

TWINS AND RESEARCH

by

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The occurrence of twins in man and in many other species, notably cattle, provides the student of heredity and environment with a unique tool. Monozygotic twins and higher multiple births correspond with isogenic strains produced so commonly in plants by asexual propagation, and in both plants and animals by inbreeding. They even possess one advantage over homozygous inbred lines, in that they may be either homozygous or heterozygous.

The value of twins as source material for research was recognized by Galton, who reported on his observations of 80 pairs in his *Inquiries Into Human Faculty*. Today the use of twins is extensively employed by scientists throughout the world. Literally scores of serological, anthropological, pathological, and psychic traits in man have been compared in monozygotic and dizygotic twins. More recently the twin method of research has been inaugurated in cattle investigations, notably in Sweden, New Zealand, and the United States.

Twins provide unparalleled material for estimating heritability, and this is the principle objective of most twin studies. Dizygotic twins also provide comparisons which enable one to check simple modes of inheritance, although they have not been extensively used for this purpose. The inheritance of the two types of twins, and the diagnosis of twins as to zygosity have received considerable attention, although these problems are only indirectly related to the twin method of research. This paper has solely to do with a discussion of the methods employed in using twins to estimate heritability, and how dizygotic twins may aid in the detection of simple modes of inheritance.

Heritability Estimates

Twin investigations in man have for the most part consisted of comparisons of the degrees of intra-pair variation in monozygotic and dizygotic twins. If the traits are qualitative, pairs are termed concordant if both members possess a trait, pairs in which only one member possesses the trait are termed discordant. Twins may also be concordant for the absence of a trait, but most comparisons have not included this class (I shall henceforth, for convenience, symbolize mo-

nozygotic twins by M, dizygotic twins by D, and concordance by C). The logic involved in comparisons of M with D is readily apparent. All intrapair variations in M must be due to environment, whereas both heredity and environment may bring about intrapair variations in D. Therefore if we assume that M and D have comparable degrees of similarity in their environments, greater intrapair variations in D as compared with M may be ascribed to differences in heredity. Allowing H to denote heritability, then $HD = \frac{CM-CD}{100-CD}$

Most investigators have reported on the percentages of C in the two types of twins, but have not made estimates of heritability. Table 1 shows HD for several traits, calculated from data collected by various investigators.

Table 1 - Degrees of concordance observed in identical and fraternal twins in respect to various traits. Concordant pairs include only those in which both members possess the trait.

Trait	Identical		Fraternal		Investigator	Heritability estimate
	Number of pairs	% Concordance	Number of pairs	% Concordance		
Club foot	35	23	133	2	v. VERSCHUER	21.43
Measles	189	95	46	87	v. VERSCHUER	61.53
Diabetes Mellitus	63	84	70	37	v. VERSCHUER	74.60
Tuberculosis	80	65	125	25	v. VERSCHUER	66.56
Scarlet fever	31	64	30	47	v. VERSCHUER	32.07
Ricketts	60	88	74	22	v. VERSCHUER	84.61
Cancer (any type)	62	61	27	44	MACKLIN	30.35
Cancer (specific type)	62	58	27	24	ROSANOFF, HANDY, PLESSET, BRUSH	44.73
Schizophrenia	21	67	37	0	LUXENBURGER	67.00
» »					ROSANOFF, HANDY,	
Maniac depressive insanity	41	69	53	18	PLESSET, BRUSH	50.00
» »	33	94	17	6	LUXENBURGER	93.61
» »	23	70	67	17	ROSANOFF, HANDY, PLESSET	63.85
Feeble-mindedness	126	87	111	45	ROSANOFF, HANDY, PLESSET	76.36
Criminality	121	72	93	34	VARIOUS INVESTIGATORS	57.55

Many traits are quantitative, in which cases various methods are used for comparing the two types of twins. Holzinger compared the intrapair coefficients of correlation in estimating HD. The formula is as follows: $HD = \frac{rM - rD}{1 - rD}$ Some of these estimates are shown in Table 2.

Table 2 - The percentages of intrapair variations in several traits among dizygotic twins estimated to be due to heredity (after Newman, Freeman, and Holzinger).

Standing height	81%
Sitting height	76%
Weight	78%
Head length	78%
Head width	75%
Finger ridges	90%
Binet IQ	68%

It is also possible to estimate the effects of different environments (E) by comparing M reared together (MT) with monozygotic twins reared apart (MA). Thus for qualitative variations $E = \frac{CMT-CMA}{100-CMA}$, and for quantitative traits $E = \frac{rMT-rMA}{1-rMA}$ (see Table 3). Unfortunately it is extremely difficult to obtain large numbers of human monozygotic twin pairs in which the members have been reared apart, or have been exposed to comparably different environmental influences.

Table 3 - Estimates of the effectiveness of the differences in environments of twins reared apart in bringing about intrapair differences in various traits.

Calculated from intrapair correlations obtained by Newman, Freeman, and Holzinger.

Trait	Amount of intrapair variation in twins reared apart due to greater differences in environment
Standing height	39%
Sitting height	12%
Weight	76%
Binet IQ	72%

Twin studies in cattle to date have been conducted somewhat differently from the conventional methods in man. The intra-pair differences are compared with the inter-pair differences of M, and significantly greater inter-pair differences are interpreted as being due to genetic factors. Cattle twin experiments are also under way in which the members of each pair are subjected to different types of feed or management, in order to evaluate the effectiveness of such environmental factors. Analysis of variance is usually employed in testing the significance of differences.

Bonnier and Hansson used a $2 \times n$ table to outline their experiments with monozygotic cattle twins, allowing n to represent the number of pairs of twins (Table 4). The letters (A and a) represent the members of each pair. As the different pairs of twins are unrelated the variations between rows are largely due to heredity, while the differences between columns are entirely due to environment. Differences resulting from non-linear interactions between rows and columns

Table 4 - (After Bonnier and Hansson)

	Twins	Total
	A_1	a_1
	A_2	a_2
	A_n	a_n
TOTAL		

may be ascribed to interactions between heredity and environment and to experimental error. Analysis of the average intra-pair variations of another group of monozygotic twins of which all animals were subjected to the same treatment was used to estimate the experimental error. The following formulae were employed to estimate heritability and environmental effects:

$$H = \frac{\text{mean sq. hered.} - \text{mean sq. interac.}}{2}$$

$$E = \frac{\text{mean sq. envir.} - \text{mean sq. interac.}}{n}$$

Table 5 shows the results of the investigations of four traits in two groups of monozygotic twins, at 6 months and at 24 months of age.

In experiment 1 the A animals were given 33% more feed, the a animal 33% less feed than normal for cattle of that age and breed. In experiment 2 the A animals were given 25% more, the a animals 25% less feed than normal. Nine pairs of twins were included in experiment 1, eight pairs in experiment 2. Note that the heritability estimates are higher in experiment 2 at both age levels for each of the four traits under consideration, indicating that the greater intra-pair differences in amounts of feed given in experiment 1 had a pronounced influence.

Some of the reasons for the differences in the use of twins as research material in man and cattle are rather obvious. Cattle are not affected by educational and cultural influence which are so important in mankind. Thus comparisons of intra-pair with inter-pair differences in cattle is an efficient method of research. Several pairs of M twins in cattle may be collected when quite young and the members

of each pair separated and subjected to different treatments. The investigator than has an excellent opportunity to compare the effectiveness of various types of treatment.

Table 5

Age	Cause of Variation	Width of head		Height of shoulders		Length of sacrum		Live weight	
		1	2	1	2	1	2	1	2
6 months	Experiment								
	Heredity	28	84	23	36	5	58	8	56
	Environment	61	—	72	26	90	20	88	40
	Interaction - error	11	16	5	38	5	22	4	4
24 months	Heredity	55	82	41	86	11	25	9	17
	Environment	35	7	51	3	88	55	89	78
	Interaction - error	10	11	8	11	1	20	2	5

Human twins who have been separated from birth are a rarity. Even when separated early in life, the differences in the environments for members of different pairs are not comparable. For that matter, differences in the home environments of twin pairs reared together vary so much from one home to another as to render difficult the interpretations of inter- versus intra-pair comparisons. The fact that D reared together have similar home environments qualifies them as good material for comparisons with M. Although D have the same degree of similarity in heredity as do siblings, their environments are more similar, due to the fact that they are contemporaries. They are likely to be in the same grade in school and to have the same teachers and playmates. Moreover, they occupy the same position with respect to birth order in the family and the same age differences exist between them and their parents, resulting in certain types of environmental similarity impossible to attain in single-born brothers and sisters.

D twins are of unique value in still another respect. Both types of twins are exposed to prenatal circumstances not encountered by the single-born. As D and sibs possess the same average degree of genetic similarity, comparisons between them provide our most efficient means of evaluating the conditions *in utero* peculiar to twins. It is for this reason that it is hazardous to draw sweeping conclusions on the heritability of a trait, based solely on twin comparisons. Comparisons of D with sibs have shown notable differences with respect to several traits. Owen (1945) found that D and higher multiple births in cattle invariably show no intra-pair differences with respect to blood group antigens. Presumably this is attributable to anastomosing of the blood vessels in the fetal membranes, resulting in interchange of primordial or *stem* cells and intermixture of blood of the members of the set. This condition, known as erythrocyte mosaicism, fortunately

does not occur in human twins. Gedda (1948) has shown that M and 85% of D in man possess remarkable similarities in the amount of glutathione in the blood cells, a condition known as *insoglutathionemia*. Yet this phenomenon is not found in sib or parent-child comparisons, but is apparently restricted to twins. Rosanoff (1937), found 46.6% C in D and 16.5% C in sibs for feeble-mindedness, indicating that the circumstances attending D before and at birth increase the chances for the condition.

There are limitations to the amount of information concerning the effects of intra-uterine conditions in twins which may be gleaned from comparisons of D with sibs, as not all of the factors associated with monozygotic twinning are present in the development of D. Thus in man the effects of the stage at which separation of the embryos occurs, the possible operation of an asymmetry mechanism, and the effects of unbalances of the blood supply are problems peculiar to M. The observation that approximately 20% of M are dichorial indicates there is some variation in the stage of embryonic development at which twinning occurs. Presumably dichorial M separated earlier than monochorial M. Price (1950) has suggested that follow-up studies of M classified as to whether monochorial or dichorial should yield important information as to how effective the stage at which twinning occurs may be upon producing intrapair differences in M.

There are additional reasons why caution must be exercised in the interpretation of heritability estimates. They may be expected to vary from one population to another, depending upon the frequencies of the genes involved, the amount of inbreeding, and other factors. (As a matter of fact, estimates of heritability have been suggested as a substitute for coefficients of inbreeding.) Comparisons of M and D tell us only the H in D, not in sibs or unrelated persons. The heritability of traits among the general population should be greater than in D or sibs, for the former have twice as much dissimilarity in genetic makeup, on the average, as do D and sibs.

Conventional twin research is based upon the assumption that twins reared together have the same degrees of similarity in environment, whether M or D. This is not strictly true, for M are so similar that people usually react to them as to a single individual, whereas D are easily distinguishable and people react to them differently. It is difficult to estimate how important this factor may be, but it should not be overlooked. The importance of it varies greatly, depending upon the type of trait under consideration. Certainly it would be of little or no importance in the investigation of such traits as body build, disease susceptibility, and visual acuity. But it may be of importance in determining degrees of C in mental and personality traits, and in criminality. This difficulty might be avoided if it were possible to compare M and D, all of whom had been reared apart.

The Inheritance of Plasticity

M differ from clones and highly inbred lines in one very important respect. Any particular clone or inbred line may consist of hundreds or thousands of individuals, whereas a pair of M consists of only two individuals. Different pairs of M correspond to different clones or populations. One hundred pairs of M are analogous to one hundred clones or highly inbred strains consisting of two individuals within each. All differences within an isogenic population of 100 individuals, regardless of whether a clone or an inbred strain, must be attributed to non-genetic factors. But differences within a population consisting of one hundred pairs of M may be due to both genetic and non-genetic factors. All intra-pair differences must be due to non-genetic factors, but inter-pair differences are the result of interactions between heredity and environment. Different pairs of M possess different genotypes, and there is good reason to believe that this accounts for the observation that some pairs of M show greater intra-pair similarities, even when exposed to somewhat similar types of environment. This variation in degree of response to various environmental stimuli may be termed plasticity. Hereditary variations in plasticity are doubtless partially responsible for the lack of complete penetrance and differences in the expressivity of many penetrant genes.

Coat color in cattle provides an example of a trait which manifests different degrees of intra-pair variation in M. A single pair of genes determines whether an animal has solid color or is spotted. The degree of spotting is largely, but not altogether, determined by heredity. Aberdeen-Angus cattle are characterized by solid black color, Holstein cattle by black and white spotting. M in Angus are more similar in coat color than are M in Holsteins, because the genotypes of the latter result in some degree of phenotypic plasticity.

Variations in functional handedness among human M appear to be determined by the same principle (Rife, 1950). A single pair of genes lacking dominance provides the genetic basis for handedness. Using *R* to symbolize the gene for right-handedness, and *r* its allele for left-handedness, M may be of the following genotypes and phenotypes:

<i>Genotype</i>	<i>Phenotype</i>
RR	Both right-handed
Rr	One right-handed, one left-handed. « Mirror-images »
rr	Both left-handed

According to this scheme, heterozygous pairs may be plastic and the prenatal conditions peculiar to twins, such as the position *in utero* or the possible operation of an asymmetry mechanism, result in one member being right-handed and the other left-handed. Homozygous M are non-plastic, being alike in handedness. Whether or not members of a pair of M are alike in handedness depends upon

their genotype. Torgersen (1950) has found evidence that a similar interaction of heredity and environment is responsible for the occurrence of *situs inversus viscerum* among twins.

All students of twins are familiar with cases of M in which amazing similarities are shown in the type and onset of diseases and abnormalities, yet other cases where members of the pair are completely discordant. May it not well be that inter-pair differences in the degrees of genetic plasticity may account for these superficially inconsistent findings? It seems probable that they are also varying degrees of plasticity or responsiveness in respect to psychic and personality traits. It is for this reason that it is inadvisable to draw sweeping conclusions on the basis of observations of a single set of monozygotic twins, triplets, quadruplets, or quintuplets. The four members of two pairs of M may have identical IQ's to begin with, yet if the members of both pairs are separated and one from each placed in a good environment, and the other members in a poor environment, the members of one pair may show greater responses to the altered environments than do the members of the other pair.

The heritability of plasticity is masked in the usual types of comparisons of concordance and correlation in M and D, in investigations of human twins. Handedness, for example, shows about the same degree of intrapair similarity in both M and D, giving HD of only 10%. The ideal test for H of plasticity is to expose the members of many pairs of M to corresponding environmental differences. If considerable and significant differences in the degree of intrapair variations occur from pair to pair, we have evidence of the heritability of plasticity. Analysis of the variance, such as that used in estimates of heritability in cattle twins, also provide a means of evaluating how much of the variation is due to the interaction of heredity and environment. Variations due to inter-pair differences in plasticity are within this category.

Dizygotic Twins and Simple Modes of Inheritance

Although investigations of M tell us nothing concerning modes of inheritance, D twins may be used for this purpose. If the modes of inheritance and the frequencies of the genes for a specific trait are known, the expected frequencies of discordant twin pairs in the total D twin population may be easily calculated. Formulae have been derived and the expected frequencies of the two kinds of concordant, as well as discordant, twins at various gene frequencies have been calculated for simple dominance, lack of dominance, triple alleles with a graded order of dominance, sex-linked, and sex-influenced inheritance (Rife 1938, 1951). Perhaps the greatest usefulness of this information is establishing limitations of the percentages of D which may be discordant for a particular mode of inheritance. The formula is $1\frac{1}{2}p^2(1-p)^2 + 2p(1-p)^3$, where p = frequency of the dominant allele, and q = the frequency of the recessive. If three alleles are involved, the following formula gives the expected percentage of discordant pairs: $1\frac{1}{2}^2(1-p)^2$

+ $2p(1-p)^3 + qr^2(1 + \frac{1}{2}q + r)$, where p = the frequency of the allele dominant to the other two, q = the frequency of the second allele in order of dominance, and r = the frequency of the recessive allele. When only two alleles are concerned, the highest percentage of discordance is approximately 27%, if three alleles are concerned the expected discordance will never exceed 40%. If significantly more than 40% of twins show discordance in a trait for which M are always concordant and for which the frequencies are the same in both sexes, we may safely assume that genes at two or more loci are involved.

Gene frequency analysis may also be used to test lack of dominance in a situation where the phenotypes of homozygous twins are solely dependent upon genotype, while the phenotype of heterozygous twins is determined by condition *in utero*, resulting in intra-pair differences or « mirror-imaging » of all heterozygous M, and also in D where both members are heterozygous. The two phenotypes occur with equal frequencies among heterozygous members of D, in which the other member is homozygous. Let p = frequency A , and q = frequency of a . The expected frequency of discordant pairs among M = $2pq$, and among D = $2pq + \frac{p^2q^2}{2}$ (Rife, 1950). The incidence of right- and left-handedness among both types of twins conforms very well to this type of analysis. Under these circumstances D can never show more than approximately 3% greater discordance than M.

D may be used in a different way to analyze the inheritance of rare traits. In such situations it is usually not feasible to collect the type of twins that are concordant for lack of the trait, but it may be possible to compare the frequency of discordant pairs to concordant pairs that are concordant for presence of the trait. If the trait is due to either a simple dominant gene, or to a pair of recessive genes, discordant pairs must result from either one of the two following matings: $Aa \times aa$, or $Aa \times Aa$. If the rare trait is due to a dominant gene, practically all discordant pairs will arise from $Aa \times aa$. Such matings produce a ratio of two discordant to one concordant pairs. If the trait is due to a pair of recessive genes, practically all discordant pairs will arise from $Aa \times Aa$. The expected ratio of discordant to concordant pairs from such matings is 6 to 1. Thus a ratio of two discordant to one concordant suggests that the trait is due to a simple dominant gene, while a ratio of six discordant to one concordant suggests it is conditioned by a pair of recessive genes.

Sibships of two may be used in the same way as D to analyze simple modes of inheritance. The advantage of using D lies in the fact that they are conventionally used in heritability estimates, and are thus available for testing modes of inheritance.

Summary

1. The uses of twins as a tool to estimate heritability are reviewed.
2. Comparisons of dizygotic twins with sibs provide a means of estimating how effective conditions *in utero* common to both types of twins may be in producing intra-pair differences.
3. Genetic variations in plasticity should be taken into consideration in the interpretation of data obtained from twins.
4. Methods for testing simple modes of inheritance by information obtained from dizygotic twins are reviewed.

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RIASSUNTO

1. Furono riesaminate le utilizzazioni dei gemelli come mezzo per valutare l'eredità.

2. Il confronto fra gemelli dizigotici e fratelli mononati fornisce un mezzo per valutare come in effetti le condizioni endouterine, comuni ai due tipi di gemelli, possano produrre delle differenze intrageminali.

3. Nella interpretazione dei dati forniti dai gemelli si potrebbero prendere in considerazione le variazioni genetiche riguardanti la plasticità.

4. Furono riesaminati i metodi per accertare i meccanismi ereditari elementari sulla base delle informazioni ottenute dai gemelli dizigotici.

RÉSUMÉ

1. On a réexaminé l'utilisation des jumeaux comme moyen d'appréciation des facteurs héréditaires.

2. La confrontation de jumeaux dizygotiques et de frères ordinaires permet d'établir comment les conditions intra-utérines, communes aux deux types de jumeaux, peuvent produire des différences intra-gémellaires.

3. Dans l'interprétation des données fournies par les jumeaux on pourrait prendre en considération les variations génétiques relatives à la plasticité.

4. On a réexaminé les méthodes propres à déterminer les mécanismes héréditaires élémentaires sur la base des informations obtenues par l'étude des jumeaux dizygotiques.

ZUSAMMENFASSUNG

1. Die Verwendung von Zwillingen zur Bewertung der Erbfaktoren wurde überprüft.

2. Der Vergleich von dizygotischen Zwillingen mit gewöhnlichen Brüdern ermöglicht es festzustellen, wie die Bedingungen im Mutterleib, welche für beide Zwillingsarten gleich sind, Verschiedenheiten zwischen Zwillingen hervorbringen können.

3. Genetische Variationen der Plastizität könnten in Betracht gezogen werden bei der Auslegung der Gegebenheiten bei Zwillingen.

4. Methoden um einfache Vererbungsweisen durch eingeholte Angaben über dizygotische Zwillinge zu erarbeiten, wurden überprüft.

(Traduzioni a cura della Redazione).