egg cell models was 120 μm), the amount of BD enclosed in the egg cell models was found to be 22.8 $\mu g/10^3$ eggs. This value coincided closely with the experimentally determined value of 22.5 $\mu g/10^3$ eggs.

Next, fluorescein-labeled proteoglycan (prepared from embryos at the gastrula stage by means of Na-pyrophosphate extraction and DEAE-Sephadex chromatography as previously described[5]) was infiltrated into cell models (8–16-cell stage) by incubation. An equilibrated state was not achieved during incubation for 2 h, and cell models seemed to accumulate the proteoglycans only gradually (fig. 1b).

Examining the distribution of dye-labeled proteoglycans in the cell models under a fluorescence-microscope, it was found that this substance was steadily concentrated into the nucleus (fig. 2), suggesting that the proteoglycans introduced into the cell models have an intense affinity with the nuclear structures. The accumulation of dye-labeled proteoglycans in the nucleus was also ascertained by examining the localization of fluorescein in subcellular structures after fractionation of cell models; accordingly, 73.3% of dye was recovered in the sedimentable portion at 900 × g (nuclei were predominant), 14.0% in the sediment at 105,000 × g (microsomal fraction) and 9.8% in the unsedimentable portion at 105,000 × g (cytosol).

The heterogenous combination of nucleus and cytoplasm can be achieved by means of transplantation of the nucleus[16,17], cell fusion[18,19], and so-called cybridization[20], and these procedures have made important contributions to elucidating the mechanism of nucleus-cytoplasm interactions. Complementary to these techniques, the use of glycerol-treated cell models has the added merit that the essential reactions between nucleus and cytoplasm are observed separately from other complicated reactions occurring in living cells. The situation is comparable to the utilization of glycerinated cell models in the study of cell motility[21].

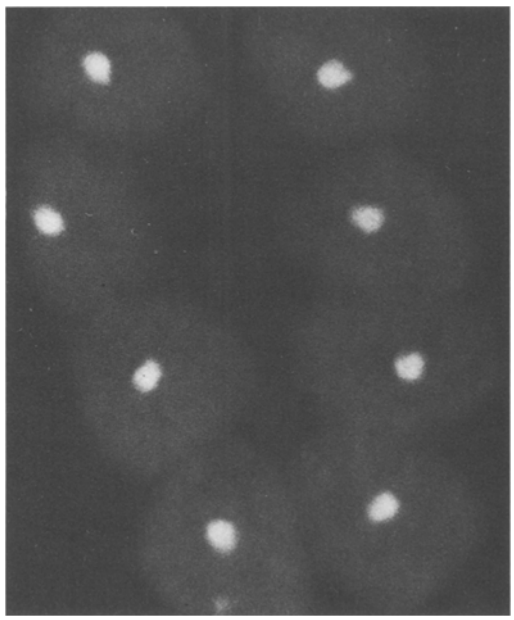

Figure 2. Cell models (8-cell stage) incubated with fluorescein-labeled proteoglycans for 1 h. Accumulation of proteoglycan into the nucleus is very noticeable. The condition of incubation was the same as in figure 1b. (× 500).

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Species-specificity of paragonial substances as an isolating mechanism in *Drosophila*[1]

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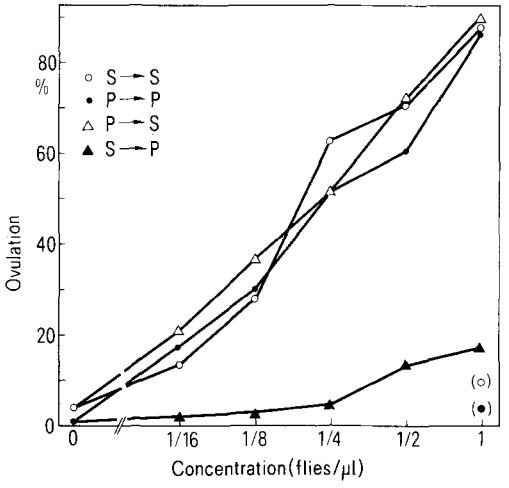
Summary. In 2 closely-related species of *Drosophila*, the oviposition stimulating effects of the substances produced in the male accessory glands (paragonia) are species-specific. A unique reproductive isolating mechanism due to this specificity is discovered.

In many insects, substances produced in the male accessory glands and transmitted to the female during copulation are known to stimulate oviposition[2]. If the effects of these substances were species-specific, they should function as reproductive isolating mechanisms by preventing the wastage of female gametes following erroneous heterospecific mating[3]. However, there has been little evidence for this type of isolating mechanism despite the extensive variations of accessory gland substances among species[4,5].

Drosophila suzukii and its close relative, *D. pulchrella*, were

the species used in this investigation. These 2 species belong to the *suzukii* subgroup of the *melanogaster* species group, and have a highly overlapping distribution in East Asia[6]. They are not only morphologically distinct, but are also qualitatively different in their courtship behaviors (Fuyama, unpublished). Nevertheless, sexual isolation between them is not complete: When *D. suzukii* males were mated with young *D. pulchrella* females, a substantial proportion of the females were inseminated. In contrast, no mating occurred in the reciprocal combination (table 1). The *D. suzukii* sperm transferred interspecifically were normally motile, although the sperm count was usually much lower than that with conspecific mating, possibly because interspecific mating was often interrupted by female rejection. However, despite considerable effort, hybrid progeny were rarely obtained. *D. pulchrella* females inseminated by *D. suzukii* males appeared to be as highly reluctant to oviposit, as were virgins. It was therefore postulated that the seminal fluid of *D. suzukii* was not an oviposition stimulus within *D. pulchrella* females.

To assess the oviposition stimulating effects of accessory gland secretions, direct counts of eggs deposited by females that had received either a glandular implant or an extract injection have traditionally been employed[7,8]. In this study, instead of counting eggs, the probability of a female retaining an ovum in her uterus, 'ovulation frequency', was used as a measure of the stimulus. It was found that 80–90% of 6–8-day-old inseminated females had an ovum in their uterus while usually less than 5% of virgin females did. This rate reflected the rate of oviposition well. This new assay method is much less laborious and more reliable than the traditional one.



Dose-response curve of ovulation frequency to paragonial extract injection. Methanolic extract of the paragonial substances were prepared as follows: 1000 virgin male flies (5–10-day-old) that had been stored at −20 °C were homogenized in 10 ml 80% methanol, and homogenates were centrifuged at 3500 rpm for 10 min. The supernatants were evaporated, the residues dissolved in 1 ml saline, and centrifuged again at 15,000 × g for 10 min. The supernatants, containing approximately 1 fly equivalent per μl, were diluted to various concentrations, and 0.25 μl each were injected into the abdominal cavity of 6-day-old virgin females. Two days after injection, the females were dissected and examined for the presence of ova in the uterus. Ovulation frequency is the proportion of females retaining ova to total females dissected. ○, Extract from *D. suzukii* male injected into *D. suzukii* female; ●, from *D. pulchrella* to *D. pulchrella;* △, from *D. pulchrella* to *D. suzukii;* ▲, from *D. suzukii* to *D. pulchrella.* Points shown in parentheses represent the corresponding figures obtained by the injections of virgin female extracts. Each point is based upon 98–103 females.

Virgin *D. suzukii* females that had received implants of conspecific paragonial gland in their thoracic regions when 4–6 days old showed, 2 days after implantation, 89.1% ovulation (n = 110), and virgin *D. pulchrella* females, 75.2% (n = 133). A significantly reduced ovulation rate of 54.5% (n = 165, $p < 0.001$) was observed when *D. pulchrella* females received implants of *D. suzukii* gland. After the reciprocal implantation, *D. pulchrella* gland to *D. suzukii* females, an ovulation rate as high as that for conspecific implantation was found (85.5%, n = 145). Implantation of an equivalent amount of testes fragments was not effective, resulting in 1.7% (n = 118) and 0.9% (n = 112) ovulation rates for *D. suzukii* and *D. pulchrella,* respectively.

To quantify the stimulus, methanolic extracts of male flies prepared according to a method similar to that described by Baumann[9] were injected using a micropipette into the abdominal cavity of virgin females, which were then examined for ovulation. The figure shows the dose-response curves to these methanolic extracts. The results confirmed those obtained with the implantation experiments. *D. suzukii* male extract injected into *D. pulchrella* females had very little stimulatory effect at any concentration, whereas the reverse injection was as effective as a conspecific one. Note that the injection direction in which the stimulus is lacking is that in which interspecific mating does occur. A plausible explanation for these asymmetries found in both mating and ovulation stimulus may be the existence of 2 different isolating mechanisms, one pre-mating and the other post-mating, which comprise a complementary isolating system. This hypothesis was tested by artificially supplementing the deficit in ovulation stimulus. *D. pulchrella* females mated at

Table 1. Sexual isolation between *Drosophila suzukii* and *D. pulchrella*

Matings	Age of females days	No. tested	No. inseminated	%
D. suzukii ♀ × *D. pulchrella* ♂:	0	178	0	0.0
	4	200	0	0.0
D. pulchrella ♀ × *D. suzukii* ♂:	0	510	42	8.2
	4	209	1	0.5

A strain of *D. suzukii* derived from several females caught at Hachijo Is., Tokyo in 1978, and a strain of *D. pulchrella* derived from a single gravid female caught at Oiso near Tokyo in 1979 were used throughout. Flies were reared on corn meal-yeast-glucose medium at 20 °C under continuous light. To test for sexual isolation, 10 virgin females of each age were mated with the same number of 4–6-day-old males in a culture vial (3 × 10 cm). After 2 days, the females were dissected and examined for the presence of spermatozoa in the ventral receptacle and/or spermathecae.

Table 2. Effect of conspecific male extract injection on the productivity of interspecific hybrids by *D. pulchrella* females

Treatments	No. of females injected	No. of females producing hybrids	No. of hybrids produced	Mean no. of hybrids/producing females ± SEM
Male extract	58	7	24	3.4 ± 1.1
Control	59	1	1	1

Newly emerged *D. pulchrella* females were mated with 5-day-old *D. suzukii* males. Copulated females were collected and, at 5–6 days old, received an injection of extract from *D. pulchrella* male equivalent to ¼ flies. The injected females were allowed to oviposit singly for the following 10 days. Control females were treated with the same volume of saline.

emergence with *D. suzukii* males and subsequently injected with an extract from their own species produced a significantly greater number of hybrid progeny than did controls (table 2). The small number of hybrids produced by even treated females was not unexpected considering the scarcity of sperm transferred during interspecific copulation, and the unusual route by which stimulant was administered. Rather unexpected was the fact that vigorous hybrids of both sexes were produced in almost equal frequency. Moreover, the female hybrids were fertile when backcrossed to the parent species, although the male hybrids were sterile.

These results indicate that the genetic incompatibility between the 2 species is very small, and that the species-specificity of the paragonial substances plays a certain role as a hybridization barrier, though the pre-mating barriers are obviously of primary importance. I would like to propose the term 'paragonial sterility' for such gametic isolation. With paragonial sterility, a female first inseminated by a heterospecific male, and then by a conspecific male will not suffer the reduction of fertility found with 'insemination reaction', another gametic isolating mechanism known in *Drosophila*[10]. Perhaps such paragonial sterility, having been favored by natural selection, will be discovered in other species, especially those whose pre-mating isolation is incomplete.

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Differential aminotransferase activity in normal, allatectomized, brain-cauterized and juvenoid treated male and female bugs *(Lohita grandis)*[1]

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Summary. L-Alanine:2-oxoglutarate aminotransferase (GPT, 2.6.1) and L-aspartate:2-oxoglutarate aminotransferase (GOT, 2.6.1) activity were different in different tissues of each sex and also in the 2 sexes. In all tissues of both sexes GPT activity was found always to be higher than GOT activity. Allatectomy leads to a decline in the activity of both enzymes in all tissues of males and females. Juvenoid treatment of the allatectomized insects reverses the effect of allatectomy. After both allatectomy and brain-cauterization and extremely high decline of the activity of both enzymes in comparison to cases in which only allatectomy had been carried out.

The literature on the effects of juvenoids, allatectomy and abalation of median neurosecretory cells at the level of the whole organism and at the biochemical level in different insect species has grown exponentially over the past few decades, but there have been few demonstrations of direct or indirect relationships between the application of a particular juvenoid, or allatectomy or brain-cauterization, and synthesis or activity of a particular enzyme[3,4]. The 1st indication of differential cellular enzyme activity in normal and sterile boll weevils was given by Chang et al.[5,6], and the occurrence of different transaminase activities in different insect tissues was reported by Bheemeswar and Sreenivasaya[7] in *Bombyx mori*, Chen and Diem[8] in *Drosophila melanogaster*, and Mandal et al.[9] in *Schizodactylus monstrosus*. A decline in the activity of transaminase resulting from allatectomy in adult insects was first reported by Wang and Dixon[10]. The application of juvenile hormone inducing the activity of acid phosphatase in different tissues was reported by Beel and Feir[11]. Such fragmentary findings demonstrate that corpora allata play an important function on the cellular enzyme level, apart from their main gonadotropic role in adult insects. Therefore it is of academic interest to know actually what role the different endocrine centres play in affecting the cellular enzyme level, and whether there are any sexual differences in their function. The present investigation was undertaken to find out the effects of brain, corpora allata and synthetic juvenoids on the transaminase activity in adult male and female *Lohita grandis* Gray (Pyrrhocoridae: Heteroptera: Hemiptera).

The insects were reared in the laboratory following the procedure described elsewhere[12] and emerging adult (4 h after emergence) males and females were used in the experiments. The necessary organ tissues for the experiments were collected by dissecting the insects under Ringer's solution mixed with phenyl thiourea. The hemolymph was collected separately in ice-cold centrifuge tubes previously coated with phenyl thiourea to inhibit the tyrosinase activity. Allatectomy was performed following the method outlined by Stay and Tobe[13], and brain-cauterization was performed using the technique of Girardie[14]. The juvenoid used here was the highly active compound of hemipteran bugs which was synthesized and bioassayed by Filho et al.[15] and Pinchin et al.[16,17]. This juvenoid compound was a derivative of N-geranylaniline and its chemical structure is N-(2,5-dichlorophenyl)-3,7-dimethyl-2,6-octadienylamine. It was injected at a dose of 20 µg/insect in 10 µl olive oil as solvent. Control insects received only the same quantity of olive oil. Insects were sacrificed after 24 and 48 h after each treatment. The enzyme was estimated following the methods described by Reitmann and Fränkel[18] and the protein content was determined by the method of Lowry et al.[19] using bovine serum albumin as standard. In each treatment 60 females and 40 males were used and all surgically treated animals were kept at