

INTROGRESSION ACROSS ISOLATING BARRIERS IN WILD AND CULTIVATED *ORYZA* SPECIES¹

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Cultivated rice species and their wild relatives are differentiated into several groups mutually isolated by various types of reproductive barriers (Chu et al., 1969a). Their natural hybrids occur across the barriers. In West Africa, two cultivated species, *Oryza sativa* L. and *O. glaberrima* Steud. are often in mixed culture, while their respective wild relatives, *O. perennis* Moench subsp. *barthii* and *O. breviligulata* Chev. et Roehr. grow sympatrically as weeds. Hybrid swarms between *glaberrima* and *breviligulata* are frequently found, but natural hybrids of other species are rare (Oka and Chang, 1964). *O. sativa* is isolated from *O. glaberrima* and *O. breviligulata* by an F₁ sterility barrier. *O. perennis* subsp. *barthii* is isolated from others by a crossing barrier and partly by F₁ weakness and sterility (Chu and Oka, 1970). However, some *barthii* populations in West Africa were found to be contaminated by introgression.

The junior author formerly reported that "the African forms of *perennis* are of two types, one with a small number of panicles per plant, a small number of spikelets per panicle and high pollen fertility, and the other with the same characters in opposite" (Oka, 1964, p. 86). Jachuck and Sampath (1967) also pointed out that some *barthii* strains varied widely in fertility and other characters and might be highly heterozygous. We often found off-types (or the second type mentioned above) of *barthii* among plants raised from seeds collected in natural

populations, though they were not found to form colonies in the natural habitats (Oka and Chang, 1964). The off-types, which we called "Obake" (a monster in Japanese), were found to be hybrids between *barthii* and other rice species.

Further, the junior author, when he travelled in Cuba in 1960, found an exceptional hybrid swarm between the American form of *O. perennis* (so-called *O. cubensis* Ekman) and *O. sativa*, which are usually isolated by distance and an F₁ sterility barrier. Studies of these natural hybrids may throw light on the role of reproductive isolation in species relationships. The purpose of the present study was to estimate the genetic make-up of those hybrid plants.

MATERIALS AND METHODS

The "Obake" types of *barthii* observed were selfed progenies of four plants, which were obtained from the seed samples collected by Dr. K. Furusato in 1959. They were classified into three families, Af11, Af64, and Af110, according to the collection number. Af11 was the progeny of a plant raised from a seed sample collected near Boro railway station, Guinea. The original seed of Af64 was obtained from several plants of a *barthii* population growing in a rice field, near the Federal Rice Research Station, Badeggi, Nigeria. Eight plants with different characters were raised from the seeds, and the selfed progenies of two of them were observed. Af110 was the progeny of a plant raised from seeds collected at the fringe of a rice field at Bathurst, Gambia.

The *perennis-sativa* hybrid swarm was found at Convento, Santa Clara, Cuba, where a large colony of *perennis* grew in a marsh partly reclaimed for rice culture a few years ago. The hybrid population

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TABLE 1. Per cent cross-fertility, F_1 seed germinability and F_1 pollen fertility between "Obake" plants and related taxa.

Cross with	Af11			Af64			Af110			<i>barthii</i>		
	Mean	Range	#	Mean	Range	#	Mean	Range	#	Mean	Range	#
Cross-fertility (using "Obake" as female parent):												
<i>barthii</i>	30	18-40	3	33	11-63	9	75	70-80	2	58	48-68	2
<i>sativa</i> and other taxa	25	8-50	12	30	13-70	9	30	11-54	11	3	0-5	17
F_1 seed germinability (using "Obake" as male parent):												
<i>barthii</i>	100		1							88	83-93	2
<i>sativa</i> and other taxa	47	20-99	8	81	0-99	17	93	86-99	2	7	0-14	104
F_1 pollen fertility:												
<i>sativa perennis</i> ,	40	0-89	13	58	40-72	10	38	3-72	11	25	0-84	13
Asia	79	60-98	4	55	1-99	12				57	13-99	9
America	8	5-10	2	12	10-14	2				17	3-46	6
<i>barthii</i>	72	50-84	5	61	5-91	19	38		1	98	97-98	3
<i>glaberrima</i>	17	10-25	4	11	0-33	4				29	0-49	4
<i>breviligulata</i>	4	3-5	2	2	0-5	4				11	0-25	5

— No. of cross-combinations tested.

occupied an area about 10 m wide and 100 m long, the water depth being about 20 cm. The original seed sample consisted of seeds of 20 individual plants. Fifteen lines were raised from the seeds and their progeny (70 plants) were used for experiments.

The plants were grown at the National Institute of Genetics, Japan, in concrete beds with automatic short-day control (12-13 hrs) or in a greenhouse. Characters measured were ligule length, primary and secondary branch numbers per panicle, spikelet number per panicle, spikelet width, and auricle size, in addition to pollen and seed fertilities. To obtain an integrated figure of their variations, hybrid indices were constructed by dividing the variation range of each character into seven classes scored from 0 to 6 and summing up the scores, as done by Anderson (1949).

The leaf-blade isozymes of peroxidase, acid phosphatase, and esterase were analysed by starch-gel electrophoresis method (Chu, 1967; Chu and Oka, 1967). For this, homogenates were made of fresh leaves 3 to 5 days after full expansion. The substrates used were: for peroxidase, 0.03% hydrogen peroxide, 0.1% benzidine ace-

tate, and 0.01 M Tris-acetic acid buffer adjusted at pH 4.0; for acid phosphatase, 0.025% sodium 1-naphthylphosphate, 0.1% fast garnet G. B. C. salt, and 1 M potassium acetate buffer at pH 5.0; and for esterase, 0.01% 1-naphthylacetate, 0.1% fast blue R. R. salt, and 1 M phosphate-acetate buffer at pH 7.0. The isozyme bands detected were numbered 1A, 2A . . . (anodal) and 1C, 2C . . . (cathodal) according to their order from the origin.

RESULTS

1) *Fertility variation of the "Obake" types of barthii.*—The eight plants from the original seeds of Af64 all showed a low pollen fertility, ranging from 1% to 8%. Two of them produced rhizomes like true *barthii* plants, while the rest did not. Twenty-five selfed progenies of plant no. 1 which had a 4% pollen and a 5% seed fertility, ranged from 0% to 70% in pollen and from 0% to 40% in seed fertility. The second-generation plants from one of them (no. 1-13; showing a 39% pollen and a 32% seed fertility) also gave a wide range of fertility. This suggests that the original

TABLE 2. Distribution of complementary lethal genes D_1 and D_2 for F_1 zygote deterioration in the progeny of "Obake" plants.

"Obake" plant tested	Cross- ferti. (%)	"Obake" × <i>barthii</i>			<i>sativa</i> × "Obake"				Genotype of "Obake" plant	Pollen ferti. (%)*
		Normal	F_1 seed Shrunken	Ratio	Cross- ferti. (%)	Normal	F_1 seed Shrunken	Ratio		
Af11-1	18	18	0	1:0	39	93	0	1:0	$d_1d_1d_2d_2$	59
Af11-4	40	8	0	1:0	30	6	0	1:0	$d_1d_1D_2d_2$	72
Af110-1-4	65	9	0	1:0	78	19	0	1:0	$d_1d_1d_2d_2$	83
Af11-2	32	5	0	1:0	47	11	6	1:1	$D_1d_1d_2d_2$	68
Af64-1-1	43	10	13	1:1	18	8	0	1:0	$d_1d_1D_2d_2$	37
Af64-1-3	48	7	5	1:1	33	7	0	1:0	$d_1d_1D_2d_2$	58
Af64-1-6	52	8	8	1:1	16	12	0	1:0	$d_1d_1D_2d_2$	83
Af64-1-9	63	16	13	1:1	12	10	0	1:0	$d_1d_1D_2d_2$	35
Af110-1-1	78	6	16	1:1	30	8	0	1:0	$d_1d_1D_2d_2$	58
Af64-1-5	38	0	21	0:1	45	13	0	1:0	$d_1d_1D_2D_2$	75
Af110-1-2	80	0	9	0:1	29	15	0	1:0	$d_1d_1D_2D_2$	40
		(barthii × "Obake")			("Obake" × <i>sativa</i>)					
Af11-1	20	3	0	1:0	25	51	0	1:0		

* Pollen fertility of the "Obake" plant tested.

Strains used for crosses: (*barthii*) Af9, Af107, Af113, ML36-4 and ML36-6, (*sativa*) 108, 563 & T.65.

plants might have been highly heterozygous. The other two families observed also showed wide variations in the fertility of selfed progenies.

2) *Distribution of complementary lethals controlling a crossing barrier.*—As reported in our previous paper (Chu and Oka, 1970), *barthii* is isolated from its relatives by a crossing barrier, which is controlled by a set of complementary lethals disturbing tissue differentiation in young F_1 seeds. The "Obake" plants generally had higher cross-fertilities and higher F_1 seed germinabilities than true *barthii*, though the values were lower than those usually found in other crosses not involving *barthii*. The mean cross-fertility, F_1 seed germinability, and F_1 pollen fertility between the "Obake" plants and other strain groups, and their variation ranges, are given in Table 1. The percentages differed markedly according to the "Obake" plants used, indicating segregation among them.

To estimate the distribution among "Obake" plants of the complementary lethal genes, 11 progeny lines of the three families were crossed with certain test-strains, and the segregation ratios for normal and shrunken F_1 seeds were examined. It was assumed that in a cross with *barthii*

($D_1D_1d_2d_2$; used as the pollen parent), if all F_1 seeds were shrunken, the plant tested had D_2D_2 ; if the F_1 seeds segregated into 1 normal and 1 shrunken, the plant had D_2d_2 ; and when normal F_1 seeds alone were produced, the plant had d_2d_2 . In the same manner, the genotypes for D_1 vs. d_1 were determined from crosses with a *sativa* test-strain ($d_1d_1D_2D_2$; used as the maternal parent).

The results of the genic analysis are given in Table 2, which shows that one of Af11 progeny lines carried D_1 , but the other 10 lines all lacked D_1 but had D_2 in homo- or heterozygous state. Some of the "Obake" plants had the double-recessive genotype, $d_1d_1d_2d_2$, which could be easily crossed with both *barthii* and *sativa* or *glaberrima*.

3) *F_1 sterility relationships of the "Obake" plants.*—The F_1 plants of the "Obake" with *sativa* or Asian *perennis* strains generally showed higher pollen fertility than those of *barthii* with the same strains, as shown in Table 1. On the contrary, their F_1 plants with the American form of *perennis*, *glaberrima*, and *breviligulata*, had lower pollen fertility than those of *barthii* with the same strains. F_1 seed fertilities also showed similar trends. As

reported elsewhere (Chu et al., 1969), the Asian form of *perennis* and *sativa* generally showed a high F₁ sterility with American *perennis*, *glaberrima* and *breviligulata*, while true *barthii* was partly fertile with them. The above tendency of the "Obake" plants to show a lower F₁ fertility with American *perennis*, *glaberrima*, and *breviligulata* than true *barthii*, suggests that they contain genes introduced from *sativa*.

To observe the variations in F₁ sterility relationships of Af64 segregants, 12 lines were each crossed with two test-strains, W106 (Asian *perennis*, annual type) and W025 (*glaberrima*). The tested lines mostly showed high F₁ pollen fertilities (above 80% except for one showing 23%) with W106 and a high sterility (lower than 20% pollen fertility) with W025. This also suggests that Af64 may have a genotype similar to the Asian form of *perennis* or *sativa*.

4) *Character variations of the "Obake" plants.*—To compare the "Obake" plants with their supposed parents, *barthii*, *sativa*, *glaberrima*, and *breviligulata*, measurements were taken with the respect to the following six traits.

Character	<i>barthii</i>	<i>sativa</i>	<i>brevi- ligulata</i>	<i>glaber- rima</i>
Ligule	Long	Medium	Short	Short
Primary branches	Few	Medium	Few	Many
Secondary branches	Few	Many	Few	Few
Spikelets/ panicle	Few	Many	Few	Many
Spikelet width	Small	Large	Large	Large
Auricle	Large	Small	Small	Small

From the data, hybrid indices for distinguishing *sativa* from *glaberrima* (X₁) and *barthii* from *sativa* and *glaberrima* (X₂) were computed. X₁ was based on ligule length and primary and secondary branch numbers per panicle. X₂ was based on ligule length, spikelet number per panicle and spikelet width. The X₁ and X₂ indices for various "Obake" lines are, together with those for the supposed parents

and their F₁ plants, shown in Figure 1. The putative parental species were clearly separated by the two indices, their F₁ plants being located midway between them. The "Obake" plants formed a continuous array of intergrades from *barthii* to *sativa*, a few approaching *glaberrima*.

Further, the X₁ index distinguishing between *sativa* and *glaberrima* was compared with pollen fertility. As mentioned, all *barthii*, *sativa* and *glaberrima* plants observed as control were highly pollen-fertile, while most "Obake" plants were infertile. It was found that the "Obake" plants approaching *glaberrima* in the X₁ index were highly sterile. Those having characters of *sativa* showed a wide range of pollen fertility. This suggests that the "Obake" plants have many genes introduced from *sativa*.

5) *Isozyme variations of the "Obake" plants.*—To estimate the genotype of the "Obake" plants, their leaf-blade isozymes of peroxidase and acid phosphatase were compared with those of the supposed parents. The results showed that some of the "Obake" plants had the same zymogram as *barthii* or *sativa*, but others had "hybrid zymograms" as found in the F₁ plants between *sativa* and *barthii* or between *glaberrima* and *barthii*. From the data, the frequencies of respective isozyme bands in the "Obake" plants were compared with those in the supposed parents and their F₁ hybrids. As shown in Table 3, about one-half of Af64 segregants had bands 2A (absent in *barthii*), 4A (characterizing *barthii*) and 5C (absent in *sativa*) of peroxidase, and three of them had 6C characterizing *glaberrima* and *breviligulata*. One plant (Af64-1-10) showed all these bands. Bands 2A and 4A are allelic and 3A represents a hybrid dimer enzyme due to 2A and 4A (our unpublished data). The occurrence of 3A in the "Obake" plants indicates their heterozygosity. The acid phosphatase zymograms also showed a similar trend. As shown in Table 4, some "Obake" plants had isozymes characterizing *barthii*, *sativa* or *glaberrima*, though most of them showed

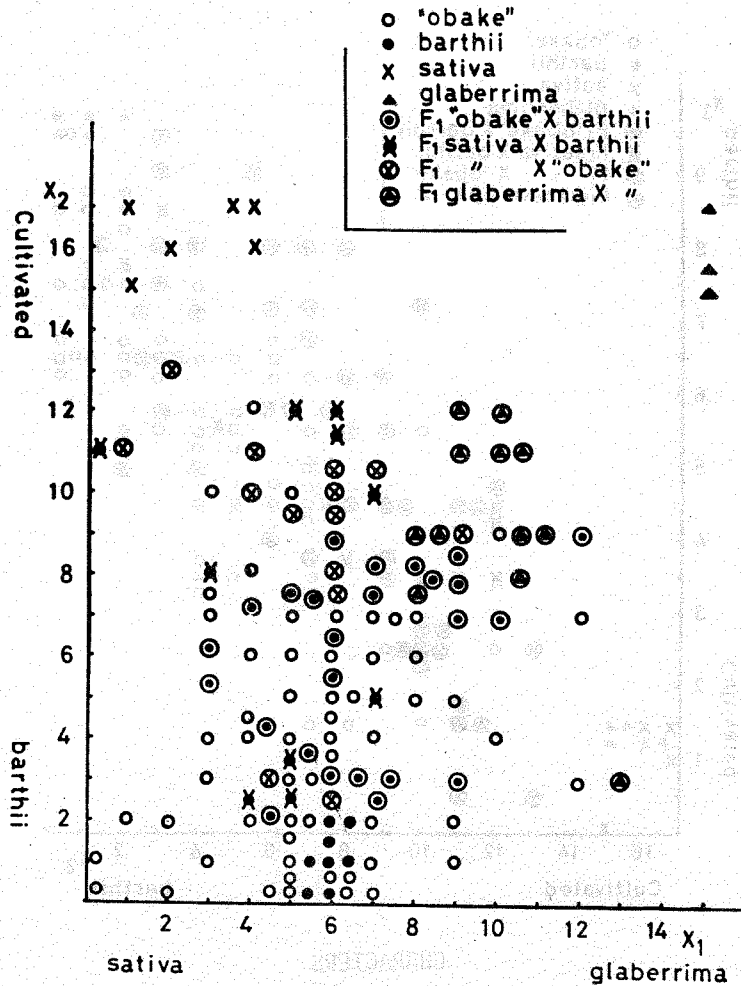


FIG. 1. "Obake" plants, their supposed parents and F₁ plants scattered by two hybrid indices, one (abscissa) for classifying *sativa* and *glaberrima*, and the other (ordinate) distinguishing *barthii* from *sativa* and *glaberrima*.

"hybrid zymograms" combining the isozymes of the parental species.

To obtain an integrated picture of the isozyme variations, a hybrid index (X₃) was constructed by scoring the presence or absence of four bands of peroxidase (2A, 4A, 5C and 6C) and five bands of acid phosphatase (5A, 1C, 2C, 3C and 4C), so as to realize the difference between *barthii* and cultivated species (*sativa* and *glaberrima*). In Figure 2, the "Obake" plants, their supposed parents and F₁ plants are

scattered by this "isozyme index" and a hybrid index for distinguishing *barthii* from cultivated species (X₂). The figure shows that the two indices were correlated.

In view of the above experimental results, the off-types of *barthii*, called "Obake," seem to be complex hybrids of *barthii* with *sativa* and *glaberrima* or *breviligulata*, produced by introgression across the reproductive barriers surrounding *barthii*.

6) *Cuban perennis-sativa* hybrid swarm. —The F₁ hybrids between the American

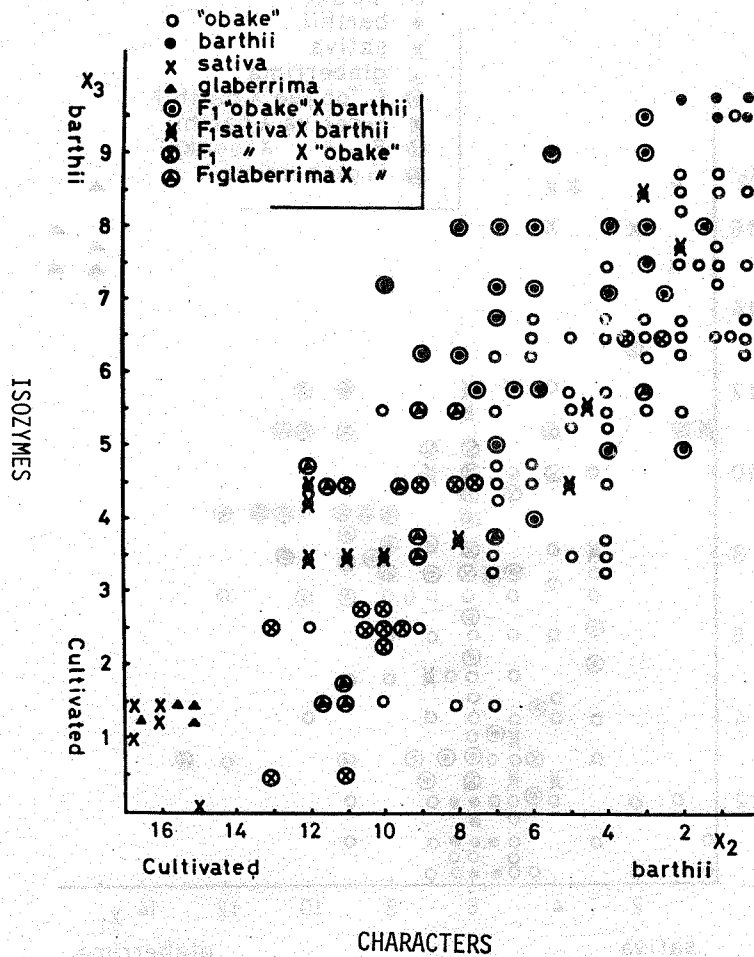


FIG. 2. "Obake" plants, their supposed parents and F_1 plants scattered by two hybrid indices, one (abscissa) combining three character values for distinguishing *barthii* from *sativa* and *glaberrima*, and the other (ordinate) scoring the presence or absence of peroxidase and acid phosphatase isozymes.

form of *O. perennis* and *O. sativa* are highly pollen-sterile, though their embryosacs are partly functional (Chu et al., 1969). The hybrid swarm found in Cuba might represent back-cross progenies of the F_1 plants between a *perennis* (W1169) and a *sativa* (C8315) population which grew side by side. The fertility variation of 15 lines (69 plants) is shown in Figure 3. The parental strains were fertile and their F_1 plants were sterile, while the plants from the swarm varied from near 0% to 100%. Twelve

plants belonging to different lines were each crossed with the parental strains to examine their F_1 sterility relationships. As also shown in Figure 3, most lines showed high F_1 fertilities with the *sativa* parent and sterility with the *perennis* parent. This suggests that the hybrid swarm might consist of plants with *sativa*-like genotypes, possibly due to back-crosses with the *sativa* parent.

The hybrid plants and the parental strains were observed regarding leaf-blade

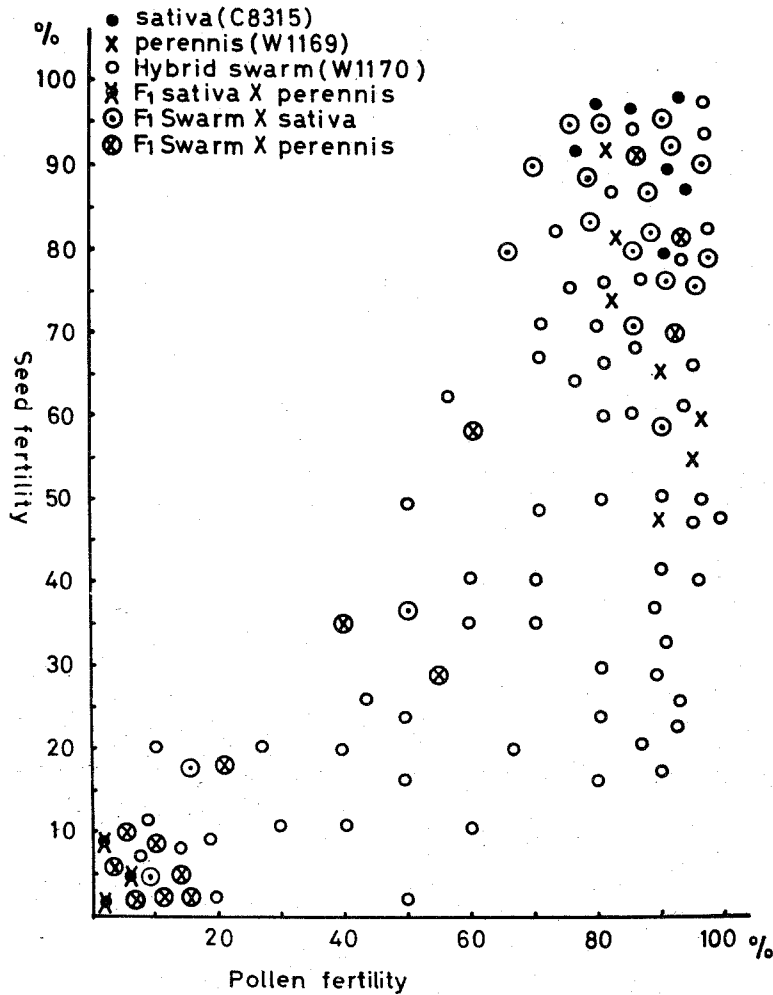


FIG. 3. Progeny lines of Cuban *perennis*-*sativa* hybrid swarm and their F_1 plants with the parental species, scattered according to pollen and seed fertilities.

isozymes of peroxidase, acid phosphatase and esterase. As shown in Table 5, in peroxidase, 10 out of 70 plants from the swarm showed "hybrid zymograms," two showed the same zymogram as the *perennis* parent, and 40 showed the same zymogram as the *sativa* parent. Similar trends of variation were also found in acid phosphatase and esterase, indicating that the hybrid swarm might have absorbed many genes from the *sativa* parent.

A hybrid index (X_4) for classifying *sativa* and the American form of *perennis* was computed from the measurements for plant height, ligule length, panicle length, awn length, anther length, number of spikelets per panicle, spikelet width and thickness, and the rate of grain shedding. Another index (X_5) based on the isozyme data was also made by scoring the presence or absence of three peroxidase (2A, 4A, and 4C), two acid phosphatase (5A and 2C)

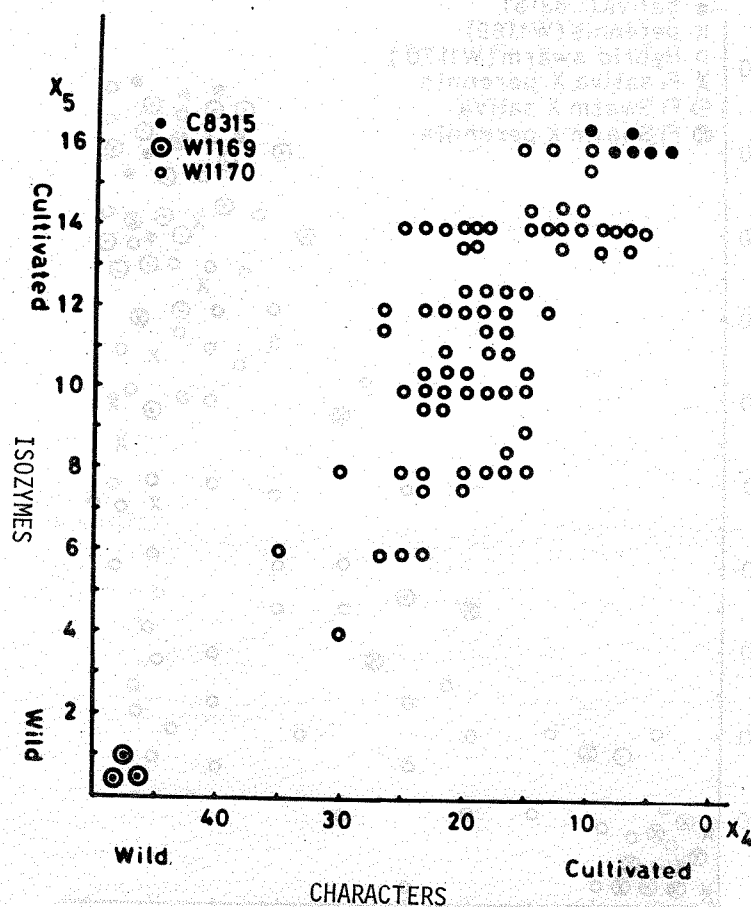


FIG. 4. Progeny lines of Cuban hybrid swarm (W1170) scattered by two hybrid indices, one (abscissa) combining nine character measurements for classifying the *sativa* (C8315) and *perennis* (W1169) parents, and the other (ordinate) scoring peroxidase, acid phosphatase and esterase isozymes.

and three esterase (7A, 9A, and 1C) isozymes, to realize the difference between the parental species. As shown in Figure 4, the two indices were highly correlated. This suggests that the genes controlling the isozymes and other characters might be "coherent," and that the hybrid swarm might be subjected to introgression and selection in a certain direction.

7) *Heterozygosity of natural hybrids.*—The "Obake" types of *barthii* as well as the plants of the Cuban hybrid swarm are descendants of hybrids. To estimate their

heterozygosity, the within-line genetic variances of ligule length, spikelet length, and spikelet number per panicle were compared with the between-line genetic variances of the same characters, using the second and third generation lines obtained from self-pollinated single plants. As shown in Table 6, the "Obake" plants had larger ratios of within-line to total genetic variance than the Cuban hybrids. They seem to have a higher degree of heterozygosity than the Cuban population.

TABLE 3. Frequencies of peroxidase isozymes found in the "Obake" plants, in comparison with their supposed parents (in %).

Group	No. of strains observed	2A	3A	4A	4C	5C	6C
<i>barthii</i>	45	0	0	100	20	100	0
<i>sativa</i>	38	100	0	0	47	0	0
<i>glaberrima</i> and <i>breviligulata</i>	42	100	0	0	100	72	93
F ₁ , <i>barthii</i> × <i>sativa</i>	12	100	100	100	42	75	0
F ₁ , <i>barthii</i> × <i>glaberrima</i>	2	100	100	100	100	100	100
"Obake," Af11	7	100	48	48	14	86	0
"Obake," Af64	61	57	11	55	79	53	3
"Obake," Af110	4	0	0	100	100	0	0
F ₁ , "Obake" × <i>barthii</i>	25	64	64	100	48	96	0
F ₁ , "Obake" × <i>sativa</i>	18	100	39	39	50	11	0
F ₁ , "Obake" × <i>glaberrima</i>	17	100	65	65	100	77	65

TABLE 4. Frequencies of acid phosphatase isozymes found in the "Obake" plants, in comparison with their supposed parents (in %).

Group	No. of strains observed	5A	6A	1C	2C	3C	4C
<i>barthii</i>	37	100	22		1	78	89
<i>sativa</i>	113	58		58	42		
<i>glaberrima</i> and <i>breviligulata</i>	8	100	100		100		
F ₁ , <i>barthii</i> × <i>sativa</i>	15	80	24		73		13
F ₁ , <i>barthii</i> × <i>glaberrima</i>	2	100	100			100	
"Obake," Af11	6	83			83	83	50
"Obake," Af64	55	86	15	13	69	64	22
"Obake," Af110	4	25		75	50		
F ₁ , "Obake" × <i>barthii</i>	25	100		16	36	84	56
F ₁ , "Obake" × <i>sativa</i>	14	100	7	7	21	14	
F ₁ , "Obake" × <i>glaberrima</i>	13	100	100		77	15	15

TABLE 5. Zymograms found in Cuban *sativa*-*perennis* hybrid swarm, in comparison with the parents.

Population	Peroxidase			Acid-phosphatase		Esterase	
	Zymogram	No. of plants		Zymogram	No. of plants	Zymogram	No. of plants
C8315 (<i>sativa</i>)	2A 4C	7		2C	7	7A 9A	7
W1169 (<i>perennis</i>)	4A	6		5A	6	1C	6
Hybrid swarm (W1170)	2A	18		2C	42	7A	19
	4A	2		5A	17	9A	20
	2A 4C	40		5A 2C	11	1C	2
	2A 3A 4A	7				7A 9A	15
	2A 3A 4A 4C	3				7A 1C	3
					9A 1C	1	
					7A 9A 1C	4	
Total number of plants		70			70		70

TABLE 6. Components of between- and within-line genetic variances in hybrid populations.

Parameter	"Obake" segregants (Af64)			Cuban hybrid swarm		
	Ligule length (mm)	Spikelet no. per panicle	Spikelet length (mm)	Ligule length (mm)	Spikelet no. per panicle	Spikelet length (mm)
Mean	28.3	63.7	7.85	26.5	123.4	8.00
Variance component, σ^2_{Bt} (Between-line)	40.1	199.1	0.002	3.83	761.4	0.184
σ^2_{Wn} (Within-line)	88.5	263.4 ^{ns}	0.363	4.28	241.2	0.008
Ratio (%) [*]	69	57	100	53	24	4
Error variance	2.29	80.4	0.021			

$$* \frac{\sigma^2_{Wn}}{\sigma^2_{Bt} + \sigma^2_{Wn}}$$

DISCUSSION

The role of hybridization in evolution was discussed by Anderson (1949) as well as by Anderson and Stebbins (1954). Its importance in maintaining genetic variability in the populations of predominantly self-pollinated plants was demonstrated by Allard and co-workers (1962; 1963). Following Anderson (1949), many workers have investigated natural hybrids in different plant groups, in order to analyze the structure of hybrid populations (Brehm and Ownbey, 1965; Levin, 1963; 1967; Ornduff, 1964; Stutz and Thomas, 1964; etc.) or to estimate the adaptability of hybrid derivatives to different environments (Benson et al., 1967; Briggs, 1962). Reproductive barriers are involved in many of the hybrids observed by these authors.

Regarding wild and cultivated rice species, Oka and Chang (1961) dealt with three hybrid swarms between the Asian form of *O. perennis* and *O. sativa*, which were found in India, Thailand, and Taiwan. They pointed out that the populations contained a large amount of genetic variation which after being released covered the whole range from wild to cultivated forms, and that such populations seemed to be established in habitats disturbed by man. Similar hybrid populations were also found in Africa between *O. breviligulata* and *O. glaberrima* (Morishima et al., 1963). In these cases, the parental species were not isolated by a reproductive barrier, and hy-

brids could be frequently produced when the parental species grew sympatrically. As the cultivated forms are predominantly self-but wild forms are cross-pollinated, wild forms may absorb genes of cultivated ones. If such wild populations grow under "cultivation pressure," a particular form that can coexist as a weed with the cultivated plants would result (Oka and Chang, 1959).

In contrast to the above, the hybrids dealt with in this paper have come into existence due to leakages in the isolating barriers. As a matter of fact, no *barthii* population containing "Obake" types with a recognizable frequency was found in Africa, and the "Obake" plants were obtained in our experimental plots only from the seeds of *barthii* populations growing in or nearby rice fields. They do not seem to be F₁ plants, as the selfed progenies of a single plant showed isozymes and other characters of both *sativa* and *glaberrima* or *breviligulata*. The *barthii* populations must have absorbed foreign genes, though the hybrids may not be easily detected in their habitats because of the dominance or the buffering effect of *barthii* genes. This wild species propagated by rhizomes is allogamous and partly self-incompatible (Chu et al., 1969b), and is isolated from its relatives by a crossing barrier. The populations containing "Obake" types may be considered as "potential hybrid swarms."

Some of the "Obake" plants were found to be double-recessive for the complemen-

tary lethals which create the crossing barrier. They could then be easily crossed with *barthii*, *sativa*, and *glaberrima* producing further hybrids. Such plants may act as a bridge for gene exchange. When hybrids are produced, barriers isolating the parental species may be gradually disintegrated.

In Asia and Africa, different rice species grow sympatrically, but in tropical America, wild and cultivated populations are generally separated by long distance (Oka, 1964, p. 76). The exceptional hybrid swarm found in Cuba seems to demonstrate that species isolated physically and sexually can be hybridized when they happen to be in contact in a disturbed habitat.

For estimating the structure of hybrid populations, we observed isozyme variations. Combining variations in different enzymes, the intergrades between species could be revealed in the correlation of zymograms with the association pattern of other characters. Possibly, genes differentiating the species might be coherent due to linkage and selection of certain advantageous assortments, as shown by Clausen and Hiesey (1960).

Reproductive isolation is a factor counteracting hybridization. It may be inferred that there is a balance between isolation and hybridization. According to Harlan (1966, p. 74), evolution in plants may be controlled by "differentiation-hybridization cycles," whose range depends upon the degree of buffering of the involved genotypes. If *barthii* had no crossing barrier its populations would soon become hybrid swarms, as it has a high hybridizing capacity and grows sympatrically with its related taxa. If it were completely isolated from its relatives, it would finally suffer from reduction of genetic variability and adaptability to changing environments. A balance between these opposing factors may play a role in the evolutionary dynamics of a species.

This balance may differ according to the breeding system. In the rice fields of West Africa, *barthii* is cross-pollinated, *breviligulata*

is partly self-pollinated, and the cultivated species, *sativa* and *glaberrima*, are selfers. The gene flow in their sympatric population might be directed to *barthii* from the other species. The crossing barrier of *barthii* might have developed in response to the gene flow.

Presumably, autogamous plants need no particular reproductive barrier for their existence, though barriers may develop as the result of differentiation. They may tolerate isolation, if they are short-lived plants having a high evolutionary rate. In allogamous plants with a high hybridizing capacity, the balance between isolation and hybridization may be subjected to selection.

SUMMARY

The African form of *Oryza perennis* Moench (subsp. *barthii*) is isolated from its sympatric rice forms by a crossing barrier and partly by F₁ weakness and sterility, but its seeds collected from the natural habitats sometimes produced off-types which we called "Obake" (monster) in this paper. Their selfed progenies were observed regarding pollen fertility, F₁ sterility relationships with *barthii* and other taxa, six morphological traits, and the leaf-blade zymograms of peroxidase and acid phosphatase. The results show that the "Obake" plants are hybrids of *barthii* with *sativa* and *glaberrima* or *breviligulata*, due to introgression across the reproductive barriers surrounding *barthii*. They had various genotypes for the complementary lethals controlling the *barthii*'s crossing barrier, some having the double-recessive combination enabling the plants to be easily crossed with both *barthii* and *sativa* or *glaberrima*. The occurrence of such plants indicates genetic diversity of *barthii* populations, which were considered to be "potential hybrid swarms." Further, a hybrid swarm between the American form of *O. perennis* and *O. sativa*, found in Cuba, was observed. In this case, the parental species are isolated by F₁ pollen sterility, the embryosacs being partly functional. The population was found to have been predominantly

back-crossed with *sativa*. The intergrades between species could be revealed in the correlation of zymograms with the association pattern of other characters, suggesting that genes differentiating the species might be coherent. It was suggested that isolation and hybridization might be in a balance under the pressure of natural selection.

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