small decrease in labeling was noted for the smallest cells; in a fraction representing less than 10 percent of the total cells a decrease of two-thirds was observed. Therefore there was a small dependence on size, but no large correlation between cell size and length of G_1 (Fig. 3).

The late dose of ^{3}H-thymidine was from 8 to 10 hours in two experiments and from 9 to 11 hours in the third experiment. No significant differences in labeling were detected for the cells of different sizes (Fig. 3).

The early dose was timed so that only large cells would have become labeled if a correlation between G_1 and size existed. Likewise, a decrease in labeling of large cells might have been

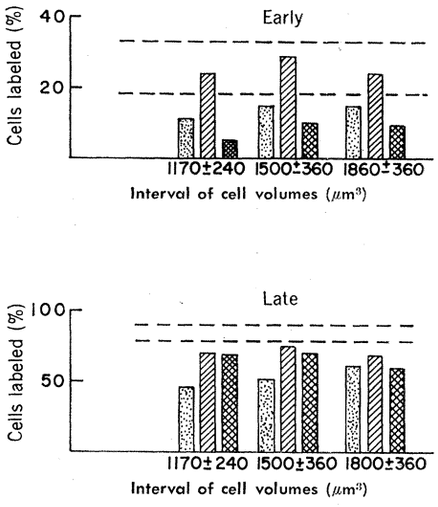

Fig. 3. Fractions of cells of each size which are in S. Shadings indicate individual experiments. Each bar represents blind counts of at least 400 cells. Dotted lines indicate the labeling of unfractionated cells for the experiments with highest and lowest labeling. Sizes of rounded cells prior to attachment to glass and flattening out were estimated by photographing cells and weighing paper cutouts of their profiles. These areas were converted to absolute volumes based upon an approximate measure of the cell diameters. Volume intervals are expressed as the average, plus or minus the standard deviation. Sized early G_1 cells were exposed for 2 hours during early and late S with 2 to 3 μc of thymidine-[methyl-^{3}H] per milliliter (New England Nuclear, final specific activity, 0.64 to 0.96 μc/nmole). After each dose of labeled thymidine, the cells were washed with cold calcium- and magnesium-free phosphate-buffered saline containing 1 mmole of unlabeled thymidine and fixed with an acetic acid, ethanol mixture (1:3). Cover slips were dried, mounted face up on slides with Permount (Fisher), and dipped in Kodak NTB-2 emulsion. These were developed after 2 to 7 days of exposure and were stained with Giemsa.

seen in the late dose. The absence of large differences in labeling patterns between large and small G_1 cells suggests that, for these cells, only a small correlation may exist between cell volume and length of G_1. The large heterogeneity in length of G_1 (Fig. 1) therefore is not primarily due to variations in cell size.

Killander and Zettterberg (*4, 5*) have suggested that a correlation does exist between the mass of a cell after mitosis and duration of its G_1 phase. They observed a smaller variation in mass of mouse L cells at early S phase than immediately after division. Furthermore, they found a large variation in age of the early S-phase cells (*4*). This suggested a critical mass for S and the possibility that G_1 of small cells would last longer than G_1 of large cells in order to reach this mass. In another investigation (*5*), different populations were derived from the same cell line. Populations with smaller average masses after mitosis had longer average G_1 periods. These two experiments only indirectly suggest a correlation between duration of G_1 and cell size. The first results are statistical, and the second results are for cells of different populations with presumably different genetic compositions (*5*). Our experiments with CHO cells do not support this hypothesis.

Petersen and Anderson have studied synchrony in wild-type and clonal CHO cells (*10*). Because they found no large differences, they concluded that genetic dispersion in the population could not account for the observed dispersion of growth rates. They and their colleagues (*11*) also concluded from mathematical considerations that cell volumes alone could not account for experimental growth patterns. Thus, both cell size and genetic contents do not account for the observed heterogeneity in cell growth. Nutritional limitations may account for the dispersion, as in *Tetrahymena* (*12*), but this factor is difficult to test with mammalian cells.

Two further hypotheses might account for variable duration of G_1. Cells might normally enter a G_0 state for variable periods between mitosis and G_1 (*13*). The G_0 state is proposed for nondivision but potential proliferation. The capacity to enter G_0 might be lost in more malignant cells, as suggested by greater desynchrony of CHO than of HeLa cells (*10*). The second hypothesis accounts for heterogeneity among daughter cells by asymmetric distribution of scarce cellular components which are unequally segregated at random, and not in parallel with total cell mass. Components associated with nuclei might be in this class, and their quantity within cells might determine growth rates.

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Sex Control by Bees: A Voluntary Act of Egg Fertilization during Oviposition

Abstract. *The alfalfa leaf-cutter bee,* Megachile rotundata, *stops abdominal contractions briefly during oviposition of female eggs but not during oviposition of male eggs. Sperm stored in the spermatheca probably is pumped onto the micropyle of the egg during this pause. The stimulus inducing fertilization seems to be associated with the depth of the nesting tunnel.*

Bees and other Hymenoptera control the sex of their offspring through arrhenotokous parthenogenesis. With some Hymenoptera, egg fertilization apparently is a random process, but in others, fertilization appears to be controlled by the female. In the honey bee, unfertilized eggs resulting in males are

laid in the larger drone cells. Fertilized eggs resulting in females are laid in the smaller worker cells or in the much larger queen cells. Other Hymenoptera lay fertilized eggs under other special conditions. The tiphiid wasp *Tiphia popilliavora* Roh. produces mostly male offspring on second instar Japanese beetle larvae and female offspring on third instar larvae (*1*). The eulophid parasitic wasp, *Coccophagus ochraceus* How., lays fertilized eggs while standing on top of the host and unfertilized eggs on the underside of the host while standing beside it (*2*).

The alfalfa leaf-cutter bee, *Megachile rotundata* (Fab.), usually produces female offspring in the inner part and males in the outer part of her nesting tunnels. Caged unmated females nest normally but produce only male offspring. The bee nests individually in tunnels 4 to 6 mm in diameter and several centimeters deep in wood or other materials (*3*). In the bottom of the tunnel she prepares a thimble-shaped cell with pieces cut from leaves and provisions the cell with a mixture of nectar and pollen. The egg is laid on the provisions, and the cell is capped with round pieces of leaf. Additional cells are completed in the tunnel at the rate of one per day until the tunnel is nearly filled. It is then plugged with several leaf disks, and the female searches for a new tunnel. She produces up to 40 eggs during her lifetime.

Females were induced to nest in transparent plastic tubes. After a bee began provisioning, the pieces of leaf were removed from the sides of the tube in order to observe nesting behavior. Most bees accepted such conditions and continued to provision the cell. Motion pictures were made of oviposition. When a bee laid an egg in the first cell in a tunnel, her behavior was different from that observed when she laid an egg in a shallower cell. The bee deposits nectar on the surface of the provisions just prior to oviposition. While doing this she cleans the tip of her abdomen by rubbing it with her hind legs. She then turns around and backs into the cell with her abdomen raised and with her sting exserted until it touches the provisions. The act of oviposition consists of a series of abdominal contractions that pump out the 3-mm long, sausage-shaped egg. When the bee is ovipositing in an inner cell, a series of abdominal contractions pump out about two-thirds of the egg. The abdominal contractions then cease for an average of 8 seconds after which they are resumed to complete the egg laying. The pause in abdominal contractions takes about one-fifth of the oviposition period. Oviposition in outer cells in the nest lacks this momentary pause and is completed in one continuous series of abdominal contractions.

Table 1. Oviposition time in seconds and sex of offspring of eggs laid by *Megachile rotundata* with (+) or without (−) a pause during oviposition, as related to site of oviposition in the nest; NO, not observed.

Nest	Oviposition time (sec) and sex of offspring in cell number from bottom of nest						
	1	2	3	4	5	6	7
1	♀ 57+	♀ NO	♀ 75+	♂34—	♂ 44−		
2	♀ 34+	♂ NO	♂ NO	♀ NO	♀ 125+		
3	♀ NO	♂ 24−	♂ 23−	♂ 20−	♂ NO	♂ NO	♂ 20−
4	* 24+	♂ NO	♂ 35+	♂ NO	♂ NO		
5	♀ NO	♀ 37+	♂ 23−	♂ NO			
6	♀ NO	♀ NO	♀ 25+	♂ NO	♂ 15−		
7	♀ 28+	♂ 23−					

* Offspring died.

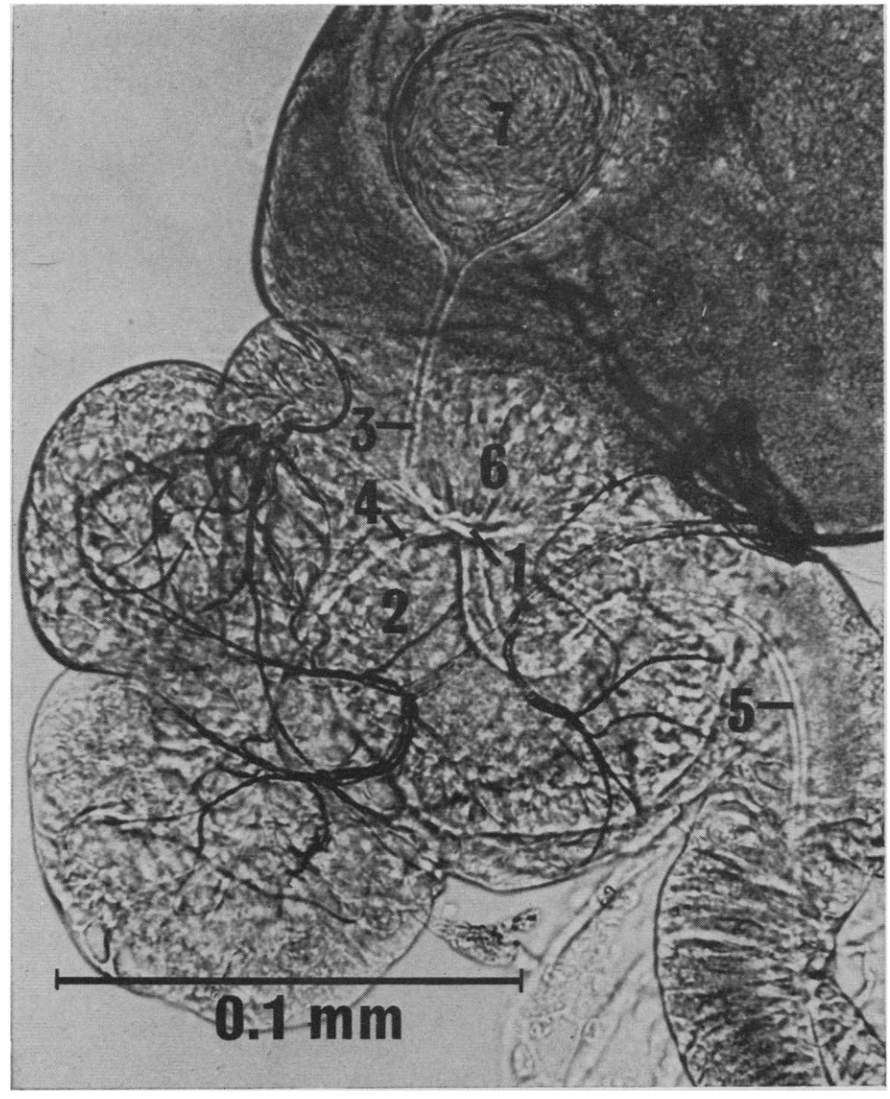

Fig. 1. Spermatheca of *Megachile rotundata*: *1*, sperm pump; *2*, globular tissue mass; *3*, spermathecal duct from sperm capsule; *4*, spermathecal gland duct; *5*, spermathecal duct leading to median oviduct; *6*, muscles which operate the sperm pump; and *7*, sperm capsule filled with sperm.

Several ovipositions were recorded as being with or without a pause, and the duration of each was timed. The eggs were reared to adults, and the sex was determined (Table 1). In all but one case each egg laid with a pause during oviposition developed into a female. Sperm may have failed to penetrate the micropyle in the one case, thus resulting in a male, or the bee may not have been mated. All eggs laid without a pause were males. With caged unmated females, only 3 of 28 ovipositions, including several in first cells, were completed without a pause, suggesting that most females may be aware of whether or not they are mated.

Fertilization of the egg thus appears to be controlled by the female during oviposition. She evidently extrudes the egg to a position in the oviduct so that the micropyle is in a proper place to receive sperm pumped from the spermatheca. She then holds the egg in that position until the sperm is expelled before she completes egg laying.

The spermatheca of *Megachile rotundata* is similar to that of the honey bee (*4*). The female leaf-cutter bee mates only once and retains enough sperm to fertilize all the eggs she can lay. The spermatheca is located dorsad to the median oviduct. The morphology of the spermatheca and its duct (Fig. 1) indicates that there is a chamber that could function as a sperm pump (Fig. 1, *1*). Two nerve bundles lead from the last abdominal ganglion to an adjoining globular mass of tissue (Fig. 1, *2*) surrounding a sperm pump. This mass twitches in Ringer solution. Within the globular mass is a chamber (Fig. 1, *1*) into which the ducts from the spermatheca (Fig. 1, *3*) and the spermathecal glands (Fig. 1, *4*) enter, and from which the spermathecal duct leads to the median oviduct (Fig. 1, *5*). Muscle fibers (Fig. 1, *6*) are inserted on one side of the sperm pump and extend to a thickened structure between the spermatheca and the globular mass. During fertilization of the egg, these muscles are assumed to contract, thus expanding the chamber and drawing in sperm and spermathecal gland fluid. Relaxation of these muscles then causes the pump chamber to contract elastically so that the sperm-fluid mixture is expelled through the larger diameter duct onto the waiting egg. It is assumed that this operation can take place within 4 to 11 seconds—the range of time of the pause during oviposition of female eggs.

The stimulus that induces the female to fertilize the egg is associated in some manner with the depth of the nesting tunnel, because very few tunnels shallower than 4 cm contain female offspring (*5*). Disturbances near the time of oviposition may cause the bee to withhold fertilization. Females sometimes laid an unfertilized egg rather than a fertilized one in an inner cell that had been cut for observation.

Because a pause during oviposition is associated with female eggs laid in deeper cells, we conclude that egg fertilization is not a random event nor the end result of a stereotyped sequence of behavior, but it is an entirely voluntary act of the female during the oviposition process. It is likely that oviposition of honey bees and other bees is of a similar nature, although we know of no reference to such a pause in oviposition.

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Opponent Color Cells in the Cat Lateral Geniculate Nucleus

Abstract. *A microelectrode survey of the cat lateral geniculate has uncovered an infrequent new type of lateral geniculate cell in layer B with "on" center responses to short wavelengths and "off" center responses to long wavelengths. The short wavelength responses are mediated by cones with peak sensitivity at about 450 nanometers, and the long wavelength responses by cones with peak sensitivity at 556 nanometers. Two of the three opponent color cells also had double opponent features.*

Cats can distinguish colors when care is taken to eliminate other cues (*1*). The receptor mechanisms underlying this ability are not well defined, as there has been no clear demonstration of more than a single type of cone in the cat retina. Studies of the cat's retinal ganglion cells, optic tract fibers, and lateral geniculate cells have demonstrated only the typical center-surround receptive field arrangement with no difference in spectral sensitivity between center and surround (*2*). In particular, the opponent color cells seen in monkey optic tract and lateral geniculate (*3, 4*) and the double opponent cells seen in the goldfish retina (*5*) and monkey cortex (*6*) have never been demonstrated in the cat.

In an earlier study (*7*) we found evidence for only one type of cone which had peak spectral sensitivity at 556 nm when the retina was illuminated with a white background sufficient to saturate the rods. Both the center and the surround of the receptive fields of units in the lateral geniculate and optic tract received input from this single type of cone; the units thus had no color opponent features. The finding of only one type of cone suggested that the two types of receptors underlying color vision in the cat were the rods and a single type of cone. However, we have recently shown that cats trained to discriminate colors in the mesopic range, where both rods and cones function, retain the ability to distinguish colors at light intensities sufficient to saturate the rods (*8*).

Since two types of receptors with different spectral sensitivities are required to discriminate color, our behavioral results are at odds with the neurophysiological evidence for only one cone type. We therefore undertook a further microelectrode survey of lateral geniculate and optic tract, with similar methods (*7*). Three adult cats were anesthetized with halothane, nitrous oxide, and oxygen. After surgery, light anesthesia was maintained with intraperitoneal thiopental. The cats were paralyzed with gallamine triethiodide and curare and were artificially ventilated with room air. Both eyes were refracted with a slit retinoscope and focused with contact lenses on a large white screen at 57 inches (142.5 cm). Extracellular recordings from sin-