Journal of Oil Palm Research Vol. 17 June 2005, p. 64-69

ILLEGITIMACY IN OIL PALM BREEDING - A REVIEW

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ABSTRACT

Shell thickness and molecular marker data indicate that illegitimacy and contamination are more widespread in oil palm breeding programmes than is usually acknowledged. Before the discovery of the mode of inheritance of shell thickness, no markers were available to detect illegitimacy. Once shell thickness could be used as a marker, it became clear that control of pollination needed improvement. However, in present day programmes anomalous segregation ratios and contamination with the wrong fruit forms still occur from time to time, and work with molecular markers shows that illegitimacy may occur even when fruit form segregation is correct. Where there is illegitimacy, family selection will be unreliable, and inadvertent inbreeding may take place. Now that molecular markers are widely available, it should be standard practice in oil palm breeding to test all crosses for legitimacy. The requirements for this are discussed.

Keywords: contamination, fruit forms, molecular markers, segregation, shell thickness.

Date received: 9 July 2004; **Sent for revision:** 3 August 2004; **Received in final form:** 9 March 2005; **Accepted:** 14 March 2005.

INTRODUCTION

In any breeding programme, it is important to know the parentage of families and individuals, for a variety of reasons. Oil palm breeders make extensive use of family performance, usually selecting the best individual plants within the best families. Such selection will only be effective if families are correctly identified, and are truly *families*; that is, all individuals have the same parentage. Progeny testing is also used, individual parent palms being evaluated on the basis of the performance of their offspring. This will not be valid if some of the offspring are illegitimate. A notable feature of oil palm breeding programmes is the very narrow genetic base of some of the ancestral populations. The palm is naturally cross-pollinating, and inbreeding causes yield depression (Hardon, 1970; Luyindula *et al*., in press). To ensure that the parents used for commercial hybrid seed production are not related, oil palm breeders rely on pedigrees (*e.g*., Rosenquist, 1986). For a pedigree to be useful, it is essential that the crosses were made correctly.

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Controlled pollination of oil palms is difficult, and prone to various sources of error; detailed descriptions of methods and the necessary checks and quality control procedures have been published (Donough *et al*., 1993; Chin, 1999; Rao and Kushairi, 1999), but it is not clear to what extent good control was practised in the past. Possible problems include damage to bags by rats, squirrels, oil palm spines or simply from repeated use; pollen-bearing weevils will enter damaged bags, causing contamination by illegitimate pollen. In some female inflorescences, the *accompanying male flowers* may produce viable pollen (Beiranert, 1935), so that some self-pollination could occur. Because of the obvious risk of errors, pollination is usually subject to careful supervision, but errors may also occur in pollen collection or storage, in labelling bunches on the palm, in the seed store or the nursery, and during field planting of trials. In this paper, I will show that illegitimacy and contamination (some illegitimate palms in a family) may have been more widespread in oil palm breeding programmes than is usually acknowledged.

SHELL THICKNESS AND ILLEGITIMACY

Shell thickness is an important yield component, whose inheritance was elucidated by Beirnaert and Vanderweyen (1941). Control is by a single gene,

and the fruit form of palms within families may reveal the existence of illegitimate or contaminated crosses in two ways. The occurrence of unexpected fruit forms is a certain indication of problems; examples include thin-shelled *tenera*s (T) in a thick-shelled *dura* x *dura* (DxD) family, Ds in a thin-shelled *dura* x *pisifera* (P) cross, or shell-less Ps in a TxD cross. In some cases, the unexpected fruit forms may not be the only contaminants; for example, if Ts in a DxD cross result from contamination with *tenera* pollen, some of the *dura*s in the family will also be illegitimate.

The second way in which fruit form can indicate contamination is by segregation ratios which differ statistically from Mendelian expectations. This is less useful, because low levels of contamination will not significantly alter the segregation ratio, unless very large numbers of palms are involved. For example, in a TxT cross with 80 palms, a typical number per family in an oil palm breeding programme, 30% contamination with *dura* pollen would give the following numbers:

Legitimate T pollen:	14 D	28 T	14 P
Illegitimate D pollen:	12D	12T	-
Total:	26D	40T	14P

These numbers, compared to an expected 20:40:20, give a Chi^2 value of 3.6 (probability of a larger value = 5.8%). Thus, more than 30% contamination must occur before the segregation ratio will differ significantly from the expected 1:2:1 D:T:P.

It is worth noting that identification of the fruit forms can sometimes be difficult. Very thin-shelled Ds may be classed as T; it is essential to look for the diagnostic mesocarp fibres in a T, seen as a ring surrounding the nut in transverse fruit sections. Another possible source of error is the existence of fertile Ps which actually have small amounts of lignified tissue adjacent to the kernel. This tissue does not completely surround the kernel to form a shell, but is discernible in fruit cross-sections.

Beirnaert and Vanderweyen's work was published over 60 years ago, but many annual reports and other publications still make no mention of fruit form segregation. As shown by the examples discussed below, it is clear that many oil palm breeding programmes have included illegitimate or contaminated crosses. However, it is not always clear to what extent this has been recognized.

Palm SP540

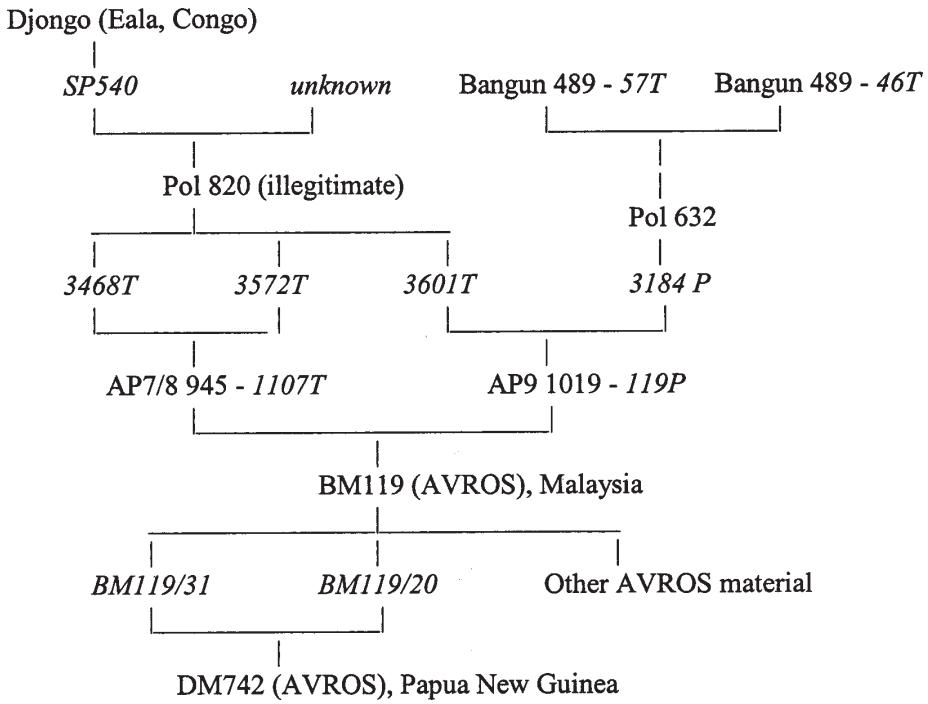
Serious breeding work started in Sumatra soon after First World War. One of the most important palms was the *tenera* SP540 at Sungei Pantjur, which was part of a consignment of seed sent by the Director of the Eala Botanic Gardens in the Congo. The seed was recorded as *var. Djongo*, indicating that it came from the Djongo *tenera* palm from the Yangambi programme (Rosenquist, 1986). A total of only 13 seedlings survived: of these, eight were Ds and five Ts, so the Djongo seed was presumably an open-pollinated TxD or TxT cross. *Figure 1* shows that SP540 was one of the ancestors of the AVROS *pisifera*s now widely used for seed production in Indonesia, Malaysia, Papua New Guinea and Costa Rica (Rajanaidu and Jalani, 1999). The pedigree, as usually published, shows family Pol 820 as a selfing of SP540. However, the family had only two Ps among 123 palms (Hartley, 1977), whereas about 30 would be expected from a T selfing. Hartley did not mention the number of Ds and Ts in the family, but a Chi^2 test shows that deviation from the expected segregation of 1:3 P:D+T is highly significant. Thus, the family Pol 820 was probably predominantly an illegitimate TxD out-cross (though some T pollen must have been present to give the two Ps in the family). At that time, the inheritance of shell thickness was not understood, so this illegitimacy was apparently not recognized. Recent work with molecular markers supports this, in that there was a greater degree of heterozygosity in AVROS family DM742 (*Figure 1*) than expected from the pedigree (Mayes, 1995).

AVROS material is sometimes referred to as being *75% Djongo*, but if SP540 was from open-pollinated seed and Pol 820 was illegitimate, there might be less than 20% of genes from the Djongo palm in present-day AVROS material.

Early Work with *Tenera*s and *Pisifera*s in Malaysia

Before the Second World War, selection work had started in the Deli *dura* population in Malaya. Publication of the *Congo Theory* of inheritance of shell thickness excited interest in Ts and Ps. Pollen was imported from Africa, and DxT and DxP crosses were made. Segregation of fruit forms in crosses made in the 1950s was often incorrect: of 24 DxT crosses in one programme, seven had segregation ratios which differed significantly from the expected 1:1 D:T, based on a Chi^2 test (Rosenquist, per. comm., 1994). In all but one of these, there was an excess of Ds. Among nearly 3000 palms from DxP crosses in the same programme, 22% were Ds.

Some of the first commercial DxP plantings were made by Oil Palms of Malaya (OPM) in 1958. A random sample of 20 palms from the 1958 OPM planting included 13 Ds (Corley, unpublished); this is a small sample, but for a binomial distribution, the 95% confidence limits for the population as a whole are between 41% and 85% D (Snedecor and Cochran, 1966). A similar 20-palm sample from a



Note: Palm numbers in italics; other numbers are progeny codes.

Source: Modified from Corley and Tinker (2003).

Figure 1. Ancestry of AVROS material.

1960 planting included 17 Ds; the confidence limits for that population are between 62% and 97% D. These plantings had a very high degree of illegitimacy, therefore. The most likely source of contamination with D pollen would have been from other Ds surrounding the female parents.

Cameroons Breeding Programme

In trials planted in the Cameroons in the mid 1960s, fruit form segregations showed numerous errors (Langham, R M, unpublished). Of 132 crosses planted in 1966 and 1967, 42 showed either significant deviations from the expected segregation ratios or contamination with incorrect fruit forms. Reasons for the errors were not always clear, but 17 DxD crosses were contaminated, with an average of 8% Ts. These crosses would have been made in blocks containing many Ts, so contamination with pollen from surrounding palms could result in Ts in a DxD cross. However, two DxD crosses included a P, which could not have arisen by contamination of a D mother palm in the field; mixing of seed, or of nursery plants, is the most likely explanation here. Two supposed DxP crosses segregated 50:50 D:T, so were probably erroneously made with T pollen instead of P.

Binga Breeding Programme, Congo

Dumortier *et al*. (1992) described a large programme in the Congo consisting of over 30 trials planted during the 1970s; their report shows that both contamination and incorrect segregation ratios were common. Among 88 DxD, TxD and TxP crosses, 52% of crosses were contaminated with wrong fruit forms, with an overall contamination rate of 3%. Ignoring contamination with wrong fruit forms, 21% of 452 TxT, TxP, TxD and DxT crosses had fruit form segregation ratios which were significantly different from expectations.

These figures suggest that control of pollination might have been poor, but in 147 DxP crosses planted at the same time, there was only 0.7% D contamination. Closer examination reveals a discrepancy: 4 DxP crosses included in trials with other types of crosses had 13% Ds, whereas the remaining 143 crosses, in trials which included only DxP crosses, had 0.3% Ds. This indicates that the main problem was probably not contamination during pollination, but mixing up of families either in the nursery or during field planting. If families were mixed in a DxP trial, that would not be detectable in the fruit forms, whereas in a trial with different types of crosses, mixing could lead to both detectable contamination, and incorrect segregation ratios.

A further problem in this programme is an excess of Ts. Averaged over all TxT crosses, in four trials with between 1500 and 2500 palms each, the proportion of Ts ranged from 54% to 58%. Based on the totals in each trial, the deviation from expectation (Chi^2 test) was highly significant. The most likely explanation of this anomaly seems to be incorrect fruit typing, but the possibility of a genetic effect, such as a lethal recessive gene closely linked to the shell-thickness gene, cannot be ruled out.

MOLECULAR MARKERS AND ILLEGITIMACY

A low level of contamination of a TxT cross, or by T pollen in a DxT cross, is not detectable by fruit form. Similarly, mixing of families would not be detectable in DxD or DxP trials. Now, though, the development of molecular markers has allowed illegitimate crosses to be identified even when the shell thickness gene is not segregating. The legitimacy of individual palms can also be tested.

Family Bg143

This family, planted in 1973 at Binga, Democratic Republic of Congo, has been extensively used in some recent breeding programmes (Rosenquist *et al.*, 1990). The family was supposed to be a self of T palm 312/3; the fruit forms segregated in the ratio 26:44:30 D:T:P, which is not significantly different from the expected 1:2:1. Yield of the family was much better than expected for a selfing, and Rosenquist *et al*. (1990) observed that palm 312/3 appeared to be *tolerant of inbreeding*. However, when molecular marker techniques were developed, it became clear that Bg143 was not a legitimate cross: of 12 palms examined, eight had marker bands not shown by 312/3, so could not have come from self pollination of that palm (Mayes, per. comm., 1993). This, rather than tolerance of inbreeding, probably explains the unexpectedly high yield of the family.

Illegitimacy in a DxP Trial

In one DxP trial, planted in the 1990s, D contamination was negligible. However, molecular marker analysis showed that six crosses out of 58 tested carried bands which were not present in either parent, and so were contaminated or illegitimate. As noted above, contamination in the field in a Deli *dura* seed garden is most likely to be with D pollen. An illegitimate DxP family which does not contain Ds must have arisen from use of pollen from the wrong *pisifera*, or from mislabelling of seed or seedlings at some stage.

DISCUSSION

Before the Second World War, most of the breeding work in Southeast Asia was with Deli *dura*. In the absence of a good marker gene, there was no way of knowing whether control of pollination was adequate. Even where Ts or Ps were used, the inheritance of shell thickness was not understood, and illegitimacy appears not to have been recognized, as in the descendants of SP540. It was only after the work of Beirnaert and Vanderweyen (1941) that it became feasible to monitor the efficacy of controlled pollination. In this respect, the early commercial plantings of DxP material in Malaysia are of interest. As noted above, the first plantings had a very high level of *dura* contamination. The 1958 planting would have started fruiting in 1961, at which point the contamination would have been recognized. It is clear that immediate and effective steps were taken to improve the control of pollination: a 20-palm sample from a 1963 OPM planting was 100% T. An important implication of this is that contamination may have been very extensive in earlier Deli *dura* breeding programmes, but was not detected because all palms were D.

Most oil palm breeders and seed producers agree that, with good control of pollination, D contamination in DxP crosses should be below 1%. From 1963 until the introduction of *Elaeidobius kamerunicus* in 1982 contamination in Malaysian commercial plantings was generally low. It appears that *Thrips hawaiiensis*, the main pollinating agent at that time, rarely gained access to bagged female inflorescences. However, *E. kamerunicus* is much more persistent, and after it was introduced D contamination became a significant problem. Rao *et al.* (1994) quoted average figures of 10%-20% for two plantation companies, while Donough and Law (1995) found up to 45% Ds in individual plantings. This problem appears to have persisted for much of the 1980s, but in a 1991 comparison of seed sources, contamination had been reduced to below 2% (Rao and Kushairi, 1999), indicating that control had been restored.

In view of the problems which arose after the introduction of the weevil to Malaysia, it is worth considering how effective control of pollination might have been in the early days of oil palm breeding in Africa, where several species of *Elaeidobius* were always present. In his programme in the Congo, Beirnaert found some crosses among his TxT crosses and T-selfs which diverged significantly from the expected 1:2:1 ratio. The proportion of *tenera*s remained constant at about 50%, but a few palms gave as much as 35% or as little as 15% Ds, with a correspondingly low or high proportion of Ps (Beirnaert and Vanderweyen, 1941). Beirnaert does not seem to have considered the possibility of contamination, but at first sight this

could explain his results. Contamination of a TxT cross with *dura* pollen would give an excess of Ds, without affecting the proportionof Ts, because both TxT and TxD crosses have 50% Ts. Similarly, contamination with *pisifera* pollen would give an excess of Ps without affecting the proportion of Ts. However, Beirnaert's results were very consistent, with a palm giving the same divergence from expectation whether it was the male or the female parent in a cross; this is not easily explained in terms of contamination.

Beirnaert did not mention the fibre ring which is characteristic of Ts, and is now regarded as diagnostic. He apparently classified fruit forms simply on the basis of shell thickness. Thus, a possible source of error in his data would be confusion between thick-shelled Ts and thin-shelled Ds. Where he found an excess of Ds, it was at the expense of Ps, not of Ts, but most of his data appear to be combined results for several families with the same parent, rather than data for single families, which could lead to further confusion. We cannot be certain that control of pollination was good in Beirnaert's time, therefore, but we do know that it was good enough to allow him to deduce how shell thickness is inherited.

Elsewhere in Africa, there were clearly problems. In Nigeria in 1954, it was stated that *in many instances, segregations ... are not at all in line with the theories* (*WAIFOR Second Annual Report, 1953-54*). No details were given, but it was noted that most of the affected crosses were made at places or times when supervision was poor. "Mixing of families in nurseries is inversely proportional to the motivation of the staff in charge, or to the distance between office and field" (Durand-Gasselin, per. comm., 2004). In 1958, Dr A G Prendergast visited Congo (per. comm., 2000); he considered that control of pollination at Yangambi was good, with female inflorescences isolated in double bags, but elsewhere he saw single bags with holes being used.

It appears, therefore, that contamination might have been a significant problem in the early days of many programmes, at least until the inheritance of shell thickness was understood and it could be used as a marker gene. Thereafter, control did improve, but we have seen that contamination and incorrect segregation were still common in programmes in the Cameroons and Congo in the 1960s and 1970s, and illegitimacy has been identified in a 1990s programme using molecular markers.

CONCLUSION

It is clear from this survey that contamination and illegitimacy have been significant throughout the history of oil palm breeding. Until now, this has perhaps not mattered too much: steady breeding progress has been made, with yields being doubled between 1950 and 1990 (Corley and Lee, 1992). Provided that yield records are accurate, mass selection of the best individuals should ensure progress, whether or not those individuals are legitimate. However, future breeding progress will rely increasingly on family selection and progeny testing, which require a high degree of legitimacy; reliable pedigrees are also necessary to avoid inbreeding.

Now that molecular markers are available, I suggest that it should become standard practice in all oil palm breeding programmes to check the legitimacy of crosses using markers. It is necessary to find markers which are polymorphic (showing different bands) between legitimate and illegitimate parents. There are several possible causes of illegitimacy, as listed below:

- use of pollen from the wrong palm. Here, markers polymorphic among all palms from which pollen is being collected are needed.
- contamination during the pollination operation, by pollen from surrounding palms. Here, markers which are polymorphic within the female parental population are needed; these may be quite difficult to find in homogeneous material such as some Deli *dura* populations, particularly if the surrounding palms are sibs of the parent palm.
- accidental self pollination by accompanying male flowers. This is probably undetectable with molecular markers, but will cause D contamination in a DxP cross.
- incorrect labelling of seeds or seedlings. Markers for both female and male parental populations will be needed here. At some stations, seed production and breeding operations are kept physically separate, with little or no risk of mixing between them. This may simplify the choice of markers.

Once polymorphic markers have been found, bulked DNA from several palms in a family can be used for testing, so the amount of work need not be onerous. Illegitimacy can be proved by the presence of marker alleles not found in either putative parent, but legitimacy is a matter of probability, and cannot be proved. The more markers tested, the greater the confidence one can have. Assuming two alleles for each marker, confidence would be 2^n, where n is the number of markers. Thus, if five markers all give results consistent with legitimacy, the chance of the family being illegitimate would be only one in 32, or about 3%, which would probably be good enough for most purposes. With microsatellites one can usually identify more than two alleles per marker,

so the number of markers needed might be fewer than five.

Lim and Rao (2004) describe the facilities required, and estimate the costs of setting up and running a marker laboratory. There are marker methods available now which are simple and cheap to apply, and a marker laboratory should form an adjunct to every serious oil palm breeding programme. In the longer term, the greatest benefits will come from the development of marker-assisted selection, but detection of contaminated and illegitimate crosses is also very important, and significant cost savings could be made by eliminating such crosses before field planting (Lim and Rao, 2004).

ACKNOWLEDGEMENT

I am grateful to several plant breeders for information, and to P L Jack for discussion of marker methods.

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