

## "Formation of Genetically Mosaic Mouse Embryos and Early Development of Lethal (t<sup>12</sup>/t<sup>12</sup>)-Normal Mosaics" (1964), by Beatrice Mintz <sup>[1]</sup>

By: Navis, Adam R. Keywords: Mice <sup>[2]</sup> Chimeras <sup>[3]</sup>

The paper "Formation of Genetically Mosaic Mouse Embryos and Early Development of Lethal (t<sup>12</sup>/t<sup>12</sup>)-Normal Mosaics," by [Beatrice Mintz](#) <sup>[4]</sup>, describes a technique to fuse two [mouse](#) <sup>[5]</sup> embryos into a single embryo. This work was published in the *Journal of Experimental Zoology* <sup>[6]</sup> in 1964. When two embryos are correctly joined before the 32-cell stage, the embryo will develop normally and exhibit a mosaic pattern of cells as an adult. Mosaics were easily characterized by [mouse](#) <sup>[5]</sup> fusions from embryos of different colors; this produced clearly visible color patterns identifying the alternate cell types. Mintz referred to the fused mice as mosaic or later as allophenic, but they are more commonly known today as [chimeras](#) <sup>[7]</sup>.

The first reported instance of a chimera was identified in cattle by comparing blood antigens of [cow](#) <sup>[8]</sup> fraternal twins. Ray D. Owen reported that an abnormal number of the twins had identical blood antigens. The adult cows with similar antigens were found to have two different populations of red blood cells suggesting that the precursors of the red blood cells, [hematopoietic stem cells](#) <sup>[9]</sup>, were transferred from one twin to the other during development. In the 1950s human [chimeras](#) <sup>[7]</sup> were also identified by examining blood antigens and later confirmed by karyotyping to reveal differences in chromosome patterns between the patient's cells. Although *Drosophila* <sup>[10]</sup> [chimeras](#) <sup>[7]</sup> had previously been produced, mammalian [chimeras](#) <sup>[7]</sup> were not successful until Mintz developed a reliable technique for the production of embryonic fusions.

[Beatrice Mintz](#) <sup>[4]</sup> conducted a previous study that examined a reproductive disorder characterized by two distinct populations of [germ cells](#) <sup>[11]</sup> in heterozygous mice. One population was a group of normal, functioning [germ cells](#) <sup>[11]</sup>, the other group did not divide and they did not migrate to the correct parts of the embryo. Mintz thought there might be a way to use dual populations of cells to study the action of the mutant cells. She wanted to apply this technique to the t<sup>12</sup> mutation, which is characterized by a developmental arrest at the [morula stage](#) <sup>[12]</sup>, when the embryo is composed of approximately thirty-two cells. She thought a fusion of a mutant embryo with a normal embryo might [rescue](#) <sup>[13]</sup> function long enough to study the mechanism of the mutation similar to the way she studied the blood disorder.

The development of a chimera was first reported by [Andrzej Krzysztof Tarkowski](#) <sup>[14]</sup>. Although he reported a novel approach to embryonic fusions, his technique was inefficient and the sole [mouse](#) <sup>[5]</sup> that survived long enough to observe the coat color did not show evidence of chimerism. At early stages, embryos are surrounded by a protective layer named the [zona pellicula](#) <sup>[15]</sup> that prevents two embryos from fusing. Tarkowski attempted to remove the [zona pellicula](#) <sup>[15]</sup> by forcing embryos through a glass needle. This often damaged the

embryos, reducing the success of the fusions. Mintz instead removed the protective layer by treating the embryos with pronase, a mixture of enzymes designed to degrade the [zona pellicula](#) [15], causing minimal damage to the embryo. Once the [zona pellicula](#) [15] was removed, she needed to fuse the embryos. This was accomplished by introducing the embryos to each other at [mouse](#) [5] body temperature. The embryos easily adhered to each other and continued to develop as a single [mouse](#) [5]. Mintz performed these fusions at different intervals in development and discovered that the embryos could form [chimeras](#) [7] if they were fused at any point until the [morula stage](#) [12]. Mintz's method was much more efficient than Tarkowski's and she was able to produce the mottled coat color pattern that was evidence of a successful fusion.

Mintz considered techniques only a means to an end. This technique was developed to study the  $t^{12}$  mutation, which arrests development at the [morula stage](#) [12]. She produced fusions of embryos homozygous for  $t^{12}$  with control mice from the same genetic line. Some embryos did not efficiently fuse and the embryo segregated itself between mutant and normal cells. Many of the fusions developed long enough to produce discernible populations of cells. Each mosaic embryo produced a different pattern of growth, but all the mutant cells began to grow larger in relation to the normal cells due to a [reduction](#) [16] in the rate of cell division. This had been previously observed and validated earlier claims. This study, more importantly, provided a successful model for the use of [chimeras](#) [7] in experimental research.

The development of a method for fusing two embryos has proved useful in many ways besides the direct study of the embryos. Mintz later used fusions of embryos with a treated [teratoma](#) [17] cell line to produce a [mouse](#) [5] model of a human disease. Teratomas are unique tumors capable of differentiating into a variety of cell types. When they produced the [germ cells](#) [11] in a fusion, they were also able to produce [viable](#) [18] offspring. The ability to introduce [embryonic stem cells](#) [19] into an embryo has also been used to create [knock-out mice](#) [20]. These mice illustrate the role of a gene in development by inactivating the gene and observing development in its absence. This study is most noted for establishing a technique to consistently produce [viable](#) [18] [chimeras](#) [7], a technique that has continued to be an integral tool for the study of developmental biology.