

The direction of pollen flow between two co-occurring rice species, *Oryza sativa* and *O. glaberrima**

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Two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* Steud., are often grown mixed in farmers' fields in West Africa. Two sympatric species were collected from two different fields. The frequency of interspecific hybridization between the two species was estimated from the seeds harvested in farmers' fields and in artificial mixtures. The two species were isolated by prezygotic isolating barriers such as autogamy and differences in time of flowering, however, the isolation was incomplete. The frequency of hybridization varied from 0.8 to 4.5 per cent. In addition, all 87 hybrids were found from the seeds which *O. glaberrima* produced. This shows that pollen flow occurs from *O. sativa* to *O. glaberrima* when they hybridize. Generally, *O. glaberrima* had a higher per cent of exerted stigmas as compared with *O. sativa*. The direction of pollen flow appeared to be explained by a higher efficiency of pollen capture in *O. glaberrima*. The genetic consequences were also discussed in relation to gene flow between two distantly related species.

INTRODUCTION

Introgression across reproductive barriers often occurs between sympatric species or semispecies into another (Anderson and Stebbins, 1954). Isolating barriers are usually much developed between distantly related taxa, reducing the possibility of gene flow between them. However, it has been repeatedly suggested that not only their wild progenitors but also more distantly related taxa have contributed to crop evolution to some extent through introgressive hybridization in maize (Mangelsdorf, 1961; Stalker *et al.*, 1877), wheat (Zohary and Feldman, 1962), sugarcane (Price, 1957) and rice (Chu and Oka, 1970). Although gene transfer was experimentally possible between these crops and their wild relatives, their distinct differences in morphology and the rarity of hybrid swarms make it difficult to establish the genetic contributions of gene transfer under natural conditions.

Oryza glaberrima is endemic in West Africa, while *O. sativa* is of tropical Asian origin. The two cultivated rice species came to be in contact with

each other after introduction of *O. sativa* into West Africa. At present, the two species are often grown mixed in farmers' fields in West Africa (Oka *et al.*, 1978). Although the interspecific F₁ hybrid is highly pollen-sterile, it can be experimentally backcrossed with the pollen of either parent (Chu *et al.*, 1978). This suggests a potential for gene flow between them, but this does not imply conclusively that introgression occurs under natural conditions. A basic problem that needs to be solved is whether or not introgression between the two distantly related species has played a role in their adaptation to the growing conditions in West Africa. In order to examine if gene transfer is taking a place between distantly related species such as *O. sativa* and *O. glaberrima*, this paper presents, as the first step, data on the frequency of natural hybridization and the direction of pollen flow.

MATERIALS AND METHODS

The two cultivated rice species, *Oryza sativa* and *O. glaberrima*, are isolated from each other by an F₁ sterility barriers although their chromosomes ($n = 12$) normally pair in the hybrid (Morinaga and Kuriyama, 1957; Chu *et al.*, 1969). They can

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be distinguished by a few distinct characteristics such as the short, tough ligules and few secondary panicle branches of *O. glaberrima* compared with the long ligules and many secondary panicle branches of *O. sativa*. The strains used were selected from seed samples collected in Nigeria in 1977 (Oka *et al.*, 1978). Strains C8840 (*O. sativa*) and C8841 (*O. glaberrima*) had been grown mixed in an upland field (Z-3) and strains C8849 (*O. sativa*) and C8848 (*O. glaberrima*) were from a rainfed lowland site (KN-1). The relative frequency of *O. sativa* was 48 per cent at Z-3 and 61 per cent at KN-1. At each site, seeds were sampled from seven to ten individuals per species and the bulked seed were used in this study.

The 1:1 mixed-planting experiment was carried out at Mishima. In early May, 18 seedlings of each strain, raised in a greenhouse for four weeks, were transplanted into two boxes (60 × 15 × 15 cm). In each box, the two sympatric strains from each site were planted alternatively at a spacing density of 5 × 5 cm. The soil was dressed with fertilizers at the rate of 6, 5 and 5 g m⁻² of N, P₂O₅ and K₂O. Seeds were harvested at maturity and the bulked seeds were used for estimating the frequency of F₁ hybrids under controlled conditions. All the plants were grown in experimentally induced short-day plots (*ca.* 12 h per day).

To look into the differences in floral traits, 14 *O. sativa* and 13 *O. glaberrima* strains including the above four strains were grown. The length of spikelet, anther, style and stigma was measured under a microscope. Six or more flowers per strain were examined. The per cent of exerted stigma in bloomed spikelets was determined from more than 100 spikelets per strain. To examine diurnal differences in flowering, flowering time was recorded on a clear day and the observation was repeated more than twice per strain. In total, 1099 spikelets were observed.

RESULTS

Frequency of interspecific hybridization

From seed samples collected in two farmers' fields (Z-3 and KN-1), 254 plants in total were grown at Mishima. Out of them, three plants showed complete sterility while all the other plants had high seed sets (53–95 per cent). All the three sterile plants were detected from the seeds collected from *O. glaberrima* plants. The sterile plants had long ligules which characterized *O. sativa*, showing that they were interspecific hybrids between the two species. The frequency of hybrids was 1.6 per cent at Z-3 and 0.8 per cent at KN-1 (table 1). This indicates that the two species naturally hybridize at both sites although the frequency is low (1.2 per cent in total).

When the sympatric strains from each site were mix-planted under controlled conditions, their flowering period partly overlapped although *O. glaberrima* tended to flower earlier than *O. sativa* (table 2). The hybridization frequency in the mixture of two upland strains seemed to be higher than that in the mixture of two lowland strains. The frequency was 4.5 per cent in the mixture of C8840 + C8841 and 0.8 per cent in the mixture of C8849 + C8848. All 84 hybrids obtained were found from the seeds which *O. glaberrima* produced. This agrees with that found from the seeds collected in farmers' fields, indicating that pollen flow occurs mainly from *O. sativa* to *O. glaberrima* when the two species hybridize.

Differences in floral characteristics

The direction of pollen flow from *O. sativa* to *O. glaberrima* might be explained by their differences in floral characteristics controlling the efficiency of pollen dispersal and capture. Anther length reflects the amount of pollen grains per anther and

Table 1 Frequencies of natural hybrids between *O. sativa* and *O. glaberrima* observed from the seeds collected in two farmers' fields where the two species coexisted

Site	Species	Strain	No. of plants examined	No. of F ₁ hybrids	Per cent of hybrids
Z-3 (Upland)	<i>O. sativa</i>	C8840	67	0	0.0
	<i>O. glaberrima</i>	C8841	62	2	3.2
		Total	129	2	1.6
KN-1 (Lowland)	<i>O. sativa</i>	C8849	57	0	0.0
	<i>O. glaberrima</i>	C8848	68	1	1.5
		Total	125	1	0.8

Note: The F₁ hybrids were identified by long ligules which characterized *O. sativa* in addition to complete sterility.

Table 2 Frequencies of interspecific F_1 hybrids between *O. sativa* and *O. glaberrima* when the two sympatric strains were mix-planted under controlled conditions

Site	Species	Strain	Flowering period	No. of plants examined	No. of F_1 hybrids	Per cent of hybrids
Z-3 (Upland)	<i>O. sativa</i>	C8840	July 24–Aug. 9	791	0	0.0
	<i>O. glaberrima</i>	C8841	July 19–July 29	798	71	8.9
		Total		1589	71	4.5
KN-1 (Lowland)	<i>O. sativa</i>	C8849	July 27–Aug. 14	795	0	0.0
	<i>O. glaberrima</i>	C8848	July 22–Aug. 3	786	13	1.7
		Total		1581	13	0.8

Note: The F_1 hybrids were identified by long ligules which characterized *O. sativa* in addition to complete sterility.

length of style and stigma are associated with the ability of pollen capture. However, table 3 shows no significant difference in length of anther, style and stigma between the sympatric strains from Z-3. In the sympatric strains from KN-1, *O. glaberrima* had a longer styles and shorter stigmas than *O. sativa*. On the other hand, the degree of exerted stigma greatly differed between the two species. *O. glaberrima* had a high per cent of exerted stigma than *O. sativa*. This difference implies that *O. glaberrima* is more efficient for pollen capture, which is consistent with the direction of pollen flow observed. Even when their flowering period overlap, they cannot interbreed unless they flower at the same time in a day. Although *O. glaberrima* tended to flower earlier than *O. sativa*, isolation due to the diurnal difference was also incomplete (table 3).

As to the floral differences, 27 strains of the two species which were from West Africa were compared under controlled conditions (table 4). Generally, *O. glaberrima* tended to have a higher per cent of exerted stigmas and shorter spikelets

than *O. sativa*. No significant difference was detected in length of anther, style and stigma between the two species. In addition, although *O. glaberrima* tended to flower earlier than *O. sativa*, isolation due to the diurnal difference was also incomplete (fig. 1) as found in the sympatric strains.

DISCUSSION

Oryza sativa was introduced into West Africa mainly after the 15th century. After introduction, *O. sativa* spread across the West African rice zone where *O. glaberrima* was well established (Porteres, 1950). The two rice species are often grown mixed at present in farmers' fields and natural interspecific hybrids occasionally occur in the fields (Oka *et al.*, 1978). Introgression between them has been assumed to occur from field observations (Borgel and Second, 1978; Steele, 1978) but has never been conclusively demonstrated. Usually, farmers use a part of their harvest

Table 3 Means and their standard deviations in parentheses for floral characteristics of sympatric strains of *O. sativa* and *O. glaberrima* collected from upland and lowland fields

Site	Species	Strain	Length in mm				Per cent of exerted stigma	Flowering time
			Spikelet	Anther	Style	Stigma		
Z-3 (Upland)	<i>O. sativa</i>	C8840	8.5 (0.3)	1.70 (0.14)	2.10 (0.22)	0.66 (0.04)	23.9	am 11:08 (18 min)
	<i>O. glaberrima</i>	C8841	7.6 (0.2)	1.68 (0.05)	2.33 (0.13)	0.74 (0.12)	53.5	am 10:28 (29 min)
		<i>t</i> value	6.114**	0.329	2.203	1.549	—	2.856*
KN-1 (Lowland)	<i>O. sativa</i>	C8849	8.5 (0.4)	1.83 (0.10)	1.88 (0.21)	1.10 (0.16)	18.9	am 10:18 (46 min)
	<i>O. glaberrima</i>	C8848	7.7 (0.5)	1.88 (0.12)	2.13 (0.17)	0.88 (0.11)	65.9	am 9:36 (18 min)
		<i>t</i> value	3.060*	0.784	2.236*	2.772*	—	2.075

*, ** show significant at 5 per cent and 1 per cent, respectively.

Table 4 Differences in floral characteristics between *O. sativa* and *O. glaberrima* collected in West Africa

Trait	Species		<i>t</i> value (df = 25)
	<i>O. sativa</i> , <i>N</i> = 14 (s.d.)	<i>O. glaberrima</i> , <i>N</i> = 13 (s.d.)	
Spikelet length, mm	8.2 (0.8)	7.3 (0.4)	3.466**
Anther length, mm	1.88 (0.29)	1.71 (0.20)	1.803
Style length, mm	0.51 (0.10)	0.50 (0.04)	0.629
Stigma length, mm	1.03 (0.25)	0.98 (0.20)	0.650
Exserted stigma, % †	18.4 (11.9)	57.2 (10.3)	8.600**
Flowering time	am 10:57 (31 min)	am 9:46 (24 min)	6.508**

** Shows significant at 1 per cent.

† Shows that data were transformed into Arcsin $\sqrt{\text{per cent}}$ before *t* test.

as the seed for the next season. The two species are randomly direct-seeded such that they are forced to coexist in farmers' field (Sano *et al.*, 1984). The present result indicates that isolation due to differences in flowering time is generally incomplete between the two species and they interbreed at a low frequency under natural conditions.

The two cultivated rice species are predominantly self-pollinated species. Autogamy prevents gene flow from other plants and acts as a mechanism of isolation. In wild rice species (*O. rufipogon* and *O. longistaminata*), Oka and Morishima (1967) estimated 20 to 100 per cent outcrossing and they observed that the level of outcrossing is associated with the timing of pollen

emission, pollen production and lengths of style and stigma although there was no significant difference in pollen size among wild and cultivated species. The two cultivated rice species shed pollen on their stigma 1–3 min after flowering, so they have a high selfing rate under natural conditions (Oka and Morishima, 1967; Virmani and Athwal, 1973). A conspicuous feature of this study is the direction of pollen flow from *O. sativa* to *O. glaberrima*. In wind-pollinated species, the pollen scatters at random in contrast to the specificity often found between plants and pollinators. Effective wind-pollination is affected by various factors including pollen production, anther and stigma exposed, size of pollen and stigma, density and shape of the plant, surrounding vegetation and distance (Levin and Kerster, 1974; Whitehead, 1969).

The present study showed that *O. glaberrima* tends to have a high per cent of exserted stigma and shorter spikelets than *O. sativa*, indicating that *O. glaberrima* has a high ability to capture pollen compared to *O. sativa*. The spikelet seems to act as an obstacle for pollen capture in *O. sativa*. Hence, the direction of pollen flow appears to result from the different efficiency for pollen capture although both species are predominantly self-pollinated species in nature. In this study, no inter-specific hybrid was found from the seeds harvested from *O. sativa*. This cannot be explained only by the difference in the per cent of exserted stigma since *O. sativa* also have some stigma exposed. Additional mechanisms such as efficient pollen transport, cross incompatibility and pollen competition must be involved. Whatever the mechanisms

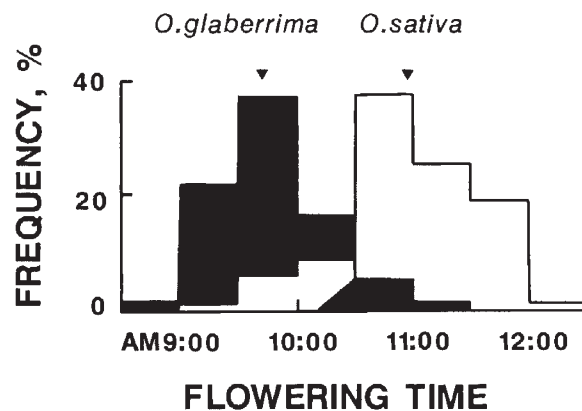


Figure 1 Diurnal difference in flowering time between *O. sativa* (14 strains, 467 spikelets) and *O. glaberrima* (13 strains, 632 spikelets).

involved are, pollen flow occurs mainly from *O. sativa* to *O. glaberrima* when they hybridize.

As mentioned before, although the F_1 hybrids are completely pollen-sterile, backcrossing is possible as the female parent since 20–30 per cent embryos remain functional (Chu *et al.*, 1969). Fertility is recovered quickly by backcrosses and BC_1F_1 and BC_2F_1 produce selfed progenies (Sano *et al.*, 1980). Further, the F_1 plants have larger stigmas than the parents suggesting a high ability of pollen capture (unpublished). Some genes were experimentally transferred between the two species by backcrossing (Yabuno, 1977) but a recombination restriction and the rapid recovery of parental phenotypes occurred in the hybrid derivatives (Sano *et al.*, 1980). The recovery of parental types might result from the M-V linkage as discussed by Grant (1981). As a consequence, they can coexist without losing their identity even after hybridization. This might make it difficult to detect hybrid derivatives or intermediate types under natural conditions even though they hybridize. However, this does not imply that any gene transfer is impossible between them after hybridization.

If the hybrid was backcrossed to the parental species, both *O. sativa* and *O. glaberrima* types would be expected in later generations. In such a case, it is also expected that the derivatives of *O. sativa* type would have the cytoplasm of *O. glaberrima* in addition to the *O. sativa* like nucleus since the maternal parent of the F_1 hybrid is likely to be *O. glaberrima* as found in the present study. It should be noted that, if pollen flow took a place from *O. sativa* to *O. glaberrima*, cytoplasmic gene flow would occur in the reverse direction.

The cytoplasm of Asian strains of *O. sativa* is different from that of *O. glaberrima* (Yabuno, 1977; Sano, 1985). Thus, the combination of the nucleus of *O. glaberrima* with the cytoplasm of Asian strains of *O. sativa* always shows cytoplasmic male sterility. However, the combination of *O. glaberrima* cytoplasm and *O. sativa* nucleus, which is expected from the direction of pollen flow, has no adverse effect on fertility. Theoretically, Takahata and Slatkin (1984) revealed that when two species hybridize it is difficult to prevent the introgression of extranuclear genomes unless there is any adverse effect from nucleo-cytoplasmic incompatibility. In other words, cytoplasmic genes could be transferred rather frequently as compared with nuclear genes which produce various abnormalities in the hybrid derivatives between the two species. Further investigations on cytoplasmic gene flow from *O. glaberrima* to *O. sativa* might offer a clear evidence of introgression between the two rice species in West Africa.

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