Chromosomal Aberrations in a Population of Ground Squirrels

Abstract. *Chromosomes were analyzed from a population of* Spermophilus beldingi *that included an adult female and three juveniles from the same burrow. All animals had a diploid number of 30. The adult and a juvenile female from the burrow had identical aberrant karyotypes containing an unpaired submetacentric and a minute metacentric chromosome. A familial aberration involving an X chromosome, without phenotypic alteration, is most probable.*

Chromosome aberrations common to several members of a family have been described repeatedly in humans. Some aberrations, particularly reciprocal translocations, are genetically balanced because such states merely represent rearrangements rather than loss or gain of chromosomal material; the phenotype of individuals affected is normal (*1*, *2*). In contrast, unbalanced aberrations, which are usually due to additions or deletions of chromosomes or their segments, lead to phenotypic abnormality; balanced and unbalanced states occasionally occur in the same family (*2*).

An unbalanced chromosome aberration was recently described in a phenotypically normal wild ground squirrel, *Spermophilus beldingi* (*3*). Examination of chromosomes from nine other specimens established the normal karyotype for this species. Because of the apparent rarity of chromosome rearrangements in wild mammalian populations, additional specimens of *S. beldingi* from the same locality were analyzed to determine the prevalence of the aberration.

Eight *S. beldingi oregonus* (Merriam) were captured alive 1.6 km southeast of Burns, Harney County, Oregon, in May 1965—1.6 km from where the aberrant *S. beldingi* was trapped in May 1964 (*3*). Four animals, including a lactating adult female, a juvenile female, and two juvenile males, were obtained simultaneously from the same burrow by flooding it with water; we shall refer to them as a family. Four more adults were trapped within 90 m of the family (Table 1). Chromosomes were analyzed from femoral marrow by use of a Colcemide, hypotonic citrate, and acetic orcein squash technique (*4*). Karyotypes were constructed from enlarged photomicrographs. Buccal smears for sex chromatin were unsuccessful.

Chromosome counts are listed in Table 1. All specimens examined had a diploid number of 30. Male karyotypes of *S. beldingi oregonus* contain eight pairs of metacentric and six pairs of submetacentric autosomes, a submetacentric X, and a minute metacentric Y (Fig. 1*a*). Karyotypes from three adult females contain autosomes similar to those of the male and two submetracentric-X chromosomes (Fig. 1*b*). Two additional females, an adult and a juvenile, belonging to the family had an aberrant karyotype characterized by eight pairs of metacentric and six pairs of submetacentric chromosomes, an unpaired submetacentric, and an unpaired minute metacentric chromosome (Fig 1*c*). The unpaired submetacentric is tentatively considered to be a morphologically normal X chromosome, while the minute is considered aberrant. Thus the aberrant karyotypes are identical with those reported earlier (*3*). The juvenile males from the family had nomal karyotypes. Gross examination of the specimens having abnormal karyotypes revealed a lactating adult female and a juvenile female, each with normal ovaries, uterine tubes, uterus, and vagina.

To our knowledge this is the first report of a probable familial chromosomal aberration arising spontaneously in a wild mammalian population. Although positive identification of a family in wild rodents is difficult, the simultaneous presence of a lactating female and three juveniles in the same burrow is good presumptive evidence of a familial relation; the fact that both animals had identical aberrations is further evidence. The aberrant female examined in 1964 (*3*) may have belonged to the same family, or may have reflected establishment of the aberration in several families.

It has been postulated that the simplest explanation of the aberration in *S. beldingi* was a double deletion of both arms of a medium-size submetacentric autosome or X chromosome to produce a minute metacentric (*3*). Exact identification of the chromosome concerned, or of the mechanism responsible for the aberration, was not possible because the X chromosomes cannot be clearly distinguished from several pairs of autosomes. Our discovery of the same aberration in (presumable) mother and daughter and its absence in the males lend support to the idea that the X chromosome is concerned.

Perhaps as significant as the familial transmission of the aberration is the observation that, despite considerable loss of chromosomal material and consequent production of an unbalanced karyotype, no phenotypic alteration is evident; the point is exemplified by the fertile, nursing, adult fe-

Table 1. Chromosome counts of *S. beldingi oregonus*. The numbers of cells counted are in parentheses. Abbreviations: A, adult; J, juvenile; f, female; m, male.

Specimen	Chromosomes (No.)	
	29	30
Af (31)*	2	29
Jf (40)*	1	39
Jm (17)	0	18
Jm (18)	1	16
Af (24)	2	22
Af (28)	1	27
Af (33)	2	31
Am (36)	3	33

* Aberrant karyotype.

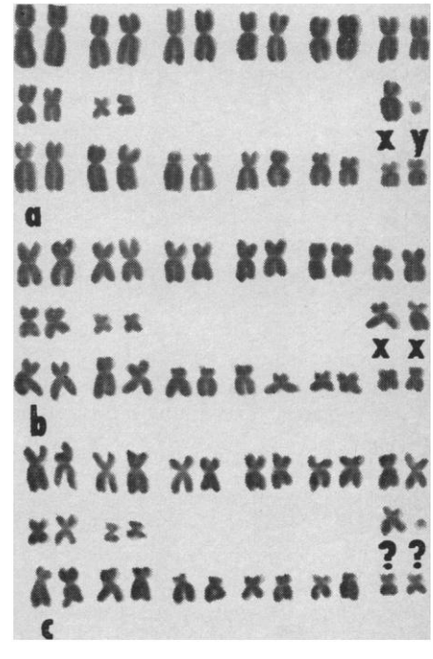

Fig. 1. Karyotypes from *S. beldingi oregonus* (× 1300). *a*, From a normal male; *b*, from a normal female; *c*, from the phenotypically normal adult female. Question marks identify the unpaired chromosomes, the minute being aberrant.

male. The "syndrome" observed in *S. beldingi* (providing that the X chromosome is aberrant and we think it is) resembles that in fertile XO mice (*5*) rather than X-chromosome deletion in humans, where there is considerable abnormality of primary or secondary sexual characteristics or of both (*6*).

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Cell Wall of Melampyrum lineare Seed: Carbohydrate Components

Abstract. *Extraction with alkali of cell wall material from the seed of* Melampyrum lineare *leaves a residue (62.7 percent) which on hydrolysis yields over 70 percent mannose. Hydrolysis of the alkali-soluble hemicellulose fraction of the cell wall also yields largely mannose. In contrast, cell wall isolated from* Melampyrum *stem contains over 95 percent of glucose residues in the alkali-insoluble fraction, and mostly xylose residues in the hemicellulose.*

The cell walls of higher plants contain various sugar residues in addition to that of D-glucose (*1*). However, most of the material containing units other than glucose can normally be extracted as "hemicellulose" with alkaline solvents. The residue, the so-called "α-cellulose," normally consists almost entirely of glucans, particularly cellulose. We report here studies of the *Melampyrum lineare* seed, whose cell wall yields an alkali-insoluble fraction consisting largely of mannose units.

Melampyrum lineare Desr. (*2*), a member of the Scrophulariaceae, is a green root parasite indigenous to the northern forests of North America. After forming root connections to a host plant, *Melampyrum* produces copious flowers and fruit, each capsule typically producing four ellipsoid seeds. The ripe seed is 1 to 3 mm in length and contains at one end an elongated embryo embedded in endosperm which occupies the remainder of the seed space. The embryo is dormant until activated (*3*), when it grows at the expense of the endosperm. The nonactivated embryo occupies up to 40 percent of the length of the seed and 10 percent of the volume, and it represents 1.5 percent of the dry weight. The soft, fibrous endosperm tissue of the ripe seed is composed of polygonal cells (average inner diameter, 61 μ) with thickened walls (average diameter, lumen to lumen, 11.2 μ). The cell wall is composed of a middle (nonpitted) lamella (about 2.8-μ thick) surrounded by inner (pitted) lamellae bordering the cell lumina. The inner lamella stained with a solution of iodine in potassium iodide followed by sulfuric acid, and also with zinc chloride iodide (*4*), but the middle lamella did not react. No part of the seed stained with phloroglucinol–hydrochloric acid (*4*), an indication of the absence of lignin.

Dormant whole seeds were cleaned and milled; they were then subjected to successive exhaustive extraction with benzene, 80 percent ethanol, and hot water. These extractions removed respectively 15.8, 22.5, and 7.7 percent of the original dry weight, and left a residue (54.0 percent) of cell-wall holocellulose. The holocellulose was then extracted with alkali (Table 1) to remove hemicellulose components. A similar extraction was carried out on unripe seeds (Table 1). These seeds were obtained from immature seed capsules at which time the seed was soft and pulpy, although the endosperm cell walls were of approximately the same thickness as those in the ripe seed. Stem holocellulose prepared similarly from fresh *Melampyrum* stems was first delignified by chlorite treatment (*5*) and then extracted with alkali.

Samples of the various holocellulose components were then hydrolyzed by digestion with 72 percent sulfuric acid at 24°C; the digest was diluted to 2*N* and autoclaved for 2 hours at 15 lb (2 atm, absolute). After neutralization, sugars were separated on paper chromatograms with a solvent mixture of ethyl acetate, pyridine, and water (8:2:1) and one of ethyl acetate, acetic acid, and water (9:2:2); they were located by spraying with *p*-anisidine hydrochloride (*6*) or aniline hydrogen phthalate (*7*). Hemicellulose hydrolyzates had the following approximate compositions. Potassium hydroxide (5 percent) extract from seed (ripe and unripe) yielded mannose, glucose, galactose, and arabinose in approximate proportions of 3:1:1:0.5, respectively. Potassium hydroxide (24 percent) plus borate (4 percent) extract from seed yielded mannose only. Potassium hydroxide (5 percent) extract from stem yielded xylose, galactose, arabinose, and rhamnose in approximate proportions of 12:1:1:0.5. Potassium hydroxide (24 percent) plus borate (4 percent) extract from stem yielded xylose, galactose, glucose, mannose, arabinose, and rhamnose (1:1:1:1:0.5:0.2).

Table 1. Fractionation of holocellulose obtained from *Melampyrum* seed and stem. Each alkali extract was the result of three successive 12-hour treatments at 24°C, each with fresh solvent.

Fraction	Weight loss (%)		
	Ripe seed	Unripe seed	Stem
Chlorite delignification			36.2
5% KOH	26.5	30.9	30.1
24% KOH + 4% borate	10.8	9.7	10.3
Alkali insol. residue	62.7	59.4	23.4

The composition of hydrolyzates from alkali-insoluble residue was determined by the method of Wilson (*7*). Results are shown in Table 2. Mannose from seed cell wall (alkali-insoluble material) was identified as the phenylhydrazone (*8*), mp 194° to 196°C; the melting point was not depressed by admixture with authentic D-mannose phenylhydrazone. Infrared spectra of the two samples were identical.

In the alkali-insoluble fraction from seed cell wall, mannose accounted for 74.4 percent (ripe seed) and 73.2 percent (unripe seed) of the sugar residues. In contrast the corresponding fraction from stem cell wall yielded

Table 2. Composition (percent) of alkali-insoluble residues from *Melampyrum* cell wall.

Sugar	Seed		Stem
	Ripe	Unripe	
Galactose	4.2	2.7	0.8
Glucose	19.0	21.9	95.1
Mannose	74.4	73.2	2.2
Arabinose	2.4	2.2	0.7
Xylose			.9
Rhamnose			.3