DETERMINATION OF TRIPLET ZYGOSITY WITH THE USE OF SEROLOGICAL AND ANTHROPOLOGICAL CHARACTERS

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This paper discusses briefly some problems involved in zygosity determination in triplets, such as the calculations of the probability of the three zygosity types, as well as the use of serological and other anthropological characters.

Triplets offer special opportunities in the elucidation of the interplay between genetic and environmental factors. Studies of triplets may throw light upon such problems as the range of variability of a given genotype or the importance of prenatal factors in the development of certain characters.

An investigation of Swedish triplets born in 1921-1950 was started in 1962 with the aim of exploring the possibilities of using triplets in genetic research. In 186 out of 402 sets the three members were of the same sex (Table 1).

Table 1. *Survey of single and multiple births (including stillbirths) in Sweden 1921-1950.*

	Sex combination				Total
Single births	M 1,643,159	F 1,544,990			3,188,149
Twin births	MM 14,125	MF 16,046	FF 13,333		43,504
Triplet births	MMM 88	MMF 126	MFF 90	FFF 98	402
Quadruplet births					5
Total number of births					3,232,060

The value of triplets in genetic studies is closely tied to the reliability of the zygosity diagnosis. Sex, serological characters as well as a few other anthropological traits are the most important adjuvants.

Discordance with regard to sex or blood groups is proof of dizygosity when twins or two members of a triplet set are compared (Table 2).

Table 2. *Zygosity diagnosis.*

	MZ	DZ
Unlike sex		+
Common chorion	+	
Blood group discordance		+
Successful reciprocal transplantation	+	
Anthropological traits:		
Dissimilarity		(+)
Similarity	((+))	

A common chorion or a successful reciprocal transplantation proves monozygosity. Reliable information about monochorial placentation is, however, rather unusual, and transplantation is only practicable in a few cases. Some selected anthropological traits, e.g., dermatoglyphics, may yield supporting evidence but cannot furnish proof of dizygosity or monozygosity.

Triplet sets with three same-sexed members with complete serological concordance may be monozygotic (MZ), dizygotic (DZ) or trizygotic (TZ). Sets with two such members may be DZ or TZ, the two concordant triplets being MZ or DZ, respectively. A set with three discordant members is TZ.

The probability of monozygosity has to be calculated in sets with two or three same-sexed

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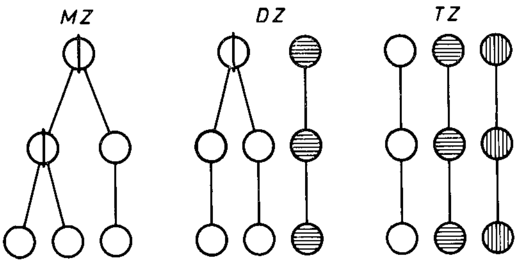
and serologically concordant members. It seems reasonable to adopt the principles used in twin studies, and to begin with an estimation of the initial probabilities of MZ, DZ, and TZ sets.

The true distribution of the three zygosity types is unknown. Their relative frequencies may be estimated by use of knowledge about twins under the assumption that the same two basic processes, zygote or embryo division and extra ovulation, are involved in twin and triplet formation. Triplets may appear if one of these processes happens more than once, or if they are combined (Figure). In the first case MZ



Figure

or TZ sets will appear. If the two events are combined, DZ sets appear. The probability of each process may be estimated from twin data.

A survey of births in Sweden in 1921-1950 includes the number of twin pairs of the same sex and of unlike sex (Table 1). According to the differential method of Weinberg (1902) it is possible to calculate the number of MZ and DZ twin pairs. The relation of the number of MZ or DZ twin births to the number of all births gives the probability of embryo splitting or extra ovulation, respectively. The problem is then to find an expression, based on the two values (a and b in Table 3), which leads to an expected frequency of triplet births and of same-sexed sets wich are not contradicted by observations.

Three prediction formulas for finding the expected frequencies of MZ, DZ, and TZ triplet births were tested (Table 3). It appears that Bulmer's formula (1958) leads to the best agreement between observation and expectation.

Table 3. *Distribution of zygosity types in triplets at birth* (estimated from twin data from Sweden 1921-1950).

Expectation according to	Formula	No. of births	Same-sex sets
	MZ DZ TZ	*exp.*	*exp.*
Jenkins 1927	$a^2 + 2ab + b^2$	585	0.398
Allen and Firschein 1957	$2a^2 + 2ab + b^2$	626	0.437
Bulmer 1958	$a^2 + 2ab + \frac{1}{2}b^2$	426	0.454
		obs.	*obs.*
		402	0.463

Estimated value of $a = 3531 \times 10^{-6}$
Estimated value of $b = 9929 \times 10^{-6}$

The initial probabilities of the three zygosity types estimated according to this formula show that DZ sets are more frequent at birth than MZ and TZ sets (Table 4). These average values are needed in the calculations of the probability of a set with two or three same-sexed and serologically concordant members being DZ or MZ, respectively.

Table 4. *Initial probabilities of zygosity types at birth in triplet sets *.*

	Of the same sex	Of unlike sex
MZ	0.208	
DZ	0.586	0.487
TZ	0.206	0.513

* Calculated according to Bulmer (1958) and data from Sweden 1921-1950.

Serological as well as some anthropological characters were studied in an unselected series of 54 same-sexed sets of Swedish triplets born in 1921-1950. One main reason for these studies was an intention to evaluate the applicability in triplets of methods used in the determination of zygosity in twins with the aid of the same characters.

The distribution of their blood (and serum) groups within 11 systems did not deviate from expectation. There were no indications of associations between zygote division or polyovulation and blood groups. The concordance observed in genetically dissimilar pairs or sets was not higher than expected. Thus, the theoretical approach seems fully acceptable in the use of blood groups in zygosity determination in triplets.

Ten selected anthropological characters, e.g., colour of eyes and bridge of nose, were studied. The intraset variations were compared with the findings in a Swedish twin series. Blood group discordant pairs or sets of triplets resembled DZ twins closely with regard to similarities and dissimilarities. In blood group identical pairs or sets the intrapair or intraset dissimilarities were not greater than in MZ twins. Consequently, it seems justified in the determination of zygosity in triplets to use odds in favour of monozygosity calculated on the basis of studies of the same characters in comparable series of twins.

The result of the final zygosity classification of the 54 same-sexed sets, based on serological and anthropological traits and on principles used in twin studies, was 16 MZ sets, 28 DZ sets, and 10 TZ sets. The proportion of MZ sets exceeds expectation.

Finger prints of the classified triplet material were analyzed in all 162 triplets with respect to pattern types and total ridge count. Comparisons between triplets from the three zygosity types and between triplets from each of them and the general population, as well as twins, showed some differences, but it seems safe to use empirical odds obtained from twin materials in the calculation of the probability of monozygosity in triplets.

Therefore, blood groups and some selected anthropological traits may be used in the zygosity diagnosis of individual sets of triplets in accordance with the principles used in twins.

This report is based on the following four papers:

Hauge M., Herrlin K.M., Heiken A. 1967. The distribution of blood groups in a series of triplets. Acta Genet. (Basel), 17: 260-274.

Herrlin K.M., Hauge M. 1967. Determination of triplet zygosity. Acta Genet. (Basel), 17: 81-95.

Herrlin K.M., Hauge M. 1968. Some anthropological traits in an unselected series of triplets. Acta Genet. (Basel), 18: 496-510.

Herrlin K.M., Hauge M., Eriksson S.A. 1970. Finger print patterns in an unselected series of triplets. Hum. Hered., 20: 336-355.

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