Hybrid zone characteristics of the intergeneric hybrid bamboo × *Gigantocalamus malpenensis* (Poaceae: Bambusoideae) in Peninsular Malaysia

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ABSTRACT. The natural intergeneric hybrid bamboo × *Gigantocalamus malpenensis* and its parental species, *Dendrocalamus pendulus* and *Gigantochloa scortechinii*, were mapped along a 4-km stretch of the Gombak valley in Peninsular Malaysia. Hybrid clumps were associated only with *D. pendulus*, which implied the latter was the seed parent, and not with *G. scortechinii*, which occupied only broader, gentler sites available for its establishment at the southwest and mid-southern parts of a southwest-to-northeast trending corridor of disturbance associated with expressway construction some three decades before. The effects of landscape-level disturbance on vegetation and plant demographic changes, gene flow and breeding dynamics are discussed.

Keywords. Bamboo, *Dendrocalamus,* disturbance, × *Gigantocalamus*, *Gigantochloa*, hybrid zone, hybridisation, Peninsular Malaysia

Introduction

A bamboo morphologically intermediate between *Gigantochloa scortechinii* Gamble and *Dendrocalamus pendulus* Ridl., two common bamboo species in the foothills of the Peninsular Malaysian Main Range, was discovered in two different localities (Perak and Selangor states) in Peninsular Malaysia (Goh et al. 2011). The hybrid nature of the morphologically intermediate taxon was demonstrated using partial Granule-Bound Starch Synthase (*GBSS*) I sequences, and this natural intergeneric hybrid was named × *Gigantocalamus malpenensis* K.M.Wong (Goh et al. 2011). Of its two known localities, the second, in the Gombak valley in Hulu Gombak, Selangor, Peninsular Malaysia, included a substantial number of clumps along a significant span of the valley, and it was decided to attempt to understand the population characteristics better, especially when its parental populations were also well represented in the same valley.

The reproductive behaviour of this hybrid, especially in comparison to its wild parental species, was of special interest. While entirely vegetative stands of both *Gigantochloa scortechinii* and *D. pendulus* are frequently encountered (Wong 1995a, b), they are known to flower gregariously, i.e., synchronised flowering of a significant number of clumps within a localised population (Burgess 1975; Wong 1995a, b), although 'diffuse-sporadic flowering' (isolated whole-clump flowering at

irregular intervals in natural populations) is also known (Wong 1995a, b). Caryopses (the one-seeded fruits of bamboos) and seedlings are easily encountered in the wild amid gregarious flowerings of these two species. However, a certain degree of self-incompatibility would be expected because caryopses and seedlings have not been found with isolated flowering clumps of *G. scortechinii*; and only a very low level of caryopsis formation was found with the flowering of an isolated clump of *G. rostrata* K.M.Wong, a related species (Wong 1995b). Goh et al. (2011) reported that a solitary cultivated clump of the hybrid from Perak had flowered quite soon after it grew into mature size and died completely afterwards, with no caryopses formed. Flowering and fruiting of the hybrid in the wild has not been recorded until the discovery of the Selangor population at Hulu Gombak.

Study site, methods and materials

The study site was in the Hulu Gombak area (101º44'30" to 101º46'E, 3º19'15" to 3º20'25"N) of Peninsular Malaysia's Selangor state. The valley was steep-sided but the terrain beside the Gombak River was around 250–400 m elevation in this area. The portion of the Gombak valley investigated resembled a southwest-to-northeast corridor, flanked by the Karak Expressway to the north of the Gombak River, and by the smaller, old Gombak Road to the south. The construction of the Karak Expressway just before 1980 was impactful, involving many slope cuts, infills and bridging across generally steep inclines. Wayside spaces abutting the Expressway and between that and the Gombak River are frequently occupied by successional stands of low vegetation, and there are patches of *Acacia mangium* Willd. cultivated for forestry or site-greening purposes but occasionally escaping.

We used a GARMIN GPSMAP 60CSx handheld GPS unit to obtain satellite readings of location coordinates for clump positions of the hybrid × *Gigantocalamus malpenensis* and its parental species, *Gigantochloa scortechinii* and *D. pendulus*, along a 4-km stretch of the Gombak valley, where these bamboos occurred in September, 2009. Clumps were noted as 'vegetative' (complete lack of flowering activity), 'flowered' (with signs of past/recent flowering but no fresh flowers seen), or 'flowering' (with fresh flowering persisting). An estimate of relative clump maturity was obtained by classifying clumps as 'mature', when there were culms exceeding 3.5 cm diameter at 1.5 m height present, or 'young', when there were no culms exceeding 3.5 cm diameter. This is based on the observation that there would be larger culm internode diameters produced with increasingly older clump age until a maximum that was representative of the species, so that younger clumps typically produced culms of smaller diameter (Holttum 1958, Wong 2004).

The coordinates were transferred from the GPS unit to a personal computer using software provided by the manufacturer. The data was then viewed in a Google Earth 5.2 scene of the Hulu Gombak area (on 1 Oct 2009) with the location coordinates obtained represented by symbols plotted onto the satellite view. A tracing of this was made (Fig. 1) to show the main Gombak River flanked by the Karak Expressway and

the Gombak Road, as well as required contours to represent the physical landform, together with plotted points showing the locations of bamboo clumps recorded.

Results

The results of the population census for the three bamboo taxa are summarised in Table 1, with locations of × *Gigantocalamus malpenensis* and its parental species, *Gigantochloa scortechinii* and *D. pendulus*, mapped in Fig. 1.

Distribution of taxa across the hybrid zone
The respective sites of the parental species were interesting: *G. scortechinii* occupied flatter or gentler, streamside places along the Gombak River, adequately represented at the southwestern and mid-southern parts of the valley, whereas *D. pendulus* typically occurred on steeper hillsides along the same valley. Hybrid individuals occurred together with *D. pendulus* clumps towards the northeastern part of the valley, and not with the *G. scortechinii* population.

Bamboo flowering and seeding during the census
There were 55 individual clumps of *D. pendulus* (incl. 1 young clump as defined for this study) and 67 clumps of *G. scortechinii* (no young clumps) recorded in the study area. All were vegetative, showing no sign of flowering.
        Among 48 clumps of the hybrid, 35 (c. 73%) were vegetative at the time of the census and 13 were recently in flower or were still flowering (Table 1). These

Table 1. Observed clumps of the hybrid × *Gigantocalamus malpenensis* and its parental species *Dendrocalamus pendulus* and *Gigantochloa scortechinii* along the Hulu Gombak valley, Peninsular Malaysia: summary of some biological attributes (vegetative vs. reproductive states; relative maturity). Clumps were noted as Vegetative, Flowered (signs of recent flowering but no fresh flowers seen), or Flowering (with fresh flowering persisting). Clump relative maturity: Mature (indicated by presence of culms exceeding 3.5 cm diameter at 1.5 m height) or Young (no culms exceeding 3.5 cm diameter present).

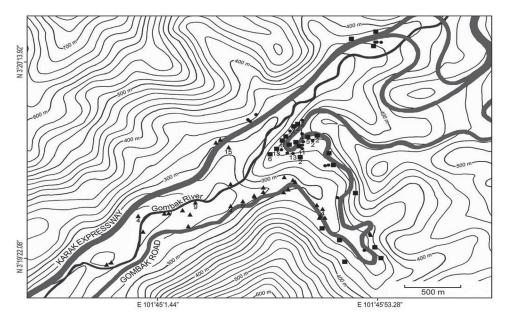| Taxon	Number of recorded clumps	Clumps in vegetative state	Live clumps that had flowered / were flowering during census	Dead clumps that had flowered
Dendrocalamus pendulus	55	55 (incl. 1 young clump)	0	0
Gigantochloa scortechinii	67	67 (incl. 0 young clumps)	0	0
Hybrid (× *Gigantocalamus malpenensis*)	48	35 (incl. 7 young clumps)	7 had flowered + 3 still flowering	3

Fig. 1. The Gombak River, Gombak Road and Karak Expressway (thick lines), and locations of the hybrid × *Gigantocalamus malpenensis* (dots) and its parental species, *Dendrocalamus pendulus* (squares) and *Gigantochloa scortechinii* (triangles), in Hulu Gombak, Selangor, Peninsular Malaysia.

vegetative clumps included only seven young clumps (i.e., clumps without any culms exceeding 3.5 cm diameter). It is reasonable to assume that this represented an establishing hybrid population originating from possibly just 1–2 parental seeding events, which must be rare, but when the flowering of both parental species on site must have coincided. Flowering in the hybrid population appears to have involved only a small proportion of hybrid individuals.

It stands to reason that the population of hybrid clumps found probably represented F1 hybrid material. Seeding of the F1 must have been absent or very limited, and seedling survival negligible, because caryopses were not found among the flowering material, and young seedlings were absent on site among the flowering hybrid clumps, in spite of a good number of these (10 out of 13) having completed flowering and not having any more fresh flowering branches on them during the census. Thus, the presence of an F2 generation was not detected although a cohort of flowering F1 hybrids was present.

Discussion

Hybrid zone characteristics
The near segregation of the parental populations was compatible with what is known about their ecological distribution: *D. pendulus* is more typical of steeper hillsides,

whereas *G. scortechinii* prefers gentler slopes and frequently spreads onto the disturbed and logged-over plains (Wong 1995b). The availability of sites suitable for *G. scortechinii* thus appeared to constrain establishment of that species to the southwestern and mid-southern parts of the study area. In particular, a protruding ridge in the middle of the study area had an abundance of *D. pendulus* established on its steep sides (Fig. 1).

The distribution of the hybrid relative to the two parental species (the hybrid being associated with *D. pendulus*) suggested that *D. pendulus* was the maternal parent for the hybrid cohort investigated. Caryopses are expected to have poorer dispersal ability compared to pollen, and bamboo pseudospikelets and caryopses mostly fall around the parent clump (Ridley 1930; Wong 1995a, b). A previous attempt at deducing the direction of the cross between the parental species, using cpDNA that was likely to have been maternally inherited and including a broader geographical sampling of the species, was equivocal probably because of complex patterns of inheritance for which past reciprocal crosses between taxa followed by introgression (sensu Anderson 1948) could not be ruled out (Goh et al. 2011).

Ecological implications

Why were these hybrids not detected earlier, although this part of the Gombak valley has been a routinely well-botanised locality? The hybrid bamboo was not observed at all during the several visits annually to the study area from 1980, when the Karak Expressway construction was being completed, to 1988, and 1996–2007 (K.M. Wong, pers. obs.). Hybrid population characteristics, particularly its distribution, suggest comparatively recent establishment. We suggest that the "corridor of change" brought about by development (i.e., opening up) of the Karak Expressway (parallel to this part of the Gombak valley) just before 1980 was a key factor that may have predisposed the hybridisation event. This enormous cleared corridor caused greater openness, spread and an increased abundance of both *D. pendulus* and *G. scortechinii* (and other pioneer or early successional plant species), as well as the removal of forest tree cover that probably served as a natural impediment to genetic exchange between the bamboo species. In the dense vegetation of tropical rain forest, high humidity could dampen pollen, increasing their difficulty in remaining airborne, and rain often removes airborne pollen; also the dense tree canopy tends to be an effective filter of airborne pollen (Turner 2001).

Greater exposure along this corridor had probably increased chances for air currents carrying pollen from *G. scortechinii* clumps in flatter terrain at one end (southwest) to find hillside patches occupied by *D. pendulus* near the other end (northeast). The tendency for both *D. pendulus* and *G. scortechinii* to flower gregariously or diffuse-sporadically from time to time probably increased the possibility of coincident flowering and cross-fertilisation.

Will the hybrid persist? At the time of the census, it could be said that the hybrid was beginning to establish. It was represented by 48 clumps, but over 70% remained vegetative while 13 clumps had come into flower. This suggests there could be some variability with respect to age at onset of flowering. The distribution of hybrid

offspring clumps among so many maternal clumps (individuals) in this case also implies a range of resulting heterozygosity in the hybrid offspring that would bring increased variability even in flowering time (or its associated vegetative longevity). There were also several clumps that had flowered but which were producing new culm shoots. The survival of such new culm shoots was not certain, but again represented a potential for regeneration following flowering, which has been noted for clumping bamboos that exhibit whole-clump flowering (Wong 1995a, b). Thus, there appears to be a good prospect for the survival of the hybrid.

Hybrids are probably more common among related species (e.g., Okada 1990, Kiew et al. 2003, Gravendeel et al. 2004) than between genera, but intergeneric crosses are also known for other plant groups (e.g., McKenzie et al. 2008). Plant taxa have also been surmised to have originated from intergeneric hybridisation events (Tara 1977, Wallace & Jansen 1995) and hybridisation between genetically divergent lines have been known to give more vigorous progeny (Edmands 2002). The potential for viable backcrosses and advanced-generation hybrids cannot be presently estimated.

Conclusion

Continuing research also examines generic boundaries among the group of bamboos classified as the Bambusinae subtribe, which includes *Dendrocalamus* and *Gigantochloa*. This includes the difficulties of classifying a mystifying complex of bamboos that have defied simple lineage studies so far (e.g., Yang et al. 2008, Stapleton et al. 2009, Yang et al. 2010, Goh et al. 2010), so that the use of more gene regions, including from both nuclear and plastid domains, would be important. The present case study of hybridisation between *D. pendulus* and *G. scortechinii* (Goh et al. 2011, this study) confirms that natural hybrids do form in the tropical Southeast Asian landscape, especially when ecological barriers between taxa change in nature. Elsewhere, Clark et al. (1989) and Triplett et al. (2010) have documented interspecific hybridisation among American bamboos. We have suggested (Wong 2004, Goh et al. 2010, Goh et al. 2011; Wong et al., in prep.) that the role of hybridisation in the evolution of Tropical Asian bamboos could have been underestimated, mostly because such hybrids are difficult to detect and confirm. Hybridisation is a significant feature of gene flow and evolutionary processes (Rieseberg 1995, Arnold et al. 2001) and extensive adaptive radiation can occur after hybridisation (Givnish 2010). It should be expected that among highly complex taxonomic groups such as the woody bamboos, hybridisation and introgression studies would hold the key to a better understanding.

In conservation terms, we have hardly any documentation of the effects of environmental change (especially through vegetation removal or degradation) on the reproductive behaviour and gene flow among plants in Southeast Asia. The primary concern is perhaps correctly placed on the decimation or reduction of plant populations in an affected area (Laurance et al. 1997), giving emphasis on potentially adverse effects associated with breakdown of long-established and naturally viable pollinator, breeding, dispersal and establishment biology (e.g., Washitani 2000). This study has

demonstrated how landscape-level changes equivalent to that seen with development in the Hulu Gombak area may predispose the plant life to changes in reproductive and ecological behaviour. In this case, the chance of hybridisation occurring between *D. pendulus* and *G. scortechinii*, the two abundant species of bamboo in the Hulu Gombak valley, was probably increased in the aftermath of landform and vegetation changes, facilitated by removal of natural physical barriers that have helped maintain reproductive isolation of the species, and the products of hybridisation began establishing only nearly 30 years following disturbance. Changes in the environment would be expected to alter relative species abundances, plant establishment, and processes or patterns (including direction and symmetry) of gene flow and breeding. Hybridisation would bring along fitness consequences influenced by the extent and quality of the breeding interaction.

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