zontal meridian (*19*). Therefore, the differential expansion seen along the vertical meridian in all the 2DG maps (for example, Fig. 1B) (*20*) is only a special case of an expansion of the cortical map perpendicular to the ocular dominance strips. This relationship resolves what would otherwise be a problem, namely that the vertical meridian is mapped over a longer distance around almost all of the elliptical circumference of the striate cortex, whereas the horizontal meridian lies along a shorter straight line connecting the ends of that ellipse (*13*).

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16. For simplicity, we ignore the distortion of a square in the cortex as a result of the quasi-logarithmic expansion.
17. The ratio of the lengths shown in Fig. 2, C and D, is about 1.25:1. This measure (and similar data from comparison of other segments) provides a lower bound to the actual anisotropy of the CMF, since the ocular dominance strips are never completely perpendicular or parallel to the 2DG ray segments. Our best estimate of the actual anisotropy is about 1.5:1.
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Sex Preselection in Mammals? Separation of Sperm Bearing Y and "O" Chromosomes in the Vole *Microtus oregoni*

Abstract. *The two sex determining sperm populations of the vole* Microtus oregoni *were separated according to DNA content by use of flow sorting instrumentation. Although the sperm were not viable, they should be useful for addressing the question of haploid expression of genes linked to sex chromosomes and for efficiently searching for biochemical markers that differentiate the two populations.*

The ability to influence the sex ratio of humans and agriculturally important animals would have profound social and economic impacts. Development of semen-based sex selection techniques has been impeded by the difficulty in identifying differences that might serve as a basis for enrichment or preferential inactivation of the sex-determining sperm populations. Although several physical, functional, and biochemical methods have been proposed and some patented (*1*), none has yet proven its efficacy in large-scale trials. The only established difference on which to base sperm separation is chromosomal constitution. In most mammals, sperm containing the X chromosome lead to female offspring, and those with the Y to males. Typically the X and Y chromosomes differ in DNA content, resulting in a 3 to 4 percent difference between the X and Y sperm in humans and agriculturally important animals (*2*). Flow cytometric techniques that are sufficiently precise to resolve these populations in many species have been developed (*3, 4*). We have used these techniques to determine the relative proportion of X and Y sperm in semen processed with several purported enrichment methods (*5*). None of the samples measured so far has been enriched.

It is reasonable to expect that appropriately adapted flow-sorting instrumentation could be used to separate sperm according to DNA content, and that these sperm might then be used to search for phenotypic differences that could be utilized for bulk separation of viable cells. However, current staining techniques adequate to resolve a 3 to 4 percent difference require decondensation of the highly compact sperm nucleus with proteolytic enzymes, substantially altering many biochemical components and generally disrupting sperm structure. The larger the difference in DNA content between the sex-determining sperm populations, the more biochemically conservative a staining protocol can be and still resolve them. For the vole *Microtus oregoni*, this difference is about 9 percent, more than double that of most mammals (*6*). We now report the successful sorting of sperm from *M. oregoni* using three preparative techniques with differing levels of cellular disruption. This represents a directly verified enrichment of the sex-determining sperm populations of a mammal.

Microtus oregoni is unusual in that it is a gonosomic mosaic (*7*); the gonadal and somatic cells have different chromosomal constitutions. Male somatic cells are XY, but the spermatogonia are OY. Thus, one of the sperm populations contains the Y chromosome and the other, here called "O," contains no sex chromosome. Only Y-linked genes are candidates for coding for markers that differentiate the two sperm classes in this animal. This may not be very different from what occurs in other mammals, since there is evidence that the X chromosome is normally inactivated during spermatogenesis (*8*).

In flow cytometry, a liquid suspension of single cells stained with a fluorochrome is made to flow in a thin stream that intersects a beam of excitation light. As each cell passes through the beam, optical detectors focused on the intersection convert the fluorescent flashes into electrical signals, which are analyzed with a multichannel analyzer. The data are displayed as histograms of the number of cells versus the brightness per cell. Multiple measurements on each cell result in multidimensional histograms.

Flow measurements of DNA content in mammalian sperm are particularly dif-

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ficult to obtain, since the sperm nucleus is highly condensed and flattened. The condensation makes stoichiometric staining difficult and causes a high cellular index of refraction (*9, 10*). The refractive index, coupled with the flatness of the sperm head, makes optical measurements sensitive to the orientation of the cell with respect to the excitation beam and detectors. Hydrodynamic forces cause cells to flow with their long axes parallel to the flow direction, but since they are usually rotationally unconstrained, special measurement techniques are required. Two measurement systems have demonstrated ability to overcome these problems (*4*). Both were used in this work; one for sorting and the other for analysis of the purity of the sorted fractions. In the analyzer (the commercially available ICP22) (*11*), sperm are measured as they flow along the optical axis and through the focus of an epi-illumination microscope. The rotational symmetry of the optics renders the measurements insensitive to variations in cell orientation. No sorting instruments with this optical arrangement are available. In the sorter (*12*), the axes of the illuminating beam and fluorescence detectors are orthogonal to the flow direction. The approximate orientation of each cell can be determined by simultaneous measurements with detectors at two angles, as in the inset to Fig. 1 (*13*). The most accurate DNA content measurements are made with the 0° detector for sperm oriented as shown (*4*). Special hydrodynamics are used to preferentially orient sperm heads so that accurate measurements can be obtained on the majority of them (*4, 13*). Tails on whole cells make current orientation methods ineffective.

Cauda epididymidal sperm for sorting were dispersed (*4*) from trapped *M. oregoni* (*14*) and stained with 20 μM 4,6-diamidino-2-phenylindole (DAPI) in 0.1M tris at pH 7.4 after preparation according to three protocols. Protocol 1: papain and dithioerythritol treatment to decondense the chromatin (*4*). Sperm tails and other cytoplasmic structures are also removed. This procedure yields the best DNA content resolution (Fig. 2a). Protocol 2: sonication followed by fixation in 80 percent ethanol. This also removes tails. Staining precision is considerably reduced (Fig. 2b). Protocol 3: fixation of intact cells in 3.7 percent phosphate-buffered formaldehyde solution. Sperm tails are not removed. Resolution is further reduced (Fig. 2c).

The sorting procedure is illustrated in Fig. 1, which is a contour plot of the correlated 0° and 90° fluorescence measurements on randomly oriented whole sperm prepared with protocol 3. The contours connect points in the histogram that contain the same number of cells. They show a general "L" shape because fluorescence is brightest when the edge of these flat cells is pointed at a detector (*13*). Thus sperm oriented as shown in the inset are relatively bright in the 90° detector but dim in the 0° detector. They appear in the vertical arm of the L. The situation is reversed for a cell measured with its edge pointing at the 0° detector. Intermediate orientations lead to intermediate signals. Exact details of this behavior depend on sperm head morphology, staining protocol, and detector numerical aperture (*4*). The two solid L-shaped lines, separated in brightness by 9 percent, schematically represent where the Y and O sperm populations might appear if the measurement perfectly resolved them. Sperm were sorted from the shaded regions of the histogram. These sort windows were chosen to select Y and O sperm with relatively high purity at the expense of ignoring a large percentage of the cells. Hydrodynamic orientation improved sorting efficiency for sperm heads (protocols 1 and 2) by increasing the proportion of cells with signals falling in the vertical arm of the L (Fig. 1). Sorting rates in these preliminary experiments were on the order of 30 cells per second for both fractions.

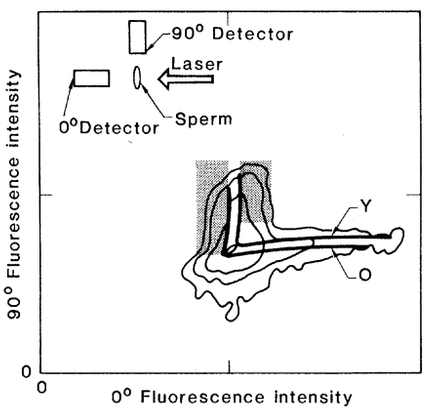

Fig. 1. Sperm sorting. Measurement geometry is shown in the inset. Cells flow in a direction perpendicular to the plane of the figure. The histogram shows the number of cells versus fluorescence intensity in both detectors for randomly oriented sperm prepared with protocol 3. Cell number is plotted normal to the plane of the figure. Points of constant cell number are connected by continuous contours, equally spaced in height. The solid L-shaped lines, separated in intensity by 9 percent, schematically represent the locus of Y and O sperm that would be observed with perfect resolution. Sperm were sorted from the two shaded regions of the histogram, O from the left and Y from the right. Laser illumination was with the 351- to 364-nm lines of an argon ion laser.

Purity of the sorted fractions was determined by restaining with protocol 1 for best resolution, measuring in the

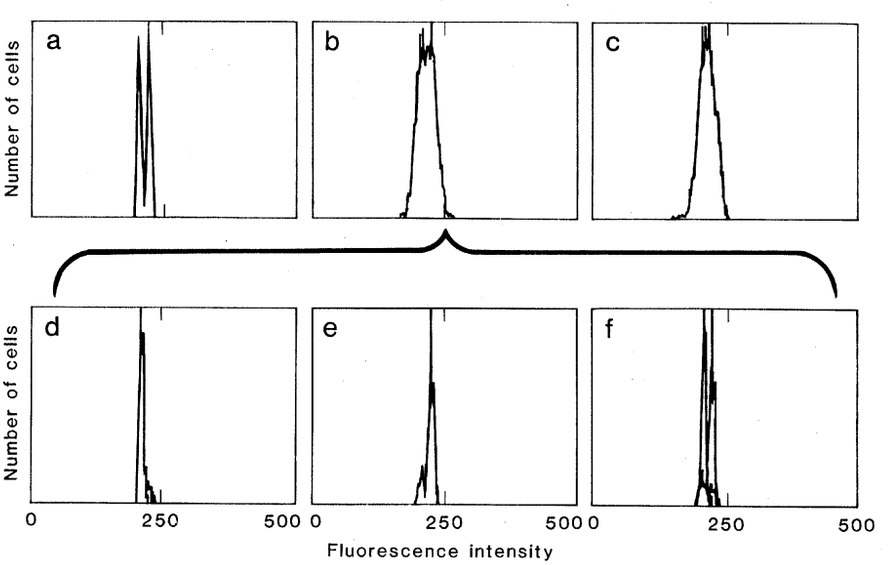

Fig. 2. Verification of enrichment. Flow cytometer (ICP22) measurements of sperm prepared with protocols 1, 2, and 3 are shown before sorting in (a), (b), and (c), respectively. In (a), the two sperm populations are almost completely resolved. The peaks are separated by 9.1 percent, and each has a coefficient of variation of 1.8 percent. Precision of DNA staining decreases from left to right. After sorting, the putatively enriched fractions obtained in each case were restained according to protocol 1 and analyzed in the ICP22. The results for the O and Y fractions sorted from a protocol 2 preparation (b) are shown in (d) and (e), respectively. Both measurements were made with the same instrumental parameters. Their photographic superposition (f) should be compared to the results from unsorted cells (a). Illumination from the mercury arc lamp of the ICP22 was selected with a UG1 filter.

ICP22, and computer-fitting a pair of normal distributions to the resulting histograms. The relative areas of the two fitted curves give the relative Y and O populations in each fraction. Figure 2d shows measurement of the O fraction sorted from the sonicated ethanol-fixed cells of Fig. 2b; the Y fraction is shown in Fig. 2e. Their photographic superposition, which should be compared to Fig. 2a, is shown in Fig. 2f. Analysis shows 95, 87, and 82 percent purity of O fractions for protocols 1, 2, and 3, respectively. The purities of Y fractions were 72, 83, and 80 percent, respectively.

Two problems currently prevent use of sorted sperm for fertilization: sorting rates are low and the stained cells are not viable. Even if vital staining techniques are developed and sorting rate is increased to that attainable with current instrumentation (about 10^3 cells per second), use of sorted sperm for inseminations in vivo will not be widespread since several million cells are required for one insemination. New instrumentation with dramatically higher sorting rates might alter this situation. Application to fertilization in vitro, where the required number of sperm is significantly lower, is more probable.

For the immediate future, flow sorting of *M. oregoni* sperm offers the possibilities of directly addressing the question of haploid expression of genes linked to sex chromosomes and efficiently searching for biochemical markers that discriminate the two sperm populations. If found, a marker might be useful for bulk separation of viable sperm, perhaps by using an antibody to bind one population to a column while allowing the other to pass through. If there is a Y-specific *M. oregoni* marker that is conserved across species, it would have general application to mammalian sex selection. In the absence of a common marker, extension to other species will require sorting of biochemically well-preserved sperm differing in DNA content by 3 to 4 percent. This will be more difficult for the human than for agriculturally important species since the human DNA content difference is at the low end of the mammalian range and the heterogeneity of human sperm nuclear condensation presents greater interference to precise staining.

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15. Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract number W-7405-ENG-48.

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Touching Textured Surfaces: Cells in Somatosensory Cortex Respond Both to Finger Movement and to Surface Features

Abstract. *Single neurons in Brodmann's areas 3b and 1 of the macaque postcentral gyrus discharge when the monkey rubs the contralateral finger pads across a textured surface. Both the finger movement and the spatial pattern of the surface determine this discharge in each cell. The spatial features of the surface are represented unambiguously only in the responses of populations of these neurons, and not in the responses of the constituent cells.*

The evolution in primates of the hand as a sensorimotor organ has doubtlessly contributed to the biological success of this order of mammals. One can trace through the prosimian and anthropoid primates an increasing capacity to manipulate, explore, and differentiate by touch objects and surfaces that are within reach (*1*). The unique tactile acuity of anthropoid primates, including man, depends in part on the high innervation density of the finger pads (*2*), but an important additional factor is the capacity to control and direct exploratory finger movements in resolving fine spatial features. Although one must normally rub the finger pads across a surface to identify it, the pattern of this exploratory movement may be varied greatly without impairing the tactile identification of the surface. From this it may be inferred that the neural representation of the spatial features of a surface at successive levels in the somatosensory pathways, although sustained by the movement of the finger pads across the surface, is largely independent of the actual pattern of finger movement.

We examined the tactile representation of simply patterned surfaces in Brodmann's areas 3b and 1 of the somatosensory cortex of the macaque. This region of cerebral cortex was chosen for analysis since its destruction, in both man and macaque, selectively impairs the subject's capacity to identify and differentiate textured surfaces with the contralateral fingers (*3*). Responses elicited in single cortical neurons when the monkey rubbed the finger pads to and fro across a ridged surface were recorded, and the effects of changing the spatial features of the surfaces touched and the pattern of finger movement used were studied systematically. The aim was to determine whether the spatial features of the surface were represented in these neural responses. A previous study (*4*) showed that the representation of these same spatial features in the monkey's digital nerve occurs only in the discharge of the population of mechanoreceptive fibers engaged by the patterned surface. Individual fibers in this population never specify the dimensions of the surface, since their responses depend not only on these dimensions but also on the pattern of movement of the skin relative to the surface. We found that this is also true for neurons in corti-

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