dually innervated tectal lobes have been produced, all or part of the projection normally destined for the ipsilateral tectal lobe has been diverted onto the contralateral one in adult animals (*2, 5, 15–18*). Thus, Levine and Jacobson demonstrated an interdigitating termination pattern in parts of dually innervated goldfish tecta that appears somewhat similar to the one we report (*16*). However, in their preparation distinctly labeled tracts could be seen in bands for the unlabeled eye when high-power microscopy was used. Electrophysiological maps of these goldfish were highly variable, with large binocular areas dispersed among single eye zones. The regular alternating eye bands predicted by the autoradiography were not seen electrophysiologically. A number of other investigators, however, using longer survival times but essentially the same preparation, have obtained electrophysiological evidence of eye-specific terminal clumping (*2, 17*). Schmidt has also demonstrated autoradiographically that clumping occurs in the same regions of the tectum where it was detected electrophysiologically (*5*). Similar clumping has also been observed following unilateral tectal damage in fetal (and neonatal) rodents (*7, 19*).

Our results differ from these previous observations most notably in the consistency of the banding pattern from animal to animal and in the fact that it is continuous across the entire tectal surface. Recent evidence in goldfish suggests that tectal "specificity" markers have a strong dependence on previous innervation patterns (*5*). Consequently, the fact that ours is a developing, rather than an established system may account for the consistency and completeness of the bands we observe.

All previous experiments on dually innervated anuran tecta have indicated a uniform termination pattern (*18*). Dual innervation has generally been produced by unitectal ablation in adult anurans (*18*). Sharma implanted a supernumerary eye above the midbrain in *Rana pipiens* embryos. A few electrode penetrations made in the tecta of four animals showed no evidence of eye-specific clumping (*20*). Hunt and Jacobson reported electrophysiological studies of three-eyed *Xenopus laevis* (*21, 22*). Their maps indicated that each eye projects continuously across the tectum in apparent disregard of the other eye's presence (*23*).

It should be possible to determine whether the anatomically observed partitioning of tectal space in our three-eyed *Rana pipiens* is equally pronounced electrophysiologically. However, the anatomical results presented here indicate that during development ganglion cell axons dramatically alter their normal continuous termination pattern in the presence of a projection from a second retina. Banding may result if afferent fibers from different retinas grow to the tectum in separate bundles and maintain this separation during termination. This seems unlikely in view of the relatively uniform distribution of labeling in the differentiating regions of tadpole tecta (Fig. 3a). Alternatively, innervation of tectal cells by one retina may increase the probability that these cells will accept subsequent innervation from the same eye.

The mechanism of banding is uncertain at present. However, the three-eyed frog preparations provide a unique opportunity to identify and to manipulate the underlying cellular interactions in order to understand their role in the normal development of the retinotectal system. Furthermore, the parallels between our experimentally induced banding and the ocular dominance columns in cat and monkey suggest that the latter termination pattern may be established through interactions which are basic to a wide variety of neuronal projections.

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Drosophila Egg Chambers Develop to Mature Eggs When Cultured in vivo

Abstract. *Egg chambers were injected into the abdomen of adult* Drosophila. *When cultured in this manner, even the earliest detectable developmental stage developed into fully mature eggs. Both isolated egg chambers and those still associated with ovarian structures developed equally well. Maturation occurred within host flies of both sexes in the absence of any hormone treatment.*

Since Clancy and Beadle's (*1*) original publication, several investigators have transplanted *Drosophila* ovaries. Frequently, larval ovaries have been transplanted into larval hosts. Bodenstein (*2*), moreover, has observed that ovaries from both late pupal and newly emerged flies are able to develop after transplantation into adult hosts. King and Bodenstein (*3*) used ovarian trans-

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plantation to determine whether defects of certain female sterile mutants that affect the ovary behave autonomously. They also showed that tumorous ovaries continue to grow when transplanted into females as well as into males.

In this report we confirm that oogenesis proceeds normally when ovaries from adult *Drosophila* are cultured in both female and male flies, and we establish the smallest unit capable of development. To this end we have cultured progressively smaller portions of the ovary and found that the youngest egg chamber [stage 1 (*4*)] can develop into an egg morphologically indistinguishable from a normal egg.

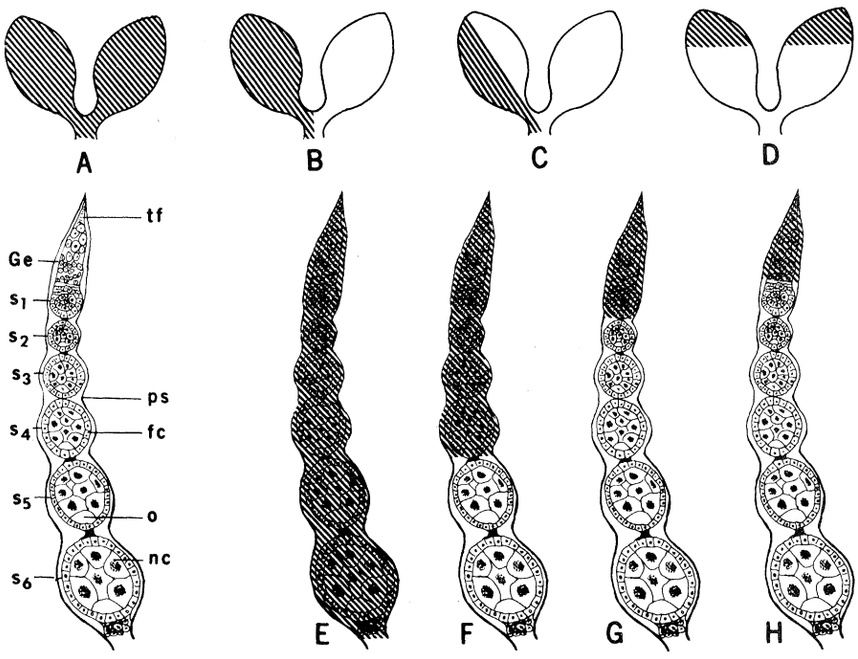

Fig. 1. Diagrams of ovaries (A to D) and ovarioles (E to H), with the parts used for transplantation shaded. The explanatory diagram of an ovariole is based on King (*4*); *tf*, terminal filament; *Ge*, germarium; *s*, egg chambers of stages 1 to 6; *ps*, epithelial sheath; *fc*, follicle cell; *o*, oocyte; and *nc*, nurse cell.

Table 1. Frequency of mature eggs obtained by in vivo culture of ovaries or portions thereof. The specified structures were cultured in the abdomen of 1- to 2-day-old hosts. Capital letters in parentheses (except for I) refer to the parts used for transplantation, as illustrated in diagrams A to H in Fig. 1. The numbers after "Ovariole" refer to the stage of oogenesis (*4*) of the most advanced egg chamber in that ovariole. The numbers after "Egg chamber" mean that the individual egg chambers were between stages 7 and 11. Ovaries A and B were from donors 0 to 1 hour old; in all other cases, donors were about 1 week old. Size measurements of egg chambers (for classification purposes) were done in physiological solution, as described by Jacobs-Lorena and Crippa (*10*).

Structure tested	Days of culture in vivo	Number of hosts with eggs/ number of hosts injected	Average number of mature eggs per injected host
(a) *Drosophila melanogaster fs(2)B* female hosts (wild-type donors)			
(A) Two ovaries	9	20/20	6.3
(B) One ovary	7	20/20	4.3
(C) One-half ovary	9	20/20	9.1
(D) One-half ovary	9	20/20	16.1
(E) One ovariole (6)	7	14/20	1.5
(F) One ovariole (4)	9	8/20	0.4
(G) One ovariole (1)	7	5/20	0.3
(H) One germarium	7	0/20	
(I) One egg chamber (7 to 11)	7	15/20	0.8
(b) *Drosophila melanogaster* male hosts (wild-type donors)			
(D) One-half ovary	6	42/42	7.9
(c) *Drosophila mercatorum* wild-type female donors and hosts			
(B) One ovary	9	10/10	6.3
(d) *Drosophila mercatorum* parthenogenetic female donors (wild-type hosts)			
(B) One ovary	8	43/43	6.4

The *Drosophila* ovary consists of a cluster of about 20 ovarioles arranged in parallel, all merging into a common oviduct. Each ovariole contains a germarium with less-differentiated cells and a vitellarium with linearly arranged egg chambers (Fig. 1). An egg chamber is composed of one oocyte and 15 nurse cells, surrounded by a layer of follicle cells. Egg chambers are formed in the germarium and migrate down the ovariole as they grow and differentiate, leaving the ovary as mature eggs through the oviduct and the uterus.

Ovaries (or parts thereof) from adult wild-type *Drosophila melanogaster* (Colmar strain) donors were injected (*5*) into the abdomen of 1- to 2-day-old females homozygous for *fs(2)B*. The germarial cells of this mutant continue dividing rather than differentiating into oocytes or nurse cells (*4*). This genetically "oocytectomized" host provides a favorable abdominal environment for subsequent development of the test tissue, since there are no host oocytes to compete with those of the implants (*3*), and we could be sure that the eggs found intra-abdominally after the culturing period originated from the implanted structures and not from the host. The same results are obtained by culturing in the wild-type fly. After culturing the ovaries for 7 to 9 days, development was assessed by dissecting the hosts and scoring the mature eggs found in the intra-abdominal cavity. These eggs were morphologically indistinguishable from normal eggs formed in the ovary.

Initially we attempted to determine whether whole ovaries (not containing mature eggs), or portions thereof, would yield mature eggs by in vivo culture. In all cases (Table 1a, A through D) the answer was positive. It is interesting that, although a larger number of egg chambers were injected when a pair of ovaries or halves of ovaries were used (Table 1a, A and B), we consistently obtained larger numbers of mature eggs in cases C and D (half of ovary or ovary tips). Moreover, in cases C and D we found the mature eggs dispersed in the host's abdomen, while in cases A and B loose mature eggs were never found—they were always associated with the injected

ovaries. It is conceivable that intact ovaries (A and B) are not able to release the mature eggs, thereby inhibiting the maturation of egg chambers preceding them in the ovariole. This phenomenon also occurs in uninjected ovaries (*6*). No such constraint is found in cases C and D.

Having determined that ovaries from adult flies were able to produce mature eggs, we proceeded to identify the smallest unit having such capability. By injecting progressively smaller portions of one ovariole (Table 1a, E through G) we found that the youngest egg chamber (stage 1) is able to fully develop into a mature egg under our culture conditions. However, when region 1 and 2 (*4*) of the germarium (that portion having no well-defined egg chambers) was injected, no mature eggs could be observed (Table 1a, H).

Table 1a, I, shows that a single egg chamber, of stages varying from 7 to 11, is capable of developing into a mature egg when cultured in vivo. This finding provides evidence for the developmental autonomy of the growing egg chambers in the absence of all surrounding ovarian structures (for example, membranes, neurons, and tracheal connections).

An interesting finding was that egg chambers are also able to develop into mature eggs when cultured in male hosts (Table 1b), which implies that the implanted ovary induced the host's fat body to produce yolk proteins. This observation complements a report by Kambysellis (*7*) who showed that male *Drosophila* do not synthesize yolk proteins but can be induced to do so by implanted ovaries. The formation of apparently normal eggs in the abdomen of male flies suggests that hormones controlling oogenesis are present in the male, or that such hormones can be induced or brought in with the transplanted ovary. Comparative measurements of the timing of oogenesis (Table 2) show that there is a time lag in the appearance of the first mature egg of about 1 day in males while female hosts show no such lag. Moreover, male hosts produce fewer eggs than female hosts do. Table 2 also shows that the first eggs appear after the first day in culture. If we assume that the most advanced egg chamber in the transplanted one-half ovary is stage 5 (*4*), appearance of the first egg should take about 1.5 days at the rate of oogenesis of wild-type females (*8*). Our results thus indicate that oogenesis in the cultured ovaries proceeds at a rate comparable to that in nontransplanted ovaries.

The eggs obtained from in vivo cultures had all the morphological characteristics of stage 14 mature oocytes (*4*), such as size, shape, structure of the micropyle, structure of the chorion, structure of the dorsal appendages, as well as the aspect of yolk. However, the ultimate proof of their viability and physiological maturity would be to obtain larvae (and adult flies) from these eggs. To our knowledge, successful artificial insemination has never been achieved with *Drosophila* eggs. Seeking the proof for viability of the eggs obtained in culture, we have used a parthenogenetic strain of *Drosophila mercatorum*. Egg chambers from this species develop normally when cultured in vivo (see Table 1, c and d). We also determined that 5.6 percent of eggs normally laid by virgin flies of the *D. mercatorum* parthenogenetic strain are able to develop, a rate that is in good agreement with Carson's 1967 data (*9*). However, of 300 eggs from the parthenogenetic strain obtained by in vivo culture of transplanted ovaries, none underwent development although about 15 larvae were expected. In view of these results, we have tested the parthenogenetic development of about 2000 mature eggs isolated from the ovaries (not laid) of the parthenogenetic strain. Again, no larvae were observed although about 100 larvae would have been expected if the eggs had been laid. This result suggests that it is essential for the eggs to pass through the genital tract in order to attain physiological maturity. We have attempted to induce in vitro maturation of "ovarian" parthenogenetic eggs in four different ways: (i) applying an extract of accessory genital organs over the eggs; (ii) exposing the eggs to a pressure of 300 atm for 10 minutes; (iii) centrifuging eggs at 23,000*g* for 20 minutes; and (iv) subjecting eggs to manual pressure, applied by rhythmical rolling of eggs, placed between a microscope slide and cover glass, in physiological solution. One thousand eggs were treated by each of the above procedures. However, no larvae were obtained. Experiments described in this section show that the lack of parthenogenetic development of eggs obtained by in vivo cultures is probably not related to culturing conditions but to some other factors. This is so because unlaid eggs from nontransplanted ovaries are also not capable of further development.

Table 2. Time course of oogenesis during in vivo culture in female and male flies. Forty *Drosophila melanogaster* females strain *fs(2)B* and 40 males were each injected with one-half ovary (Fig. 1D). On each of the four subsequent days, ten females and ten males were dissected, and the number of mature eggs was scored.

Day	Average number of mature eggs per host	
	Female	Male
1	2.0	0
2	6.0	3.2
3	12.2	3.6
4	15.0	5.5

The formation of a mature egg involves a complex developmental program. Among major events are several mitotic or endomitotic cycles of the associated follicle cells and nurse cells, a very large increase in the volume of the egg chamber, intake of yolk proteins and other nutrients from the surrounding milieu, formation of the chorion, and degeneration of nurse cells at the end of oogenesis. It is remarkable that differentiation through all these stages takes place in an autonomous fashion, outside the ovary, even from the youngest egg chamber. Male hosts also seem to provide a favorable environment for development, even though one might have expected them to lack proper hormonal balance or to be unable to produce yolk proteins. Physiological maturation, however, seems to depend upon the influence of an unknown factor exerted during the passage of the egg through the terminal portion of the genital tract, as evidenced by the development of laid parthenogenetic eggs but not of "ovarian" eggs or those that have matured in in vivo culture.

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