h	0.1190	0.0018	0.1173	0.0015	0.2313	0.0029	0.2343	0.0045
2 h	0.2385	0.0033	0.2291	0.0027	0.4421	0.0044	0.4409	0.0052
4 h	0.4372	0.0036	0.4471	0.0029	0.4420	0.0043	0.4420	0.0053
6 h	0.6405	0.0035	0.6455	0.0027	0.4413	0.0041	0.4416	0.0053

Left hand figures show feeding on sugar solution (control) in self-conditioning experiment or on a single compound in cross-conditioning experiment. S/L indicates prefeeding of bees on sugar and post-feeding on linalool. C/L indicates prefeeding on D(+)-Carvone and post feeding on linalool and so on.

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Table 2 (contd.)

Duration of exposure	Uptake by feeding (mg)							
	Linalool constant							
	Self-conditioning Sugar				Cross-conditioning Linalool			
	D(+)-Carvone		Terpineol		(D+)-Carvone		Terpineol	
	S	L	S	T	L	C	L	T
30 min	0.0536	0.0018	0.0561	0.0014	0.1218	0.0020	0.1233	0.0020
1 h	0.1120	0.0040	0.1143	0.0020	0.2106	0.0019	0.2423	0.0033
2 h	0.2270	0.0049	0.2222	0.0037	0.4592	0.0030	0.4497	0.0044
4 h	0.4450	0.0050	0.4430	0.0037	0.4582	0.0026	0.4520	0.0042
6 h	0.6425	0.0047	0.6572	0.0039	0.4594	0.0025	0.4512	0.0043

Duration of exposure	Terpineol constant							
	Self-conditioning Sugar				Cross-conditioning Terpineol			
	Linalool		D(+)-Carvone		Linalool		D(+)-Carvone	
	S	L	S	C	T	L	T	C
30 min	0.0556	0.0018	0.0578	0.0009	0.1090	0.0013	0.1107	0.0046
1 h	0.1127	0.0025	0.1072	0.0010	0.2104	0.0023	0.2240	0.0068
2 h	0.2251	0.0048	0.2264	0.0022	0.4410	0.0047	0.4332	0.0120
4 h	0.4436	0.0047	0.4457	0.0022	0.4422	0.0045	0.4319	0.0120
6 h	0.6552	0.0049	0.6533	0.0018	0.4423	0.0046	0.4319	0.0120

Since a single load of chemical vapours was inadequate to provide repellency beyond a 2 h duration, it was considered desirable to change the course of experiment in order to obtain repellency, if possible, up to at least 4 h. It was presumed that if the sensory organs are loaded with a double effect of chemical vapours in different combinations, probably it might yield the required results. Therefore, cross conditioning experiments with all the three compounds were carried out. In these experiments, a single load of chemical caused the bees to feed less than that of SW solution, but the feeding was definitely more when compared with double load effect of the chemical vapours (Table 1, 2). The observations suggest that prefeeding with CS solution had a relatively lower effect and compared nearly with effects obtained by feeding simple SW solution in self conditioning experiments. This was comparable until 1 h of duration of feeding, but the situation remarkably changed after 2 h of feeding. Feeding SW solution kept on increasing while first CS feeding attained uniformity. A pronounced effect was obtained by transfer of the bees to a second load of chemical vapours. But surprisingly, the total effect obtained was limited to a certain level, which was seemingly common, whether the bees were exposed to a single chemical effect or to a double. This limit is probably an indicator of the maximum holding capacity of sensory cells. But this level remained uneffected in reciprocal experiments and also whether the first exposure of the bees was with alcohols e. g linalool, terpineol, and the second exposure with ketone e. g D(+)-carvone. All these conditions did not change, if the two exposure were with alcohols e. g linalool and terpineol. Treatments with any of these combinations or with 2 variable concentrations did not stretch the adaptation limit of the bees to the compounds beyond 2 h. On comparing, these results with the semi-field experiments carried out by BHARDWAJ (1974), it is observed that adaptive experiments carried out by him in the field indicate an almost similar kind of results. The bees got adapted to 3-heptanone, 2-heptanone and acetophenone, within almost 2 h. The only exception was the essential oils extracted from the leaves of *Ocimum sanctum* which repelled bees nearly for 3 h. According to CAIN (1968) a much longer duration of constant stimulation would be required to show a decline in sensitivity which was characteristic of olfaction.

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Zusammenfassung

Es ist anzunehmen, daß sich Arbeiterinnen von *Apis florea* an einen Lebensraum mit chemischen Dämpfen akklimatisieren. Es wurden Versuche unternommen, um festzustellen, wie lange Linalool, Terpineol und D(+)-carvon bei Konzentrationen von R_c50 und R_c60 wirksam bleiben. Bei Selbstanpassungsversuchen wurden Bienen für Zeiträume von 30 Minuten, 1 Stunde, 2 Stunden, 4 Stunden und 6 Stunden mit 30%iger Zuckerlösung gefüttert und dann für den gleichen Zeitraum in eine Atmosphäre mit einem chemischen Dampf gebracht. Sie wurden dann für den gleichen Zeitraum einem zweiten chemischen Dampf ausgesetzt. In jedem Fall wurden reziproke Versuche vorgenommen. Bei den Selbstanpassungsversuchen erhöhte sich die Nahrungsaufnahme von reiner Zuckerlösung mit der Zeit, aber die von einer Mischung von Zuckerlösung und Chemikalie blieb zwei Stunden lang ungefähr gleich. Bei der Überkreuz-Anpassung erhöhte sich die Nahrungsaufnahme von der vorher verwandten Chemikalie ebenfalls bis zu 2 Stunden und blieb danach gleich. Dasselbe Verhalten war zu beobachten, wenn die Bienen zur nächsten Chemikalie überführt wurden. Daraus wird geschlossen, daß Linalool, Terpineol und D(+)-carvon maximal 2 Stunden eine abweisende Wirkung ausüben.

Summary

It is likely that *Apis florea* workers become acclimatized to an environment containing chemical vapours. Experiments were conducted to investigate how long linalool, terpineol and D(+)-carvone remain effective at R_c50 and R_c60 concentrations. In self-conditioning experiments, bees were fed on 30% sugar solution for 30 min, 1 h, 2 h, 4 h and 6 h and were then transferred to an atmosphere containing a chemical vapour for the same periods. They were then exposed to a second chemical for the same periods. Reciprocal experiments were performed in each case. In selfconditioning experiments, feeding on pure sugar solution increased with time but feeding on a mixture of sugar solution and chemical behaved similarly for 2 h. In cross-conditioning, feeding on pre-exposed chemical also increased up to 2 h and maintained uniformity afterwards. Same behavior was observed when the bees were transferred to the next chemical. It is concluded that linalool, terpineol and D(+)-carvone can have a repellent effect to a maximum of 2 h.

Резюме

Предполагают, что рабочая пчела *Apis florea* акклиматизируется к среде с химическими парами. Проводили опыты по изучению времени эффективности линалоола, терпинеола и Д(+)-карвона при концентрациях R_c50 и R_c60. В опытах по адаптации пчел к химикатам кормили их 30%ным раствором сахара в течение 30 минут, 1 часа, 2, 4 и 6 часов, а затем помещали их в среду с химическими парами на такой же период. После этого пчелы на такой же период были подвержены влиянию второго химиката. В каждом случае проводили обратные опыты. В опытах по адаптации пчел активность поедания на чистом растворе сахара возросла, в то время как активность поедания на смеси из раствора сахара и химического вещества в течение 2 часов осталась почти неизмененным. При перекрестной адаптации активность поедания на заранее примененном химическом веществе тоже возросла в течение 2 часов, а потом осталась на достигнутом уровне. Тоже самое наблюдалось тогда, когда пчелы подвергались последующему химическому веществу. На основе этого сделают выводы, что эффективность линалоола, терпинеола и Д(+)-карвона, т. е. их отпугивающее действие составляет не более 2 часов.

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