A simple choice test was devised which compared host-wood attraction with that of a sample plus host-wood by observation of the number of eggs laid in each case. The method[6] involved placing 2 groups of 5 blocks ($15 \times 5 \times 2$ cm) a distance of 20 cm apart in a glass bioassay tank (30 cm $\times$ 30 cm $\times$ 60 cm fitted with a glass top), 1 group of which acted as a control. The blocks in each group were separated by spacers to produce a 0.4 mm gap and held together by 2 elastic bands. All bioassays were conducted in a room held at 70% relative humidity and 27 °C, the temperature having been observed to be critical for this bioassay. Chemical samples (5 µl pure substance) were introduced to 1 group of blocks by continuous diffusion from a glass capillary tube (1.4 mm i.d., sealed at one end). This technique is known to produce a constant rate of volatilization after an initial high rate of diffusion[7]. Wood blocks were of a single origin and the block surfaces were smoothed with glass paper in order to minimize tactile stimuli. Each bioassay test was run simultaneously as one of a group of 4 with the position of sample and blank blocks being changed alternately to eliminate the effects of external factors such as uneven lighting. Because the number of eggs laid by an individual female is subject to uncontrollable variation, it was decided to employ a nonparametric Wilcoxon matched-pairs sign-ranked test. 4 sets of experiments were conducted: a) p-cymen-8-ol against control, b) (−)-verbenone against control, c) (−)-verbenone + p-cymen-8-ol (1:1) [this being the approximate ratio of co-occurrence in frass] against (−)-verbenone and d) (−)-myrtenol against control. 10 replicates were run in each test (8 in d) using 2 unmated males and 2 unmated females. The results of these tests clearly indicate that (−)-verbenone is an oviposition stimulant with respect to the host wood ($N = 10, T = 1, p < 0.005$), whereas p-cymen-8-ol ($N = 10$, $T = 28$, $p > 0.05$) and (−)-myrtenol[8] ($N = 8$, $T = 21$, $p > 0.05$) have no activity alone. However, the activity of (−)-verbenone is synergized by the presence of p-cymen-8-ol, as evidenced by experiment c ($N = 10$, $T = 0$, $p < 0.005$).

In earlier studies, Becker reported that whereas a number of monoterpenoid hydrocarbons have a stimulating effect on oviposition in *H. bajulus*, oxygenated monoterpenes in general were either neutral or repellent. The apparent discrepancy between Becker's work and our own is probably explained by concentration effects since the amounts of test compounds employed in the former study were very much larger than those used here[6]. Indeed, we found in our preliminary studies that high concentrations of (−)-verbenone resulted in an oviposition preference for untreated control blocks, suggesting a repellent effect in such cases. Further bioassay studies are in progress which include tests of the role of other monooxygenated monoterpenes, and also the discrimination between attraction and stimulation with respect to oviposition.

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2 D. A. Evans and M. D. Higgs, Tetrahedron Lett. *1975*, 3585.
3 R. Berry, Int. Biodeterioration Bull. *8*, 141 (1974).
4 M. A. Floyd, D. A. Evans and P. E. Howse, J. Insect Physiol. *22*, 697 (1976).
5 We acknowledge the assistance of Mr S. W. Bolwell in this assay, and are particularly grateful for his help with the testing of **3**.
6 G. Becker, Z. vergl. Physiol. *30*, 253 (1943).
7 E. O. Wilson, W. H. Bossert and F. E. Regnier, J. Insect Physiol. *15*, 597 (1969).
8 The chirality or enantiomeric purity of the myrtenol present in frass has not been determined due to the small amount of material available.

DNA sequence relatedness of some *Neurospora* isolates

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Summary. DNAs from several isolates of *Neurospora* were characterized by thermal denaturation and DNA-DNA hybridization. All the DNAs were found to have 3 components. Based on the percentage of DNA hybridization and the degree of base pair complementation with reference DNAs it has been possible to assign some of the isolates to the known species groups.

Quantitative data on genetic relatedness have been successfully obtained through the use of heterologous duplex formation in vitro, thereby, comparing a large number of shared genes[2–6]. The midpoint temperature of dissociation (Te) of hybrid duplexes is used as a criterion of the degree of complementation of DNA-strands from 2 different sources[2–6]. Homologous hybrids are assumed to be perfectly matched[7]. Thus, difference in the Te (ΔTe) of homologous and heterologous hybrids gives an estimation of the DNA-sequence divergence between the 2 species[8]. For the purpose of establishing a phylogenetic tree of different known species or to assign an unknown isolate to an authentic species group, reciprocal DNA-DNA hybridization in all combinations should be employed[9]. In a previous attempt[4], however, using 2 reference species the DNA-sequence divergence among some authentic species of *Neurospora* was estimated and their positions on a phylogenetic tree were depicted. *Neurospora* isolates collected from nature have been analyzed for their taxonomic status based on the crossing behavior[10]. This communication, based on

1 Present address: Laboratory of Pathophysiology, Bldg. 10, National Institutes of Health, Bethesda, Md. 20014, USA.
2 D. E. Kohne, J. A. Chiscon and B. H. Hoyer, J. Human Evolut. *1*, 625 (1972).
3 P. K. Chakrabartty, J. Hered. *66*, 213 (1975).
4 P. K. Chakrabartty, J. gen. appl. Microbiol., Tokyo *23*, 77 (1977).
5 B. D. Cowie, R. J. Avery and S. P. Champe, Virology *45*, 30 (1971).
6 G. F. Shields and N. A. Straus, Evolution *29*, 159 (1975).
7 R. J. Britten and D. E. Kohne, Science *161*, 529 (1968).
8 B. J. McCarthy and R. B. Church, A. Rev. Biochem. *39*, 131 (1970).
9 S. K. Dutta, Mycologia *68*, 388 (1976).
10 D. D. Perkins, B. C. Turner and E. G. Barry, Evolution *30*, 281 (1976).

Table 1. Thermal denaturation profile data of *Neurospora* isolate DNAs

Isolates	Low temperature denaturing population		Medium temperature denaturing population		High temperature denaturing population	
	Fraction (%)	Tm in °C (moles % GC)	Fraction (%)	Tm in °C (moles % GC)	Fraction (%)	Tm in °C (moles % GC)
N. sp. (Mysore)	13	81.1 (29)	13	86.5 (42)	74	92.5 (57)
N. sp. (Gianjor)	7	80.6 (28)	17	86.5 (42)	76	92.2 (56)
N. sp. (Lahore)	10	79.6 (25)	30	85.7 (40)	60	91.5 (54)
N. sp. (Kuala Lumpur)	6	83.0 (33)	10	87.2 (45)	84	92.2 (56)

Table 2. Percentage DNA-hybridization and thermal stability measurements of *Neurospora* isolates

Source of ^{32}P-DNA	Source of unlabeled DNA	Normalized percentage hybridization	Te of labeled product (°C)		ΔTe (°C)	
			Low temperature eluting fraction	High temperature eluting fraction	Low temperature eluting fraction	High temperature eluting fraction
N. crassa	*N. crassa*	100	84.3	91.4	0.0	0.0
N. crassa	*N.* sp. (Mysore)	96	80.0	88.3	4.3	3.1
N. crassa	*N.* sp. (Gianjor)	83	78.0	87.5	6.3	3.9
N. crassa	*N.* sp. (Lahore)	83	76.5	86.7	7.8	4.7
N. crassa	*N.* sp. (Kuala Lumpur)	84	76.5	86.2	7.8	5.2
N. sitophila	*N. sitophila*	100	82.3	91.2	0.0	0.0
N. sitophila	*N.* sp. (Mysore)	86	78.8	89.1	3.5	2.1
N. sitophila	*N.* sp. (Gianjor)	89	80.5	89.4	1.8	1.8
N. sitophila	*N.* sp. (Lahore)	88	78.2	87.3	4.1	3.9
N. sitophila	*N.* sp. (Kuala Lumpur)	86	78.3	87.3	4.0	3.9

shared DNA-sequences with the reference species, attempts to make a parallel survey of the taxonomic status of some of the natural isolates of *Neurospora*.

In this study *Neurospora* species (Mysore-1e a, India; DDP P287), *N.* sp. (Lahore-1A, Pakistan; DDP P349), *N.* sp. (Gianjor-1 A + a, Bali; DDP P202), *N.* sp. (Kuala Lumpur-1e a, Malay; DDP P274); all obtained from Dr D. D. Perkins, Stanford University, California, USA, were used. Representative strains are also available from Fungal Genetics Stock Center[11] (FGSC, California State University, Humboldt, Arcata, California 95521, USA). Reference ^{32}P labeled DNAs were isolated from *N. crassa* 74A, FGSC 987; and *N. sitophila* 10 B-A, FGSC 580 (previously known as *N. intermedia*[4, 9, 12]).

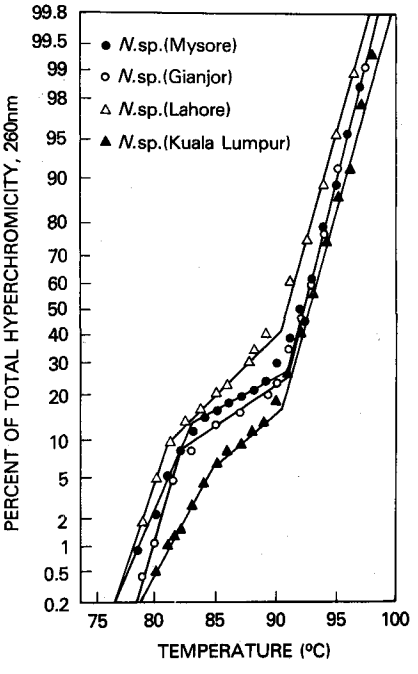

Fig. 1. Normal probability plot of thermal denaturation of DNAs of *Neurospora* isolates in 0.13 M phosphate buffer.

All the DNAs used were characterized by their base composition as calculated from the 50 percentile point of the hyperchromic transition (Tm)[13]. On a normal probability plot thermal denaturation profiles of all the classified *Neurospora* sp. DNAs were found to be triphasic indicative of the presence of 3 components of DNA[4]. This was also found to be true for the *Neurospora* isolate DNAs. Figure 1 presents the thermal denaturation profiles of DNAs from *Neurospora* isolates. Table 1 summarizes the thermal denaturation profile data. From the base composition of their DNAs it seems that all the isolates form a homogeneous group. Next, attempt was made to study the nucleotide sequence homologies of the isolates. The details of methods have been published[3, 4]. In this work ^{32}P labeled DNAs from *N. crassa* of from *N. sitophila* reacted with the unlabeled DNAs from the same source or from the isolates. The separated hybrid duplexes adhering to a column of hydroxylapatite were subjected to a stepwise increase in temperature. Figure 2 shows the thermal elution profiles of the reaction products as measured by radioactivity. The elution profiles are biphasic when plotted on a normal probability plot in agreement with the previously published observation[4]. Table 2 presents the thermal elution data of hybrid duplexes.

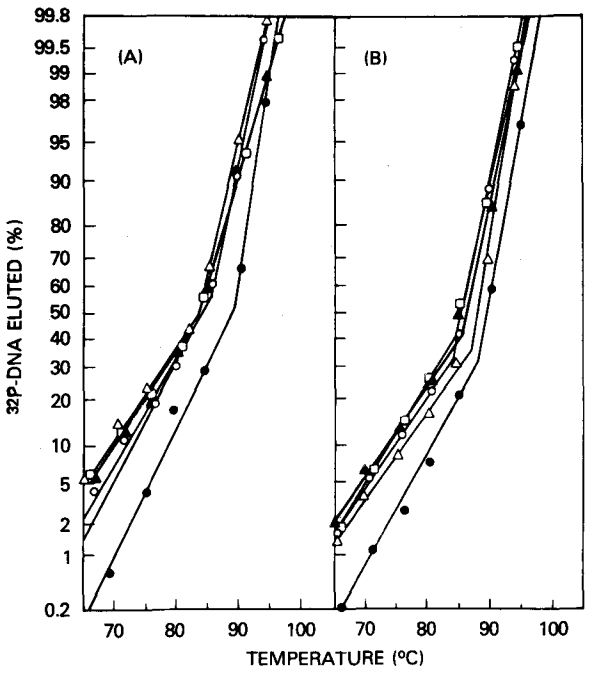

Fig. 2. Normal probability plot of thermal elution profiles of duplexes formed between reference ^{32}P-DNA and excess of unlabeled DNAs from different sources. *A* Between *N. crassa* ^{32}P-DNA and ○, *N.* sp. (Mysore); △, *N.* sp. (Lahore); ▲, *N.* sp. (Gianjor); □, *N.* sp. (Kuala Lumpur); and ●, *N. crassa* DNAs. *B* Between *N. sitophila* ^{32}P-DNA and ▲, *N.* sp. (Mysore); ○, *N.* sp. (Lahore); △, *N.* sp. (Gianjor); □, *N.* sp. (Kuala Lumpur); and ●, *N. sitophila* DNAs.

Considering the base composition and the sequence divergence data together, from the present work and from my previous work[4], it seems that *N.* sp. (Gianjor) occupies a position superimposing on or very close to that of species group *N. tetrasperma* on a phylogenetic tree. *N.* sp. (Mysore) is almost equidistantly related to both *N. sitophila* and *N. crassa* and seemingly occupies a position in the species group *N. intermedia*. The present study has been unable to assign the isolates *N.* sp. (Lahore) and *N.* sp. (Kuala Lumpur) to any particular reference species group. However, they seem to be very close to each other and relatively closer to *N. sitophila* than to *N. crassa*. Based on crossing behavior, however, all the strains of *N.* sp. (Lahore) were tentatively assigned to the species group *N. crassa*[10]. Assignments of the isolates to the known species groups, in this work, is treated as only tentative and certainly not final. But results in this study conform largely to the established strain assignments[10] of the isolates. It is believed that the data presented here will be helpful to other workers interested in *Neurospora* genetics and evolutionary biology.

11 R. W. Barratt and W. N. Ogata, Neurospora Newl. *21*, 33 (1974).
12 D. D. Perkins and B. C. Turner, Neurospora Newl. *20*, 41 (1973).
13 M. D. Knittel, C. H. Black, W. E. Sandine and D. K. Fraser, Can. J. Microbiol. *14*, 239 (1968).

Neurosecretory control of Corpora allata activity in cockroach, *Periplaneta americana* L.

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Summary. The brain neurosecretory material is involved in the control of Corpora allata activity during post-embryonic development. A high concentration of neurosecretory material within the Corpora allata restrains the activity of the gland.

It is well known that the neurosecretory cells (NSC) of the protocerebrum, prothoracic gland (PTG) and Corpora allata (CA) govern the development of insects. However, while the control mechanism of brain NSC and PTG are understood that of CA is not quite clear as yet. Most of the references about the control of CA are concerned about their activity in relation to reproduction. Engelmann[2] held that the activity of CA during ovarian development in *Leucophaea maderae* is regulated purely by nervous stimuli from the brain and suboesophageal ganglion. But Highnam[3] in *Schistocerca gregaria* established that the median NSC of pars intercerebralis liberate a substance which is involved in the activation of the CA of adult females. The median NSC have been found to control protein metabolism which may allow an activation of CA. Strong[4] in *Schistocerca paranensis* suggested that lateral NSC activate the CA. Scharrer[5–7] demonstrated experimentally that the inhibition of CA is brought about by the protocerebral NSC. She also suggested the involvement of neurosecretory components in the regulation of cyclical activity of CA[8]. Khan and Fraser[9] gave histological evidence that the neurosecretory material restrains the activity of CA. The aim of the present study was to examine in more detail the correlation between the neurosecretion and the activity of CA, and to investigate further the role of neurosecretion in the control of CA activity during development of *Periplaneta americana*.

Material and methods. The different instar nymphs were reared at 25 °C temperature. The heads of the nymphs of known ages were fixed in aqueous Bonins fluid and em-

1 Present address: P. G. Department of Zoology, Patna University, Patna, Bihar.
2 F. Engelmann, J. Insect Physiol. *1*, 257 (1957).
3 K. C. Highman, Q. J. microsc. Sci. *103*, 52 (1962).
4 L. Strong, J. Insect Physiol. *11*, 271 (1965).
5 B. Scharrer, Anat. Rec. *96*, 577 (1946).
6 B. Scharrer, Biol. Bull. Woods Hole *102*, 261 (1952).
7 B. Scharrer, Biol. Bull. Woods Hole *121*, 370 (1961).
8 B. Scharrer, Gen. Comp. Endocr. *2*, 30 (1962).
9 T. R. Khan and A. Fraser, Mem. Soc. Endocr. *12*, 349 (1962).