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GENETIC DIVERSITY AND GENETIC STRUCTURE OF AN ENDANGERED SPECIES, *ERIOCAULON NUDICUSPE*, GROWING IN ARTIFICAL DISTURBING HABITATS

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ABSTRACT: *Eriocaulon nudicuspe* (Eriocauloaceae) is an endangered species in Japan. Habitats of the species are lost by city development. So many conservation areas are established in Aichi Prefecture, and protection activities are done by many natural protecting groups, and the extinction rate in recent years is being decelerated. But many protecting groups often transplanted from some other places, and genetic disturbance was a problem. We sampled 12 populations of the species from nature reserved areas, and studied them for allelic variation at 17 enzyme loci. There was no significant correlation between the real distance of conserved areas and genetic distances, suggesting that the gene disturbances occurred in these areas especially in frequently managed areas. On the other hand, the degree of the genetic differentiation at strictly conserved area where conservation management is done only once a year was high and there was no evidence about genetic disturbances. There was a possibility peculiar genetic disappearance of each habitat for genetic disturbance, and the necessity with which a guideline of protection activity is made was indicated.

Keywords: Genetic disturbance, Genetic distance, Conservation management, Nature reserved area, Endangered species

1. INTRODUCTION

Ueda (1989) defined Tokai hilly land element, 14 species plants, a lot of local endemic, semi-endemic and relict taxa growing in small wetlands in the Central Japan [1]. The habitats of the elements have been destructed by the urban growth, and all species are defined endangered species. Tomita (2004) reported the distribution change of *Eriocaulon nudicuspe* for 25 years, almost 50% areas are disappeared [2]. So Aichi Prefecture supported the conservation activities of the species.

For the conservation, the moderate disturbance is very important, so many nature protecting groups keep being active for decades [3][4]. The extinction rate of the endangered species is less than 5 % for the activities, and another population was appeared. Everyone easily collects seeds of the species and grows up by sowing. Sometimes people can easily move seeds of the species attaching the sole.

But there is another problems occurred in the disturbance. Some researchers pointed out the genetic disturbance affect serious damage of the endangered species [5][6]. The conservation activities sometimes transplant the endangered species from one habitat to another habitat. This manipulation causes hybrid breakdown, dilution and outbreeding depression [7]-[9].

Tsumura and Suyama (2015) published the book "The guideline for transplanting of trees seedling" where the genetic boundaries were shown in maps. But there is no herbaceous plants guideline about transplantation because of the difficulties of the samplings. Then we investigated the genetic structure and genetic diversity of *Eriocaulon nudicuspe* and analyzed the effect of the conservation activities with 3 questions, 1) Is there a relationship between the location and genetic distance? 2) Is there a relationship between the conservation activities and genetic diversity? 3) Is there a relationship between population size and genetic diversity? It was paid attention to the three points and analyzed.

2. MATERIALS AND METHODS

2.1 The Study Site

The study was carried out on the wetland at Aichi Pref. and Shizuoka Pref. (Table 1). 36 habitats of the species reported in the previous research [2], but growing could be confirmed only in 17 habitats in 2007. The investigation was done at 12 populations that can secure the enough number of individuals. Fig.1 shows the locations of 12 populations that were investigated. The capital letter of each location exhibits same river systems.

Table 1 The characters of study site, location

Study site	location North	East
A1	34° 44’ 45”	137° 27’ 09”
A2	34° 42’ 10”	137° 24’ 58”
A3	34° 38’ 38”	137° 16’ 30”
B1	34° 55’ 04”	137° 46’ 20”
B2	34° 49’ 38”	137° 45’ 54”
B3	34° 48’ 56”	137° 45’ 56”
C1	34° 56’ 06”	136° 56’ 14”
C2	34° 55’ 11”	136° 53’ 00”
C3	34° 54’ 00”	136° 52’ 15”
C4	34° 52’ 07”	136° 53’ 53”
C5	34° 50’ 09”	136° 53’ 10”
D1	35° 08’ 56”	137° 04’ 57”
D2	35° 06’ 10”	137° 00’ 05”
E1	35° 14’ 43”	137° 03’ 08”
E2	35° 14’ 08”	137° 02’ 35”
F1	35° 06’ 01”	137° 14’ 23”
F2	35° 07’ 52”	137° 09’ 30”

The same capital indicates the same water system

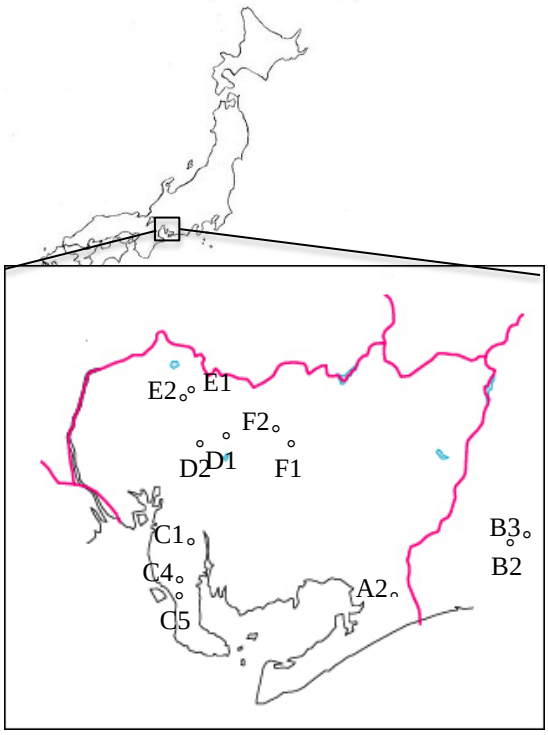

Fig. 1. Distribution of the population examined.

2.2 The Study Plant

Eriocaulon nudicuspe Maxim. (Fig. 2) is an annual plant distributed in circum-Ise Bay area, the center of Japan [1]. However its population has been decreasing and Red Data Book of Japanese vascular plants listed the species a ‘vulnerable’ level species [10][11]. This species is distributed in acid marsh with low nutrient, wetland around paddy fields and edge of reservoirs around Ise-Bay area. The seeds of the species emerge in spring and bloom late summer to early autumn. An individual has 1-10 inflorescence and produces 0-12 seeds per inflorescence.

Fig. 2 The flowers of *E. nudicuspe*.

2.3 Electrophoresis

Fresh leaves were collected from 30 individuals per population at June in 2007. Leaves were kept on ice during 2 hours transportation to the laboratory. The following enzyme systems were examined: aconitase (ACO), sikimate dehydrogenase (SKDH), iso-citrate dehydrogenase (IDH), malate dehydrogenase (MDH), acid phosphate (ACP), phospho-glucose isomerase (PGI), phospho-glucomutase (PGM) and menadione reductase (MR). Leaves were used to resolve the following 17 putative loci: aco-1, aco-2, skdh, idh, mdh-1, mdh-2, mdh-3, acp-1, acp-2, acp-3, acp-4, acp-5, pgi-1, pgi-2, pgh-3, pgm and mr. Samples were ground in a cold extraction buffer described by Odrzykoski and Gottlieb [12]. The enzymes were resolved on 10.8% starch gel. System 5 of Soltis et al. [13] were used. Staining procedures followed previous works [13]-[15].

2.4 The Statistical analysis

For each population the number of alleles per locus (A), proportion of polymorphic loci (P), and gene diversity (h) were calculated. We used all loci data in the calculation of A, and regarded a locus as polymorphic if the frequency of its most frequent alleles is under 0.95. In addition, total gene diversity [16] was calculated for species level. The population genetic structure was analyzed by

initially calculating Nei's G_{ST} value [16]. Values for genetic identities (I) and standard genetic distance (D) were computed for each pairwise comparison of all populations. The neighbor joining method [17] based on D was used for constructing a phenogram for E. nudicuspe.

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3. RESULTS

3.1 The Study Site

The number of individuals and the frequency of conservation activities were shown in Table 2. The number of individuals ranged from 20 to 50000. C5 and F2 wetland are left, and few persons hardly enters. C4 and F1 are managed severely, and protection activity is permitted only once a year. And in the other area conservation activity is performed, and reaping and organic matter removal are conducted periodically.

Table 2. The number of individuals of each population in 2007, Geographical Features, and frequency of conservation activities within a year

Study site	No. of individuals	Geograhical Features	Freq. of act.
A1	1000	silt, clay, gravel	4
A2	1000	Terrace sediment	10
A3	200	Terrace sediment	4
B1	50000	silt, clay, gravel	12
B2	1500	silt, clay, gravel	4
B3	30000	silt, clay, gravel	4
C1	1430	silt, clay, gravel	1
C2	20	mud marsh	1
C3	800	silt, clay, gravel	4
C4	5190	silt, clay, gravel	1
C5	3000	silt, clay, gravel	0
D1	7200	kaoline, gravel	12
D2	4000	granite	12
E1	1000	silt, clay, gravel	1
E2	5000	silt, clay, gravel	1
F1	7000	silt, clay, gravel	1
F2	5200	silt, clay, gravel	0

3.2 Genetic Diversity

Seventeen loci were scored: aco-1, aco-2, skdh, idh, mdh-1, mdh-2, mdh-3, acp-1, acp-2, acp-3, acp-4, acp-5, pgi-1, pgi-2, pgh-3, pgm and mr, fifteen loci were polymorphic. In all population aco-1 and mr were monomorphic. Allele frequencies at the polymorphic loci are listed in Appendix.

Table 3 summarizes the resultant values of *A*, *P* and *h* for each population.

And total gene diversity (H_T) of the species was 0.293. The levels of genetic diversity in *E. nudicuspe* was almost same that of other endangered species, for example, *Aster kantoensis* growing in the river bed were 0.36(*P*), 1.53 (*A*) and 0.142 (*h*) [18]. And other endangered species showed, *P* (0.199 to 0.65), *A* (1.44 to 2.01) and *h* (0.037 to 0.43) [19]-[22]. But the index of river wetland endangered species, *Penthorum chinense*, is higher than that of *E. nudicuspe* [23], these were 2.42 (*A*), 0.75(*P*) and 0.308 (*h*).

Table 3. Mean number of polymorphic loci (*A*), proportion of polymorphic loci (*P*), and gene diversity within a population *(h*) at 17 loci for examined populations of *E. nudicuspe*

Population	*P*	*A*	*h*
A2	0.647	2.059	0.156
B2	0.765	2.118	0.165
B3	0.706	1.941	0.129
C1	0.800	2.400	0.350
C4	0.818	2.636	0.473
C5	0.765	1.941	0.141
D1	0.294	1.471	0.078
D2	0.588	1.941	0.194
E1	0.412	1.529	0.078
E2	0.588	1.765	0.116
F1	0.706	2.059	0.157
F2	0.471	1.529	0.079

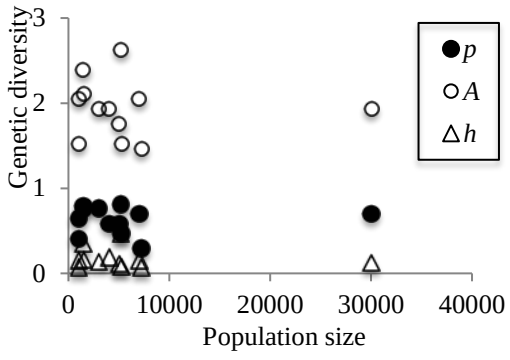

Fig. 3. The relationships between population size and genetic diversity indexes *p, A* and *h*

Fig. 3 showed the relationships between population size and genetic diversity. There are no significant relationships between the population size and genetic diversity.

Fig. 4 showed the genetic distance among population. The genetic distance between the nearest populations were small, but the longest distant population. Especially B2, B3 are the edge of the distribution of the species, but they close to the center of the distribution. It is suggested that gene flow occurred constantly among the populations.

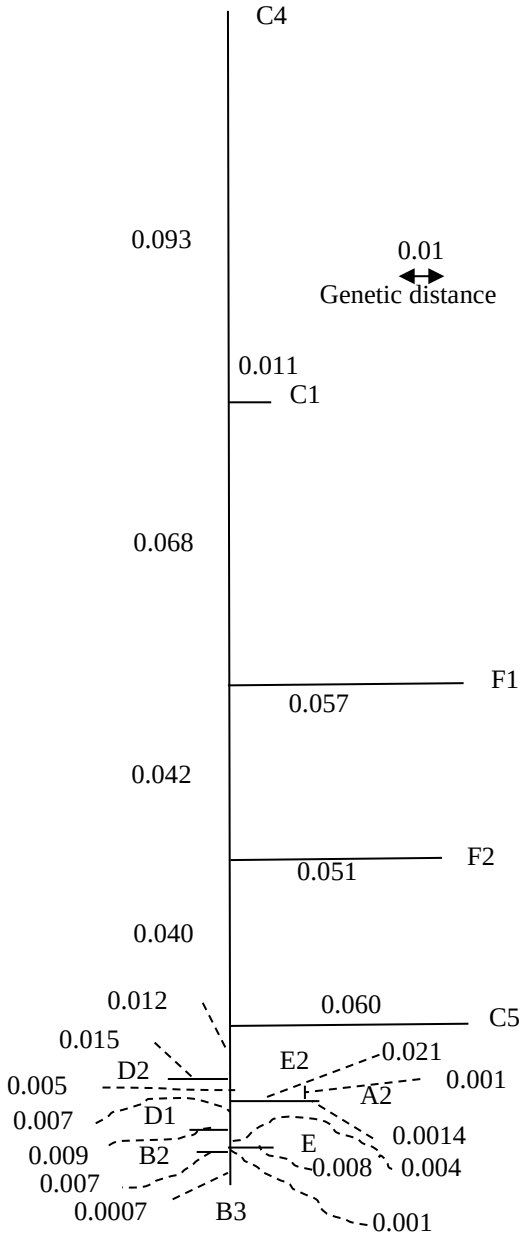

Fig. 4. Phenogram using the neighbor joining method based on Nei's (1987) standard genetic distance.

The result of total population genetic structure G_{ST} was 0.236. The value of G_{ST} suggested that middle level differentiation occurred. It is always observed isolated populations (no differentiation: 0-0.05, low level differentiation: 0.05-0.15, middle level: 0.15-0.25, high level: 0.25-).

4. DISCUSSION

No significant correlation existed between any of the genetic diversity parameters and the actual size of populations, which indicates that the effective population size is independent of the actual population size. Several factors are known to reduce the effective size of population [24]: (1) fluctuation in population size, (2) variation in fecundity among individuals, (3) overlapping generations, (4) geographic dispersion of populations, and (5) unequal numbers of males and females. Factors 2-5 are not plausible as cause of the observed lack of correlation between the genetic diversity and actual population sizes. Fluctuation in population size represented by genetic drift severely reduces the effective population size of population, which is the harmonic mean of the actual number of individuals in the last t generations [24]. For an endangered species like *E. nudicuspe* nursing by many conservation groups, the bottleneck effect is a primary factor acting to reduce the effective size of populations. The species rapidly establishes new populations by conservation activities. Thus even large population with N > 5000 are likely to have a high probability of being of recent origin and remain influenced by the bottleneck effects.

Because *E. nudicuspe* only occurs in the wetlands along the river system, its distribution is disjunct, and as a result, inter-river gene flow is expected to be less than intra-river gene flow. Sometimes wild animals occur the gene flow, but many constructions, road, buildings and railways, prevent long distant movements. *E. nudicuspe* is mainly pollinated by small sap chafers with limited flight ability (personal obs.) and its seeds have no specialized mechanism for long distance dispersal, inter river gene flow is considered feasible due to their geographical proximity.

But the hierarchical analysis of the population gene structure of the species showed the relationships among the river system nor real distance system. Especially B2 and B3 are placed the east end of the distribution, near to E or D population that are placed the center of the distribution. D1, D2 and A2 populations are well managed by three conservation groups. The members of the groups sometimes visit another conservation area, so we guess that the people make the gene flow among the different river

system. On the other hand, among strictly conserved area, C1, C4, C5 F1 and F2, the gene flow aren't almost observed. Genetic distance is over 0.07 among the populations. It was suggested that high Gst was dependent on the isolated 4 populations. The conservation activities cause unexpected gene flow.

Generally each population is exposed to natural selective pressure each area and adapts itself to it. Therefore, if an artificial gene flow occurred, human disappears an adaptive gene of to each habitat. Sometimes annual fluctuation of environment selects adaptive genes, another gene disappear the adaptive gene by their reproductive ability when the selective event did not occur. It is suggested that the artificial gene flow decrease the gene diversity of endangered species. It is suggested that we must not occur artificial gene disturbance in order to conserve endangered species. The conserved area should be protected strictly. There were some populations with gene disturbance about *E. nudicuspe* observed. Among these populations we cannot recover from gene disturbance. We make a proposal that in order to avoid the inbreeding depression high level gene flow management among the populations. That cause the uniformity of gene diversity, but there is low inbreeding depression expected, so among populations the individual has high fecundity.

5. CONCLUSION

We get three main conclutions.

1) There is no relationship between the real distance and genetic distance.
2) There is a relationship between the conservation activities and genetic structure.
3) There is no relationship between population size and genetic diversity.

Based on the conclusions, two proposals about the conservation activities

1) The conservation activities should be strictly managed, because of unexpected genetic disturbances.
2) We should make the networks among the habitats where the genetic disturbance occurred, the networks cause high level gene flow and avoid the inbreeding depression, because the recovering from genetic disturbance is very difficult.

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Appendix Allel frequencies at 15 loci of 12 examined populations of *Eriocaulon nudicuspe.*

		population											
locus	allele	A2	B2	B3	C1	C4	C5	D1	D2	E1	E2	F1	F2
aco-1	N	19	22	28	30	28	28	14	19	30	30	30	30
	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
aco-2	N	19	22	28	-	-	28	14	19	30	30	30	30
	a	0.921	0.932	0.911	-	-	1.000	0.929	0.947	0.983	0.933	0.967	0.950
	b	0.079	0.068	0.089	-	-	0.000	0.071	0.053	0.017	0.067	0.033	0.050
skdh	N	19	22	28	-	-	28	14	19	30	30	30	30
	a	0.947	0.932	0.929	-	-	1.000	1.000	0.921	1.000	0.950	1.000	0.967
	b	0.053	0.068	0.071	-	-	0.000	0.000	0.079	0.000	0.050	0.000	0.033
idh	N	19	22	28	-	-	28	14	19	30	30	30	30
	a	0.026	0.000	0.000	-	-	0.946	0.000	0.132	0.000	0.000	0.017	0.000
	b	0.974	0.955	0.839	-	-	0.054	1.000	0.868	0.950	0.917	0.933	1.000
	c	0.000	0.045	0.161	-	-	0.000	0.000	0.000	0.050	0.083	0.050	0.000
mdh-1	N	19	22	28	30	28	28	14	19	30	30	30	30
	a	0.526	0.909	0.839	0.733	0.143	0.946	1.000	0.763	1.000	1.000	0.133	0.950
	b	0.474	0.091	0.161	0.267	0.482	0.054	0.000	0.237	0.000	0.000	0.867	0.050
	c	0.000	0.000	0.000	0.000	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mdh-2	N	19	22	28	29	25	28	14	19	30	30	30	30
	a	1.000	0.864	0.804	0.690	0.600	1.000	1.000	0.763	0.967	0.950	1.000	0.950
	b	0.000	0.136	0.196	0.310	0.400	0.000	0.000	0.237	0.033	0.050	0.000	0.050
mdh-3	N	19	22	28	-	-	28	14	19	30	30	30	30
	a	1.000	0.977	0.982	-	-	0.911	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.023	0..018	-	-	0.089	0.000	0.000	0.000	0.000	0.000	0.000
acp-1	N	46	49	28	-	-	28	12	49	30	30	30	30
	a	0.957	0.980	0.893	-	-	0.839	1.000	1.000	1.000	0.983	0.917	1.000
	b	0.043	0.020	0.107	-	-	0.161	0.000	0.000	0.000	0.017	0.083	0.000
acp-2	N	38	49	28	26	28	28	17	49	30	30	30	30
	a	0.947	0.959	1.000	0.288	0.173	0.821	1.000	1.000	1.000	1.000	0.950	1.000
	b	0.053	0.041	0.000	0.346	0.500	0.179	0.000	0.000	0.000	0.000	0.050	0.000
	c	0.000	0.000	0.000	0.308	0.288	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	d	0.000	0.000	0.000	0.058	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000
acp-3	N	43	44	28	28	28	28	17	47	30	30	30	30
	a	0.154	0.000	0.052	0.444	0.411	0.036	0.265	0.021	0.000	0.000	0.367	0.467
	b	0.231	0.193	0.328	0.556	0.589	0.339	0.176	0.149	0.000	0.100	0.633	0.450
	c	0.286	0.409	0.121	0.000	0.000	0.464	0.118	0.447	0.433	0.400	0.000	0.083
	d	0.176	0.227	0.628	0.000	0.000	0.161	0.206	0.266	0.400	0.217	0.000	0.000
	e	0.154	0.170	0.172	0.000	0.000	0.000	0.235	0.117	0.167	0.283	0.000	0.000
acp-4	N	46	49	28	-	11	28	17	49	30	30	30	28
	a	0.239	0.153	0.339	-	0.318	0.036	0.029	0.643	0.283	0.283	0.167	0.071
	b	0.457	0.837	0.661	-	0.455	0.964	0.971	0.357	0.717	0.717	0.75	0.929
	c	0.000	0.01	0.000	-	0.227	0.000	0.000	0.000	0.000	0.000	0.083	0.000

acp-5	N	26	26	27	19	-	28	16	30	30	30	30	30
	a	1.000	1.000	1.000	0.500	-	1.000	1.000	1.000	1.000	1.000	0.783	1.000
	b	0.000	0.000	0.000	0.500	-	0.000	0.000	0.000	0.000	0.000	0.217	0.000
pgi-1	N	19	49	17	29	30	28	12	15	30	29	30	30
	a	0.000	0.112	0.152	0.534	0.283	0.054	0.000	0.092	0.017	0.000	0.067	0.967
	b	0.921	0.755	0.783	0.414	0.433	0.786	1.000	0.618	0.983	0.966	0.267	0.033
	c	0.079	0.133	0.065	0.052	0.283	0.161	0.000	0.289	0.000	0.034	0.667	0.000
pgi-2	N	19	22	28	28	0	0	12	49	30	30	30	30
	a	1.000	1.000	1.000	0.117	0.071	1.000	1.000	1.000	1.000	1.000	0.629	1.000
	b	0.000	0.000	0.000	0.300	0.286	0.000	0.000	0.000	0.000	0.000	0.371	0.000
	c	0.000	0.000	0.000	0.350	0.411	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	d	0.000	0.000	0.000	0.150	0.214	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	e	0.000	0.000	0.000	0.083	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000
pgi-3	N	19	48	28	-	-	28	11	49	23	21	29	30
	a	0.053	0.000	0.000	-	-	0.036	0.000	0.000	0.000	0.000	0.241	0.000
	b	0.026	0.063	0.000	-	-	0.304	0.255	0.255	0.017	0.033	0.172	0.867
	c	0.737	0.531	0.964	-	-	0.500	0.510	0.510	0.800	0.850	0.276	0.133
	d	0.184	0.000	0.036	-	-	0.161	0.235	0.235	0.183	0.117	0.310	0.000
pgm	N	35	49	28	30	28	28	0,	34	30	30	30	30
	a	0.348	0.061	0.000	0.150	0.536	0.679	0.000	0.000	0.000	0.000	0.000	0.000
	b	0.485	0.776	0.893	0.850	0.464	0.286	0.542	0.468	1.000	1.000	0.017	1.000
	c	0.167	0.143	0.107	0.000	0.000	0.036	0.458	0.456	0.000	0.000	0.850	0.000
	d	0.061	0.020	0.000	0.000	0.000	0.000	0.000	0.076	0.000	0.000	0.133	0.000
mr	N	19	22	28	30	30	28	12	49	30	30	30	30
	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

N; No. of examination, –; non signal