Satellite Association and Structural Anomalies in Two North Indian Populations—Rajputs and Punjabis

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Abstract An attempt has been made to study the morphological variability of the human chromosomes in two Indian populations—Rajputs and Punjabis with respect to satellite association and the structural anomalies.

INTRODUCTION

Morphological variability of the human chromosomes are restricted to the heterochromatic regions, especially in the short arms of the acrocentric chromosomes. To study this chromosome polymorphism in Rajputs and Punjabis, two important parameters viz. satellite association and structural anomalies were investigated in detail.

I. Satellite association: The distributional studies of human chromosomes in metaphase plate have shown that the acrocentric chromosomes tend to lie near the middle of the nucleus (BARTON *et al.*; 1965, GALPERIN, 1968). These acrocentric chromosomes show an association pattern which was first reported by FERGUSON-SMITH and HANDMAKER (1961) and confirmed by MERRINGTON and PENROSE (1969), REITALU (1965) and BISHUN (1966).

The only population study available with respect to satellite association is that of COHEN and SHAW (1967) who have studied five ethnic groups and have reported non-significant differences in between these groups. An attempt has been

made in the present study to use satellite association as a criterion for studying population differences.

A number of factors like technique, physical and chemical agents, age, sex and clinical disorders have been reported by different authors and these are said to increase or decrease the frequency of acrocentric chromosomal association.

With regard to the technique used, BACK and ZANG (1969) have reported significantly higher association pattern in macro-culture as compared to microcultures but, on the contrary, HANSSON (1970 b) has reported microcultures showing an increase in the percentage of cells with association complexes when compared with macro-cultures. NANKIN (1971) has observed that the reduction of sodium citrate concentration in the hypotonic solution results in the decrease of satellite association frequency. NANKIN (1970) and MUELLER (1971) have also reported that the occurrence of association depends on the duration of culture. The association of acrocentric chromosomes also depends on the type of tissue, viz., blood or skin used in culture (FROLAND and MIKKELSEN, 1964;

PROKOFIEVA-BELYOVSKAYA *et al.*, 1968).

Chemical and physical agents are strong factors influencing the association phenomenon considerably as reported by MERZ and PREMPRE (1966), LUCHSINGER *et al.* (1969), MCQUARRIE *et al.* (1970) and HOEHN *et al.* (1971). The capacity of the acrocentric chromosomes to form association depends on the stage of ontogenesis (ARAGATSUNI, 1967, 1969; PROKOFIEVA-BELGOVSKAYA *et al.*, 1968; BOGOMAZOV and DOROSHENKO, 1968; MUELLER, 1971; COOKE, 1972). ZANG and BACK (1968) observed non significant sex differences in the satellite association pattern. On the contrary, GALPERIN (1968) and ROSENKRANZ and HOLZER (1972) have observed the association tendency to be greater in females as compared to males.

An increased frequency of satellite association were observed in clinical cases arising from mitotic and meiotic non-disjunction (LYONS *et al.*, 1965; ZELLWEGER *et al.*, 1966; LUCHSINGER *et al.*, 1969; ROSENKRANZ and FLECK, 1969; ZHDANOVA, 1971; ROSENKRANZ and HOLZER, 1972). Contrary to this, FROLAND and MIKKELSEN (1964) and BACK and ZANG (1969) have found no difference in frequency of associations in cases arising from non-disjunction error as compared to normal controls. HANSSON (1970 a) observed association complexes to be more heterogeneous in mentally retarded patients as compared to normal controls. NANKIN *et al.* (1968) have observed that the satellite association occurs in normal and hyperthyroid females with equal frequency. The Ph^{+1} and Ph^{1-} cells have been found

to differ significantly in their association pattern in a female patient with chronic myelogenous Leukemia (KITCHIN and SHAW, 1971).

Analysis of metaphases for satellite associations from women taking oral contraceptives and those taken as control show a significantly higher frequency of D-D associations in women taking the "pill" compared to the controls. (MCQUARRIE, 1970).

Non-random participation of acrocentric chromosomes have been reported by NAKAGOME and BLOOM (1970), COOKE (1971), PATIL and LUBS (1971) and NAKAGOME (1973). In contradiction with this, SHAW *et al.* (1969) have reported that the D group chromosomes do randomly involve, both in DD and DG associations.

II. Structural anomalies: It is usual for a small number of cells in the normal cell lines of an individual to show some structural anomalies like chromatid or chromosome gap or break. The cause of the existence of this kind of cells in normal individuals is not known, but a variety of chemical and physical mutagens (to which the civilized man is exposed) might be one reason (BLOOM *et al.*, 1970). Over 80 physical, chemical and biological agents have been shown to induce chromosomal aberration in human somatic cells (SHAW, 1970). Many mechanisms by which damage is caused to chromosomes have been postulated. Some of these include a direct virus "attack" or its combination with chromosome or else interference with DNA synthesis (NICHOLS, 1966).

The structural anomalies of one or the other type in normal populations have been reported by different authors (BLOOM and TJIO, 1964; COURT BROWN *et al.*, 1966, LUBS and SAMUELSON, 1967; AMAROSE and SCHUSTER, 1971; BROGGER, 1971; MÜLLER, 1971, MEHES *cf.* MÜLLER, 1971 and LITFIELD and GOH, 1973).

BLOOM *et al.* (1970, 1973) have studied the structural anomalies in the Yanomama and Piaroa Indians of Venezuela and have observed that there is a progressive decline in the percentage of cells with structural anomalies in successive years 1969, 1970 and 1971 for which they have concluded that this decrease in the frequency is a result of the elimination of chromosomally damaged cells after exposure to a chromosome breaking agent.

MATERIALS & METHODS

Rajputs and Punjabis are two endogamous groups of North and North-West India. Rajputs are divided into several subcastes or gotras which intermarry. Khattris and Aroras are two subcastes in Punjabis among whom inter-marriage is prevalent in recent times. Blood samples of 100 Rajputs and 100 Punjabis (Khattris and Aroras) have been collected from Blood Bank Transfusion Centre, Army Base Hospital, Delhi Cantonment during August, 1971 and December, 1973. In order to study the morphological variability of human chromosomes in these two groups, metaphase preparations were made from leucocyte cultures of these samples according to the method of MOORHEAD *et al.* (1960) with slight modifications. TC

199 was used as culture medium supplemented by 20% fetal calf serum. 1000 units of penicillin and 1000 mcg of streptomycin each were used as antibiotics. Phytohemagglutinin-M (0.2ml) was used to initiate cell division. The culture were incubated at pH 7.3-7.4 for 72 hours at a temperature of 37°C. At 70 hours 0.2 µg/ml. of calcemide was added to the culture and it was further incubated for 2 hours. Hypotonic treatment was then performed for 30 minutes using 0.075 M KCl. The materials were then fixed in 1:3 acetic acid-methyl alcohol fixative. The slides were made by air-drying method and stained in buffered Giemsa. Photomicrographs of five wellspread metaphases from each individual have been taken. The scoring of the metaphases for satellite association and structural anomalies were done as follows:

Two or more acrocentric chromosomes were considered "associated" when the following conditions laid down by ZANG and BACK (1968) were fulfilled:

- 1) The distance between the two acrocentric chromosomes did not exceed the length of the long arm of the largest G chromosome of the mitosis in question.
- 2) If the short arms of these chromosomes were connected by clearly visible thread like structures, larger distances were accepted.
- 3) Larger distances, up to the length of the long arm of a D-chromosome, are also accepted when the associating patterns lies exactly on the same longitudinal axis.
- 4) The short arm of the second, or any further associating acrocentric chromo-

somes, pointed towards those of the first and did not lie below the "centromere-line" of the first chromosome. ZANG and BACK (1968) defined "centromere-line" as the line that crosses the centromere perpendicular to the chromosomal longitudinal axis.

Spontaneous structural anomalies in the normal cell line were scored in the following manner: A gap was recorded when the chromosome showed an achromatic segment in the course of the chromatids, without displacement of the distal chromatid part, when only one of the chromatids showed an achromatic segment in the same region it was called a chromatid gap. A break was defined by a complete separation of continuity in the course of the chromatids with a diverging axis of the two chromatid parts concerned and/or a displacement of the distal part. When only a single chromatid was involved, it was called a chromatid break, but when both the sister chromatids appeared to be separated in the same region and displaced from the parent it was called a chromosome break.

Cells were also classified as hypomodal and hypermodal according whether the number of chromosome is less than or

more than the modal number 46 respectively.

RESULTS AND DISCUSSION

I. Satellite Association: A total of 434 and 413 associations of acrocentric chromosomes have been observed in a set of 500 metaphases from 100 Rajputs and 500 metaphases from 100 Punjabis respectively. The percentage of cells showing at least one association of acrocentric chromosomes is found to be 65.0 per cent among Rajputs and 61.0 per cent among Punjabis (Table 1). The highest percentage of cells showing association of acrocentric chromosomes is reported by COHEN and SHAW (1967) in 96.4 per cent of the total metaphases studied by them and the lowest by ZELLWEGER *et al.* (1966) in 13.3 per cent of the total metaphases (Table 2).

The maximum number of cells show only one association of acrocentric chromosomes (46.0 per cent in Rajputs and 41.4 per cent in Punjabis), followed by two associations of acrocentric chromosomes (16.2 per cent in Rajputs and 17.8 per cent in Punjabis) as shown in table 3. Majority of the associations are formed by two chromosomes. (77.8 per cent in

Table 1. Frequency of cells showing association of acrocentric chromosomes.

Population	No. studied	No. of metaphases observed	Cells showing association of acrocentric chromosomes	Cells with no association of acrocentric chromosomes
Rajputs	100	500	325 (65.0)	175 (35.0)
Punjabis	100	500	305 (61.0)	195 (39.0)

Figures in parenthesis indicate percentage.

Table 2. Percentage of association of acrocentric chromosomes in human lymphocyte metaphases as reported by different studies.

No.	Authors	Percentage of cells with association
1.	ZELLWEGER <i>et al.</i> (1966)	13.5
2.	NANKIN <i>et al.</i> (1968)	43.5
3.	FERGUSTN-SMITH and HANDMAKER (1961)	60.0
4.	Present study-Punjabis	61.0
5.	Present study-Rajputs	65.0
6.	PRAKOFIEVA-BELGOVSKAYA <i>et al.</i> (1968)	68.4
7.	ZANG and BACK (1968)	87.1
8.	NANKIN (1970)	89.9
9.	COHEN and SHAW (1967)	96.4

Table 3. Distribution of cells with different number of association of acrocentric chromosomes.

Type of Cells	Rajputs	Punjabis
Cells with no AAC	176 (35.0)	195 (39.0)
Cells with 1 AAC	230 (46.0)	207 (41.4)
Cells with 2 AAC	81 (16.2)	89 (17.8)
Cells with 3 AAC	14 (2.8)	8 (1.6)
Cells with 4 AAC	—	1 (0.2)
Total	500 (100.0)	500 (100.0)

Rajputs and 79.42 per cent in Punjabis) as shown in Table 4. Other studies show the same pattern except that of COHEN and SHAW (1967) in which the majority of the associations are formed by four chromosomes (38.33 per cent).

The involvement of D and G chromosomes in association of acrocentrics is shown in Table 5. It may be seen from Table 5 that the D/G type of association predominate (41.01 per cent in Rajputs and 43.58 per cent Punjabis), followed by D/D (23.04 per cent in Rajputs and 23.49

per cent in Punjabis) and G/G (13.82 per cent in Rajputs and 12.35 per cent in Punjabis) type of associations. All other types of associations combined together account for 22.12 per cent of associations in Rajputs and 20.58 per cent of the associations in Punjabis. The same trend is observed in other studies except that of COHEN and SHAW (1967) and COOKE (1972) where the frequency of "others" type of associations is 73.261 per cent and 61.392 per cent respectively.

Rajputs and Punjabis are compared with

Table 4. Number of chromosomes per association of acrocentric chromosomes.

Type of association	Rajputs	Punjabis
Association formed by two chromosomes	338 (77.88017)	328 (79.41882)
Association formed by three chromosomes	73 (16.82026)	54 (13.07504)
Association formed by four chromosomes	16 (3.68662)	26 (6.29539)
Association formed by five chromosomes	5 (1.15206)	4 (0.96852)
Association formed by seven chromosomes	2 (0.46052)	—
Association formed by eight chromosomes	—	1 (0.24213)
Total	500 (100.0)	500 (100.0)

Table 5. Distribution of the association of the acrocentric chromosomes by the group to which associating chromosomes belong.

Type of association	Rajputs		Punjabis	
	No.	%	No.	%
DD	100	23.04147	97	23.48668
DG	178	41.01382	180	43.58353
GG	60	13.82488	51	12.34866
DDD	11	2.53456	6	1.45278
DDG	41	9.44700	26	6.29539
DGG	17	3.91705	18	4.35835
GGG	4	0.92165	4	0.96852
DDDD	1	0.23041	—	—
DDDG	1	0.23041	—	—
DDGG	11	2.53456	17	4.11622
DGGG	3	0.69124	1	0.24213
DDDDG	—	—	1	0.24213
DDDGG	1	0.24213	1	0.24213
DDGGG	4	0.92165	1	0.24213
DGGGG	—	—	1	0.24213
DDDDGGG	1	0.23041	—	—
DDDGGGG	1	0.23041	—	—
DDDDGGGG	—	—	1	0.24213
Total	434	99.99993	413	99.99995

each other in relation to the distribution of association complexes (Table 6). The distribution of the association complexes made of two chromosomes like D/D, D/D and G/G are taken separately whereas the

rest of the associations are put together and named as "others". Rajputs and Punjabis show statistically non-significant differences among themselves in relation to the distribution of association com-

Table 6. Comparison of Rajputs and Punjabis in relation to the distribution of association complexes.

Population	Association type				Total AAC	Chi-square	P (d. f.=3)
	DD	DG	GG	"Others"			
Rajputs	100	178	60	96	434	0.9350	.90 > P > .80
Punjabis	97	180	51	85	413		

Table 7. Comparison of Punjabis with other world populations in

No. Population	Authors	No. of individuals studied	No. of metaphases observed
1. Russian	PROKOFIEVA-BELGOVSKAYA <i>et al.</i> (1968)	38♂	1235
2. English I	FERGUSON-SMITH and HANDMAKER (1961)	5♂	250
3. Sweedish I	HANSSON (1970 a)	7♂. 4♀	1100
4. Sweedish II	HANSSON (1970 b)	2♂. 5♀	700
5. English II	COOKE (1972)	*	1613
6. Indians Japanese Negroes Jews Non-jews	COHEN and SHAW (1967)	100♂	1000
7. Mexican			
8. Punjabis	CUEVAS-SOSA (1970)	1♂. 1♀	300
	Present study	100. ♂	500

* Not known. Figures in parenthesis indicate percentage.

Table 8. Comparison of Rajputs with other world population in

No. Population	Authors	No. of individuals studied	No. of metaphases observed
1. Russian	PROKOFIEVA-BELGOVSKAYA (1968)	38♂	1235
2. English I	FERGUSON-SMITH and HANDMAKER (1961)	5♂	250
3. Sweedish I	HANSSON (1970 a)	7♂. 4♀	1100
4. Swedish II	HANSSON (1970 b)	2♂. 5♀	700
5. English II	COOKE (1972)	*	1613
6. Indians Japanese Negroes Jews Non-Jews	COHEN and SHAW (1967)	100♂	1000
7. Mexican			
8. Rajputs	CUEVAS-SOSA (1970)	1♂. 1♀	300
	Present study	100. ♂	500

* Not known. Figures in parenthesis indicate percentage.

plexes (chi-square=0.9350, d.f. =3:90>P statistically significant differences with >.80). Swedish (HANSSON, 1970 a, b), English (CO-

When Rajputs and Punjabis are compared with other populations in relation by combining Indians, Japanese, Negroes, to the distribution of D/D, D/G, G/G and Jews and Nonjews (COHEN and SHAW, "others" type of associations they show 1927). They also show statistically non-

relation to the distribution of association complexes.

Association types				Total AAC	Chi-square	P (d. f.=3)
DD	DG	GG	"Others"			
348 (26.646)	531 (41.658)	173 (13.247)	254 (19.449)	1306	2.2572	.70>P>.50
47 (21.560)	98 (44.954)	34 (15.596)	39 (17.890)	218	1.9361	.70>P>.50
261 (23.759)	516 (46.952)	144 (13.123)	178 (16.197)	1099	4.1987	.30>P>.20
180 (27.565)	298 (45.636)	90 (13.017)	85 (13.017)	653	11.3274	.02>P>.01
729 (11.992)	1214 (19.970)	404 (6.646)	3732 (61.392)	6079	268.0745	P>.001
44 (6.800)	95 (14.683)	34 (5.255)	474 (73.261)	647	282.3994	P>.001
151 (24.754)	260 (42.623)	66 (10.820)	133 (21.803)	610	0.8920	.90>P>.80
97 (23.487)	180 (43.584)	51 (12.349)	85 (20.581)	413		

relation to the distribution of Association complexes.

Association types				Total AAC	Chi-square	P (d. f.=3)
DD	DG	GG	"Others"			
348 (26.646)	531 (40.658)	173 (13.347)	254 (19.449)	1306	2.8909	.50>P>.30
47 (21.560)	98 (44.954)	34 (15.596)	39 (17.890)	218	2.2433	.70>P>.50
261 (23.759)	516 (46.952)	144 (13.123)	178 (16.197)	1099	8.7188	.05>P>.02
180 (27.565)	298 (45.636)	90 (13.783)	85 (13.017)	653	16.3177	P>.001
729 (11.992)	1214 (19.970)	404 (6.646)	3732 (61.392)	6079	258.3840	P>.001
44 (6.800)	95 (14.683)	34 (5.255)	474 (73.261)	647	273.5273	P>.001
151 (24.754)	260 (42.623)	66 (10.820)	133 (21.803)	610	2.4133	.50>P>.30
100 (23.042)	178 (41.014)	60 (13.825)	96 (22.120)	434		

significant differences with the Russian (PROKOFIEVA-BELGOVSKAYA *et al.*, 1968), English (FERGUSON-SMITH and HANDMAKER, 1961) and Mexican (CUEVAS-SOSA, 1970) series (Table 7 and 8).

The functional significance of the phenomenon of "the association of acrocentric chromosomes" is still not clear. EDWARDS (1961) reports that the association of acrocentric chromosomes seems to be predominantly due to the association of the satellites. FERGUSON-SMITH and HANDMAKER (1963) report that the association phenomenon may indicate the position of active nucleolus organiser sites. They also report that the chromosome associations are result of nucleolus fusion during interphase. COOKE (1972) reports that pattern of association is determined by the nucleolar organizing ability of the chromosomes.

"Nucleolus organiser sites are largely concerned with the mass production of ribosomal RNA, and are composed of repeated sequences which code for ribosomal precursors. Thus non-homologous

chromosomes may have regions of homology at their nucleolus organiser sites. Non-homologous associations at meiosis could represent true synapsis between such regions, which then organise a common nucleolus. If true synapsis occurs, there is the possibility of rare accidental crossing-over between non-homologous which would lead to just the sort of rearrangement and polymorphism encountered" (FERGUSON-SMITH, 1967, 1972).

II. Structural anomalies and aneuploidy : Lymphocyte metaphases have been scored for aneuploidy as shown in Table 9. It may be seen from Table 9 that 98.00 per cent of Rajputs and 97.20 per cent of Punjabis have model cells while 1.80 per cent Rajputs and 2.40 per cent Punjabis have hypomodal cells. It is quite difficult to interpret the existence of aneuploid cells in normal cell lines. Technical factors seem to be the reason for the existence of hypomodal cells. The missing chromosomes being detached during preparation. Further, only two tetraploid sets have been noticed in Punjabis, and

Table 9. Incidence of aneuploidy in the normal cell lines of Rajputs and Punjabis.

Population	Total number of cells observed	No. of chromosomes			
		44	45	46	47
Rajputs	500	—	9 (1.80)	490 (98.00)	1 (0.20)
Punjabis	500	1 (0.20)	12 (2.40)	486 (97.20)	1 (0.20)

Figures in Parenthesis indicate percentages.

Table 10. The incidence of structural anomalies in Rajputs and Punjabis.

Population	No. of individuals	No. of metaphases analysed	Cells with structural anomalies	
			No.	%
Rajputs	100 males	500	19	3.8
Punjabis	100 males	500	24	4.8

two endoreduplication stages-one each from Rajputs and Punjabis have also been encountered.

The frequency of structural anomalies in Rajputs and Punjabis are listed in Table 10. Structural anomalies are recorded in 3.8 per cent of the metaphases in Rajputs and 4.8 per cent in Punjabis. Variable frequencies of structural anomalies as reported by different studies in normal controls are listed in Table 11. The minimum frequency of structural anomalies are reported by BLOOM and

TJIO (1964) as 0.5 per cent in 1910 control cells whereas the maximum frequency of 10.7 per cent of structural anomalies were observed out of a sample of 3720 cells derived from five male and five female controls (LUBS and SAMUELSON, 1967).

The incidence of different types of structural anomalies in Rajputs and Punjabis are listed in Table 12. Out of a total of 19 cells with aberrations in Rajputs, 11 have been found with chromatid gaps, 3 with chromosome gaps, 3 with chromatid

Table 11. Frequency of structural anomalies as reported by different studies.

References	No. of individuals studied	No. of metaphase analysed	Cells with structural anomalies	
			No.	%
BLOOM and TJIO (1967)	*	1810	9	0.5
BLOOM <i>et al.</i> (1970)	174 Japanese	16035	157	1.0
BLOOM <i>et al.</i> (1973)	27 Piaros I Indians	2660	85	3.2
COURT BROWN <i>et al.</i> (1966)	468 Individuals	12420	417	3.3
MEHES C.F. MUELLER (1971)	11 males 11 females	6800	235	3.4
AULA (1965)	10 Individuals	1567	59	3.8
Rajputs-Present study	100 males	500	19	3.8
BLOOM <i>et al.</i> (1970)	49 Yanomama Indians	4875	200	4.1
MUELLER (1971)	36 children	4754	226	4.8
Punjabis-Present study	100 males	500	24	4.8
LITTLEFIELD and GOH (1973)	10 males 21 females	29709	1794	6.3
LUBS and SAMUELSON (1967)	5 males 5 females	3720	398	10.7

* Not known.

Table 12. The incidence of different types of structural anomalies in Rajputs and Punjabis.

Population	Cells with structural anomalies			
	Chromatid		Chromosome	
	Gap	Break	Gap	Break
Rajputs	11	3	3	1
Punjabis	14	2	4	2

Table 13. The frequency of different types of structural anomalies as reported by various studies.

References	No. of metaphases analysed	Chromatid Gap	Chromosome Gap	Chromatid Break	Chromosome Break
BLOOM and TJIO (1964)	1810	9			
BLOOM <i>et al.</i> (1970)	16035			105	23
BLOOM <i>et al.</i> (1973)				45	22
COURT BROWN <i>et al.</i> (1966)	12420	277	83	38	
MEHES C.F. MUELLER (1969)	6800	93	15	102	13
AULA (1965)	1567				59
BLOOM <i>et al.</i> (1970)	4875			112	33
MUELLER (1971)	4754	162	11	32	8
LITTLEFIELD and GOH (1973)	29709				
LUBS and SAMUELSON (1967)	3720	165		221	32
Rajputs-Present study	500	11	3	3	1
Punjabis-Present Study	500	14	4	2	2

break and 1 with chromosome break. Further, 1 dicentric chromosome has been found in this series. Punjabis, on the other hand show 14 chromatid gaps, 4 chromosome gaps, 2 chromatid breaks, 2 chromosome breaks and 2 diacentric chromosomes out of a total of 24 cells with aberrations.

The frequency of different types of structural anomalies as reported by different studies of the world are listed in Table 13. It is observed from this table that exists a lot of spontaneous chromosome damage in the normal cell lines of normal individuals.

Chromosome breaks occur during the G₁ phase while the chromosome is still functionally single while the chromatid breaks occur after chromosome reduplication during the G₂ phase and the S phase. The exact mechanism by which these damages to chromosomes are caused is still unknown but cumulative exposure to a variety of agents in a total environment

of man may be an important factor.

SUMMARY

In this paper, an attempt has been made to study the morphological variability of the human chromosomes in two Indian populations-Rajputs and Punjabis with respect to satellite association and the structural anomalies. The results are discussed in the light of findings revealed by different studies.

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北部インド人二集団，ラジパット族とパンジャブ族の 付属性連合と構造的異常

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ラジパット族とパンジャブ族の付属性連合と構造的異常を調べる目的で，この二集団の人の染色体の形態的変異を調べた。またその結果を，これまで他の研究によって解明された知見と照して論じた。