ygen consumption in stage 4 respiration was measured in a medium containing 50 mM KCl; 25 mM Tris-HCl at pH 7.4; 10 mM KH_2PO_4/Na_2HPO_4 buffer at pH 7.4; 8 mM $MgCl_2$; 70 mM sucrose and 0.05% bovine serum albumin. The following NAD^+-linked substrates were used: 5 mM pyruvate; 5 mM pyruvate plus 5 mM malate; 10 mM glutamate; 10 mM glutamate plus 5 mM malate, 10 mM glutamate plus 5 mM pyruvate. Succinate at a concentration of 5 mM or with 3 $\mu mole \cdot l^{-1}$ rotenone was used to study succinate: ubiquinone reductase linked oxygen uptake. Finally 5 mM ascorbate plus 0.25 mM tetramethylphenylenediamine (TMPD) and 1 $\mu g \cdot mg^{-1}$ protein of antimycin A was used to study the effect of camphor on cytochrome c linked respiration.

Synthetic grade d,l-camphor supplied by BDH was used. Stock solutions of camphor were prepared in 30% ethanol/water solvent to overcome the low solubility of camphor in water. The camphor solution was added in aliquots of 25–50 µl. Runs where equivalent amounts of the solvent alone were added were done as blanks. No detectable changes in the rates of oxygen consumption were observed in these blank runs.

Results and discussion. The rate of oxygen uptake with the NAD^+-linked substrates, was decreased by the addition of camphor. Inhibition as a function of camphor concentration is shown in the figure. The rates were approximately halved by the addition of 4–5 mM camphor which is equivalent to 4–5 $\mu mole \cdot mg^{-1}$ protein.

When succinate without rotenone was employed as the substrate, maximum inhibition corresponding to a 50% decrease in the rate of oxygen consumption occurred at a camphor concentration of about 8 mM. The results were the same as those reported previously for rat kidney mitochondria[6]. When rotenone was present with succinate as substrate, camphor, at concentrations < 8 mM, did not produce any decrease in the rate of oxygen consumption suggesting that at these concentrations, camphor specifically inhibits NAD^+-linked respiration. The decrease in oxygen consumption observed for the succinate substrate in the absence of rotenone may result from some initial conversion of succinate to malate in the citric acid cycle with the subsequent effect of camphor on malate linked oxygen uptake.

With succinate substrate, concentrations of camphor > 8 mM caused a large increase in the rate of oxygen consumption. A similar effect was observed when 5 mM of succinate was added to mitochondrial suspensions which had been respiring on NAD^+-linked substrates and which contained > 8 mM camphor. Oxidation of NAD^+-linked substrates was not enhanced by these concentrations of camphor. These results may be explained in terms of currently accepted pathways of electron transport and energy transfer[7–13].

Camphor at concentrations < 8 mM is postulated to block electron transport from NADH to ubiquinone or block energy transfer at site I and this is consistent with the results of the experiments with camphor at concentrations < 8 mM. If higher concentrations of camphor uncouple at site II, an increased rate of oxygen uptake will result with the succinate substrate, but not with NAD^+-linked substrates whose electron transport path to site II has been inhibited.

When ascorbate plus TMPD were used as the substrate in the presence of antimycin A, camphor caused a relatively small decrease, $< 25\%$, in the rate of oxygen consumption at concentrations > 10 mM. This indicates that camphor partially blocks electron transport from reduced cytochrome c or energy transfer from site III. High camphor concentrations (> 8 mM) are required to observe measurable decreases in oxygen consumption. Therefore, the result of this blockage of electron transport or energy transfer is masked by uncoupling by camphor at site II when the succinate substrate is employed.

1 Present address: Department of Human Metabolism and Clinical Biochemistry, Medical School, University of Sheffield, Beech Hill Road, Sheffield S10 2RX (England).
2 Acknowledgment. D.F.G.-C. is grateful to the New Zealand Cancer Society (Wellington Branch) for financial support. We thank Mrs E. Dye for technical assistance and Dr W. Jordan for valuable discussions.
3 To whom reprint requests should be addressed.
4 M.A. Cardullo, Ph.D. Thesis, Boston College 1971.
5 V.A. Jegede, Ph.D. Thesis, Boston College 1974.
6 D.F. Guilland-Cumming and G.J. Smith, Experientia *35*, 659 (1979).
7 B.C. Pressman, J. biol. Chem. *238*, 401 (1963).
8 J.B. Chappel, J. biol. Chem. *238*, 410 (1963).
9 B. Chance and G. Hollunger, J. biol. Chem. *238*, 432 (1969).
10 R.J. Guillory and E.C. Slater, Biochim. biophys. Acta 105, 221 (1965).
11 B. Chance and G. Hollunger, J. biol. Chem. *238*, 418 (1963).
12 L. Ernster and C.P. Lee, A. Rev. Biochem. *33*, 729 (1964).
13 C. Bhuvaneswaran and K. Dakshinamurti, Biochemistry *11*, 85 (1972).
14 W.C. Schneider, in: Manometric Techniques, p. 188. Ed. W.W. Umbreit, R. Burris and J.E. Stauffer. Burgess Publishing Co., Minneapolis 1965.

The antennae and mating behaviour of *Drosophila* females[1]

B.N. Singh, O.P. Singh and J.P. Gupta

Genetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi-221005 (India), 23 January 1981

Summary. The females of *Drosophila bipectinata* and *D. malerkotliana* are able to discriminate between their own and alien males in the absence of antennae. Thus mate recognition seems to depend on contact chemoreceptors in these 2 species.

The role of antennae in the mating behaviour of females has been studied in many species of *Drosophila*. Mayr[2] found that the removal of the antennae of *D. melanogaster* females reduced their receptivity to courtship, which led him to suggest that the antennae of females act as receptors in the chain of stimuli-response courtship reactions. The removal of the antennae of females not only reduces their receptivity to courtship but it also removes sexual isolation between *D. pseudoobscura* and *D. persimilis*[2,3]. However, Manning[4] has found that the removal of antennae has no effect on sexual isolation between *D. melanogaster* and *D. simulans* which suggests that the females of these species discriminate between their own and alien males through contact chemoreceptors. An interesting case has been

Number of females dissected and percentage inseminated in 'multiple choice' experiments between *D. bipectinata* (b) and *D. malerkotliana* (m)

Crosses Females	Males	No. pairs of ♀♀	Percent females inseminated Homogamic	Heterogamic	χ^2	Isolation index**
Experimental b+m	b	88	89.77	7.95	117.88	0.84
Cont. b+m	b	95	88.42	4.21	135.48	0.91
Experimental b+m	m	95	86.31	13.68	100.84	0.73
Cont. b+m	m	97	89.69	16.49	104.33	0.69

*Calculated from a 2×2 contingency table. **Stalker's isolation index.

reported by Ehrman[3], who found that the removal of female antennae causes complete cessation of mating in sibling species of the *D. willistoni* species group.

D. bipectinata and *D. malerkotliana* are 2 closely related and sympatric species of the *bipectinata* species complex. Interspecific hybridization occurs in the laboratory[5–7] as well as in nature[8]. The purpose of the present article is to report the results of 'multiple choice' experiments designed to investigate the role of antennae in mating behaviour of females of these 2 species.

Materials and methods. The local strains of both species are being maintained in our laboratory on regular *Drosophila* food medium. Virgin females and males were collected and aged for 7 days. The antennae of the females were amputated 2 days before making the crosses. To study the mating behaviour and sexual isolation between the 2 species, 1 female of each of the 2 species was placed together with 1 male of one of the species in a food vial. After exposing the females to the male for 5 days, both females were dissected and their sperm receptables were examined for the presence or absence of sperm. Isolation index was calculated following the formula of Stalker[9].

Results. The results are presented in the table. It is evident from the data that there is no considerable change in the frequency of matings in experimental crosses with deantennated females when compared to that of controls. Homogamic matings are more frequent than heterogamic matings and sexual isolation remains pronounced in all the crosses studied. The χ^2-values indicate that the deviation from randomness of matings is statistically significant ($p < 0.001$). This shows that the females of both species are able to discriminate between their own and alien males in the absence of antennae.

Discussion. Since in *Drosophila* generally the females rather than the males are responsible for discrimination, the data presented here clearly show that the removal of antennae neither reduces the receptivity of females to courtship nor removes sexual isolation between *D. bipectinata* and *D. malerkotliana.* Thus these 2 species of the *bipectinata* complex are different in this respect from other species of *Drosophila* in which the role of the antennae in mating behaviour of females has been demonstrated by different investigators. In the *D. willistoni* species group, the removal of female antennae causes complete cessation of mating[3]. In *D. melanogaster* the amputation of the antennae of females reduces their receptivity to courtship to a marked degree but not their ability to discriminate[2]. On the other hand, the antennae of females not only act as receptors but also as organs of species discrimination in *D. pseudoobscura* and *D. persimilis*[2,3]. Therefore, it is evident that the role of antennae in mating behaviour of females varies in different species groups of *Drosophila.*

There is evidence to show that contact chemoreceptors play an important role in sex stimulation and mate recognition. In many species groups of *Drosophila,* males tap the females with their forelegs and the mating takes place due to the exchange of chemical stimuli during this tapping[10]. Similarly, sex stimulation and species recognition seem to depend on contact chemoreceptors in *D. bipectinata* and *D. malerkotliana.*

1 We are grateful to the Head of the Department of Zoology, B.H.U. for providing the necessary facilities. Thanks are due to the U.G.C., New Delhi, India for awarding a junior research fellowship to O.P.S.

2 E. Mayr, Evolution *4,* 149 (1950).
3 L. Ehrman, Evolution *13,* 147 (1959).
4 A. Manning, Anim. Behav. *7,* 60 (1959).
5 R.D. Narda, J. Genet. *60,* 1 (1970).
6 J.P. Gupta, Experientia *29,* 224 (1973).
7 I.R. Bock, Aust. J. biol. Sci. *31,* 179 (1978).
8 J.P. Gupta, Y.N. Dwivedi and B.K. Singh, Experientia *36,* 290 (1980).
9 H.D. Stalker, Genetics *27,* 238 (1942).
10 H.T. Spieth, Bull. Am. Mus. nat. Hist. *99,* 401 (1952).

Detection of nucleolar organizing regions in the chromosomes of *Nigella damacena*

M. Hizume, A. Tanaka and H. Shigematsu[1]

Biological Institute, Faculty of Education, Ehime University, Matsuyama 790 (Japan), 10 June 1981

Summary. Nucleolar organizing regions (NORs) of *Nigella damacena* were detected at the secondary constricted regions on 3 pairs of chromosomes by the silver staining method. The size of the NORs were found to be correlated with the size of the nucleoli.

Secondary constrictions have been observed on 3 pairs of *Nigella damacena* chromosomes and thought to be the nucleolar organizing regions (NORs)[2]. To confirm that these secondary constrictions are actual NORs, a recently-developed staining method that can specifically detect NORs has been applied to chromosomes of *Nigella damacena.*

Materials and methods. Seeds of *Nigella damacena* L. were purchased from a commercial source (Sakata Seed Co., Yokohama) and incubated on moistened filter paper in