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.*

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

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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

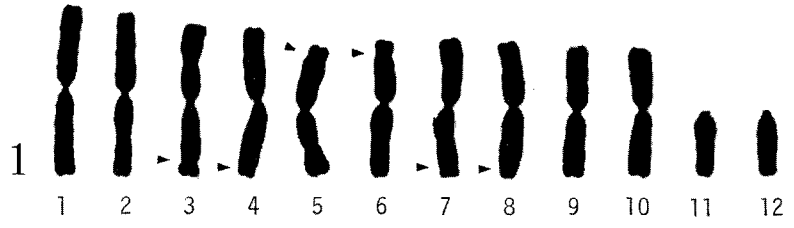
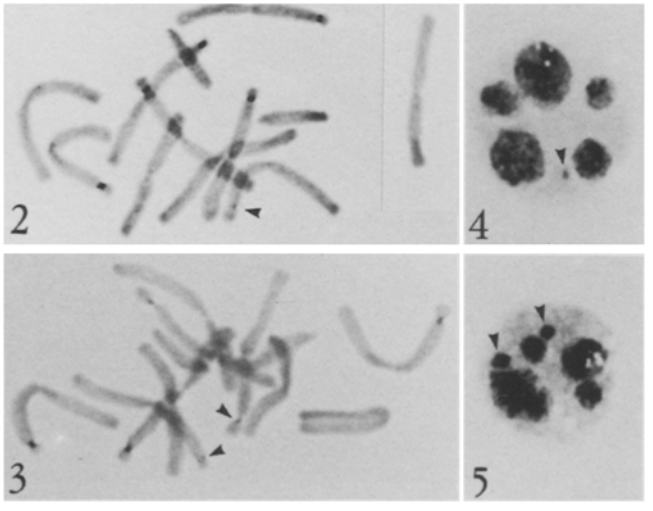
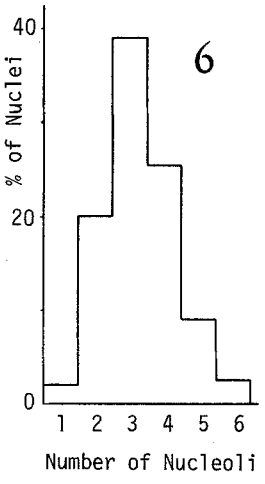


Figure 1. Metaphase chromosomes of *Nigella damacena* stained with Giemsa. Arrow heads indicate the secondary constrictions. × 1200.





Figures 2 and 3. Metaphase chromosomes of *N. damacena* stained with silver nitrate. The smallest stained regions, indicated by arrow heads, varied in number among seedlings. × 1450.
Figures 4 and 5. Interphase nuclei of *N. damacena* stained with silver nitrate. Arrow heads indicate the smallest nucleoli. × 1100.
Figure 6. Histogram of frequency of nuclei with each nucleolar number. Nucleoli were examined from 1032 of 10 seedlings.

Petri dishes at 20 °C in the dark. After a week, actively growing primary root tips were harvested and pretreated in 0.05% colchicine solution at 20 °C for 2.5 h, then fixed in an ethanol-glacial acetic acid (3:1) mixture at 5 °C for 1 h. The fixed materials were treated with both cellulase and pectinase prior to flame-drying. The slides were stained with a 50% $AgNO_3$ solution at 60 °C for 1–3 h (see Hizume et al.[3] for details). Some slides were stained with 2% Giemsa solution for 10 min for standard karyotype analysis.

Results and discussion. The chromosome complement (2n = 12) was composed of 5 pairs of metacentric chromosomes and a pair of telocentric chromosomes. The secondary constrictions appeared at or near terminal regions in 3 metacentric pairs of chromosomes (No. 3–8, fig. 1). After Giemsa staining in some cells of some clones the secondary constrictions were not apparent, but after silver staining all the 6 secondary constrictions were always heavily stained (figs 2 and 3). While the size of the stained regions in the complement was typically variable, 1 or 2 regions were larger than the others. The smallest stained region seemed to be located in the 4th pair of chromosomes. 1–6 nucleoli, usually 2–4, are visualized as darkly-stained spherules in the nucleus after staining with silver nitrate (fig. 6). In the nucleus with 6 nucleoli their size was graded from large to small, and 2 nucleoli were typically larger while 1 or 2 were typically smaller (figs 4 and 5). This appearance of nucleoli was strongly correlated with the size of the silver stained region on the metaphase chromosomes. The maximum number of nucleoli per nucleus is coincident with the number of the silver stained regions and the secondary constrictions; therefore, we concluded that all the secondary constrictions of *Nigella damacena* chromosomes were NORs.

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Cytochemically rich neuroglia invest metacercariae in the brain of *Phoxinus phoxinus* L.

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Summary. Histochemical determination of neuroglia investing parasites in naturally infected fish depends upon recognition of the highly soluble or diffusible nature of the cytoplasm.

Remarkably, minnows survive although the brain is parasitized by the trematode, *Diplostomum phoxini* (Faust, 1917). Cercariae invade fish, migrate to the brain[2] and grow from 0.22 × 0.03 mm^3 to metacercariae, 0.35 × 0.13 mm in size[3] or larger[4]. They live anywhere in the brain but most lie beneath the ependyma of the IVth ventricle[2]. Parasitized fish live for more than 2 years in the laboratory[5]; all mature minnows in a population can be infected[2–6], while reports of parasite-induced aberrant behaviour[7,8] or death of the host[3] are rare.