"EQUIVOCALNESS" AND OTHER EMPIRICAL METHODS IN ZYGOSITY ASSESSMENT

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Zygosity determination is generally carried out by different methods in small or large twin samples. The probability method based on sex and genetic markers is limited to relatively small samples, as a consequence of its cost. The empirical questionnaire method is applied in several large twin registers. Its margin of error is low enough for population studies, its cost is negligible, but its accuracy is insufficient when zygosity of twin pairs included in definite samples must be individually assessed. Efforts to bridge the distance between the two methods should be made, and they may take either direction: (1) find new, inexpensive genetic markers, or (2) increase the number and accuracy of empirical methods. The accuracy of a number of empirical methods applied to a twin sample of established zygosity has been compared. One modification of the "two peas in a pod" method, originally called "equivocalness method", appears to warrant inclusion in questionnaire methodology. Also, compound probability as expressed by several empirical methods may reach an acceptable level of accuracy in zygosity assessment.

Although some twin study methodologies can do without, the classification of twin pairs as MZ or DZ is a generally important and often indispensable prerequisite. Sex and blood groups traditionally represent the foundation on which reliable zygosity determinations are based. However, such a method too may provide insufficient indications, especially when it is not extended (as it very seldom is) to include parental phenotypes. As an example, two twins who are concordant as to sex and to phenotypes O, M, rh, have a probability of being DZ of 0.22 according to the tables of Maynard-Smith and Penrose; yet this probability approaches 0.70 if both parents are found to be 0, M, rh.

To this we must add the high cost of blood group determinations (especially if extended to rare systems) and the difficulty in securing blood samples (especially if the twins are not easily reached by the researchers.)

This of course justifies the continued search for alternative methods of twin zygosity diagnosis involving lower costs and possibly dispensing with blood sampling.

We should not forget that up to a few years ago twin pairs were classified as identical or not if they were found (or often simply reported) to be respectively monochorionic or not. A recent study at the Mendel Institute, reported in a degree thesis, compared different criteria for zygosity diagnosis: the error of the fetal-membrane method was found to be 27.1%, as compared to 8-9%[1] for the alternative methods of "equivocalness" and "first-sight".

A recent study involving a sizable sample of MZ pairs was the occasion for us to proceed to a further comparison between different methodologies of twin zygosity diagnosis, as listed below.

1. The first method was the classic one: sex and blood groups (A_1A_2BO, MN, Rh-Hr). Discordant pairs were obviously discarded. We considered "MZ" pairs with $p\text{DZ} \leq 0.05$, and "unknown" (= ?) pairs in which $0.05 < p\text{DZ} < 0.1$. Referring to the initial letters of the names of the Authors (Maynard-Smith and Penrose) this criterion was identified as MSP.

2. A "First-Sight" diagnosis was made by experienced workers, classifying the pairs as "MZ", "DZ", "?".

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3. "Identicalness": the Italian version of the "two peas in a pod" test ("were the twins as alike as two drops of water?") was administered to the mother and to each twin (answers: "yes", "no", "don't know").
4. The "Equivocalness" criterion was similarly applied by asking mother and each twin appropriate questions (i.e., whether the twins had ever been mistaken one for the other, and if so by whom: relatives, outsiders, nobody, "don't know"). For purposes of homogeneity with the other methods, the first two alternatives were pooled in the "yes" — i.e, MZ — group.
5. Finally, the combined variability of three Qualitative Traits was considered (eye color, hair color, skin color) using the international standards. Only cases of total concordance were considered as MZ; discordance of one degree for one trait was considered as "unknown" (= ?); discordance of over one degree or more than one trait was considered as DZ.

Table 1 lists the results with all five methods for all the 55 pairs that were found to be concordant as to sex and blood groups.

Table 1. *Zygosity of 55 twin pairs according to five different criteria*

Twin register no.	First sight	Ident.	Equivoc.	Qual. traits	MSP	Twin register no.	First sight	Ident.	Equivoc.	Qual. traits	MSP
10918	MZ	MZ	MZ	MZ	MZ	9880	MZ	DZ	MZ	MZ	MZ
70918	MZ	MZ	MZ	MZ	MZ	9098	?	MZ	MZ	DZ	?
10413	MZ	MZ	MZ	MZ	MZ	9525	MZ	MZ	MZ	MZ	MZ
10685	MZ	DZ	MZ	MZ	MZ	9081	MZ	MZ	MZ	MZ	MZ
9067	MZ	MZ	MZ	MZ	MZ	10502	MZ	MZ	MZ	MZ	?
10335	MZ	MZ	MZ	MZ	MZ	11765	MZ	MZ	MZ	MZ	MZ
71274	MZ	MZ	MZ	MZ	MZ	10938	MZ	MZ	MZ	MZ	MZ
6770	MZ	MZ	MZ	MZ	MZ	6889	MZ	MZ	MZ	DZ	?
8666	MZ	DZ	MZ	MZ	MZ	9865	MZ	?	MZ	DZ	MZ
11721	MZ	MZ	?	MZ	MZ	8766	MZ	MZ	MZ	MZ	?
6742	MZ	MZ	MZ	MZ	MZ	9065	MZ	?	MZ	MZ	MZ
4546	MZ	MZ	MZ	MZ	MZ	10180	MZ	MZ	MZ	MZ	MZ
8692	MZ	DZ	MZ	MZ	MZ	10951	MZ	DZ	MZ	DZ	MZ
11184	MZ	MZ	MZ	MZ	?	10878	MZ	MZ	MZ	MZ	MZ
4596	?	DZ	DZ	DZ	MZ	6924	MZ	DZ	MZ	MZ	MZ
9520	MZ	MZ	MZ	MZ	MZ	6832	MZ	MZ	MZ	MZ	MZ
10150	MZ	MZ	MZ	MZ	MZ	11323	MZ	DZ	MZ	MZ	MZ
8664	MZ	MZ	MZ	MZ	MZ	9147	MZ	MZ	MZ	MZ	MZ
11113	MZ	MZ	MZ	DZ	MZ	9935	?	DZ	MZ	MZ	MZ
10413	MZ	MZ	MZ	MZ	MZ	12901	MZ	?	MZ	?	MZ
12636	MZ	DZ	MZ	MZ	?	6997	MZ	MZ	MZ	MZ	MZ
9448	MZ	MZ	MZ	MZ	MZ	10464	MZ	?	MZ	MZ	MZ
11069	MZ	MZ	MZ	MZ	MZ	10603	MZ	DZ	DZ	?	MZ
10445	MZ	?	MZ	MZ	MZ	10285	MZ	MZ	MZ	MZ	MZ
8667	MZ	MZ	MZ	MZ	?	13031	MZ	MZ	MZ	MZ	MZ
10370	MZ	MZ	MZ	MZ	MZ	11734	MZ	DZ	MZ	MZ	MZ
8793	MZ	MZ	MZ	?	?	11229	MZ	DZ	MZ	MZ	MZ
11993	MZ	DZ	MZ	MZ	?						

We see first of all that in 9/55 cases the MSP reference criterion gave a doubtful response. We then see that in 27/55 cases all criteria agree on the MZ diagnosis. In 17/55 cases, four criteria agreed on the MZ diagnosis while one criterion differed. Assuming that these cases were in fact MZ, we see that the Identicalness criterion gave 8 wrong and 3 doubtful diagnoses: the MSP criterion gave 4 doubtful diagnoses; the Qualitative Traits criterion gave one error; the Equivocalness criterion gave one doubtful diagnosis; the First-Sight criterion gave all correct diagnoses.

Table 2. *Comparison of diagnostic criteria*

	First sight			Identicalness			Equivocalness			Qualitative traits		
	MZ	DZ	?	MZ	DZ	?	MZ	DZ	?	MZ	DZ	?
MSP:												
MZ	44	—	2	29	12	5	43	2	1	40	4	2
?	8	—	1	7	2	—	9	—	—	6	2	1
Equivocalness:												
MZ				35	12	5						
DZ				—	2	—						
?				1	—	—						

In only one case was the situation reversed; the MSP criterion diagnosed as MZ one pair which was assessed as DZ by three criteria and doubtful by the other. This pair does in fact have discordant electrophoretic patterns (Milani-Comparetti M., Saccucci F. 1976. Plasma protein variability in MZ twins. Acta Genet. Med. Gemellol. (Roma), 25: 154-156).

In 8 cases three criteria gave an MZ diagnosis while the other two differed: the dissenting or doubtful criteria were Identicalness (6 times), Qualitative Traits (5 times), MSP (4 times), First Sight (once).

In 2 cases only two criteria favored MZ (First Sight with MSP once, Identicalness with Equivocalness another time).

Table 2 shows each of the four empirical criteria compared with MSP, as well as a comparison between the two methods adaptable for questionnaire diagnosis (Identicalness and Equivocalness).

The per cent frequencies of MZ, DZ, and doubtful diagnoses, for each of the criteria on our sample of pairs assumed to be MZ, are then compared in the Figure.

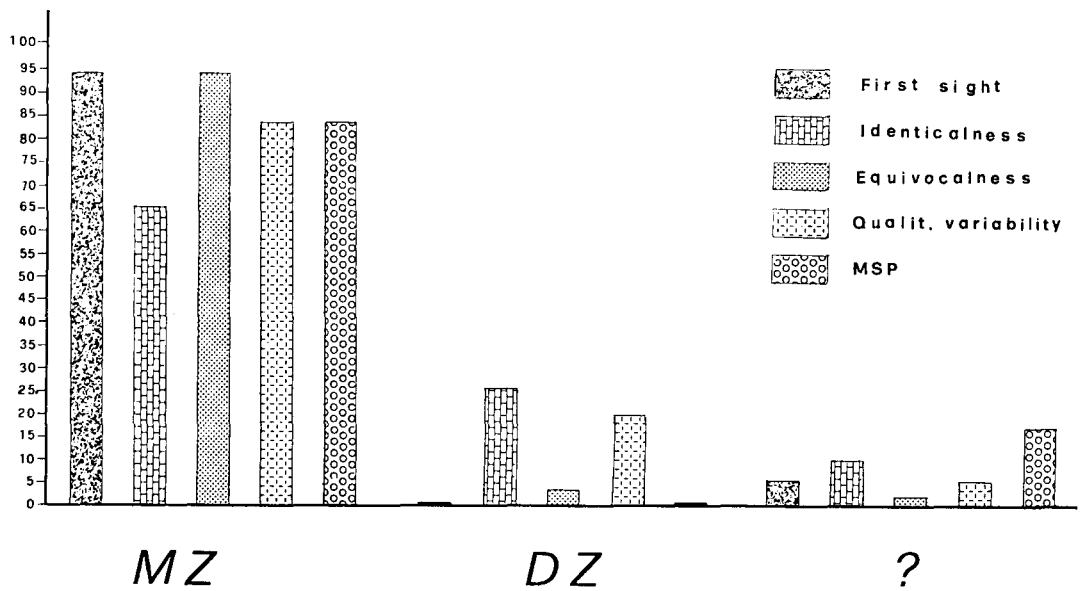

Figure. Histogram of per cent frequencies of MZ, DZ, and doubtful diagnosis by the five criteria.

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By assuming maximum probability value for concordance of all criteria, high probability value for concordance of four criteria out of five, and low probability value for concordance of three criteria out of five, empirical reliance scale would place in top position the First-Sight criterion for direct examination and Equivocalness for questionnaire methods.

We believe that the study should be extended, with similar methodology, to a sample representing the population distribution of both types of zygosity. Meanwhile, we believe that our results tend to favor the inclusion of the Equivocalness criterion in questionnaire zygosity diagnosis.

More complete experimental results may lead to the definition of reliability values for each criterion, providing the basis for the calculation of compound probabilities in zygosity diagnosis.

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