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of biowastes generated from various economic sectors as feedstock for the production of bio-fuels. Bio-fuels need not be THE only option. They may and should be backed up or complemented by other economically viable energy options.

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Biology of a Long-necked beetle (*Cycnotrachelus* sp.) in Peninsular Malaysia

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Abstract The Long-neck beetle *Cycnotrachelus* sp. was found on a specific wayside plant (*Bridelia tomentosa*) in an urban environment. Its breeding cycle was completed only on this plant. When more than three beetles were present on a plant, males often outnumbered females. The males had distinctively longer necks and were bigger in size than the females. There were variations to courting and mating in terms of approach, time taken to attract the female's attention, number of attempts at mounting, duration of copulation, challenge by other males and posturing locations on the leaf. The complete metamorphosis (all within the cradle) from the egg to the emergence of adult from the cradle was estimated to be 28-35 days.

Keywords Long-necked weevil/beetle – *Cycnotrchelus* sp. – behaviour – life cycle – *Bridelia tomentosa*

INTRODUCTION

There is no information of the Long-necked or Giraffe-necked weevils/beetles in Malaysia except for a brief report in The Naturalist [1]. Members of these leaf rolling weevils belonging to the genera *Cycnotrachelus* and *Trachelophorus* of the family Attelabidae are relatively new to Malaysian biologists. The adult beetle is unique in appearance, with a long, distinct neck.

The authoritative literature on Leaf-rolling weevils has been published in a 523 pages monograph by Lagalov [2]. Other than this book the literature has been limited to publications by Russian, S. American and Japanese entomologists. According to the website http://zipcodezoo.com there are currently 18 species in the genus *Cycnotrachelus*. There are similar but not identical looking weevils (photographs without labels) from Taiwan and Papua-Indonesia. An impressive member of the group is the large species found in Madagascar.

This paper describes the biology of a *Cycnotrachelus* sp. not uncommonly found on wild *Bridelia tomentosa* (Euphorbiaceae) plants growing along the roads and in open spaces in rural and urban areas. It is part of a continuing long-term study being carried out by the author.

MATERIALS AND METHODS

The study was mainly based on field observations with the naked eyes and macro-photography recordings of infested *B. tomentosa* plants in the Damansara Heights and Bukit Tungku areas, Kuala Lumpur. The visible population was small and the beetles were detectable primarily on plants that had their cradles. Sightings of the beetles were recorded by examining the foliage of individual plants from the top to the bottom at about the same time (10.00-12.00 hr) at least once a week between August and December of 2007.

The host plant is commonly found along the roadsides of urban Kuala Lumpur. It is a typical tree of waste ground which could grow up to 18 m in height. The leaf is narrowly elliptic. In young plants the leaves tend to be broader than narrower. The upper side is light to dark green while the under side is silvery or glaucous [3]. Depending on its immediate environment the outline of its crown, the branching and the surface of the bark may be variable.

Limited work in the laboratory on the morphology was performed at the Parasitology Laboratory of the Faculty of Veterinary Science and scanning electron microscopy at the Institute of Biosciences, Universiti Putra Malaysia. Rearing of larvae and pupae were

carried out at home from cradles (nests) taken primarily from four colonies consisting of 36 plants. Before incubation at room temperature (25-30°C) the cradles were carefully opened to identify the stage of development of the insect and then re-rolled and put into a 7x1.5 or 6x2.5 cm glass vials. The vials were placed in the upright position to simulate a hanging cradle and kept in a semi-dark room. Cradles with larvae were opened every four days and with the pupae every two days. Measurements of insect parts were made through the eye-piece micrometer calibrated with a stage micrometer at 15x, 30x, 45x and 60x magnification. Cradles were measured with a standard ruler.

Specimen collections were limited as the population was small and to ensure a reasonable crop for the continuing studies.

RESULTS

Host plant and beetle population

The Long-necked beetle's breeding cycle was completed only on the *B. tomentosa* plant although it was detected resting or stopping on other plants between flights to the host plant. Cradles of the beetle were found on plants of various sizes that had leaves suitable for their construction. Old plants with very small leaves were not favoured.

The observations in 2007 indicated that the beetle's breeding activities coincided with the full sprouting of young leaves (flushing) beginning late July to about end of October. The population of the beetle as indicated by sighting was small. The beetles tended to colonize plants within a group that was not more than 300-400 meters away from each other. Table 1 shows the sightings of adult beetles and also the number of nests at the peak period. Due to the size of the beetle and the full leaf bloom of the plant, the sightings indicated the minimum number. When the sighting was more than three beetles on a plant, males often outnumbered females. Of the 70 sightings, 42 were males.

Table 1. Sighting of Long-necked beetles on nine plants in Jalan Setiamurni 5, Kuala Lumpur from August to December 2007. *Nests/cradles as counted on 12 September 2007 at 12:00 hr. Plant A1 was not observed during this period.

Date	Plant									Total
	A	B	C	D	E	F	G	H	I	
08 Aug	1	3	1	-	2	-	-	-	4	11
16 Aug	4	4	1	1	1	1	1	1	5	19
22 Aug	-	-	-	-	2	-	4	-	2	8
29 Aug	-	2	-	1	1	-	1	-	3	8
05 Sep	-	-	-	1	3	-	-	-	2	6
12 Sep	-	-	-	-	1	-	-	-	3	4
19 Sep	-	-	-	-	-	1	1	-	2	4
26 Sep	-	-	-	-	1	-	-	1	1	3
03 Oct	-	-	-	-	1	N	-	-	1	2
10 Oct	-	-	1	-	3	N	-	-	-	4
02 Dec	-	-	-	-	-	N	-	-	1	1
No. nests*	45	43	12	35	15	5	18	0	76	249

Adult morphology

Adult males

The males had distinctively longer necks and were visibly bigger in size than the females of the same age (Fig. 1). Males measured 10.3-12.6 mm from end of the last abdominal segment to the tip of the mandibles and weighed from 19.8-30.0 mg (n=12). The length of the head and neck ranged from 3.8-5.7 mm. The broadest ventral part of the body was 3.8 mm. The cuticle of the neck was shallowly folded into rings, suggesting shortening and lengthening function. Mature males in courtship phase exhibited metallic blood red colour when in bright sun lighted spots. Their ventral rear ends were more pointed compared to those of the females.

The antenna measured from 2.4-2.8 mm. The terminal antennal segment was slimmer and more pointed than that of the female. The 11 segments of the antenna were covered with hair sensillae which were very dense on the last three segments (Fig. 2). The terminal segment consisted of a cylindrical base and a conical top which appeared like two separate segments. At least four types of sensillae could be recognized on the last three segments (Fig. 3). The other segments showed distinct cuticular plates with sparsely distributed spike-like sensillae. Many more types of sensillae were distributed all over the anatomy of the adult including their legs. It is known among insects that each type of sensilla perform specific function as mechanosensory, contact chemosensory, air-borne chemosensory (olfactory), proprioceptive, thermosensory and hygrosensory receptors. Further scanning electron microscopy studies of the various sensillae are being carried out as part of the ongoing study of this beetle

Adult females

The necks of females were distinctively shorter than adult males (Fig. 4). The lengths of adult females measured 8.6-9.5 mm from the tip of the abdomen to the tip of the mandibles and weighed from 19.5-19.8 mg (n=11). The greatest width of the body was 3.5 mm ventrally and 4.0 mm dorsally. The length of the head and neck ranged from 2.0-2.4 mm. The ring folds of the neck cuticle were less pronounced than in the males. The conical tip of the antenna was less slim than the male and the 11 segments measured 2.0-2.1 mm. As in the male the last three segments were densely covered with hair sensillae. The ventral rear end of the female was blunt and characterized by a slit.

Courtship and mating

Fully matured males flew in a zigzag or spiral manner between branches or around a plant to land on the upper or underside of a leaf. It would remain stationary with the head and long neck stretched to the full (posturing) for 2-45 minutes before flying to a few more leaves nearer to the leaf with a female or directly to one with a female posturing on it or the one with a female starting to build a cradle. Posturing by males at the tip or top of a leaf appeared to give it vantage position in relation to other males and females on the plant. Sighting of each other was rather difficult as the plants chosen were usually in full leaf bloom and the number of individuals on a plant was not more than 4 or 5 individuals at any one time. It was most likely that posturing was for the male to detect the female sex pheromones and in the female to extend the distribution of her pheromones.

Once on the leaf with the female, the male bowed a few times touching the leaf surface with its mandibles. This could be followed by both the sexes remaining stationary facing each other or one behind the other with the heads and necks parallel to their bodies for periods of 1.5 to 12 minutes. In this position the necks were not stretched to the full as in posturing. After this the female moved off to continue working on the leaf to construct the cradle. The male might follow her immediately or after a few seconds to a minute.

Most females did not accept the males in his first few attempts to mount her. It was when the female was at the tip of the leaf starting to roll it that the male grabbed her to mount and copulate (Fig. 5). The female showed more interests to build the nest but the male persisted by following behind her or by waiting patiently on the same leaf before making more attempts. During the waiting period the male postured exhibiting his neck and form. One to five mountings without mating were noted before a female accepted a male. Most of the successful mountings took place at the tip of the leaf when the female was busy making the first roll of the leaf.

Occasionally a second male might fly (3 out of 12 observations) onto the leaf to challenge the first male. The fight that ensued was rather mild and might just be a neck stretching contest and a bit of neck wrestling (Fig. 6). The vanquished was than chased around the periphery of the leaf, occasionally pecked and finally pushed off the leaf. The courting of the female than continued and within less than a minute copulation took place and they remained locked for 12-28 minutes. During this time the female continued to perform the chore of cradle construction with a load on her back. After the male was disengaged the female continued to work on the cradle until it was finished.

There were variations to courting and mating in terms of approach, time taken to attract the female's attention, number of attempts at mounting, duration of copulation, challenge by other males and posturing locations on the leaf.

Cradle construction

The construction of a cradle was entirely done by the female though some males might pretend to fold the edges of the leaf on which a female was working on. Six stages of construction of a cradle could be recognized: leaf inspection and selection; incising or cutting of leaf blade; folding of leaf blade; inward bending of the leaf edges; leaf rolling; and collar seal to complete cradle construction.

The first step was an inspection routine to select a leaf by walking over both its upper and lower surfaces and touching various points with her mandibles. She might walk over one to five leaves usually within a radius of not more than 60 cm before deciding on the leaf of her choice. The leaf chosen was invariably the young, light-green, soft, second to the fourth terminal leaf. The size of the leaf appeared less important than the quality of the leaf on the plant that was chosen.

The chosen leaf was then incised or cut with her mandibles across at about its upper fifth, past the central vein and then slightly curving upward leaving a few secondary veins intact (Fig. 7). She might take a rest at the top of the leaf or continued with the

third stage of building, i.e., folding the leaf blade by clamping it along the central vein from the underside with her mandibles. This was followed by the inward folding of the leaf blade edges along the lower one third (Fig. 8). Occasionally the leaf-blades folding was preceded by the folding of the leaf edges. She might rest again inside the folded leaf where a male might be posturing and waiting to mount her. Then she went to the tip of the leaf and crimped it to create the crater for her single egg. Some females were not seen to take this step.

At this time, she might be mounted by the male. With or without a male on her back, she continued the next phase of rolling the leaf tip upwards and also folding the edges inwards. The strenuous work of rolling the leaf continued until the roll reached the upper third of the leaf. The final stage consisted of folding the incised part of the leaf to form a collar of darker green upper surface against the glaucous underside of the rest of the completed cradles. Table 2 shows the dimensions of completed cradles taken from three plants (n=36) and the bottom row of Table 1 indicates the number of cradles at the peak of the breeding period at Jalan Setiamurni 5, Damansara Heights, Kuala Lumpur in 2007.

After the completion of the cradle she walked over it then stood on top or side of it to rest for a while (4-8 minutes) before going off to a nearby leaf to feed. The time taken to complete a cradle ranged from 24 to 68 minutes (n=6). Various factors such as interference by the male, strong wind, leaf size and experience of the individual influenced the time taken and the finished quality of the cradle. Well-built cradles were watertight (Fig. 9). Though the attached portion of the cradles might be as narrow as 2 mm (2-15 mm, n=28) they could withstand relatively strong wind.

A female might incise several (<4) leaves before settling for a suitable leaf or might come later or the next day to use them for making more cradles. Such incised leaves were within 25 cm of each other. One female built one or two cradles a day, possibly three. The total number of cradles built by a female during the breeding period was not known from the field observations. Sighting of females on a plant on any given day was not more than five and the highest number of cradles on a plant was 76 nests over 30-day period (infested plants observed n=9).

At least 50% of the cradles remained green or mainly green and attached to the branches throughout the life-cycle of the beetle. A small percentage (n=4) of nests turned yellow and fell to the ground at pupal stage. All four pupae (different ages) kept at room temperature in the kitchen laboratory hatched after 1-7 days.

Table 2. Dimension (mm) of cradles constructed by the Long-necked beetle on three plants in Jalan Setiamurni 5, Kuala Lumpur in 2007.

Cradle	Plant A	Plant A1	Plant D
Length			
mean±SD	15.17±3.07	14.25±2.34	14.75±3.31
range	12-20	11-19	10-20
Top width			
mean±SD	5.83±1.53	5.92±1.16	5.25±1.54
range	4-9	5-8	3-8
Bottom width			
mean±SD	4.42±1.38	5.59±1.11	4.83±1.03
range	3-8	4-7.5	3-6.5

Metamorphosis

The complete metamorphosis (all within the cradle) from the egg to the emergence of adult from the cradle was estimated to be 28-35 days.

Egg-laying

The female laid a single egg which was glued to the inside of the fold of the selected leaf blade located close to the tip which was then rolled up to form the cradle. It was oval in shape, bright yellow in colour and measured 1.27 x 1.0 mm to 1.32 x 1.0 mm (Fig. 10). The shell was smooth. It was easily dehydrated on exposure to air and readily burst when it came in contact with water. The shell of unhatched eggs which might be due to infertility or infestation turned brown in colour, became wrinkled and brittle.

Hatching of the egg

Limited observations on plants (n=5) and in bottle incubated cradles (n=3) indicated the incubation period to be 72-144 hours. The newly hatched curve grey larva was about half the size as the egg.

Larval stages

The artificial rearing of larvae was not very successful. Most of them dried up or became infested by fungus after a few days. The tiny larva (0.66 x 0.33 mm) that broke out from the egg shell was curve in shape

and grayish in colour. The light brown mandibles were already visible. The apod/vermiform larva grew in length and width rapidly by consuming the inside folds of the cradle (Fig. 11). As it ate it passed out pelleted faeces (frass) which were encased in a thin membrane, extruding like sausages from its anal opening. Several instars preceded the pupa stage. The larval stage was estimated to last 14-21 days.

Pupa stage

The artificial rearing of pupa was relatively more successful. The exarate pupa was yellow in colour with prominent black eyes (Fig. 12). Most of the external features of the adult beetle could be clearly distinguished – the mouth parts, the head and neck and all the three pairs of legs were positioned close to the thorax and abdomen.

The development of the wings and the shortening and rounding of the abdominal segments followed. The pupa stage was estimated to last for 4-7 days. After this phase, the young beetle appeared with its membranous wings trailing beyond the last abdominal segment. The cuticle covering the beetle at this stage was yellow and light brown.

Juvenile

Newly transformed beetle from the pupa stage was yellowish in colour and it remained in the cradle for 4-6 days until it turned light orange. Young adult then emerged from the cradle by chewing an exit hole through the folds from inside to the outside. It was dark orange in colour and about 0.5-0.75 mm, smaller in length than mature adult. Males had distinctively longer and slender necks than females. Except for lighter colour and size the juvenile had all the features of the adult beetle.

DISCUSSION

This paper provides the initial field observations primarily from data collected in 2007 on the breeding behaviour of a long-necked beetle, also referred to as the giraffe-necked weevil found feeding and breeding only on a specific plant, *B. tomentosa*, in an urban environment. Another common name given to this group of beetles is the Leaf-rolling weevils/ beetles. The first two common names relate to their appearance while the latter to their laborious task of cradle building. These insects do not just roll leaves but they actually perform rather intricate work to construct a watertight barrel that houses the entire developmental stages of the next generation. They certainly deserve more recognition than just as rollers of leaves.

Descriptions by Kobayashi and Kato [4, 5] on the general behaviour of *Cycnotrachelus roelofsi* found on the plant *Styrax japonica* in the construction of cradles is quite similar to the species being described. As these weevils are host plant specific there is no doubt that this Malaysian species whose host plant is *B. tomentosa* is a different species.

Field observations on the behaviour of this beetle in an environment that is subjected to changes wrought by man, pose challenges in interpreting such observations. The host trees, for example, may suddenly be trimmed or chopped off by the City Hall gardeners. Fogging to control mosquitoes is also carried out at unknown intervals. These factors would affect both the host plants and the population of insects. The estimates on the length of time taken to complete each developmental stage will be tested in an experiment planned to be carried out in a green house.

Observations made in 2007 and 2008 indicate that the beetles' breeding peaked during the months of August and September. However, the location of highly infested plants in 2007 (Setiamurni) was different from 2008 (Bukit Tengku). Though the plants infested in 2007 were still present, very low infestation was noted as compared to plants in another area with similar but not identical environment. One factor that might have caused low infestation in 2008 in the Setiamurni plants was the heavy attacks on the leaves of the plants by other species of phytophagus beetles. Consequently few suitable healthy leaves were available for cradle construction. Longer-term observations would provide confirmation of the seasonal nature of breeding peaks and the relationships to leaf flushing of *B. tomentosa*.

In *C. roelofsi* found on *Styrax japonica* in Japan, there are two types of cradle/nest, suspended cradles and cut-off cradles which appeared to be influenced by climatic changes and survival factors [4]. The proportions of the two cradle types changed over time; the suspended type was dominant early in the season but was gradually replaced by the cut-off type. The survival rates were always higher in the cut-off type than in the suspended type.

No cut-off cradles have been noted in this study though an insignificant number of dropped cradles

were noted due to yellowing of leaves. Such dropped cradles could continue to support the last phase of metamorphosis of the beetle but unlikely at the egg-incubation and larval stages as the later requires green fodder as food to develop.

The predominant cause of beetle mortality in *C. roelofsi* [4] was highest at the egg stage and caused by parasitism by two minute trichogrammatid wasp species, *Poropoea morimotoi* and *P. cunabulintrans*. In the current study the causes of mortality at various stages were due to fungus, larvae of other insects and poor cradle construction. The parasitic larvae have not been identified to date. In another study also with *C. roelofsi* [5] the cradles constructed using mature, tough leaves were more effective against terrestrial cradle herbivores than those constructed using new, soft leaves.

In the current study in general well built cradles that were watertight were less impaired by the attacks by other beetles, wasps and fungus even when young soft leaves were used. Most cradles were constructed using young, soft leaves at the 2nd to the 4th apical nodes. Older cradles were found on older and harder leaves at the 5th to the 8th nodes. These cradles when built were at the 2nd to the 4th nodes but due to growth after a month were pushed back towards the trunk. Such cradles normally house late larval stages or pupae.

The host plant which provided feeds for the adult beetles and their larval stages as well as cradle building materials did not appear to be adversely affected by the infestation as all infested plants showed full leave bloom a month or two after the infestation.

The various types of hair sensillae observed on the antennae and other parts of the anatomy of this beetle deserve special attention and should stimulate further studies on micro-receptors structure and functions. Their roles in the breeding behaviour of the beetle need to be investigated.

Many questions remain unanswered with regards to the biology of this Malaysian Long-necked beetle. Among them are: (1) Is the seasonal peak the same throughout Peninsular Malaysia? (2) Do females share the same host tree? (3) How many nests does one female build and how many per day? (4) Are there cut cradles as in some of the temperate beetles? (5) Why does this beetle have such a long neck? (5) How many species are there in Malaysia? Studies are underway to find some of the answers.

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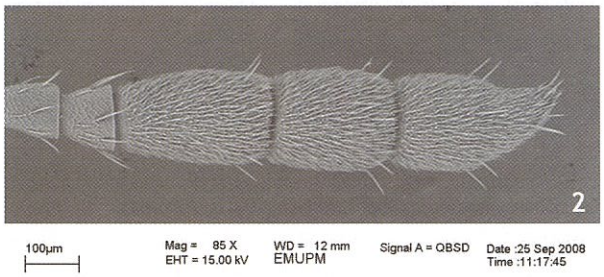


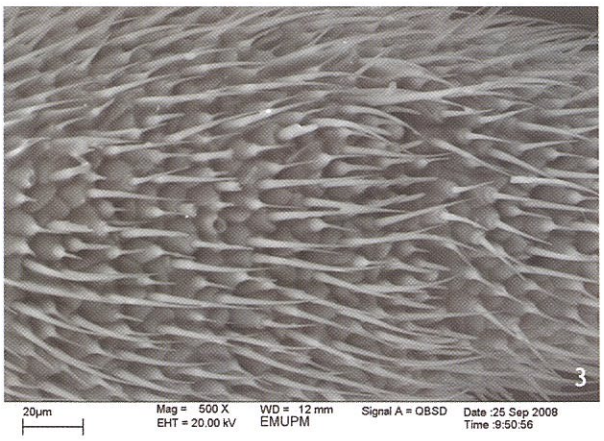
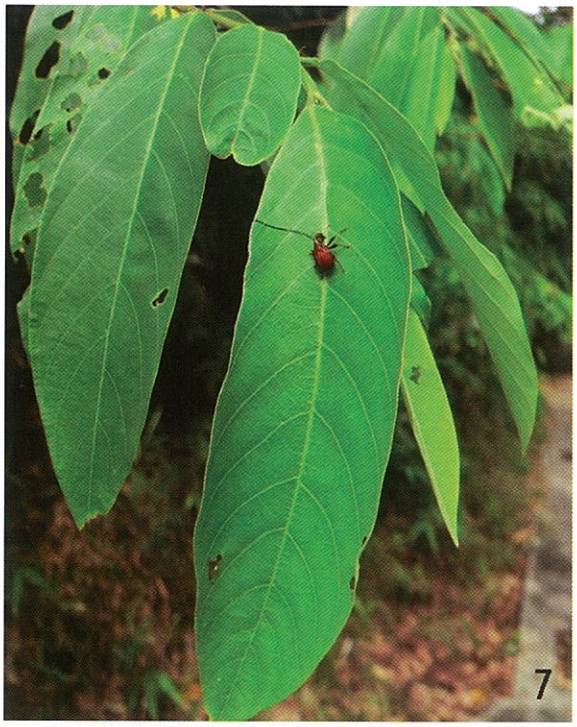
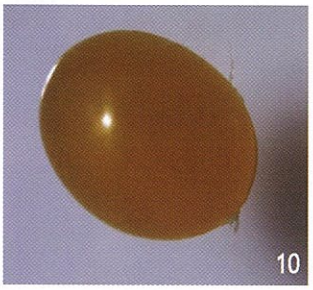
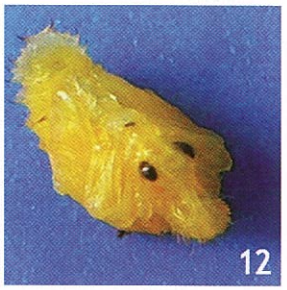


Figure 1. Adult male of *Cycnotrachelus* sp. – note the distinctively longer neck.
Figure 2. The last three segments of the antenna of *Cycnotrachelus* sp. are densely covered with sensillae.
Figure 3. Different types of sensory sensillae on male antenna of *Cycnotrachelus* sp.
Figure 4. Adult female of *Cycnotrachelus* sp. – note shorter and stouter neck.
Figure 5. Mating of *Cycnotrachelus* sp. while the female was building the cradle.

Figure 6. Two males of *Cycnotrachelus* sp. in a neck stretching contest as part of the courtship battle for a female.







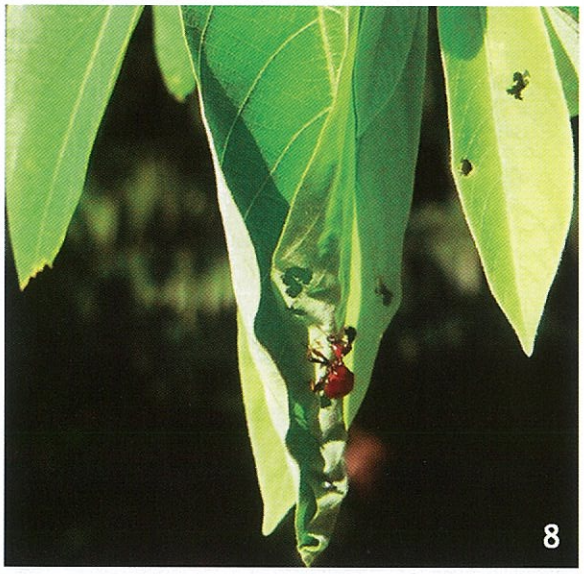


Figure 7. First stage of cradle construction by *Cycnotrachelus* sp. female – incising the leaf.
Figure 8. Third to fourth stage of cradle construction by *Cycnotrachelus* sp. female – folding leaf margins and tip.
Figure 9. Completed cradle constructed by *Cycnotrachelus* sp.
Figure 10. Fresh egg of *Cycnotrachelus* sp. taken from a cradle.
Figure 11. Larval stages of *Cycnotrachelus* sp. – fresh specimens.
Figure 12. Pupa of *Cycnotrachelus* sp. – live specimen.

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Telamonia dimidiata and *Phintella versicolor* (Arachnida: Salticidae): two new records for Peninsular Malaysia

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Abstract Two species of jumping spiders – *Telamonia dimidiata* (Two-striped Telamonia) and *Phintella versicolor* (Multi-coloured Phintella) – were collected in the garden of the Institute of Biological Sciences, University of Malaya. They are new records for Peninsular Malaysia.

Keywords jumping spiders – new records – Malaysia – *Telamonia dimidiata* – *Phintella versicolor*

INTRODUCTION

There are more than 40,000 known species of spiders in the world, grouped into some 3694 genera in 109 families [1]. The exact number of species in Malaysia and the surrounding region is not known, but most certainly runs into hundreds, and many species await to be discovered and named [2, 3]. Recently, a new genus (*Malayozodaria*) and four new species (*Mallinella gombakensis*, *Mallinella maruyamai*, *Mallinella tunidifemoris* and *Malayozodaria hoiseni*) of the Family Zodariidae were described from Peninsular Malaysia [4]. Many specimens at hand are believed to be unnamed species. This paper reports two new records of jumping spiders (*Telamonia dimidiata* and *Phintella versicolor*, Salticidae) for Peninsular Malaysia.

MATERIALS AND METHODS

For over three decades in the course of documenting the fauna (particularly odonates, lepidopterans and tephretid fruit flies) of the University of Malaya campus, spiders were observed, photographed and collected when needed for further taxonomic confirmation. While studying the colour polymorphism of the larvae of a damselfly in October 2008, two male *Telamonia dimidiata* (Fig. 1) and one male *Phintella versicolor* (Fig. 2) were sighted and photographed in the garden of the Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia. One male *T. dimidiata* and the only *P. versicolor* were collected and preserved in alcohol. The specimens were identified with existing literature [e.g. 5, 6].

RESULTS AND DISCUSSION

Only male spiders were seen in the garden of the Institute of Biological Sciences. They were found moving about on garden plants. The two male *T. dimidiata* (Fig. 1) were observed on different occasions. Likewise the male *P. versicolor* (Fig. 2) was seen on separate occasion.

The jumping spiders (Family Salticidae) are represented by some 560 genera and 5188 species in the world [1]. Salticid spiders of the genus *Telamonia* Thorell, 1887 are beautifully coloured. They are represented by some 36 nominal species and are distributed in the Oriental region [1]. The recorded species for Malaysia are: *T. annulipes* Peckham & Peckham, 1907 – Borneo, Sarawak; *T. bombycina* (Simon, 1902) – Borneo, probably Sarawak; *T. leuteocincta* (Thorell, 1891) – Malaysia; and *T. resplendens* Peckham & Peckham, 1907 – Borneo, Sarawak [1]. In addition, *T. festiva* Thorell, 1887 has been recorded to occur from Myanmar to Java and *T. hasselti* (Thorell, 1878) from Myanmar to Sulawesi. These species are therefore probably present in Malaysia. Indeed *T. festiva* (Jolly Telamonia) has been found in the University of Malaya campus [Yong, unpublished data] and it has been recorded in Singapore [5].

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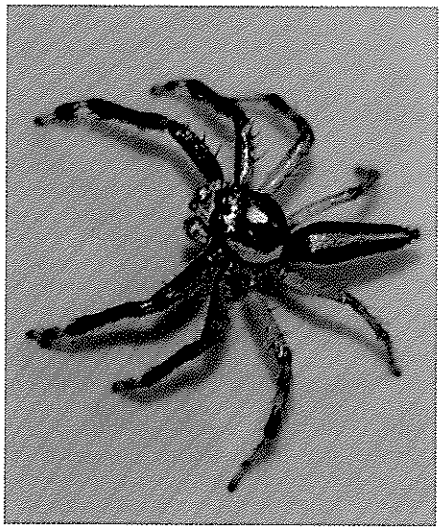

Figure 1. Male *Telamonia dimidiata.*

Figure 2. Male *Phintella versicolor.*

Telamonia dimidiata (Simon, 1899), commonly known as the Two-striped Telamonia, was originally described as *Viciria dimidiata*. Its type locality is Sumatra (Painan), Indonesia. It occurs in India, Bhutan and Sumatra [1]. As it has been reported in Singapore [5], its occurrence in Malaysia is not totally unexpected. The present finding records its presence in Peninsular Malaysia. In 2002, it became the subject of an email hoax – lurking under toilet seats and portrayed as a 'killer spider'. It is however non-venomous. The male spider is very dark, with white markings, and red hairs around the eyes. It measures 8-9 mm in length and the female 9-11 mm.

Salticid spiders of the genus *Phintella* Strand, 1906 are moderately small spiders, about 3-7 mm long with slender body. They are represented by some 41 species [1]. Of the species present in this region, the type locality of *P. suavis* (Simon, 1885) is Peninsular Malaysia; it occurs from Nepal to Malaysia [1]. The Banded Phintella, *Phintella vittata* (C. L. Koch, 1846), occurs from India to the Philippines [1] and has been reported for Singapore and Malaysia [5]. It occurs in the University of Malaya campus [Yong, unpublished data]. There are two other species which probably occur in Malaysia – *P. debilis* (Thorell, 1892) in Sumatra [6]; and *P. versicolor* (C. L. Koch, 1846) in China, Korea, Taiwan, Japan, Sumatra and Hawaii [1]. The Multi-coloured Phintella, *P. versicolor*, was originally described as *Plexippus versicolor*. It has been recorded in Singapore [5]. Its occurrence in Peninsular Malaysia is therefore to be expected.

The present report forms only a very small part of the numerous unrecorded and unnamed spiders of Malaysia. The University of Malaya campus alone is a treasure house of spiders as well as other animal life [7].

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Pre-logging survey of herpetological and mammal fauna at Lakum Forest Reserve, Raub, Pahang, Malaysia

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Abstract An extensive herpetological and mammal survey was undertaken from July 2002 through April 2003 at Lakum Forest Reserve, Raub, Pahang, Peninsular Malaysia prior to logging for the construction of a dam. A total of 190 species from these two taxa was identified. The herpetological fauna comprised 44 species of amphibians from 5 families and 56 species of reptiles of 11 families. The mammal fauna comprised 90 species of large and small mammals from 29 families.

During the process of clear-felling of the forest the amphibian group would be most impacted with a complete loss of all species in the area. Eighty-eight percent of the 56 species in the reptile group would also suffer the same fate as that of the amphibians with only the larger reptiles such as the pythons, rat-snakes and monitor lizards comprising 12% of reptiles having the ability to move into adjacent forest. The volant mammals would be the least affected, with only those that are residents in the under-storey stratification decimated. The non-volant mammals, the residential ground species such as rodents, some of the under-storey squirrels and insectivores would be totally impacted, while most of the larger mammal species such as the giant squirrels, tapir, tiger and small carnivores would have moved out during de-forestation.

Keywords pre-logging studies – herpetological species – mammal species – Lakum Forest Reserve – Pahang

INTRODUCTION

The Lakum forest reserve (LFR), Raub, Pahang, Peninsular Malaysia were to be inundated for a proposed dam for raw water transfer from Pahang to Selangor. This called for an extensive survey on the flora and fauna to assess species diversity prior to logging. The different disciplines in flora and fauna were undertaken by expert groups of scientists. We undertook the survey on herpetological and mammal taxa from July 2002 through April 2003, the results of which are presented herewith.

STUDY AREA

The Lakum forest reserve is about 15,552.60 ha in area. It is a logged over forest that was last logged in the 1980s as evidenced by the numerous old logging trails still in existence. The general habitat is one of a regenerating forest type covered with thick undergrowth forming a rich under-storey with an abundance of bamboo and rattan. Deeper into the forest the vegetation is more a good standing forest type where large and medium timber trees abound. There are numerous small and large streams with patches of swampy areas. During the arid period most of the smaller streams would dry out, with the swamp areas remaining swampy and moist.

Four representative study plots within LFR were selected for the survey (Fig.1). Plot 1 (3° 37.20' N 102° 00.36'E) was situated along Sg. Bakap, Plot 2 (3° 36.21' N, 102° 00.44' E) along Sg. Semuku, and Plot 3 (3° 35.08' N, 102° 01.02' E) along Sg. Temengat; these plots were adjacent to Felda Krau Empat. They represented the northern, middle and southern section of the forest respectively. Each of these plots measuring 2 km in area was spaced 1.5 km apart between each of the plots. Plot 4 (3° 35.50' N,

102° 08.08' E) was situated at the northeastern tip of LFR of which one-third of the study plot was in Krau Game Reserve bordering Sg. Tigris, a pristine forest. The plot was similar in size to Plots 1-3 (Fig. 1).

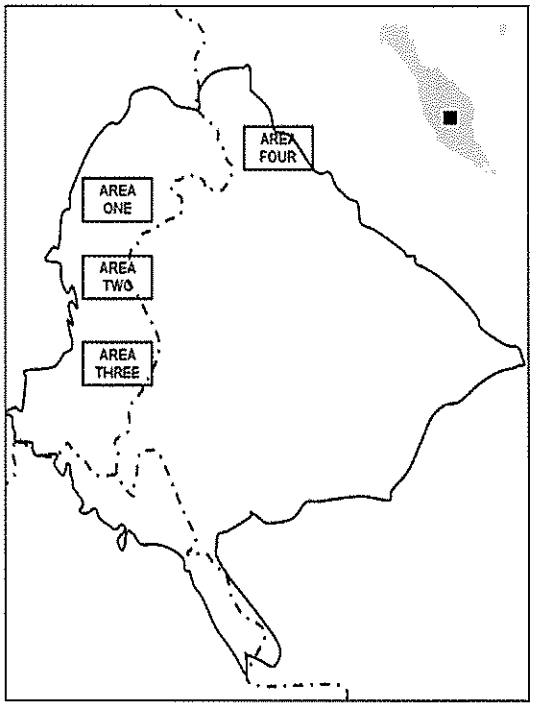

Figure 1. Study plots at Lakum Forest Reserve, Raub, Pahang.

Habitats of survey plots

The habitats of the survey plots 1-3 were similar to the overall habitat of the LFR, being a disturbed secondary forest. Plot 4 was supposedly a pristine forest, but due to rampant illegal logging and resident Orang Asli families (Che Wong tribe) there, the forest both at the LFR side and the one-third at the Krau Wildlife Reserve side was a highly disturbed secondary forest, similar in habitats to Plots 1-3.

METHODOLOGY

Five professionals, herpetologist, mammalogist, parasitologist and two para professionals supported by four game rangers and two Orang Asli composed the field team. Five field trips were carried out during the period from 31 July 2002 to 3 April 2003 by the same individuals of the field teams. In Plots 1, 2 and 3, four field trips were made from 31 July to 6 August 2002, 25 September to 1 October 2002, 6-12 January 2003, and 16-22 March 2003. Only one field trip from 28 March to 3 April 2003 was carried out in Plot 4.

Amphibians and reptiles

During daylight hours, the search for herpetological species was carried out in the forest by overturning dead logs and removing forest litter on the forest floor in search of terrestrial species, such as toads, burrowing snakes, skinks etc. Any reptiles such as snakes, lizards, flying lizards, geckos when sighted were recorded. Attempts were also made to capture the amphibians and reptiles by hand when possible. In the night the collection of toads and frogs was made along forest streams, swamps, mud pools and on the forest floor, and reptiles such as snakes, lizards and geckos clinging onto branches and trunks of trees sighted were recorded and hand-caught when possible.

Mammals

Large mammals

The large mammal survey was conducted based on direct and indirect observation. Indirect observation was based on signs left by wildlife such as faeces, wallows, tracks, vocalization and other obvious signs. The 'large mammals' included the elephant as the largest, and primates as the smallest. Other families included the deer, gaur, tapir, serow, bear and pig. 'Small mammals' include all other mammals in size smaller than primates.

Small mammals

The small mammal survey was conducted by using 100 collapsible traps to capture ground-dwelling under-canopy animals, particularly rodents. The traps were baited with a variety of fruits and they were not systematically placed, but in habitat niches with a higher chance of catches. For the capture of bats, 4 mist nets and 3 harp traps were deployed. These were placed on possible fly-ways of bats along the forest trail.

The harp traps were visited in the morning hours. The mist nets were opened from 8-11 pm while the harp traps were opened throughout 24 hours. Mist nets and harp traps were checked at 15 minutes interval till 11 pm, with the latter checked again during the morning hours together with the rodent traps. All animals trapped were transferred into cloth bags and taken to the field station for identification

and then released. While visiting the mist nets and harp traps, any nocturnal animals sighted were noted. During daylight hours, the search for diurnal mammals, particularly squirrels including any other animal species were also noted, and if possible were identified to either species or generic levels.

Voucher specimens of 1-2 individuals of amphibians and reptiles and bats were taken as reference collection. These were all deposited in the Museum at the Institute of Biodiversity of the Wildlife and National Parks at Bukit Rengit, Lanchang, Pahang.

RESULTS AND DISCUSSION

A total of 190 species comprising 100 species of herpetiles and 90 species of mammals was identified in the study plots 1-4 during the five survey trips from July 2002 to April 2003. The herpetological fauna comprised 44 species of amphibians from 5 families (Appendix1) and 56 species of reptiles from 11 families (Appendix 2). The 90 species of mammals were represented by 29 families (Appendix 3).

AMPHIBIANS

Bufonidae Four of the five species (Appendix 1: Nos.1-2, 4-5) of toads in this family were common toads of forest habitats. The fifth species *Bufo melanostictus* (Appendix 1: No.3) was associated with human habitation. Its presence in the forest trail might have been associated with the oil palm plantation neighbouring the forest where this toad was very common [1].

Megophryidae All three species of frogs in this family were common species of forest habitat (Appendix 1: Nos. 6-8). The very distinct sound of the Horn toad, *Megophrys nasuta* (Appendix 1: No. 6) was frequently heard at various parts of the forest suggesting the species was rather common.

Ranidae This family had the highest species diversity with 19 species collected from the study plots (Appendix 1: Nos. 9-27). All these species with the exception of one species are common ranids in forest habitats [1]. The exception was *Rana siberu* (Appendix 1: No. 19), a new record for Peninsula Malaysia [2].

Rhacophoridae All nine species of rhacophorid frogs collected in the study plots (Appendix 1: Nos. 28-36) are common species in forested habitats [1], with the exception of two species viz. *Rhacophorus tunkui* and *Theloderma* sp. (Appendix 1: Nos. 31, 35). *Rhacophorus tunkui,* first recorded from Sungai Jasin, Ulu Endau, Johor and Kuala Tahan, Taman Negara, Pahang was described as a new species to Peninsular Malaysia [3]. Subsequently in 1991 this frog was collected by the senior author in Ulu Gombak forest reserve (specimen in the Medical Museum, I.M.R. K.L; unpublished). With the present specimen, this frog is now known from four localities, two from Pahang, one each from Johor and Selangor. The *Theoloderma* sp. was a significant finding being the second specimen collected which constituted new locality records for LFR. The first and third specimens were obtained from Ulu Muda forest reserve, Kedah by UKM scientists and the fourth specimen was from Taman Negara, Pahang by Dennis Yong. The species was later described as *Theloderma licin* [4].

Microhylidae Of the eight species of microhylid frogs collected in the study plots, the most common were *Microhyla heymonsi* and *M. butleri* (Appendix 1: Nos. 39-40). Both these are commensal species which occur in gardens of human habitation, grassland, fields to forest habitats [1]. The less common species (Appendix 1: Nos. 37-38, 41-44) in the study plots had been recorded elsewhere in forested areas to be fairly common [1, 3; 5; 6].

REPTILES

The reptiles identified in the study plots comprised three taxonomic groups. The lizards were represented by five families, snakes by four families and turtles by two families (Appendix 2).

Lizards

Geckonidae The nine species of geckos (Appendix 2: Nos. 1-9) were all common inhabitants of forest habitats [7, 8]. Some of these species, *Gecko monarchus, Gehyra mutilata, Hemidactylus frenatus, H. garnotii* (Appendix 2: Nos. 2, 6-8) are commmensal species that inhabit human habitations [9].

Eublepheridae The only single species *Aelurocabates felinus* of this family was collected in one of the study

plots (Appendix 1: No. 10). This species is common in forest habitats but nowhere abundant [9]. This gecko is a collector's item by animal dealers.

Varanidae Three of the four varanid monitors of this family (Appendix 2: Nos. 11-13) were sighted in the study plots. The Water-monitor *Varanus salvator* and the Clouded monitor *V. nebulosus* are common in forest habitats and both these are commensals inhabiting a wide variety of habitats ranging from human habituated surroundings, plantations, swamps, grassland and plantations. The Harlequin monitor *V. rudicollis* is restricted to forest habitat and is nowhere abundant [10].

Agamidae All the nine species of agamid lizards (Appendix 2: Nos. 14-22) identified in the study plots are common in forested habitats [7]. One of these the Green-crested lizard *Broncocela cristatella* (Appendix 2: No.16) is also common around human habitations [9].

Scincidae Four species of skinks (Appendix 2: Nos. 23-26) were either sighted or collected. All these skinks are common in forested areas, while two of these viz. *Lygosoma bowringi* and *Mabuya multifasciata* (Appendix 1: Nos 24-25) are also commensals [9].

Snakes

The land snakes identified comprised two groups. The harmless group belonged to the families Colubridae and Pythonidae with the poisonous group from the families Elapidae and Viperidae.

Colubridae The colubrid snakes of this family have the highest species diversity with about 90 species known in Peninsular Malaysia [11]. Only 16 species were sighted and/or collected in the study plots. All the 16 species (Appendix 2: Nos. 27-42) are common species in forested areas [11]. Three of these snakes, the Oriental Whip snake *Ahaetulla prasina,* Common Malayan racer *Elaphe flavolineata* and the Indo-chinese Rat snake *Ptyas korros* (Appendix 2: Nos. 27,34,39) are commensal species, and also inhabit human surroundings [11].

Pythonidae The only two species in this family were both sighted and collected in the study plots (Appendix 2: Nos. 43-44). The Reticulated python *Python reticulatus* is very common while the Short-tailed python *P. brongersmai* though common is nowhere abundant [11]. Both the species according to the Orang Asli there were being collected as a food resource and also for commercial trading. Both species are 'PROTECTED' under the Protection of Wildlife Act 72 (Act 76), i.e. a licence is required to collect them.

Elapidae Three of the 10 species in this family were sighted and/or collected in the study plots (Appendix 2: Nos. 45-47). All three species are common in forested areas [11]. Two of these species, the Common cobra *Naja sumatrana* and the Banded Krait *Bungarus fasciata* are also common around human habituated surroundings, plantations and swamp forest. The Blue Malaysian Coral Snake is confined to forest habitat [12].

Viperidae Three species were collected in the study plots of 11 species recorded in this family in Peninsular Malaysia [11, 13]. Among them, the Sumatran pit viper *Parias* (*Trimeresurus*) *sumatranus* (Appendix 2: No. 48) is not common [11, 12], thus the presence of this species in the area constitutes an additional locality record in Peninsular Malaysia. The other two species *P.* (*Trimeresurus*) *hageni* and *Tropidolaemus wagleri* (Appendix 2: Nos. 49-50) are common in forested areas [11].

Turtles

The identification of the six species of turtles were from empty shells collected from the Orang Asli residents in one of the study plots, and a few life specimens from other study plots. They belonged to two families, the hard-shelled Bataguridae and the soft-shelled Trionychidae.

Bataguridae All the five species of turtles identified of this family (Appendix 2: Nos. 51-55) in the study plots are common in forested areas [14]. Based on the empty shells of these species found in the Orang Asli houses, it suggests that turtles in general are commonly collected as a food resource. According to the Orang Asli they also collected these turtles and any other species that they chanced upon for trading to animal dealers in the town. None of these species are protected under the Protection of Wildlife Act 72 [Act 76].

Trionychidae The Asian Softshell turtle *Amyda cartilaginea* (Appendix 2: No. 56) was identified on a carapace still fairly fresh in an Orang Asli's house in study plot 4. According to the Orang Asli, this turtle was highly sought for as a food resource and also for trading to the animal dealers in Raub. This species according to them was quite common in muddy rivers at LFR and could weigh anywhere from 10-30 kg and fetched high prices in Raub and Bentong. The species is 'TOTALLY PROTECTED' under the Protection of Wildlife Act 72 (Act 76).

MAMMALS

A total of 90 species of small and large mammals was identified in the study plots at LFR. They comprised 29 families from ten orders viz. Insectivora, Dermoptera, Chiroptera, Scandentia, Primates, Pholidota, Rodentia, Carnivora, Perissodactyla and Artiodactyla respectively (Appendix 3: Nos. 1-90).

Order Insectivora

Erinaceidae The Moonrat *Echinosorex gymnurus* (Appendix 3: No. 1) was sighted in one of the study plots in a swamp area. The species is fairly habitat specific, and is always associated with water bodies in forested areas. Though insectivorous in habit, it has a preference for fish [15].

Order Dermoptera

Cynocephalidae The single species, Flying lemur *Cynocephalus variegatus* in this family (Appendix 3: No. 2) was sighted in one of the study plots. Although only a single individual was sighted, according to the Orang Asli there, the flying lemur was often sighted by them during their visits to the forest collecting forest products. It feeds on a variety of leaves and in coconut plantations feed on the flower of the coconut [16].

Order Chiroptera

A total of 40 species of bats was netted in the study plots. These comprised nine species of fruit bats in the family Pteropodidae. The 31 species of insectivorous bats belonged to seven families, viz. Vespertionidae (13 species), Hipposideridae (8 species), Rhinolophidae (6 species) and one species each from the families Emballonuridae, Nycteridae, Megadermatidae and Molossidae (Appendix 3: Nos. 3-42).

Pteropodidae All the nine species identified (Appendix 3: Nos. 3-11) in the study plots are common species of forested areas [17] except the Short-nosed fruit bat *Cynopterus sphinx* (Appendix 3: No. 5) which is not common and can be easily mistaken as the Horsefield's fruit bat *C. horsefieldii,* but differs by the lack of peg-like cusp on lower cheek teeth. The distribution pattern of *C. sphinx* is not well known, thus it requires further study.

Emballonuridae, Nycteridae, Megadermatidae, Mollossidae A single species each from these families was netted in the study plots. All these species i.e *Emballonura monticola, Nycteris tragata* (=*N. javanica*), *Megaderma spasma* and *Mops mops* (Appendix 3: Nos. 12-14, 42) are widely distributed throughout the country [18].

Rhinolophidae Six species (Appendix 3: Nos. 15-20) were identified in the study plots. All are common under-storey forest bats. One of these *Rhinolophus affinis* (Appendix 3: No. 15) is more confined to caves [18].

Hipposideridae Eight species were identified (Appendix 3: Nos. 21-28). Like the rhinolophid bats, all the hipposiderid bats are common forest species. All are cave dwellers [18] with the exception of *Hipposideros ridleyi* (Appendix 3: No. 25) which normally roosts in culverts and in crevices of dead tree trunks and fallen logs. They roost in groups of 2 to 15 individuals. The species was once thought to be uncommon, however in recent years they were found to be fairly common in lowland forest (B. L. Lim, unpublished data).

Vespertilionidae This family has the highest species diversity with at least 42 species in Peninsular Malaysia [18]. Thirteen species were identified (Appendix 3: Nos. 29-41) in the study plots. Of these seven species (Appendix 3: Nos. 30-31, 34-35, 39-41) were found to be not as common in the area. However none of the 13 species are considered rare, generally they are common in forested areas with widespread distribution throughout the country [17].

Order Scandentia

Tupaiidae The Common Tree-shrew *Tupaia glis* (Appendix 3: No. 43) was very common in the

area. This species once was confined to secondary and primary forests. In the recent two decades, this species is now a common commensal animal within human habitation both in urban and rural areas. Its adaptability to landscape and environmental changes enhances its survival like any commensal animal species, such as the house and field rats in nature [19].

Order Primates

Lorisidae The single species of this family in Peninsular Malaysia [17] was sighted in one of the study plots (Appendix 3: No. 44). Though only a single individual was sighted, according to the Orang Asli the animal had been occasionally collected for medicinal and charm purposes. The species is common but nowhere abundant [20, 21]. It is 'TOTALLY PROTECTED' under the Protection of Wildlife Act 72 (Act 76).

Cercopithecidae Four of the six species of monkeys in this family [17] were sighted in all the study plots (Appendix 3: Nos. 45-48). All these species were fairly common in the area, the most common and abundant was the Long-tailed macaque *Macacca fascicularis* (Appendix 3: No. 45). The two species of leaf monkeys, the Banded and Dusky leaf monkeys (Appendix 3: Nos. 47-48) were often hunted for food by the Orang Asli there. All the four species of monkeys are 'TOTALLY PROTECTED' under the Protection of Wildlife Act 72 (Act 76).

Hylobatidae The presence of the Lar gibbon *Hylobates lar* (Appendix 3: No. 49) was based on vocalization heard during some of the morning and late afternoon calls. According to the Orang Asli there, this animal was uncommon in the area. The species is 'TOTALLY PROTECTED' under the Protection of Wildlife Act 72 (Act 76).

Order Pholidota

Manidae The Scaly anteater *Manis javanica*, only species in this family (17), was based on scales collected in one of the study plots (Appendix 3: No. 50). According to the Orang Asli there, the animal was quite common in the forest and often captured for food. It is a 'TOTALLY PROTECTED' animal under the Protection of Wildlife Act 72 (Act 76).

Order Rodentia

Sciuridae The species diversity of the lowland diurnal tree and ground squirrels was exceptionally high at LFR. All 11 lowland species (Appendix 3: No. 51-61) were present in the area where 9 species were trapped and 2 species of Giant squirrels were sighted. All these species are common and widely distributed throughout the country [17]. Only *Ratufa bicolor, R. affins* and *Callosciurus prevosti* (Appendix 3: Nos. 51-52, 54) are 'TOTALLY PROTECTED' by law.

Pteromyidae Three species of flying squirrels were identified in the study plots (Appendix 3: Nos. 62-64). Of these only *Petinomys verdermanni* was caught in a trap whereas the other two larger ones i.e. *Petaurista petaurista* and *Aeromys rephromelas* were sighted. All the three species are common in forest areas with none anywhere abundant. They are all widely spread throughout Peninsular Malaysia [17] and are 'TOTALLY PROTECTED' by law.

Rhizomyidae The Common bamboo rat *Rhizomys sumatrensis* was sighted in the study plot (Appendix 3: No. 65). This species though common in forest of bamboo habitat is nowhere abundant [22]. The species in the last two decades is fast dwindling in numbers due to hunting by the Orang Asli as food. This animal should be 'TOTALLY PROTECTED' failing which there will be a lack of gene pool in any forest types to replenish the stock in nature.

Muridae Eight species were trapped in the study plots (Appendix 3: Nos. 66-73). All are common forest species with the exception of the Wood rat *Rattus tiomanicus* (Appendix 3: No. 66) which is a commensal species. Its presence in the disturbed forest is influenced by the neighbouring oilpalm plantations where this species is very abundant and is also the primary vertebrate pest.

Hystricidae The Common porcupine *Hystrix brachyrus* (Appendix 3: No. 74) was identified by the quills found in the study plots. The presence of the quills suggests that the species had been poached by hunters. According to the Orang Asli there, this porcupine was fairly common in LFR. Like the Bamboo rat *Rhizomys sumatrensis* (Appendix 3: No. 65), this porcupine is a favourite food item for the Orang Asli. This species is 'PROTECTED' by law.

Order Carnivora

Ursidae The presence of the Malayan bear *Ursus malayanus* (Appendix 3: No. 75) in one of the study plots was based on claw marks and the stripped bark on the tree trunk. According to the Orang Asli, this creature was quite common in LFR. However, during our entire period there, this was the only sign of the Honey bear. This species is 'TOTALLY PROTECTED' by law.

Felidae Three species of wild cats were identified in the study plots. The tiger *Panthera tigris* (Appendix 3: No. 76) in the area was based on footprints sighted both on the trail and in swampy areas. The identification of the tiger footprint was confirmed by Game Rangers and the Orang Asli. The Panther *P. pardus* and the Leopard cat *Prionailurus bengalensis* (Appendix 3: Nos. 77-78) were sighted by team members and Game Rangers. According to the Orang Asli there the tiger was not common, panther was more often seen and the leopard cat was quite common in the forest. The leopard cat has in recent years adapted itself well to more open habitat and is often reported as a predator to poultry farms around human habitation in rural areas [23]. All the cat species are 'TOTALLY PROTECTED' by law.

Mustelidae Two species, the Small-clawed otter *Aonyx cinerea* and Yellow-throated marten *Martes flavigula* (Appendix 3: Nos. 79, 80), were sighted during daylight hours. Two individuals of otters were sighted playing in the stream but according to the Orang Asli there this otter was quite common in the area. The Yellow-throated marten, sighted bouncing through the jungle path, is an uncommon animal [17]. Both the species are 'TOTALLY PROTECTED' by law.

Herpestidae Only the Brush-tailed mongoose *Herpestes brachyura* (Appendix 3: No. 81) was sighted by team members along the forest stream during the day. This species is the next most common among the four species recorded in Peninsular Malaysia [17].

Viverridae Five of the 11 species recorded in Peninsular Malaysia were sighted (Appendix 3: Nos. 82-86). The Common Palm civet and Small-toothed civet (Appendix 3: Nos. 82-83) were sighted on tree branches in the night. The other three species, the Masked Palm civet, Banded Musang and Little civet were sighted during the day along forest paths and forest streams. All three civets are common in forest areas with the exception of the Banded Musang which is uncommon in Peninsular Malaysia [17, 24].

Order Perissodactyla

Tapiridae The Tapir *Tapirus indicus* present in the study area was based on footmarks identified by the Game Rangers and Orang Asli. The footmarks were found in the swampy area of one of the study plots. According to the Orang Asli there, the tapir was occasionally sighted by them during their routine visits to the forest in search of forest products. However, based on our sighting of the footprints it did not give the impression that the animal was common in the area. This species is 'TOTALLY PROTECTED' by law.

Order Artiodactyla

Suidae, Tragulidae, Cervidae A species each from these three families was identified in the study areas. The presence of the wild pig *Sus scrofa* (Appendix 3: No. 88) was based on footprints, while the mouse-deer *Tragulus javanicus* and Barking deer *Muntiacus muntjak* (Appendix: 3: Nos. 89-90) were sighted during the night. All the three species according to the Orang Asli there were quite common often hunted by the Orang Asli and hunters. During our period there, we came across empty cartridge shells in the forest trails. These animals are classified as 'Game animals' whereby a Game permit is granted to hunt them during open seasons. They are 'PROTECTED' by law.

HABITAT NICHES

Within any one habitat it is possible to divide the animals by the different zonation niches at which they find food, and whether they are active by day or night [25]. Such a classification aids in understanding why some animals can survive the destruction of the forest while others cannot. Based on this classification, it is possible to divide the mammal and herpetological fauna at LFR into four layers of occupational habitats as follows:

(1) Upper-air Community: Insectivorous bats which hunt above tree level e.g the Free-tailed bat and the Flitter mice of the families Molossidae and Vespertilionidae (Appendix 3: Nos. 29-42).

(2) Canopy community: Animals confined to the

crowns of trees feeding predominantly on leaves, fruits, nectar or insects and mixed-feeders. The diurnal mammals include the Leaf monkeys, Lar gibbon, Giant-tree squirrels of the families Cercopithecidae, Hylobatidae and Sciuridae (Appendix 3: Nos, 47-48, 49, 51-52). The nocturnal mammals are the Flying lemur, Fruit bats, Slow loris and Flying squirrels of the families Cynocephalidae, Pteropodidae, Lorisidae and Pteromyidae (Appendix 3: Nos. 2, 3-11, 44, 62-64). Diurnal reptiles are the Flying lizards of the family Agamidae (Appendix 2: Nos. 17-20).

(3) Middle-zone: Flying animals exclusively insectivores such as the Sheath-tailed bat, Slit-faced bat, False vampire bat, Horseshoe bats, Round leaf Horseshoe bats of the families Emballonuridae, Nycteridae, Megadermatidae, Rhinolophidae and Hipposideridae (Appendix 3: Nos. 12-28).

Sensorial animals range up and down the trunks and lianes entering both the canopy and the ground layers. The smaller members are all mixed feeders on both fruit and insects with the larger ones carnivorous in habit.

The diurnal mammals include the Tree shrews, Macaques, Tree squirrels and Bear of the families Tupaiidae, Cercopithecidae, Sciuridae and Ursidae (Appendix 3: Nos. 43,45-46, 53-59, 75). The nocturnal mammals are the Tree rat, marten, civets of the families Muridae, Mustelidae and Viverridae (Appendix 3: Nos. 70, 80, 82-85). The diurnal reptiles are the Monitor lizards, Crested lizards, Tree-snakes and Python of the families Varanidae, Agamidae, Colubridae and Boidae (Appendix 2: Nos. 11-13, 14-16, 21-22, 27-33, 35-26, 42-43). Geckos and Vipers of the families Geckonidae, Eublepharidae and Viperidae (Appendix 2: Nos. 1-10, 48-50) are nocturnal reptiles. The nocturnal amphibians are the Tree frogs of the family Rhacophoridae (Appendix 1: Nos. 28-36).

(4) Terrestrial-zone: Large animals living on the ground, without climbing activity, feeding by browsing on leaves or on fallen fruits or tubers together with attendant large carnivores (this group does not lend itself to a division into diurnal and nocturnal forms). Tiger, Panther, Tapir, Pig, Mousedeer, Barking deer of the families Felidae, Tapiridae, Suidae, Tragulidae and Cervidae (Appendix 3: Nos. 76-77, 87-90).

Small mammals that burrow in or search the litter and perhaps the lower part of tree trunk are predominantly either insectivorous or mixed feeders, but some entirely vegetable feeders with attendant carnivores.

The diurnal animals are represented by (a) mammals – Ground squirrels and otter of the families Sciuridae and Mustelidae (Appendix 3: Nos. 60-61, 79); and (b) reptiles – Skinks of the family Scincidae (Appendix 2: Nos. 23-26).

The nocturnal animals are represented by (a) mammals – Moonrats, Anteater, Bamboo-rat, forest rats, porcupine, leopard cat, civet and mongoose of the families Erinaceidae, Manidae, Rhizomyidae, Muridae, Hystricidae, Felidae, Viverridae and Mustelidae (Appendix 3: 1,50, 65, 66-69, 71-73, 74, 78, 81, 86); (b) reptiles – Colubrid snakes, Python, Elapid snakes and turtles of the families Colubridae, Pythonidae, Elapidae, Bataguridae and Trionychidae (Appendix 2: Nos. 34, 37-41, 44, 45-47, 51-56); and (c) amphibians – Bufonids, Megaphryids, Ranids, and Microhylids of the families Bufonidae, Megaphryidae, Ranidae and Microhylidae (Appendix 1: Nos. 1-5, 6-8, 9-27, 37-44).

The association of mammal and herpetological fauna within the different layers of the forest with estimated impact upon the mammal and herpeto-fauna by distribution of the forest is shown in Table 1.

Table 1. Feeding habits of the different taxa of animals at different layers of the forest. UAC, Upper air community; MZ, Middle-zone; CC, Canopy community; TZ, Terrestrial –zone; EM%, percentage of estimated mortality.

Animal taxa	No. spp.	UAC	CC	MZ	TZ	EM %
Volant mammals (bats)	40	14	9	17	-	18 (45%)
Non-volant mammals (Non-flying mammals)	50	-	10	17	23	27 (54%)
Reptiles (Lizards, Snakes, Turtles)	56	-	4	32	20	49 (88%)
Amphibians (Toads and Frogs)	44	-	-	9	35	44 (100%)
No. of species	190	14	23	75	78	138 (73%)

Volant mammals None of the species at the Upper Air Community (Appendix 3: Nos. 29-42) will be affected by the clear-felling of the forest. All these species are aerial-feeders on insects and can easily move out of the destructive forest to the neighbouring forest areas. However, in the canopy zone and middle zone layers, at least 6 out of 9 species of fruit bats (Appendix 3: Nos. 3-4, 8-11), and 15 of 17 species of insect bats in the middle zone layer (Appendix 3: Nos.12-20, 24-27) would be affected. All these species roost among crevices of rock boulders and hollows of tree trunks. The 3 species of fruit bats and 4 species of insect bats (Appendix 3: Nos. 5-7, 21-23, 28) not affected by deforestation are cave roosters which are visitors to the forest in search of food. Thus, of the 40 species of bats identified at LFR, 18 or 45% of the species would perish due to clear felling of the forest.

Non-volant mammals In the canopy zone 7 of the 10 species, the leaf monkeys, lar gibbon, Giant Tree squirrels and Giant Flying squirrels (Appendix 3: Nos. 47-49, 51-52, 62-63) would not be affected, as all these highly mobile species would move out when the forest is in the process of felling. The Flying lemur, Slow loris and the Small flying squirrel (Appendix 3: Nos. 2, 44, 63) would be impacted by the clear felling of the forest as these species are under-storey inhabitants. In the middle zone 9 of the 18 species, the macaques, bear, marten and civets (Appendix 3: Nos. 45-46, 75, 80-85) would have moved out to adjacent areas of the forest during the clearing of the forest for these species are highly mobile. The inhabitants of the terrestrial zone would suffer the highest fatalities as most of the species are either semi- or permanent residents. Of 23 species identified, only 7 species, the tiger, panther, leopard cat, marten, tapir, pig and barking deer (Appendix 3: Nos.76-78,80,88,90) would have moved out to adjacent forest areas. The remaining 16 species of small mammals, the Moonrat, Anteater, Bamboo rat, Forest rats, porcupine, otter and the small civet (Appendix 3: Nos. 1, 50, 65-69, 71-74, 79, 86, 89) would fall victim to the clear felling of the forest. In all, of the 50 species identified at LFR, 27 of these would perish due to the clear felling of the forest.

Reptiles All four species of flying lizards in the canopy zone (Appendix 2: Nos. 17-20) would be affected by the clear felling of the forest as these species have rather short flight range and normally rest on the trunks in the under-storey of the forest. In the middle zone at least 7 of 32 species (Appendix 2: Nos. 12-14, 29-31, 43) would move out during the destruction of the forest. These are large reptiles of high mobility, while the remaining 25 species of geckos, crested lizards, tree snakes and vipers are under-storey inhabitants with very short range of movement would perish due to the clear felling of the forest. Similarly, in the terrestrial zone all 20 species of skinks, colubrid snakes, short tailed python, elapid snakes and turtles would also perish as all of them are living as burrowers, under forest litters, holes on the ground and in streams. Altogether 49 or 88% of reptile species identified at LFR would perish due to the clear felling of the forest.

Amphibians All 9 species of tree frogs in the middle-zone and the 35 species in the terrestrial zone would perish upon the total destruction of the forest, giving 100% decimation of the taxa identified at LFR (Appendix 1: Nos 1-44). This is because these amphibian species are fairly restricted within their habitats with many of them fairly static in their mobility range.

Based on the analysis of the different stratification of habitat-niches of the mammal and herpetological fauna at LFR, it is without doubt that the overall expected impact on both these groups of animals caused by clear felling within the dam area is most serious. Animal taxa such as the amphibian group which are residents with limited range of mobility would be totally wiped out. None of the 44 species of amphibians identified at LFR in the middle or terrestrial zones is expected to survive under such traumatic phenomena. Similarly, the reptile group, 49 or 88% of 56 species recorded in the three stratification zones would suffer the same fate as that of the amphibians. The 9 species that could survive are large reptilian species with a wider range of mobility.

In contrast, the volant species are least impacted by clear felling of the forest. This is due to most being bat species that are transient species where the forest is but part of its flight range in search of prey. In LFR, only 18 or 45% of the 40 species would fall as victims as all these species are residents of the under-storey of the forest. The non-volant mammals, on the other hand, are more vulnerable to habitat loss than the volant mammals. In LFR, 27 or 54% of the 50 species within the three stratification zones would be

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Editorial

With the joyous celebration of Christmas, it is relevant to consider the "Joy of Discovery" by explorers and more so by scientific explorers – the research scientists. At the recent BBC round-table discussion of the newly-elected Nobel Laureates, two of the Nobel minds have shared their experiences in making scientific discoveries – the exhilarating feelings of great joy at the moment of truth. I am certain that many research scientists have encountered similar experiences. This is indeed the prize of researchers especially in fundamental work.

Prior to discovery, one may wonder what prompts a researcher to undertake a research project. Let me take an example of organic synthesis and in the words of Robert Burns Woodward (1965 Nobel Prize for Chemistry):

> "Should I ask here explicitly why the chemist synthesizes things? From the point of view of pure science the question answers itself and, beyond that, there are obvious practical reasons for such activities, which are certain to become more compelling in the future. But I should like to mention here a basis for action more related to the spirit of man. The structure known, but not yet accessible by synthesis, is to the chemist what the unclimbed mountain, the uncharted sea, the untilled field, unreached planet, are to other men. The achievement of the objective in itself cannot but thrill all chemists, who even before they know the details of the journey can apprehend from their own experience the joys and elations, the disappointments and false hopes, the obstacles overcome, the frustrations subdued, which they experience who traverse a road to the goal. The unique challenge that chemical synthesis provides for the creative imagination and the skilled hand ensures that it will endure as long as men write books, paint pictures, and fashion things which are beautiful, or practical, or both."

Academician Emeritus Professor
Tan Sri Datuk Dr Augustine S. H. Ong
Co-Chairman

impacted by the clearing of the forest, for most of the small mammal species are residential species, such as the shrews, tree squirrels and forest rats. The larger mammal species i.e. tigers, leopards, bear etc. which are transient species and highly mobile would not be impacted.

In sum, the impact on the mammal and herpetological fauna by the clear felling of LFR would account for 73% decimation of the 190 species identified for the area.

During the five weeks survey at the Lakum Forest Reserve (LFR) from July 2002 to April 2003, the species diversity of amphibians, reptiles and mammals was assessed as quite high, with a total of 190 species. All the 100 species of amphibians and reptiles and 90 species of small and large mammals are common in lowland forests elsewhere throughout Peninsular Malaysia [11, 12, 17, 18, 26]. Two species of amphibians are considered significant discovery. They are *Rana siberu,* a first record for Peninsular Malaysia [2] and *Theloderma licin*, a new species [4]. The rest of the 42 species of amphibians are common species throughout the lowland forests in the country [1, 6, 27, 28].

As with many comprehensive surveys, there are bound to be differences with respect to species diversity that can be found in the respective areas. This is attributed to varying factors, such as methodologies employed, time span, season, number of personnel and condition of the forest, etc. A comparison of the vertebrate species diversity from three other forest areas where the senior author participated is presented in Table 2.

The duration of research activities varied from 5 to 48 weeks in these areas. In Weng Subcatchment, Ulu Muda Forest Reserve, Kedah, the 48 weeks activities were over a period of 3 years, that of Krau Wildlife Reserve, Pahang of 16 weeks over a period of 2 years, and that of Temenggor Forest Reserve, Perak of 14 weeks in 2½ years compared to Lakum Forest Reserve of 5 weeks within 1 year. The four component animal taxa (amphibians, reptiles, volant mammals and non volant mammals) in Weng Subcatchment totaled 196 species [29, 30], Temenggor Forest Reserve 195 species [31-34], Krau Wildlife Reserve 221 species [35-38] and the present study at Lakum Forest Reserve 190 species.

Comparing the results from the four forest reserves, the species diversity of the four component taxa is more or less equally diverse. The number of species for each of the component taxa also shows no marked difference ($p>0.05$) irrespective of the time expended in each of the forest areas. The results from this comparison indicate the 190 vertebrate species found in the Lakum Forest Reserve is probably approaching the asymptotic level within the five weeks of intensive research activities (Fig. 2). There were 221, 195 and 196 vertebrate species in KWR, TFR and UMFR covering a period of 18, 14 and 48 weeks of field activities respectively. The mean total catch for the four FRs is about 200 species. KWR has a slightly higher total (but still not significantly

Table 2. Comparison of animal taxa from four different areas in Peninsular Malaysia.

Areas	Amphibians	Reptiles	Volant mammals	Non-volant mammals	Total catch (% of 802)	No. of working weeks
Lakum Forest Reserve, Pahang (LFR)	44 (25.00%)	56 (33.70%)	42 (22.80%)	48 (21.20%)	190 (23.70%)	5
Krau Widlife Reserve (KWR)	47 (26.70%)	55 (33.10%)	51 (27.70%)	68 (37.10%)	221 (27.50%)	16
Temenggor Forest Reserve (TFR)	44 (25.00%)	52 (31.30%)	42 (22.80%)	57 (25.20%)	195 (24.30%)	14
Weng subcatchment, Ulu Muda Forest Reserve Kedah (UMFR)	41 (28.10%)	53 (31.20%)	49 (23.30%)	53 (23.40%)	196 (24.40%)	48
Total	176	216	184	226	802	

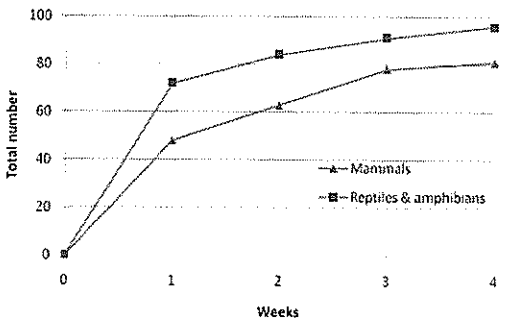

Figure 2. Total cumulative number of species captured over time.

different) which is attributed to its relatively less disturbed state and presence of a wider altitudinal range.

In conclusion, this simple analysis indicates that a minimum time span of at least 4-5 weeks field activities spanning over a period of not less than 4 months is essential to obtain a reasonable database of vertebrate fauna in forested areas. This minimum period of assessment could also be applied to the Rapid Assessments for EIA (Environmental Impact Assessment) for development projects undertaken by Government agencies and private sectors to ensure that at least 80-85 % of the vertebrates are sampled.

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Appendix 1. Amphibian species examined and sighted at Plots 1-4, Lakum Forest Reserve during the period April 2002 – April 2003. Figures, hand caught; Figures in bracket, number counted through vocalization; V, vocalization; VSC, Voucher specimen collected.

	Species	Plot 1	Plot 2	Plot 3	Plot 4	Remarks
Family Bufonidae						
1	*Bufo asper*	7	9	13	3	Along bank of streams and in streams: (2 VSC)
2	*B. parvus*	2	2	2	3	On forest floor and banks of streams (4 VSC)
3	*B. melanostictus*	-	-	1	3	Orang Asli compound (1 VSC)
4	*Leptophryne borbonica*	-	-	1	3	On stream banks (2 VSC)
5	*Pedostibes hosii*	2	-	-	-	On forest floor and banks of streams (1 VSC)
Family Megophryidae						
6	*Megophrys nasuta*	2	V (1)	V (1)	1	Highly vocalised in the forest: on forest floor (1 VSC)
7	*Leptobrachium hendricksoni*	1	-	1	2	On forest floor (2 VSC)
8	*L. nigrops*	-	1	-	-	Peat swamp habitat (1 VSC)
Family Ranidae						
9	*Limnonectes blythi*	15	19	10	5	In forest streams (4 VSC)
10	*L. paramacrodon*	4	3	1	-	In forest streams (2 VSC)
11	*L. laticeps*	2	2	-	-	In water puddles on open space. (2 VSC)
12	*L. malesianus*	-	-	-	1	Peat swamp
13	*L. plicatellus*	-	1	-	-	Stream bank (1 VSC)
14	*Fejervarya limnocharis*	-	5	-	5	Shallow pools at edges of stream. (2 VSC)
15	*Rana erythraea*	6	7	9	1	On banks of streams. (1VSC)
16	*R. glanulosa*	5	5	4	1	Highly vocalised in forests (2 VSC)
17	*R. baramica*	4	-	2	-	River banks and in streams (2 VSC)
18	*R. signata*	10	6	4	2	In forest streams and highly vocalised. (4 VSC)
19	*Rana siberu*	1	-	-	-	On branch of tree near pool side.(1VSC)
20	*R. hosii*	1	-	-	-	In forest streams and highly vocalised. (1 VSC)
21	*R. chalconota*	14	12	16	-	River banks and on leaves of bushes. (2 VSC)
22	*R. hascheana*	1	-	-	-	River banks and on leaves of bushes. (1 VSC)
23	*R. miopus*	2	4	3	6	River banks (2 VSC)
24	*R. nigrovittatus*	2	-	3	-	River banks (2 VSC)
25	*R. nicobariensis*	1	3	-	1	On forest floor near streams. (2 VSC)
26	*Occidozyga laevis*	3	2	2	7	In water puddles on forest trails (2 VSC)
27	*Amolops larutensis*	1	-	-	-	In water puddles on forest trails (1 VSC)
Family Rhacophoridae						
28	*Polypedates leucomystax*	8	6	7	14	On bushes along open trails and highly vocalised. (2 VSC)
29	*P. macrotis*	6	7	2	-	In bushes and on branches of low trees (2 VSC)
30	*P. colletti*	3	3	-	-	On branches of low trees (2 VSC)
31	*R. tunkui*	-	1	-	-	On bushes of forest trail (1 VSC)
32	*Rhacophorus nigropalmatus*	1	2	-	1	On bushes of forest trail. (1VSC)
33	*R. appendiculatus*	7	8	13	-	On tree branches and in bushes beside streams (5 VSC)
34	*R. pardalis*	1	1	-	-	On branches of low tree (2 VSC)
35	*R. reinwardtii*	-	-	-	3	On branches of tall trees 15 ft high (1 VSC)
36	*Theloderma licin*	1	-	-	-	On river bank and leave of plant (1 VSC)
Family Microhylidae						
37	*Microhyla inornata*	-	-	2	-	Banks of streams (2 VSC)
38	*M. borneensis*	1	-	-	1	Bank of streams (2 VSC)
39	*M. heymonsi*	6	2	3	3	Water puddles on trails (2 VSC)
40	*M. butleri*	1	1	2	-	Water puddles on trails (2 VSC)
41	*M. berdmorei*	1	1	-	-	On forest floor (2 VSC)
42	*Kaloula baleata*	1	2	-	-	On forest floor beside dead log (1 VSC)
43	*M. palmipes*	1	-			On forest floor beside dead log. (1VSC)
44	*Kalophrynus pleurostigma*	1	-	-	-	On forest trail under dead leaves (1 VSC)
	No. of species	36	27	21	20	
	No. of individuals	125	117	96	62	

Appendix 2. Reptile species examined and sighted at Plots 1-4, Lakum Forest Reserve, during the period April 2002-April 2003. S, sighted; V, vocalisation; Figures, hand caught; Figures in ( ), no. sighted; C, common (based on collection from two surveys); VC, very common (based on collection from three surveys); NC, not common; VSC, voucher specimen collected. The nomenclature of reptile species followed that of Indraneil Das and Norsham Yaakop (2005), L. Lee Grismer (2005) and Lim and Indraneil Das (1999).

Species	Plot 1	Plot 2	Plot 3	Plot 4	Remarks
LIZARDS					
Family Gekkonidae					
1 *Gekko smithii*	4	1	V (1)	V (2)	Canopy dweller-highly vocalised (2 VSC).
2 *G. monarchus*	1	-	-	1	
3 *Cytodactylus quadrivirgatus*	10	7	5	4	On tree trunks and on leaves of small trees (4 VSC)
4 *C. consobrinus*	4	2	S (1)	-1	On tree trunks (3 VSC)
5 *C. elok*	4	-	-	-	On tree trunks (4 VSC)
6 *Gehyra mutilata*	1	1	-	-	On leaves at fringe forest (1 VSC).
7 *Hemidactylus frenatus*	-	1	-	-	Trunk of tree at campsite (1 VSC)
8 *H. garnotii*	-	-	1	-	Trunk of tree in forest (1 VSC)
9 *Cnemaspis kendalli*	-	2	2	-	On leaves of bushes along open trails (2 VSC)
Family Eublepharidae					
10 *Aelurocalabotes felinus*	1	-	-	1	On forest floor and tree trunk (2 VSC)
Family Varanidae					
11 *Varanus salvator*	S (1)	S (3)	S (2)	S (1)	Along forest trails, along banks of streams and forest floor.
12 *V. nebulosus*	S (1)	S(1)	S (1)	S (1)	On forest floor and on tree trunks.
13 *V. rudicollis*	-	S (1)	S (1)	-	On forest floor.
Family Agamidae					
14 *Acanthosaura armata*	-	1	S (1)	-	On branches of tree.
15 *Aphaniotis fusca*	1	1	2	S(1)	On leaves of bushes (1 VSC)
16 *Bronchocela cristatella*	1	S (4)	2	1	On leaves of bushes and branches of trees (1 VSC)
17 *Draco melanopogon*	1	S (2)	1	1	On tree trunks (1 VSC)
18 *D. maximus*	-	-	S (1)	-	On tree trunks
19 *D. quinquefasciatus*	S (2)	1	S (1)	S (1)	On tree trunks (1 VSC)
20 *D. (volans) sumatranus*	S (4)	1	S (3)	S (1)	On tree trunks (1 VSC)
21 *Gonyocephalus belli*	2	-	-	1	On tree trunks (1 VSC)
22 *G. grandis*	2	2	-	2	On tree trunks nr stream (2 VSC)
Family Scincidae					
23 *Dasia olivacea*	S (1)	1	S (1)	S (1)	On dead logs (1 VSC)
24 *Lygosoma bowringii*	-	-	S (2)	1	In bushes along open trail (1 VSC)
25 *Mabuya multifasciata*	4	2	2	S (3)	On forest floor and in bushes along trails (2 VSC)
26 *M. longicauda*	-	1	1	-	On forest floor
SNAKES					
Family Colubridae					
27 *Ahaetulla prasina*	S (1)	S (2)	1	1	On branches of small plants (photograph).
28 *A. mycterizans*	1	1	-	-	On branches of tree (1 VSC)
29 *Boiga dendrophila*	S (2)	2	-	S(1)	On branches of trees (photograph)
30 *B. cynodon*	S (1)	S (1)	-	-	On forest floor
31 *B. nigriceps*	-	S (1)	-	-	On branch of tree

32	*Dendrelaphis formos*	-	-	1	-	On branch of tree (1 VSC)
33	*D. caudolineatus*	-	-	S (1)	S (1)	On branch of tree
34	*Elaphe flavolineata*	-	1	S (2)	S (1)	On forest floor (1 VSC)
35	*E. taeniura*	-	S (1)	-	-	On forest floor
36	*Gonyosoma oxycephalum*	S (1)	1	S (1)	-	On branches of tree (photograph)
37	*Macropisthodon rhodomelas*	S (1)	-	-	1	On forest floor (1VSC)
38	*Xenochrophis trianguligerus*	1	-	1	-	On bank of stream (1 VSC)
39	*Ptyas korros*	S (1)	S (1)	-	S(1)	On forest floor
40	*P. carinata*	S (1)	-	-	S (1)	On forest floor (sighted part of body and tail)
41	*Psuedohabdion longiceps*	1	1	-	-	Under dead log
42	*Aplopeltura boa*	-	-	-	1	On tree branch (1 VSC)
Family Pythonidae						
43	*Python reticulatus*	1	S (1)	-	S(1)	On forest floor.
44	*P. brongersmai*	S (1)	-	-	-	On forest floor.
Family Elapidae						
45	*Naja sumatrana*	1	S (1)	S (1)	S(1)	On forest floor.
46	*Calliophis bivirgata*	S (1)	-	S (1)	-	On forest floor.
47	*Bungarus. fasciatus*	S (2)	-	-	-	On forest floor.
Family Viperidae						
48	*Parias (Trimeresurus) sumatranus*	1	-	1	S(1)	On forest floor (1VSC)
49	*P. (Trimeresurus) hageni*	1	1	-	1	On tree branch (1 VSC)
50	*Tropidolaemus wagleri*	2	-	-	1	On tree branch (photograph)
TURTLES Family Bataguridae						
51	*Cyclemys dentata*	-	1	-	(1)shell	In stream and shell in Orang Asli house
52	*Heosemys spinosa*	1	1	-	(1)shell	On forest floor near stream and shell in Orang Asli house
53	*Cuora amboinensis*	-	-	-	(1)shell	Orang Asli's house
54	*Notochelys platynota*	-	-	-	(1)shell	Orang Asli's house
55	*Manouria emys*	-	-	-	(1)shell	Orang Asli's house
Family Trionychidae						
56	*Amyda cartilaginea*	-	-	-	(1)shell	Orang Asli's house
	No. of species	37	34	28	36	
	No. of individuals	67	62	41	43	

Appendix 3. List of mammal species examined and sighted at Plots 1-4, Lakum Forest Reserve during the period April 2002-April 2003. N, Mist net and Harp trap; TP, Totally Protected; VSC, Voucher specimen collected; S, Sighted; P, Protected; T, Trapped; FP, Foot print; NP, Not Protected; V, Vocalization.

Species	Plot 1	Plot 2	Plot 3	Plot 4	Legal Status	Remarks
INSECTIVORA						
Family Erinaceidae						
1 *Echinosorex gymnurus*		S(1)			NP	Swamp area
DERMOPTERA						
Family Cynocephalidae						
2 *Cynocephalus variegatus*			S(1)			Tree branch
CHIROPTERA						
Family Pteropodidae						
3 *Cynopterus brachyotis*	N-4	N-7	N-5	N-4	NP	(1-VSC)
4 *C. horsefieldii*	N-1	N-2	N-4	N-2	NP	(1-VSC)
5 *C. sphinx*	-	-	-	N-1	NP	(1-VSC)
6 *Eonycteris spelaea*	N-5	N-1	N-4	N-5	NP	(2-VSC)
7 *Penthetor lucasi*	-	N-2	-	-	-	(1-VSC)
8 *Chironax melanocephalus*	-	-	N-2	N-1	NP	(1-VSC)
9 *Megaerops ecaudatus*	N-1	N-2	N-1	-	NP	(1-VSC)
10 *Balionycteris maculatus*	N-2	N-1	N-1	N-1	NP	(1-VSC)
11 *Macroglossus sobrinus*	N-1	N-1	N-2	N-1	NP	(1-VSC)
Family Emballonuridae						
12 *Emballonura monticola*	-	-	N-2	-	NP	(1-VSC)
Family Nycteridae						
13 *Nycteris javanica*	N-1	-	-	N-1	NP	(1-VSC)
Family Megadermatidae						
14 *Megaderma spasma*	-	N-1	N-2	-	NP	(1-VSC)
Family Rhinolophidae						
15 *Rhinolophus affins*	N-3	N-4	N-2	N-2	NP	
16 *R. stheno*	N-1	N-1	N-1	N-2	NP	(2-VSC)
17 *R. lepidus* (=*R. rufulgens*)	-	N-2	N-3	-	NP	(2-VSC)
18 *R. sedulus*	N-1	-	N-1	-	NP	(1-VSC)
19 *R. trifoliatus*	N-2	N-1	N-3	N-2	NP	(1-VSC)
20 *R. luctus*	-	N-2	N-1	-	NP	(1-VSC)
Family Hipposideridae						
21 *Hipposideros bicolor*	N-1	-	N-2	-	NP	(1-VSC)
22 *H. cineraceus*	-	N-1	-	N-1	NP	(1-VSC)
23 *H. cervinus*	-	N-1	N-1	-	NP	(1-VSC)
24 *H. larvatus*	N-2	N-1	N-1	N-1	NP	(1-VSC)
25 *H. ridleyi*	N-1	N-1	N-1	-	NP	(1-VSC)
26 *H. diadema*	N-1	N-1	N-2	N-1	NP	(1-VSC)
27 *H. armiger*	-	-	N-2	-	NP	(1-VSC)
28 *H. galeritus*	-	N-1	N-1	-	NP	(1-VSC)
Family Vespertilionidae						
29 *Myotis muricola*	N-1	N-1	N-2	N-1	NP	(1-VSC)
30 *M. horsefieldii*	-	-	N-1	-	NP	(1-VSC)
31 *M. siligorensis*	-	-	N-1	-	NP	(1-VSC)
32 *Kerivoula papillosa*	-	N-1	N-1	-	NP	(1-VSC)
33 *K. intermedia*	N-1	-	-	N-1	NP	(1-VSC)
34 *K. minuta*	N-1	N-1	N-1	-	NP	(1-VSC)

35	*K. pellucida*	-	-	N-2	-	NP	(1-VSC)
36	*Murina suillus*	-	N-1	N-2	-	NP	(1-VSC)
37	*M. cyclotis*	N-1	N-1	-	N-1	NP	(1-VSC)
38	*Tylonycteris robustula*	N-1	-	N-2	-	NP	(1-VSC)
39	*T. pachypus*	-	N-2	N-2	-	NP	(1-VSC)
40	*Glischropus tylopus*	-	-	-	V-2	NP	(1-VSC)
41	*Pipstrellus tenus*	-	-	N-1	-	NP	(1-VSC)
Family Molossidae							
42	*Mops mops*	-	-	N-1	-	NP	(1-VSC)
SCANDENTIA							
Family Tupaiidae							
43	*Tupaia glis*	T-4	T-5	T-2	T-3	NP	released
PRIMATES							
Family Lorisidae							
44	*Nycticebus coucang*	-	-	S(1)	-	TP	On tree branch in forest
Family Cercopithecidae							
45	*Macaca fascicularis*	S(4)	S(7)	S(4)	S(3)	TP	By Orang asli & field team
46	*M. nemestrina*	S(1)	S(2)	S(4)	S(3)	TP	-do-
47	*Presbytis femoralis*	S(2)	S(3)	S(4)	-	TP	-do-
48	*Semnopithecus obscurus*	S(4)	S(2)	S(2)	-	TP	-do-
Family Hylobatidae							
49	*Hylobates lar*	-	V	-	-	TP	Vocalization
PHOLIDOTA							
Family Manidae							
50	*Manis javanica*	-	Scales	-	-	TP	Collected by Orang Asli in forest
RODENTIA							
Family Sciuridae							
51	*Ratufa bicolor*	-	S(1)	S(2)	-	TP	Sighted by Orang Asli & field team
52	*R. affinis*	-	-	S(1)	-	TP	Sighted by field team
53	*Callosciurus notatus*	T-7	T-4	T-6	T-4	NP	released
54	*C. prevostii*	-	-	T-1	-	TP	released
55	*C. caniceps*	T-1	-	T-3	-	NP	released
56	*C. nigrovittatus*	T-1	T-1	T-2	-	NP	released
57	*Sundasciurus tenuis*	T-1	T-4	T-3	-	NP	released
58	*S. lowii*	T-2	T-1	T-2	T-1	NP	released
59	*S. hippurus*	T-2	T-1	T-2	T-1	NP	released
60	*Lariscus insignis*	T-2	T-1	T-2	-	NP	released
61	*Rhinosciurus laticaudatus*	T-1	T-1	T-2	-	NP	released
Family Pteromyidae							
62	*Petaurista petaurista*	-	S-2	S-1	-	TP	Sighted by Orang Asli & field team
63	*Aeromys tephromelas*	-	-	S-1	-	TP	-do-
64	*Petinomys sp.* (? *vordermanni*)	-	-	S-1	-	TP	-do-
Family Rhizomyidae							
65	*Rhizomys sumatrensis*	-	S-1	-	-	NP	-do-
Family Muridae							
66	*Rattus tiomanicus*	T-2	T-3	T-1	T-1	NP	released
67	*Sundamys muelleri*	T-2	T-1	T-3	T-1	NP	released
68	*Berlymys bowersii*	-	-	T-1	-	NP	released

69	*Leopoldamys sabanus*	T-4	T-3	T-7	T-2	NP	released
70	*Niviventer cremoriventer*	-	-	T-2	-	NP	released
71	*Maxomys rajah*	T-2	T-3	T-4	T-1	NP	released
72	*M. surifer*	T-1	T-1	T-3	T-1	NP	released
73	*M. whiteheadi*	-	-	T-1	-	NP	released
Family Hystricidae							
74	*Hystrix brachyura*	Quills	S(1)	Quills	-	TP	Quills collected by Orang Asli
CARNIVORA							
Family Ursidae							
75	*Ursus (Helarctos) malayanus*	ST	-	ST	-	TP	Scaring 'Footprint sign' on trunk of trees
Family Felidae							
76	*Panthera tigris*	-	-	FP	-	TP	Footprint nr swamp area & on trail identified by Game ranger & Orang Asli (cast)
77	*P. pardus*	S(1)	-	S(1)	-	TP	Sighted by Game Rangers
78	*Prionailurus bengalensis*	S(1)	S(1)	S(2)	-	TP	Sighted by Game Rangers, Orang Asli & field team
Family Mustelidae							
79	*Aonyx cinerea*	-	S(2)	-	-	TP	Sighted by Orang Asli & field team swimming & playing in river.
80	*Martes flavigula*	S(1)	-	-	-	TP	On the forest floor
Family Herpestidae							
81	*Herpestes brachyurus*	-	S(1)	S(1)	-	TP	Sighted by Orang Asli & field team
Family Viverridae							
82	*Paradoxurus hermaphroditus*	S(1)	S(1)	S(2)	Skin	P	Sighted by field team, Orang Asli & Game Rangers
83	*Aretogalidia trivirgata*	-	-	S(1)	-	TP	-do-
84	*Paguma larvata*	S(1)	-	-	-	TP	Sighted by Game Ranger on the forest floor
85	*Hemigalus derbyanus*	-	-	S(1)	-	TP	Sighted by Orang Asli & field team on forest floor
86	*Viverricula indica*	-	S(1)	-	-	TP	-do-
PERISSODACTYLA							
Family Tapiridae							
87	*Tapirus indicus*	FP	FP	FP	-	TP	Along swamp areas identified by Orang Asli
ARTIODACTYLA							
Family Suidae							
88	*Sus scrofa*	FP	FP	FP	FP	P	Along edge of swamp areas
Family Tragulidae							
89	*Tragulus javanicus*	S(1)	S(1)	S(2)	-	P	Sighted by Orang Asli & field team
Family Cervidae							
90	*Muntiacus muntjak*	-	-	S(1)	-	P	Sighted by Game Rangers
	No. of species	48	57	80	31		
	No. of individuals	69	83	117	40		

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Pharmacogenomics of tocotrienol/tocopherol complex in the small intestine of Nrf2 Knockout and C57BL/6J mice

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Abstract Vitamin E family is recognized not only as the major chain-breaking antioxidant, but also as an anti-proliferating agent. Recently, it has also been considered as a nutrition-based strategy to prevent various aging-related disorders. Nrf2 is a redox-sensitive transcription factor that plays a pivotal role in cancer prevention and neurodegenerative diseases. However, studies on the interactions between tocotrienol/ tocopherol complex and Nrf2 are lacking. In the present study, we show the global gene expression profiles regulated by Nrf2 in the small intestine of C57BL/6J (wild type) and C57BL/6J/Nrf2-/- (knockout) mice given 0.2% Tocomin® – a mixture of tocotrienol and tocopherol in the diet. The profiles were analyzed using Affymetrix mouse genome 430 2.0 array and Genespring 7.2 software. The microarray results were validated by real-time PCR analyses. Clusters of genes that were either induced or suppressed more than 2 fold were identified as Nrf2-dependent Tocomin®-induced or -suppressed genes, respectively. Based on their biological functions, these genes can be classified into diverse categories responsible for cellular homeostasis control such as cell adhesion, cell growth and differentiation, DNA associated proteins (replication and transcription), detoxifying enzymes, apoptosis, mRNA processing and splicing, transport, cell cycle, electron transport, biosynthesis and metabolism, carbohydrate homeostasis, G-protein coupled receptors and G-protein signaling, inflammatory and immune response proteins, calcium homeostasis, cytoskeleton, extracellular matrix and smooth muscle associated proteins, kinases and phosphatases, heat shock proteins, and ubiquitination and proteolysis. Overall, tocotrienol and tocopherol mixture supplementation upregulated expression of 144 Nrf2-dependent genes, while it was associated with a higher number of suppressed genes. Interestingly, phase II detoxification/ antioxidant genes are not the major group of Nrf2-dependent genes modulated by Tocomin®-treatment. Results from this study have provided potential insight into the promising link between *in vivo* biological effects of Tocomin® and the transcription factor Nrf2-mediated gene expression profile that could potentially contribute to the overall beneficial health effects of Tocomin® against aging-related disorders.

Keywords Tocotrienols – Tocopherols – Tocomin® – Nrf2 – Small intestine – Gene expression profile

INTRODUCTION

Vitamin E family includes 2 groups of closely related fat-soluble compounds, tocotrienols and tocopherols. In nature, there are eight vitamin E analogs including α–, β–, γ–, and δ–tocopherol, and α–, β–, γ–, and δ–tocotrienol. They possess a 6-membered, aromatic chroman-6-ol ring structure and aliphatic side chain. Tocotrienols and tocopherols differ only in their aliphatic tail. While tocotrienols have an unsaturated isoprenoid or geranylgeranyl side chain possessing three double bonds, tocopherols have a saturated phytyl side chain. Tocopherols are found abundantly in oils extracted from soybean, olive,

cotton seed, and sunflower seeds and referred as 'the classical vitamin E', whereas tocotrienols are found in appreciable levels only in palm oil and rice bean oil and inadequately studied.

Vitamin E is recognized as the major chain-breaking antioxidant preventing the propagation of oxidative stress. The oxidative stress from uncontrolled production of free radicals is considered to be an important event leading to the aging process and aging-related diseases [1-3]. Therefore supplementation of vitamin E is considered to be a nutrition based strategy to prevent various disorders such as cardiovascular and neurodegenerative diseases [4,5]. Recently, tocotrienols have gained more interest in their biological properties that are unique and not shared by the classical vitamin E. Tocotrienols have been found to inhibit the growth of several tumor cell lines in culture, including human breast cancer cells [6] and hepatocarcinoma cells [7]. The mechanism of this effect is not yet completely understood, but tocotrienols could interrupt cell cycle, resulting in upregulation of apoptosis [8] and inhibit proliferation of bovine aortic endothelial cells, resulting in anti-angiogenic effect [9]. Tocotrienols also alter expression of broad functional genes, in breast cancer, such as immune modulatory genes as well as genes involved in cell cycle control [10]. However, there is no report about the effect of tocotrienol/tocopherol complex on the global gene expression profile *in vivo*.

Nuclear factor E2 related factor 2 (Nrf2) is a basic leucine zipper, redox-sensitive transcription factor that is pivotal to the antioxidant response and homeostasis. It has been extensively studied to gain an insight into its role in chemoprevention. Nrf2 is known to be involved in the regulation of antioxidant response element (ARE) mediated transcription of several genes including antioxidant enzymes such as glutathione-S-transferase and glutamylcysteine synthetase as well as detoxifying enzymes such as phase II metabolizing enzymes. Activation of the Nrf2/ARE pathway is believed to be an important mechanism of action of several potentially dietary chemopreventive agents. Recently, Nrf2 knockout mice model has been used to better understanding the compound-regulated cancer pharmacology and toxicological effect mediated through transcription factor Nrf2 *in vivo*.

Given the important roles of vitamin E in aging-related diseases and the Nrf2/ARE axis in chemoprevention, this present study is aimed to explore the potential role of tocotrienol/tocopherol complex in modulating Nrf2 function *in vivo*. By using the microarray expression profiling, the global gene expression profiles elicited by oral administration of tocotrienol/tocopherol complex in small intestine of Nrf2 knockout (C57BL/6J/Nrf2-/-) and wild type mice (C57BL/6J) is investigated and the tocotrienol/tocopherol complex-regulated, Nrf2-depedent genes are identified.

MATERIALS AND METHODS

Animal care and treatments

The protocol for animal studies was in accordance with the NIH Guide for the Care and Use for Laboratory Animals, and was approved by the Rutgers Institutional Animal Care and Use Committee (IACUC). Nrf2 knockout mice Nrf2 (–/–) (C57BL/SV129) have been described previously [11]. Nrf2 (–/–) mice were backcrossed with C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME). To confirm the genotype from each animal, DNA was extracted from the tail and analyzed by polymerase chain reaction (PCR) using primers: (3'-primer, 5'-GGA ATG GAA AAT AGC TCC TGC C-3'; 5'-primer, 5'-GCC TGA GAG CTG TAG GCC C-3'; and lacZ primer, 5'-GGG TTT TCC CAG TCA CGA C-3'. The second generation (F2) of male Nrf2 knockout mice was used in this study. Age-matched male C57BL/6J mice were purchased from The Jackson Laboratory. Nine- to 12-week-old mice were used and housed at Rutgers Animal Facility and maintained under 12-hour light/dark cycles. All animals were allowed water and food *ad libitum*. After 1 week of acclimatization, mice were put on AIN-76A diet (a special diet free of any antioxidants) (Research Diets Inc., NJ, USA) for another week. The mice were then divided into 4 groups – Group I: Nrf2 (-/-) mice, AIN-76A control diet; Group II: Nrf2 (-/-) mice, 0.2% Tocomin® diet; Group III: wild type mice, AIN-76A control diet; and Group IV: wild type mice, 0.2% Tocomin® diet.

Mice were sacrificed after 1 week of treatment and small intestines were retrieved and stored in RNAlater (Ambion, Austin, TX). Tocomin® was a gift from Carotech Bhd (Perak, Malaysia) and it has a standard composition of ~50% tocotrienol/tocopherol complex including *d*-γ-tocotrienol (~20%), *d*-β-tocotrienol (~1.5%), *d*-δ-tocotrienol (~5.5%), *d*-α-tocotrienol (~11%) and *d*-α-tocopherol (~10%).

More information about this product can be obtained at www.carotech.net. Tocomin® diet was prepared by adding 0.2% of Tocomin® into AIN-76A diet (Research Diets Inc., NJ, USA).

Sample preparation, microarray hybridization, and data analysis

Total RNA from the small intestine was isolated by using a method of Trizol (Invitrogen, Carlsbad, CA) extraction coupled with the RNeasy® Mini kit from Qiagen (Valencia, CA) according to the manufacturer's protocol. After RNA isolation, all the subsequent technical procedures, including quality control and concentration measurement of RNA, cDNA synthesis and biotin labeling of cRNA, hybridization and scanning of the arrays, were performed at CINJ Core Expression Array Facility of Robert Wood Johnson Medical School (New Brunswick, NJ). Affymetrix mouse genome 430 2.0 array containing > 45,101 probe sets representing > 34,000 well-substantiated mouse genes was used to probe the global gene expression profile in mice following treatment. Briefly, each array was hybridized with cRNA derived from a pooled total RNA sample from four mice per treatment group. After hybridization and washing, the intensity of the fluorescence of the array chips was measured by the Affymetrix GeneChip Scanner. The expression analysis file created from each sample (chip) was imported into GeneSpring 7.2 software (Agilent Technologies Inc., Palo Alto, CA) for further data characterization. A new experiment was generated after importing data which was normalized to the 50th percentile of all measurements on that array. Data filtration based on flags present in at least one of the samples was generated. Lists of genes that were either induced or suppressed >2-fold between treated *versus* vehicle group of same genotype were created by filtration-on-fold function within the presented flag list. By using color-by-Venn-Diagram function, lists of genes that were regulated >2-fold only in C57BL/6J mice were created.

Quantitative real-time PCR for microarray data validation

To validate the microarray data, seven genes including glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene were selected from different categories for quantitative real-time PCR analyses. The specific primers for these genes designed by using Primer Express 2.0 software (Applied Biosystems, Foster City, CA) are listed in Table 1. Instead of using pooled RNA from each group, RNA samples isolated from individual mice were used in real-time PCR analyses. First-strand cDNA was synthesized using 4 µg total RNA following the protocol of SuperScript III First-Strand cDNA Synthesis System (Invitrogen) in a 40 µl reaction volume. Real-time PCR was done as described previously [12]. The amplicon specificity was determined by first-derivative melting curve analysis and values for each gene were normalized by the values of corresponding GAPDH gene expression.

Table 1. Oligonucleotide primers used for quantitative real-time PCR.

Gene name	GenBank Accession No.	Forward/Reverse primer
ATP-binding cassette, sub-family B (MDR/TAP), member 1A (Abcb1a)	M30697	F: 5'-TTCAGGGCTTCACATTTGGC-3' R: 5'-GGAGTCGCTTGGTGAGGATCT-3'
Cyclin D1 (Ccnd1)	NM_007631	F: 5'-AGTCATCAAGTGTGACCCGGA-3' R: 5'-TCAATCTGTTCCTGGCAGGC-3'
Cytochrome P450, family 3, subfamily a, polypeptide 44 (Cyp3a44)	AB039380	F: 5'-GTCCTGCTGGCAATCATCCT-3' R: 5'-TGTACGGGTCCCATATCGGT-3'
Glutathione S-transferase, alpha 3 (Gsta3)	NM_010356	F: 5'-ATGTTCCAGCAAGTGCCCAT-3' R: 5'-CTGCACCAGTTTCATCCCGT-3'
Hepatic nuclear factor 4, alpha (Hnf4a)	NM_008261	F: 5'-GGCAATGAAGAAATCAGTTCCC-3' R: 5'-CCCCAGTGTGTACCCTGTCAC-3'
Leptin receptor (Lepr)	U42467	F: 5'-CCTTCTCATGGCCCATGAGTA-3' R: 5'-AAGCACTGAGTGACTCCACAGC-3'
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	NM_008084	F: 5'-CAGGAGCGAGACCCCACTAA-3' R: 5'-ATACTCAGCACCGGCCTCAC-3'

The fold changes in expression levels of treated samples over control samples were calculated by assigning unit value to the control (vehicle) samples. The correlation between corresponding microarray data and real-time PCR data was evaluated by the coefficient of determination, r^2 as reported elsewhere [13,14].

RESULTS

Tocotrienol/tocopherol complex (Tocomin®) modulated gene expression patterns in small intestine

In order to analyze the global gene expression profiles in the small intestine of mice treated with Tocomin®, the oligonucleotide microarray analysis was used. Genes that were regulated by Tocomin® in C57BL/6J (wild type) mice but not in Nrf2 (-/-) (knockout) were considered to be Nrf2-dependent genes. We report here that 7 days post-administration of Tocomin® diet was able to alter 503 Nrf2-dependent genes. Amongst these gene, it induce the expression levels of 144 Nrf2-dependent genes more than 2 fold and also suppressed the expression levels of 359 Nrf2-dependent genes (Fig. 1).

Quantitative real-time PCR for microarray data

To validate the data generated from the microarray studies, seven genes from different categories (Table 1) were chosen to confirm the tocomin® regulation effects by using quantitative real-time PCR analyses as described in detail under Materials and Methods. Computation of the correlation statistic showed that the data generated from the microarray analyses are highly correlated with the results obtained from quantitative real-time PCR (coefficient of determination, $r^2 = 0.992$; Fig. 2).

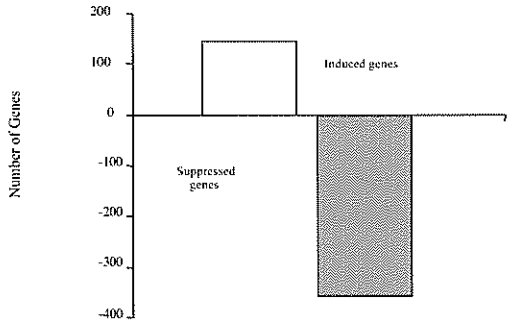

Figure 1. Regulation of Nrf2-dependent gene expression by Tocomin® in mouse small intestine. Gene expression patterns were analyzed after 7 days administration of Tocomin® diet; Nrf2-dependent genes that were either induced or suppressed >2-fold were selected. The positive numbers on the Y-axis refer to the number of genes being induced; the negative numbers on the Y-axis refer to the number of genes being suppressed.

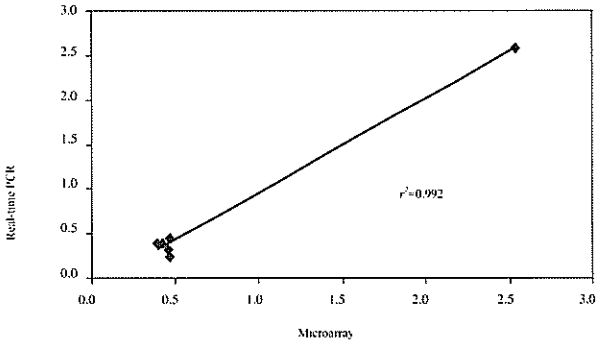

Figure 2. Correlation of the microarray data and quantitative real-time PCR data. Fold changes in gene expression obtained by oligonucleotide microarray were plotted against corresponding gene expression fold changes measured by quantitative real-time PCR. Data obtained from the two methods were highly correlated (r2=0.992).

Tocotrienol/tocopherol complex (Tocomin®) induced Nrf2-dependent genes in small intestine

Genes that were induced only in the Tocomin®-treated wild type mice but not in the Tocomin®-treated Nrf2 (-/-) mice were designated to be Tocomin®-induced Nrf2-dependent genes. Based on their biological functions these genes were classified into categories such as cell adhesion, cell growth and differentiation, DNA associated proteins (replication and transcription), detoxifying enzymes, apoptosis, mRNA processing and splicing, transport, cell cycle, electron transport, biosynthesis and metabolism, carbohydrate homeostasis, G-protein coupled receptors and G-protein signaling, inflammatory and immune response proteins, calcium homeostasis, cytoskeleton, extracellular matrix and smooth muscle associated proteins, kinases and phosphatases, heat shock proteins, and ubiquitination and proteolysis. The ability of Tocomin® to induce many of those genes has not been shown earlier, therefore these inductive effect are novel, however the involvement of Nrf2 in the regulation of these genes is still unknown. Table

2 lists genes relevant to our interest.

In response to Tocomin® treatment, many genes of DNA associated proteins (replication and transcription) were upregulated. Representative members included zinc finger protein 458 (Zfp458), ADP-ribosylation factor-like 10A (Arl10), scleraxis (Scx), T-box 2 (Tbx2), nuclear factor I/A (Nfia), zinc finger protein 644 (Zfp644), zinc finger protein 114 (Zfp114). Interestingly, several important transport genes were identified as Tocomin®-regulated, Nrf2-dependent. These included synaptotagmin I (Syt1), rho-related BTB domain containing 3 (Rhobtb3), solute carrier family 25 (mitochondrial carrier, Aralar), member 12 (Slc25a12), solute carrier family 34 (sodium phosphate), member 2 (Slc34a2), chloride channel 5 (Clcn5). Other categories of genes induced by Tocomin® in an Nrf2-dependent pattern included cell adhesion (integrin alpha 2b and cadherins 2, 22), cell growth and differentiation (myocardin and forkhead box K1), mRNA processing and splicing (synaptojanin 2), biosynthesis and metabolism (aldehyde dehydrogenase 18 family, member A1 and 1-acylglycerol-3-phosphate O-acyltransferase 3), G-protein coupled receptors and G-protein signaling (inositol 1,4,5-triphosphate receptor and gastrin releasing peptide receptor), cytoskeleton, extracellular matrix and smooth muscle associated proteins (dentin matrix protein 1 and forming 2), kinases and phosphatases (proviral integration site 1 and AXL receptor tyrosine kinase). However, for the detoxifying enzymes, Tocomin® could only upregulate the expression of glutathione S-transferase, mu 6 (Gstm6). Hence, it clearly demonstrates that Tocomin®– a mixture of tocotrienol and tocopherol is not a potent inducer of detoxifying enzymes.

Tocotrienol/tocopherol complex (Tocomin®) suppressed Nrf2-dependent genes in small intestine

As shown in Table 3 which lists the relevant genes to our interest, Tocomin® could inhibit the expression of many categories of Nrf2-dependent genes. Major DNA associated proteins (replication and transcription) identified as Tocomin®-modulated genes included several zinc finger proteins, hepatic nuclear factor 4, alpha (Hnf4a), general transcription factor IIF, polypeptide 2 (Gtf2f2), D site albumin promoter binding protein (Dbp). Other major categories of genes affected included transport, ubiquitination and proteolysis, inflammatory and immune response proteins, biosynthesis and metabolism, kinases and phosphatases, G-protein coupled receptors and G-protein signaling, and cytoskeleton, extracellular matrix and smooth muscle associated proteins. Interestingly the phase II detoxifying genes glutamate-cysteine ligase catalytic subunit (Gclc), glutathione S-transferase, alpha 3 (Gstm3) and aldo-keto reductase family 1, member C13 (Akr1c13) found to be inhibited by Tocomin® at 7 days post-administration. In additionally, phase I genes such as cytochrome P450 family members Cyp2c50 and Cyp4a10 were also modulated in response to Tocomin®-treatment.

Several members of solute carrier family (Slc4a7, Slc11a2, Slc15a1, Slc16a9, Slc23a1, Slc28a2, Slc35a3, Slc35b2, Slc43a2), which may be regarded as phase III genes were also downregulated in an Nrf2-dependent manner after Tocomin® treatment. A variety of genes involved in ubiquitination and proteolysis were also found to be inhibited by Tocomin®. These mainly include ubiquitin D (Ubd), ubiquitin specific peptidase 2 and 15 (Usp2, Usp15) and proteasome (prosome, macropain) subunit, alpha type 6 (Psma6). A number of inflammatory and immune response genes such as chemokine (C-C motif) ligand 9 (Ccl9), chemokine (C-X-C motif) ligand 13 and 16 (Cxxl13, Cxcl16), immunoglobulin (CD79A) binding protein 1 (Igbp1) and interferon induced transmembrane protein 1 (Ifitm1) were found to be downregulated too.

Besides these categories, a number of genes belonging to biosynthesis and metabolism such as high density lipoprotein (HDL) binding protein (Hdlbp), ribosomal protein L37a (Rpl37a), Acyl-Coenzyme A oxidase 2, branched chain (Acox2), kinases and phosphatases such as serine/threonine kinase 17b (apoptosis-inducing) (Stk17b), PTK2 protein tyrosine kinase 2 (Ptk2), G-protein coupled receptors and G-protein signaling such as regulator of G-protein signaling 1 and 18 (Rgs1, Rgs18), prostaglandin E receptor 4 (subtype EP4) (Ptger4) and cytoskeleton, extracellular matrix and smooth muscle associated proteins such as myosin IB (Myo1b), stomatin (Stom) and many more were found to be Nrf2-dependent. Futhermore, cell cycle genes including cyclin B1, D1 and E2 (CcnB1, CcnD1, CcnE2) and cell division cycle associated 8 (Cdca8) were also observed to be modulated in response to Tocomin® treatment *via* Nrf2.

DISCUSSION

Recent works have shown that vitamin E (both tocotrienols and tocopherols) has anti-tumor activity in several experimental systems [6,7,15] and this inhibitory effect on cell growth of vitamin E, however, may be independent of its antioxidant activity [1,3]. Although, the mechanism of action is not yet completely understood, tocotrienols, not tocopherols, could modulate a number of intracellular signaling pathways involved in apoptosis [8] and mitogenesis [9,10]. In the present study, we aimed to identify Tocomin®-regulated Nrf2-dependent genes in mice small intestine by using C57BL/6J (wild type) and C57BL/6J/Nrf2-/- (knockout) mice and genome-scale microarray analyses. The potential role of Tocomin® in modulating Nrf2 function as a transcriptional activator *in vivo* was explored.

Results obtained show that diverse gene categories responsible for controlling cellular defense and homeostasis in small intestine tissue are altered after oral administration of Tocomin®. However, the total number of Tocomin®-regulated, Nrf2-dependent genes (503) is lower than those of other chemopreventive agents reported previously [12,14,16-18]. These could have three possible explanations: (1) the time of exposure is different. Indeed, Nrf2 is a redox-sensitive transcription factor, therefore 7 days administration of Tocomin®, which has strong antioxidant activity, may lower cellular oxidative stress level leading to decrease of Nrf2 transcriptional activity; or (2) the anti-tumor activity of Tocomin® may not exclusively be *via* Nrf2-regulated pathway; or (3) the concentration of Tocomin® in small intestine may be not enough to exert their biological effects and may be dependent on the pharmacokinetic disposition of Tocomin® in the gut and the resultants exposure parameters such as Cmax, Tmax and AUC. As previously reported by Ikeda *et al.* (2001) that tissue uptake and transport of vitamin E isoforms is different and their tissue deposition is extremely low [19].

Although, the role of Nrf2 in the transcriptional activation of phase II detoxifying/antioxidant enzymes is well-recognized as the major chemopreventive mechanism of several chemopreventive agents, however, Tocomin® could upregulate only the expression of Gstm6, while suppressed the expression of Gclc and Gstm3. Thus it clearly demonstrates that Tocomin® has no major effect on phase II detoxifying/antioxidant system at 7 days post-administration. Surprisingly, the downregulation of phase II detoxifying/antioxidant genes observed in this study parallels that observed with ER stress inducer Tunicamycin which has also been shown to be regulated through Nrf2 [13]. As well, as noted with NQO1 earlier [14], there could possibly be a temporal control of phase II genes expression which explains our results with Tocomin® from a biological standpoint.

Interestingly, Tocomin® could alter expression of a huge battery of genes belonging to the solute carrier family. These transport genes, which may be regarded as phase III genes, might have significant pharmacological effects by modulating the excretion/efflux of reactive carinogens/metabolites.

One of the successful mechanisms by which chemopreventive agents attain anti-tumor properties is by modulating the cell cycle and cell growth/differentiation as well as apoptosis processes. Modulation effects of tocotrienols on expression of several genes involving apoptosis had been previously reported [10,15]. We also found that several cell cycle, cell growth and differentiation and apoptosis genes are Tocomin®-regulated, Nrf2-dependent. Inhibition of cell cycle-related genes particularly Ccnb1 and other cyclin proteins by Tocomin® is consistent with earlier report that tocotrienols could downregulate Ccnb1 in breast cancer cells [10]. These cyclins play an important role in regulation of cell cycle by acting through the formation of enzymatic complexes with different cyclin-dependent kinases. Overexpression of cyclins was found in various tumor tissues [20], therefore suppression of these proteins could result on inhibiting of tumor cell growth.

Other major mechanisms by which tocotrienols exerts chemopreventive action may be by modulating the transcriptional machinery (DNA associated proteins (replication and transcription) and mRNA processing and splicing related proteins) and regulating some of the key signal transduction pathways (kinases and phosphatases). In addition, several genes associated with the ubiquitin/proteasome pathway are regulated in response to Tocomin® in an Nrf2-dependent manner. Additionally, many genes related to inflammatory and immune response were also seen to be regulated through Nrf2 and modulated by Tocomin®. Amongst others, Ifitm1, a membrane protein implicated in the control of cell growth [21] has been found to be suppressed by Tocomin®. Consistent with our result, inhibitory effect of tocotrienols on Ifitm1 expression

had been previously reported in breast cancer cells [10]. Interestingly, Tocomin® could modulate the expression of key calcium homeostasis genes [13], a feature that has previously been reported to distinguish ER stress inducer Tunicamycin from other Nrf2 activators such as EGCG, curcumin, sulforahane and PEITC [12,13,16-18,22]. Furthermore, the downregulation of high density lipoprotein (HDL) binding protein and leptin receptor, on the other hand, and the concomitant activation of arachidonate 5-lipoxygenase activating protein and low density lipoprotein (LDL) receptor-related protein 11 point to an Nrf2-regulated link between Tocomin®-induced oxidative stress and lipid/cholesterol metabolism that has not been explored earlier.

In conclusion, this is the first report *in vivo* examining the potential role of Tocomin® in modulating Nrf2 function as a transcriptional activator of the global transcriptome expression profiling. Our study shows that Tocomin®- a mixture of tocotrienols and tocopherols selectively alters the expression of diverse functional group of Nrf2- dependent genes. Of interest is its ability to modulate phase III transport genes, transcriptional machinery genes, some of the key signal transduction pathway as well as genes involved cell cycle, cell growth and differentiation. In addition, Tocomin® may also exert its anti-tumor effects by Nrf2-independent pathway. However, more studies are warranted to better understand the role of Tocomin® in chemoprevention strategy.

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Valve leakage monitoring using acoustic emission

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Abstract This paper describes a method of valve leakage condition monitoring using acoustic emission technique. Acoustic emission (AE) signals were detected by a piezoelectric transducer mounted on the surface of the gate valve. The AE dynamic response 'rf' enveloper was extracted from the raw signal and used as characteristic features to represent a gate valve operating condition. In this experiment, artificial leak conditions from out-feed valve were investigated and they corresponded to each step size which was varied at 10 percent increment step of the leakage rate. To obtain the calibration curve, the pattern of AErms signals was collected from in-feed valve operating from its closure to fully opened condition. The relationship between the AErms and the leakage rate was correlated by using the 6th order polynomial root determination. The results were very promising in terms of revealing the leakage condition of the gate valve.

Keywords acoustic emission – calibration – condition monitoring – leak – valve

INTRODUCTION

Gate valve is one of the most typical components being widely used in petrochemical and power plant industries. The failures of gate valves have resulted in significant production effort such as over pressurization of pressure system. Therefore, it may lead to energy loss and damage to flow system components. Monitoring the condition of valves has obtained considerable attention over the past decades due to its particular importance of safety, environment and the economical reasons [1,2]. A reliable condition monitoring system will reduce the failure and unplanned maintenance. In addition, the huge attendant cost due to the loss of energy and machine downtime can be reduced.

Nowadays there are many kinds of conventional methods available for the valve leak detection [3]: visual inspection using dye penetration, hydro-static testing, flow or pressure measurement, and ultrasonic testing. However, these methods are time consuming and can reveal the leakage of valve only after it had occurred. The objective of this research is to demonstrate that a condition-based monitoring using acoustic emission (AE) can provide not only timely detection of valve leakage but also the rate of the leakage so that maintenance or replacement can be performed prior to the loss of safety function. Therefore the use of acoustic emission method has been proposed to be used for valve monitoring instead of those conventional methods (Fig. 1).

Acoustic emission is a natural phenomenon of stress wave generation and propagation spontaneously when a material is subjected under stress [4]. Plastic deformation and growth cracks are the primary sources of acoustic emission in metals. The acoustic

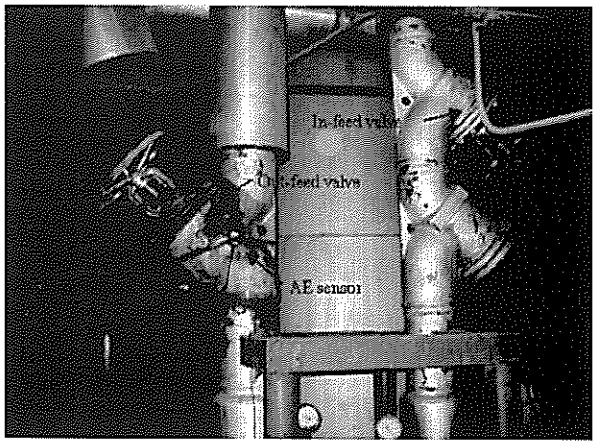

Figure 1. Gate valve with AE monitoring in petrochemical industry.

signal can be detected by a piezoelectric transducer, which converts the mechanical energy carried by the elastic wave into an electrical signal.

When the valve operates, the strain caused by the fluid can be produced by the applied internal pressure due to its flow which acts upward through the thickness of the material and causes the strain [5]. The change in strain produces the physical change in acoustic emission waves that propagate through the valve.

THE PROPOSED APPROACH

A systematic approach to classify the dynamic responses of AE signatures associated with the valve operating condition is performed in this study. The proposed approach is focused on the calibration, analysis and understanding of the capability of the AE technique to provide diagnostic information on the gate valve operating conditions. Figure 2 illustrates the systematic diagram of the proposed valve monitoring using AE technique.

The gate valve needs be calibrated in order to obtain the characteristic curve for its operating condition. The calibration involves only opening the gate valve from its close status to fully open status while acquiring the AE signal. The relationship of the AE_{rms} and valve leakage rate can be performed by using polynomial expansion [6].

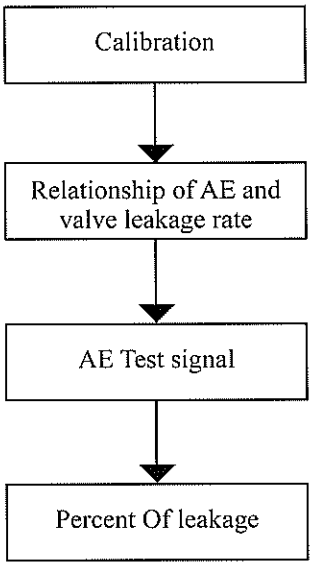

Figure 2. The systematic diagram of valve monitoring

In this study, the AE_{rms} is used as representative for acoustic emission signals collected from different valve operating conditions of the out-feed valve. This is because of the assumption that the energy of AE signal in terms of its AE_{rms} can be used as the signature representation of the different valve leakage condition. Since acoustic emission activity is attributed to rapid release of energy in material. The root-mean-square of the AE signal can be mathematically expressed [7] as:

$$AE_{rms} = \sqrt{\frac{1}{T}\int_{t_0}^{t_0+T} v^2(t)dt}$$

where $v(t)$ is the electrical voltage signal obtained from piezoelectric transducer, t_o is the initial time, T is the period of the AE activity.

The drawing of gate valve is illustrated in Figure 3. The gate valve operation pressure drop ΔP will be varied by the cross section area of the valve plunger which can be adjusted between 0-100%. The energy loss K_i may be in the form of heat and sound energy that will be calculated by the equation:

$$\Delta P = \sum_{i=1}^{i=n} K_i \cdot \left[\frac{128 \cdot \mu \cdot L \cdot Q}{\pi^2 \cdot d_L^4}\right]$$

where μ is kinemetric viscosity of the working fluid [m^2/s], L length of the conduit passage[m], Q the volumetric flow rate [m^3] and d_L the inlet pipe diameter[m^2].

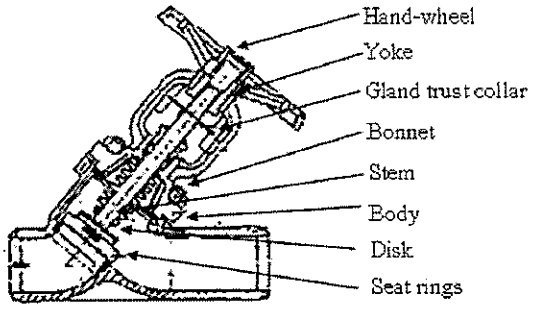

Figure 3. The drawing of gate valve.

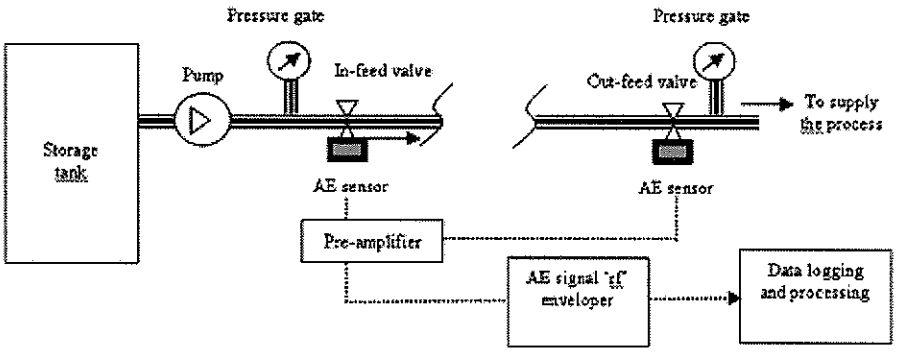

Figure 4. The gate valve test.

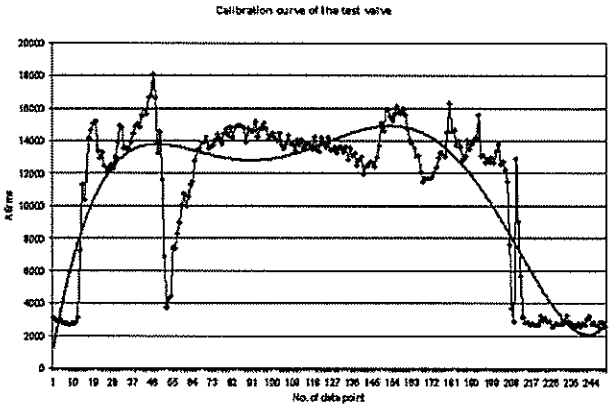

Figure 5. Example of calibration curve of the test valve.

EXPERIMENTAL RESULTS AND DISCUSSION

To verify the proposed approach, the experiment on the out-feed gate valve was performed as shown in Figure 4. The resonant type of acoustic emission transducers (Holroyd Instruments, UK: Model ASS-1) were mounted on the circumference of the surface of the valve. It provided the 100 kHz of resonant frequency which responded well with the material degradation and valve leakage. The acquired signal was then amplified with 60 dB gain pre-amplifier. The AE signal enveloper converted the amplified signal to 'rf' signal which was digitized to personal computer for further data logging and processing.

To obtain the calibration curve, the in-feed valve was fully opened from its closure condition. The pattern of AE_{rms} signal during the calibration process is shown in Figure 5. The investigation of the relationship between the AE_{rms} and the leakage rate was performed by using the 6^{th} order polynomial root determination. The result was investigated and its relationship can be mathematically expressed as:

$$y=(1.446 \times 10^{-9})x^6+(3.138\times10^{-8})x^5-(4.306 \times10^{4})x^4+ (1.336\times10^{-1})x^3-(1.568\times10)x^2+ (7.682\times10^{2})x+539.9 \quad (1)$$

where y is the AE_{rms} in x10 micro volt, x the leakage rate in percent. However, when a part of the valve is changed, the previously founded equation of calibration must be recomputed.

In the experiment, artificial leaks from incomplete closure of the out-feed valve were used to simulate the leakage. A centrifugal pump was used to generate the internal pressure about 2.0 bars within the system. To vary the leakage of the out-feed valve, each step size of the incomplete closure was varied at 10 percent increment step of the leakage rate. At each step of the closure of the valve, the AE signals from the piezoelectric sensor were captured, pre-processed and recorded into data logger. The AE_{rms} is therefore computed in order to correlate its energy of the AE signal with the leakage rate that can be obtained from the calibration curve.

The experimental result is shown in Figure 6. It illustrates that the time domain signal of the leakage rate at the 5 % gave rise to the abrupt change in

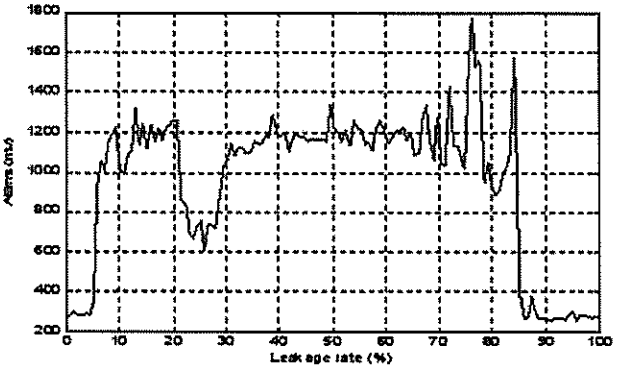

Figure 6. Results of AErms versus leakage rate (percent).

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Are bio-fuels a red herring or a sustainable option?

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Abstract With the world's fossil fuels depleting in supply, crop options were examined for the production of bio-fuels – either bio-diesel or bio-ethanol. In humid tropical Malaysia, the best option as a bio-diesel source is oil palm (surpassing all oil-producing crops), followed by coconut and Jatropha. However, Jatropha is yet unproven locally and harvesting the seed is very labour-intensive. For bio-ethanol production, Brazil has proven sugarcane to be the highest yielder. However, if two crops are raised on the same land in a year, sweetpotato can surpass sugarcane in ethanol yield. Looking at net energy ratio, bio-diesel from palm oil and bio-ethanol from sugarcane and cassava top the list, coupled with substantial reductions in greenhouse gas emissions. R&D may be able to improve the productivity and cost-efficiecny of Jatropha (for bio-diesel) and nypah palm (for bio-ethanol), but the future lies in giving research focus to the production of cellulosic alcohol from biowastes. Cellulosic alcohol production will eliminate the worry of tying up arable land meant for food and feed crops with crops grown for bio-fuels.

Keywords bio-diesel – bio-ethanol – crop yields – net energy ratio – Malaysia

INTRODUCTION

Without doubt, the world's fossil fuel reserves are depleting. It has been estimated that a decline in worldwide crude oil production will become apparent before 2010 [1]. That is only two years down the road! This is not helped by the ever-increasing demand for petrol and diesel. It seems like everyone wants to own a car ... or two ... or three!

What are bio-fuels? They are fuels derived from biomass, i.e. produced by living organisms such as plants, animals and microorganisms, or from metabolic byproducts of such organisms (e.g. cow manure). More important, they are a renewable energy source.

Basically, there are two types of bio-fuels: bio-diesel and bio-ethanol. We should also not forget bio-gas. A quick word about bio-gas (as I shall not be delving on this matter in this paper): bio-gas is typically a gas produced by anaerobic digestion or fermentation under anaerobic conditions of organic matter (which can be manure, sewage sludge, municipal solid waste, biodegradable waste or any other biodegradable feedstock). Bio-gas is made up principally of methane and carbon dioxide. It has a lot of potential because the production of bio-gas helps to recycle wastes.

BIO-DIESEL

With the escalating prices of petrol and diesel, bio-diesel is a hot topic in the news these days. Factories are sprouting up in Malaysia, competing with each other to convert palm oil into bio-diesel. We are indeed lucky to have palm oil at our disposal as the oil palm is arguably the highest oil-yielding crop in the world. Elsewhere, people are using coconut oil, soybean oil, rapeseed oil, sunflower seed oil, groundnut oil, etc. Indeed, any vegetable oil (even used cooking oil) can be used as bio-diesel; all that is required is that its fatty acids be first converted to esters. The acids used in the conversion are very expensive, but Toda *et al.* [2] at Tokyo Institute of Technology using common, inexpensive sugars have come up with a recyclable solid acid which costs one-tenth to one-fiftieth that of conventional catalysts.

The momentum to produce bio-diesel from palm oil has ground almost to a halt: of the 92 approved licensees, only six are operating, producing 350,000 tonnes per year. Why is this so? Earlier this year, the

higher amplitude of the AE 'rf' signal about 1.2 Volt from the background level about 0.3 mVolt (about 40 times higher). This was due to the small step sizes of the incomplete closure of the out-feed valve which caused the turbulent flow within the valve, hence the greater the AE activities. However, at 85 % of the incomplete closure of the valve the amplitude averaged about 0.4 Volt. This is because the flow of the fluid was becoming laminar flow, hence smaller amount of AE activities.

The methodology described in this paper has been shown to work well for condition monitoring of valve operating conditions. The method involves extracting the estimated calibration curve from the reference valve as a mean to identify the leakage rate of the test valve which is of the same type. Overall the method has the advantage that it can provide timely detection of the presence of leakage and efficient to implement and can be readily adapted to predict accurately from flow characteristics and their behaviour of the valve leakage in industry.

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Data collection using a real time production monitoring system for factors affecting production lines

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Abstract The ability of the real time production monitoring system to collect production data on real time basis would enable the production team to respond in a timely manner to solve any production related issues that may arise. Information must be collected at each shift end and disseminated accurately in order to meet the production goals. The task of a real time production monitoring system is to assist the production team to produce their best within the available resources. Apart from that real time production monitoring system helps in improving quality matters and reducing overheads. This system should also proactively detect and react to the faults by informing the relevant personnel in the departments before they escalate. Data collected should be used in analysis and should be ranked for further action. The data gathered should be accurate in order to identify the various faults at production level and attempts are made to immediately rectify them in order to improve the efficiency of the production line. An accurate data management system and a reliable production monitoring system are both equally important in improving production performance. Accurate monitoring of production lines enables better utilization of the available resources and hence efficiency. Studies have shown that information from production line is vital in producing valuable report for enhancing the production yield.

Keywords Real time production monitoring system – production data – production team – available resources – production line – data management system – production yield

TYPICAL PRODUCTION SYSTEM

A production line is a set of sequential operations established on a factory shop floor whereby materials are put through a refining process to produce an end product that is suitable for onward consumption or components are assembled to make finished goods. In general a production process involves a moving platform or conveyor to move partially completed products to workers who perform simple repetitive tasks designed to permit very high rates of production per worker.

There are typically three types of production line in industries which are robot and automated machines, semi automated machines (human and machines), and manual/work bench (human).

Production process using automated machineries or robots (Fig. 1) are capital intensive as it uses a high proportion of machinery in relation to workers. Machineries for automated production process such as robots have high installation costs. Thus, mass

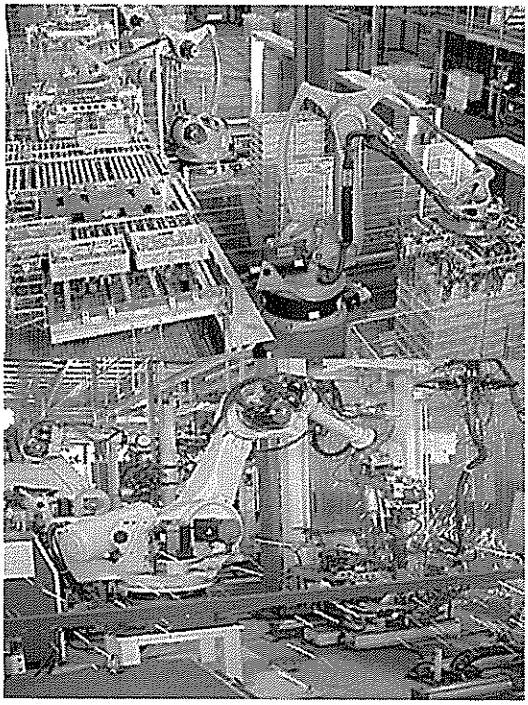

Figure 1. Automated production lines.

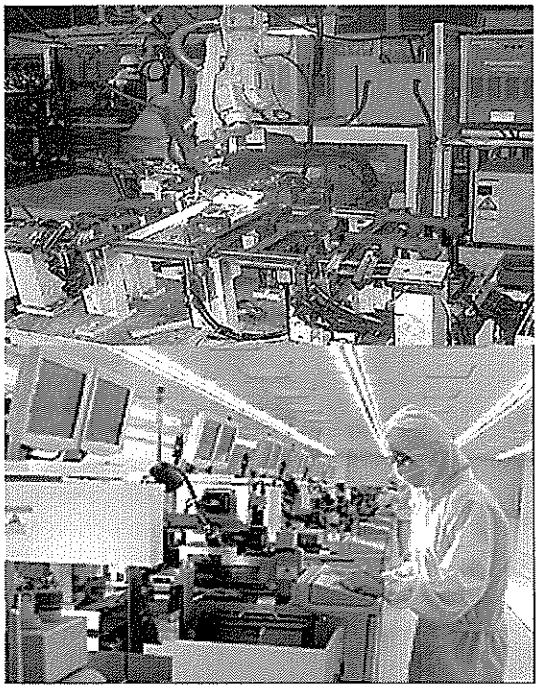

Figure 2. Semi automated production lines.

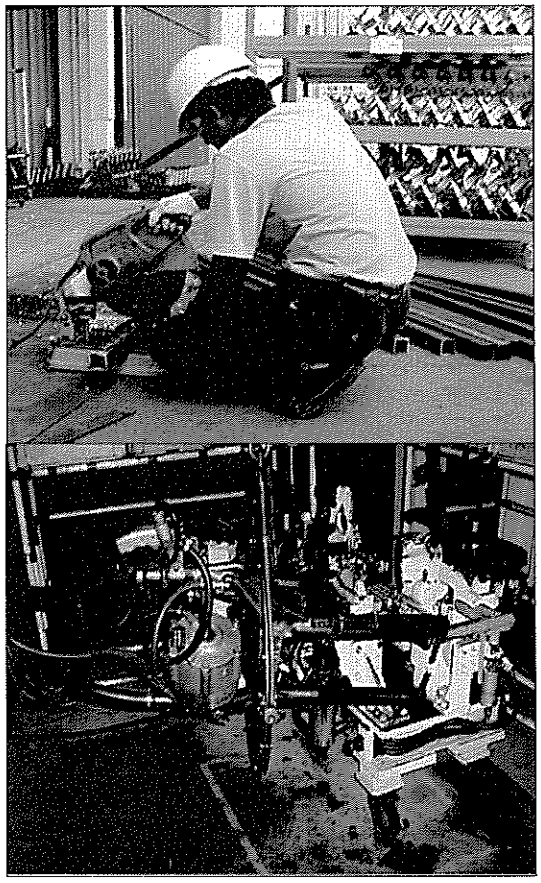

Figure 3. Manual/work bench production lines.

production is ideally suited to serve large, relatively homogeneous populations of consumers, whose demand would satisfy the long production runs required by this method of manufacturing.

Semi-automated production lines (Fig. 2) consist of human and machineries working together to accomplish certain task. Such production lines are practiced for moderate production output. The production process involves repetitive task for both the humans and machineries to establish products within definite standards.

Most of the work station has its craftsmanship in the workbench. Skilled workers are required to follow procedures in producing parts. Such production lines are practised for small scale production and when it comes for manual assembly process. The manual production line (Fig. 3) is focused on handmade products or requires specific method on producing the parts. Such production lines are operated base on low scale production lines and the production output is not consistent.

THE URGE FOR REAL TIME PRODUCTION MONITORING SYSTEM ON PRODUCTION LINES

The picture of the perfect shop floor is one that utilizes a real time production monitoring system to achieve optimized performance and minimal unscheduled downtime. Direct connection to machine controls is used to continuously collect data, monitor and analyze production related parameters without human intervention. This enables manufacturers to uncover opportunities for improvement and ensure that machines and man power are operating within defined specifications. Being able to view and analyze monitored production parameters from the shop floor of industries at any time enables the management and the production team to strive towards a better production yield [1-5].

A real time production monitoring system can save manufacturers thousands of Ringgit by increasing their production yield and optimizing the available resources. A real time production monitoring system is a tool that helps the management gather and distribute production data/information to anyone on the shop floor as events are happening. The ability to monitor dozens of process line parameters such as set goals, actual production output, cycle time, planned stop, rejection rate, non production time etc and alert

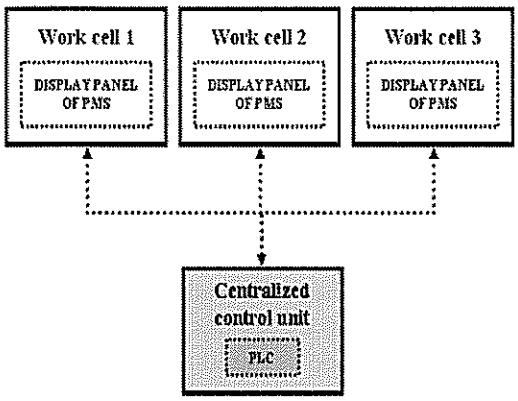

Figure 4. Layout of the real time production monitoring system.

plant staff to changing conditions can significantly decrease down time. Continuous monitoring gives the workers and the supervisors a direct line of sight into process line activity and enables them to keep up to the set goals. Apart from that production monitoring system enables the respective personnel to solve production problems quickly as event happens. A real time production monitoring system is essential in helping industries achieve realistic production goals, reduced down time and increase in production yield.

A real time production monitoring system consists of a real time display panel for monitoring production line problems. A systematic and accurate online data collecting system for production lines can be established with the help of this production monitoring system. The production monitoring system is useful for displaying the production line targets, acts as a calling unit and be able to inform the management on production line faults along the production process. The state-of-the-art electronics is used to build the entire production monitoring system (Fig. 4).

FACTORS AFFECTING PRODUCTION LINES

Analysis are carried out by the management on production line problems almost daily and counter measures are brought to light to further strengthen the performance of this production line. Analysis is made easy when data is translated into various categories base on critical factors which affect the production lines. The factors affecting production lines can be categorized into three – man power utilization, machine efficiency, and other factors. Each of these factors will result in various consequences towards the production lines.

Man power utilization

Competitive pressures and changing production management paradigms have increased the importance of reliable and consistent production equipment and have led to the popularity of Overall Equipment Effectiveness (OEE). Research that evaluates the various decisions in OEE development will help to drive towards a better productivity and yield. Production monitoring system is designed to maximize equipment effectiveness and improving efficiency of workers by establishing a comprehensive monitoring system covering the entire shop floor of industries. The design of the system enables users from all nature to interact with the system at any event of faults on production lines. The aim of production monitoring system is to bring together management, supervisors and workers to take rapid remedial actions as and when required.

Improper monitoring of workers without a production monitoring system will result in low standards of production output and will increase the maintenance of machineries. Data such as discussed in the case study below is used to visualize the actual performance of the workers. Human performance varies from time to time depending on their capability and duration of work. When the performance of a worker drops, the production output also drops. Improper monitoring of workers will result in low standards of production output and will increase the maintenance of machineries. A major factor contributing to this is the attitude of the workers themselves. From a study conducted in Company A, most of the workers tend to perform in an average manner and for most of the time there will be less productivity and resulting towards wastage of the production time. The only solution for this problem is to have better supervision on them during working hours [6].

Machine efficiency

Machine efficiency is one of the factors that are overlooked by the management and this can lead towards losses which reduces the yield [7, 8]. Even though the ultimate production calculation tool, OEE was brought to light ages ago, the awareness and the impact of OEE is not realized. OEE is a powerful production tool which compromises on equipments

performances specifically [1, 8].

Improper maintenance of machines will result in low standards of production output and will increase the maintenance of machineries. Machines are meant to work efficiently but in some circumstances, machines can be less productive due to improper preventive maintenance. Preventive maintenance is a key factor which keeps the machine running efficiently. The maintenance activity on machineries needs extra attention by the management and responsible personnel to ensure the optimum usage of machineries and to eliminate unwanted wastages due to machine stoppage.

Other factors affecting production lines efficiency

The amount of time taken to solve the faults on production lines plays an important role in maximizing the production output. Close attention on production line is necessary by appointing someone watching the whole shop floor without a blink of the eye to avoid all the problems from getting to the peak. On the race to meet the targets there will be unwanted breaks on the machines where it will delay the production process. The process to convey message to the respective departments on the faults is another obstacle in the existing industrial environment. The stops cause losses in production output. Adding to it is the calling process base on the departments availability is another factor of unmet production output.

Essentially human capitalize nearly all the processes on the industrial shop floor from the management to the layman (operators). Visualizing an industrial environment there are a big number of people from various departments working together in meeting the set goals. When it comes to unmet goals, fingers are not to be pointed to an individual whereby the supporting department also has their contribution on this matter. Monitoring of supporting departments in industries is another factor which should be taken in account for improving the production performance. The real time production monitoring system is designed to show the performance of all the related departments for each shift. By knowing their performance, the departments can be aware of the problems arising and counter measures can be taken to further improve their working quality. The supporting departments play an equal role as the production team in order to maintain the consistent pace of work on the industrial shop floor.

There are three departments in industries, viz. total quality management (TQM), production planning and control (PPC), and maintenance and others (vendors) which is the major contributor for production interruption.

The TQM involves all the quality matters of the parts produced. When a machine is not calibrated base on specification or irregular inspection on the parts produced it will result in higher rate of rejection or parts near to perfect. Rejection is a restricted word in industries because they will lead towards cost factor. Rejection can be reject rework which usually falls on parts that can't be repaired to maintain base on the specification of customers. The second rejection is reject scrap which is a total lost part which will directly result to waste. Such facts will affect the output when it comes to tight end.

The PPC involves in planning the production process and supplies base on orders. When parts are not ordered base on demand then the production process will be affected when the raw parts are running short. The most crucial task of this department is to plan the production process base on daily targets. Wise production line management is important for this team to sustain.

The maintenance department is responsible for all technical matters on the industrial shop floor. Preventive maintenance is the important task for this department whereby the machine has to be checked on timely basis to ensure optimum performance of the machines. This is to reduce machine parts replacements. Apart from that the most critical matter on industries is safety. When a machine is out of shape, the higher chances for the human who operates it will be injured.

CASE STUDY: COMPANY A

The real time production monitoring system was used to collect sample data from production lines of company A for analysis. The raw collected data from the production line is translated from machine language to management language which explains the content of the data base on the listed factors. Machine language here is the collected data on the performance of a certain machine and the data is later analyzed and interpreted for the management to understand their true capacity and performance. Base on the factors affecting the production line, data collected are categorized into 3 categories.

The performance of the worker was 55.88% (and 44.22% non utilized time) on a particular shift. Base on this information, the management can analyze the

Table 1. Factors affecting overall equipment effectiveness (OEE).

Factor	Effectiveness (%)
Availability	83.96
Performance	55.51
Quality	57.89
Overall	26.98

Table 2. Factors affecting the performance of department.

Department	Performance (%)	
	Target	Actual
Total Quality Management	98.11	85.85
Production Planning and Control	95	100
Others	95	100
Maintenance	98.11	98.11

work nature of the worker time to time to maintain a consistent work pace through the production process.

The overall equipment effectiveness of a machine (Table 1) was 26.98% on a particular shift. This finding shows that the machine was in a mint condition and the performance was far below average. The detail analysis on the machine is also visualized using the OEE formation and this further helps to uncover the crucial factor which drives the machine to less productivity.

On a particular shift, the performance of two departments (the production planning and control department, and others actual) was at 100% (Table 2). Next to it was the maintenance department at 98.11% and the worst was the total quality management department at 85.85%. With the help of a real time production monitoring system detail break down analysis is easily attempted.

By analyzing all the collected data, it clearly shows that the most crucial factor falls under machineries, followed by workers and the supporting departments. Such truthful data help the management to uncover the problems which reduce the production yield. Using the collected data, the root cause of the problem can be easily traced and counter measure could be taken base on necessary needs. There are lots of rooms for improvement and counter measures can be monitored using a real time production monitoring system.

CONCLUSION

The real time production monitoring system developed is an essential production tool in industries for both the management and the production team. It captures and distributes unadulterated production information at all levels along the production process without human intervention. With the collected data, realistic production goals can be achieved. Events occurring can also be displayed with the help of a real time production monitoring system. Production faults can be rectified instantly. A real time production monitoring system enables the production team to operate efficiently optimizing all available resources towards better production.

The real time production monitoring system works alongside OEE. The awareness of OEE is vital when it comes to decision making. Companies have begun to value the great strength of OEE in its ability to help the management improve the overall operation performance of their machineries. With OEE decision making is made easy. The simple metrics of OEE brings to light all the valuable information required by the management.

One of the greatest strengths of industries is human capital, whereby they are considered as the major role player on the development of industries. The real time production monitoring system helps the management to efficiently monitor the workers and drive towards optimum man power utilization which is in line with the set requirements of industries. Information on human capital will further strengthen the true capacity of the workers performance not only on the production lines but also to the supporting departments involved in the production process. When man power utilization is being optimized this will boost morale towards a better production yield.

With the limited resources available on the industrial shop floor, the practice of using real time production monitoring system is crucial. The real time production monitoring system should be fully utilized so that whatever resources available within the industrial sector were not wasted but used to the optimum to improve the production yield. By taking these necessary steps industries can improve and maintain a more efficient production line.

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Inverse identification of viscoelastic material properties using the biaxial bubble inflation test

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Abstract An inverse method based on the Levenberg-Marquardt algorithm and the finite element method has been applied to the bubble inflation technique. The method was used to identify viscoelastic constitutive properties from the bubble pressure–piston displacement relationship. The strain dependent behaviour was characterised by a two-parameter Mooney Rivlin hyperelastic model while the time dependent behaviour was characterised by the Prony series. To overcome difficulties of implementing the Prony series in an inverse method due to its large number of variables, the Prony series was fitted with a three-parameter modified power law equation. The inverse method was evaluated using numerical experiments and it was found that good estimates of the viscoelastic properties could be obtained using only one set of bubble pressure–piston displacement data.

Keywords biaxial – bubble inflation – viscoelastic – inverse identification

INTRODUCTION

The bubble inflation technique has long been used to characterise the mechanical properties of soft materials including rubbers, polymer melts, food materials and tissues [e.g. 1-8]. The technique is attractive because it approximates the biaxial tensile conditions attained in practice for these materials. For example, the technique is popular for characterising the behaviour of dough as it is perceived to simulate the expansion of the air cells in the dough during the baking process [5].

In the bubble inflation technique, a sample in the shape of a thin disc or a membrane is held by a clamp at its circumference and then inflated to a bubble using pressurised air [5]. The pressurised air can be achieved via for example a piston mechanism. Typically, the pressure inside the bubble and the bubble height are measured experimentally. An analytical solution which relates the stress-strain relationship to these measurements has been derived by Bloksma [3]. However, recent studies [5, 6] have suggested that the assumptions regarding the bubble geometry in Bloksma's solution are inaccurate and that it is necessary to measure the bubble geometry experimentally to calculate the stress and strain. Some of these geometrical measurements, such as the thickness of the material at the pole of the bubble, are tedious and very difficult to obtain.

With the advancement of computer technologies, inverse methodologies have been increasingly used to identify material properties from non-standard experiments data where the deformation field is not homogeneous and analytical solutions are not available. In the inverse method, the stress-strain properties are changed in an iterative manner until the predicted mechanical response matches the experimental measurements. The predictions are normally obtained numerically such as using the finite element method. For the bubble inflation technique, an inverse estimation of material parameters has been attempted previously [7]. In that work, the material was assumed to be characterised by a hyperelastic constitutive equation and time-dependent effects were not included [7]. However, it has been shown [5, 9] that there is a large variation of the strain rate as the bubble expands. For a highly viscoelastic material, the time dependent properties should be included in the analysis to provide a realistic characterisation of the material behaviour [4].

There are various mathematical models which are used to characterise the time dependent behaviour of viscoelastic materials. The Prony series, which consists of N number of exponential functions, is used widely in numerical codes since it can be efficiently implemented [10, 11]. However, to achieve a globally smooth, broadband viscoelastic behaviour across a large time span, a large number of exponential functions are required. Furthermore, the larger the number of parameters that needs to be characterised, the larger will be the computational expense of the inverse method [12]. An alternative to the Prony series is the power law equation [13, 14] which contains a smaller number of parameters but which is more difficult to implement in numerical codes.

The objectives of the current work were twofold. The primary objective was to apply an inverse methodology to obtain strain and time dependent viscoelastic properties from measurements of bubble pressure and piston displacement in the bubble inflation test. The second objective was to combine the benefits of both the power law equation and the Prony series to facilitate the inverse identification of the time dependent behaviour. To evaluate the feasibility of the methodology, a numerical experiment was performed since the correct solutions are known.

MATERIALS AND METHODS

Finite Element Model

The finite element analyses were performed using the commercial package ABAQUS [15]. The geometry of the simulation is shown schematically in Figure 1 [9]. The material was modelled to be in contact with a gas which was enclosed in a cylinder and driven with a piston at a predetermined speed. The material was modelled using axisymmetric, eight-noded elements while the gas was modelled using bi-nodal hydrostatic elements. Both the cylinder and piston were modelled as rigid bodies. Unless otherwise stated, the simulations were performed assuming a piston speed of 25 mm/min, which is of the same order of magnitude as the speed used in previous experiments [5]. The total piston displacement was 300 mm and the time duration was 12s.

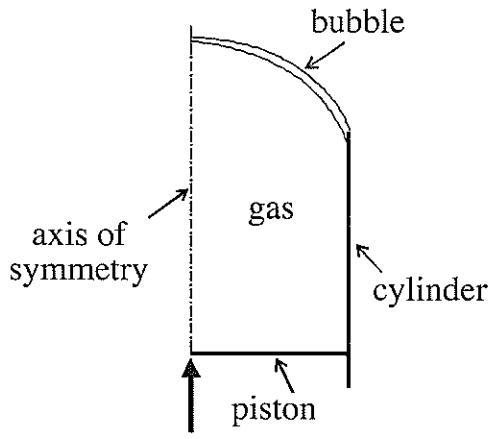

Figure 1. Schematic of finite element model to simulate the bubble inflation test.

From the simulations, values of bubble pressure and piston displacement were obtained from the ABAQUS code variables PCAV and U2 respectively. These variables relate to experimental measurements of pressure in the bubble and piston displacement that can normally be obtained without significant experimental set-up.

The inverse methodology pursued in this work was based on matching predicted bubble pressure–piston displacement using guesses for the material properties to 'experimental' data. The 'experimental' data here refer to exact finite element computations of PCAV and U2 using known values of material properties.

Material Model

Simple models to characterise viscoelastic behaviour typically assume that the strain and time dependent behaviour is separable such that under a step-strain relaxation test, the stress is described by:

$$\sigma(\varepsilon,t)=\sigma_0(\varepsilon)g(t) \quad (1)$$

where σ is the stress at true strain ε and time t. The strain dependent function, $\sigma_0(\varepsilon)$, has dimensions of stress and the time dependent function, $g(t)$, is dimensionless.

Two common models for $g(t)$ were of interest in this work. The first model was the Prony series which can be written as:

$$g(t)=g_\infty+\sum_{i=1}^{N} g_i \exp\left(-\frac{t}{\tau_i}\right) \quad (2)$$

where τ_i are time constants, g_∞ and g_i are dimensionless constants; and

$$g_\infty+\sum_{i=1}^{N} g_i = 1 \quad (3)$$

with g_∞ corresponding to the equilibrium component. The time constants are usually unknown, but for the approximation of experimental data, a popular technique is based on Schapery's collocation method where the range of τ_i falls within the duration of the experiment and each τ_i is associated with a decade of time apart [16, 17].

The second model was the power law equation [13, 14], which can be written as:

$$g(t) = \left(\frac{t}{t_r}\right)^{-n} \quad (4)$$

where n is the power law exponent and t_r is a reference time. Equation (4) is of limited use due to the singularity when $t \to 0$. In parallel to the Prony series where the concept of instantaneous and equilibrium (long-term) stresses are incorporated, the modified power law equation [17] can be used instead,

$$g(t) = g_\infty + (1 - g_\infty)\left(1 + \frac{t}{t_r}\right)^{-n} \quad (5)$$

where g_∞ defines the equilibrium component in the same way as in the Prony series.

Comparing between equations (2) and (5), it can be seen that the modified power law equation requires only three independent variables to characterise the time dependent behaviour, compared with N variables in the Prony series (assuming that the time constants are selected a priori). The fewer and fixed number of variables make the modified power law equation a more convenient choice for use in inverse methods. However, since the Prony series is used in ABAQUS, a fitting procedure has to be performed to convert equation (5) into equation (2).

The strain dependent behaviour of the material was assumed to be described by the Mooney-Rivlin hyperelastic potential, where the true stress in the uniaxial deformation state is given as:

$$\sigma_0(\lambda) = 2\left(C_{10}\lambda + C_{01}\right)\left(\lambda - \frac{1}{\lambda^2}\right) \quad (6)$$

where C_{10} and C_{01} are constants. Incompressibility of the material was assumed.

Inverse Method

The inverse method was used to determine the two strain dependent parameters, C_{10} and C_{01}, and the three time dependent parameters, n, t_r and g_∞. The Levenberg-Marquardt algorithm as described in [19] was utilised to minimise the objective function, Φ, which is given by:

$$\Phi = \frac{1}{2}\sum_{i=1}^{100}\left[r_i(\mathbf{p})\right]^2 = \frac{1}{2}\mathbf{r}^T\mathbf{r}$$

where $\mathbf{p}^T = \{C_{10}, C_{01}, n, t_r, g_\infty\}$ and $\mathbf{r} = \mathbf{P}^* - \hat{\mathbf{P}}$, where $\mathbf{P}^*$ and $\hat{\mathbf{P}}$ are respectively the predicted and experimental values of bubble pressure corresponding to the same piston displacement. The total piston displacement was divided into 100 points and the objective function was summed over all the points.

A general outline of the inverse method is presented in Figure 2. Guess values were initially assigned to the material parameters C_{10}, C_{01}, n, t_r and g_∞. The modified power law parameters were then fitted with the Prony series in a least squares error method in a Microsoft Excel spreadsheet using the Solver function. To perform this fitting, the number of exponential terms in the Prony series was predetermined. In addition, the time constants τ_i for these exponential terms were also selected a priori. The Prony series and the strain dependent parameters were then input into the finite element models of the bubble inflation to obtain the predicted bubble pressure-piston displacement relationship. This was repeated in subsequent iterations as the material parameters were changed in the algorithm to match the predicted relationship to the experimental measurements. If no solution was found after twenty iterations, the inverse method was stopped and the last computed material parameters were reported.

The inverse method was implemented in a Python script which interfaced with the ABAQUS software to perform the finite element simulations and with Microsoft Excel to fit the Prony series to the modified power law equation. In order to maintain stability of the material models and obtain physically meaningful values, the following constraints were applied: $C_{10} \geq 0$, $C_{01} \geq 0$, $t_r > 0$, and $g_\infty \geq 0$

These constraints may be implemented by means of the penalty method [7, 19] but in this work, an alternative implementation was used. Each of the parameters was assumed to be the squared product of another variable which was to be determined in the inverse method, e.g. $C_{10} = \left(C_{10}^*\right)^2$ where C_{10}^* replaced C as the free parameter in the inverse algorithm.

price of crude palm oil shot through the roof, making it uneconomical to consider bio-diesel. The potential market for bio-diesel in Europe has 'disappeared' due to the 'green' lobby in Europe which raised doubts over the eco-friendly status of palm oil, citing the destruction of rainforests – habitat to the orang utan – for planting more oil palm. Indeed, it is now evident that the bio-fuel industry in any country needs government support and commitment to succeed.

Recently, there has been a lot of excitement and interest in a 'new' oil-producing crop, *Jatropha curcas* or physic nut. Jatropha oil has a low iodine value (IV) of 13 compared to palm oil (54). Generally speaking, an IV of less than about 25 is required for long-term use in unmodified diesel engines. High IVs reflect a tendency for the oil to solidify whereas oils with low IVs can be used as fuel without any further processing other than extraction and filtering. Jatropha has also been touted as being able to grow in arid and rather infertile conditions – thus, should not be competing for fertile, arable land used for food crop production. However, we should approach the cultivation of Jatropha with caution at the present moment:

1. Harvesting the fruit is a major cost (35-50% of the total production costs) because it is necessarily a manual operation. We can ill-afford to embark on the cultivation of a labour-intensive crop.
2. Unlike annual oil crops like soybean, sunflower, groundnut and rapeseed, fruit ripening is not uniform and ripe fruit clusters are distributed all over the tree.
3. Harvesting is very labour-intensive: Jatropha requires about 500 man-hours to harvest one tonne of seed compared to only about 16 man-hours for oil palm.
4. Bio-diesel yield from oil palm at about 4,750 litres/hectare is unsurpassed by any other oil crop, whereas bio-diesel yield from Jatropha is a mere 1,500 litres/hectare [3] – less than one-third (Fig. 1).
5. Furthermore, there is as yet no supporting local data on the bio-diesel yield of Jatropha.

It may be seen that oil palm surpasses all oil-crops at the present moment in bio-diesel production. Coconut with a bio-diesel yield roughly half that of oil palm ranks as a poor second, with Jatropha coming in third.

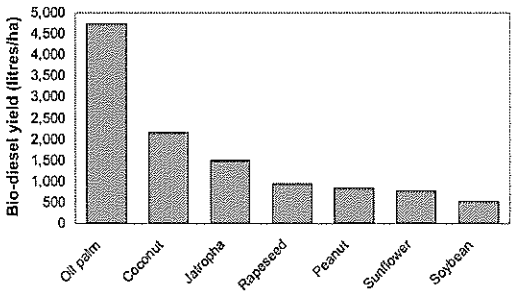

Figure 1. Comparative bio-diesel yields of selected oil crops.

BIO-ETHANOL

Essentially, bio-ethanol refers to alcohol fuel produced from crops. Usually crops which concentrate sugar or store starch are the obvious choices as alcohol is produced when sugar is fermented. For starch crops, an extra step is required to hydrolyze the starch into sugars first. Currently, ethanol is the alcohol of choice and petrol cars can take up to 10% ethanol without the need to modify their engines.

Brazil is a shining example of the success of bio-ethanol. It takes the lead in the use of ethanol made from sugarcane as a bio-fuel since its ethanol fuel programme began 30 years ago. There, ethanol is used interchangeably with petrol in specially modified car engines called Flex cars. USA uses corn as the feedstock for alcohol production, and gasohol (a mixture of alcohol and gasoline) is already available at petrol pumps in some states of USA. Other countries which have jumped on the bio-ethanol bandwagon include China (using cassava and molasses), Thailand (using cassava) and Canada (using corn).

From Figure 2, it may be seen that, on a given piece of land, the highest yielding crop in a year is sugarcane, followed closely by sweet sorghum and sugar beet. Sweetpotato can be almost as high-yielding if two crops (taking 3½ to 4 months per crop) are raised per year. Indeed, the ethanol yield per hectare from two crops of sweetpotato surpasses that of sugarcane. In any case, in Malaysia, climate conditions are too wet to raise sugarcane with a high sugar content, while sweet sorghum is more suited to the subtropics and sugar beet to the temperate zone. Thus, sweetpotato becomes the most likely choice for the production of bio-ethanol.

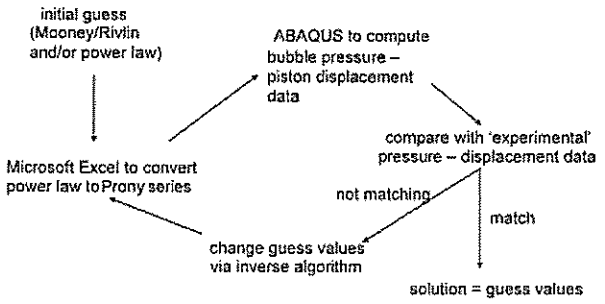

Figure 2. Outline of the inverse method.

Numerical Experiments

To evaluate the feasibility of applying the inverse method to the bubble inflation technique, numerical experiments were conducted, i.e. finite element computations of the bubble pressure-piston displacement based on known parameters were used as the 'experimental' data. The different experiments, referred to as 'cases', are summarised in Table 1.

In Cases 1 to 4, the time dependent behaviour was not included in the analysis. Thus, the inverse method was used to identify the values of C_{10} and C_{01} from the bubble pressure–piston displacement relationship. The experimental data were computed using the Mooney-Rivlin constants from [4].

In Cases 5 to 6, only the time dependent material parameters were subjected to identification in the inverse method. The experimental data were computed using the material parameters from [18]. Since a different hyperelastic potential was used in [18], the material parameters were recalculated for the Mooney-Rivlin hyperelastic potential. Two exponential terms were used in the Prony series corresponding to τ_i = 1s and 100s respectively [18]. Case 7 was similar to Case 5 except that the analysis was based on a piston speed of 0.25 mm/min rather than 25 mm/min.

In Cases 8 to 11, all five material parameters were subjected to identification from the inverse method. Similar to Cases 5 to 7, two exponential terms in the Prony series were used. In Cases 12 to 15, five exponential terms were used in the Prony series corresponding to time constants τ_i = 0.1s, 1s, 10s, 100s and 1000s. The larger number of exponential terms is preferable in practice since no information about the time dependent behaviour is normally known a priori.

RESULTS

The initial guesses and the final values of the material parameters, C_{10}, C_{01}, n, t_r and g_∞, for all Cases are shown in Table 1.

For Cases 1 to 4 where only the strain dependent parameters were evaluated, it can be seen that the inverse method could identify accurately the material parameters if reasonable initial guesses were used. Similar success has also been found previously [7].

For Cases 5 and 6, the final values for n, t_r and g_∞ were different even though their corresponding bubble pressure–piston displacement relationships matched the experimental data very well (Fig. 3). The discrepancies between Cases 5 and 6 were due to two factors. Firstly, the limited number of the exponential terms and the selected values of τ_i in the Prony

Table 1. Summary of values for the different cases in the numerical experiments. The units are kPa for C_{10} and C_{01} and seconds for t_r. Both n and g_∞ have no units. Where applicable, the values are expressed up to three decimal places.

Case No.	Input values					Initial guesses					Final values from inverse method					Itera-ions
	C_{10}	C_{01}	n	t_r	g_∞	C_{10}	C_{01}	n	t_r	g_∞	C_{10}	C_{01}	n	t_r	g_∞	
1	0.367	0.010				1.0	1.0				0.367	0.010				8
2	0.367	0.010				1E-06	1E-06				0.367	0.010				11
3	0.367	0.010				100.0	25.0				0.367	1.010				11
4	0.367	0.010				1E+04	2500.0				5188	2514				20
5	1.214	0.000	-0.468	0.396	0.000			-0.500	1.000	0.003			-0.596	0.669	0.003	8
6	1.214	0.000	-0.468	0.396	0.000			-0.100	100	0.010			-0.213	0.026	0.000	10
7	1.214	0.000	-0.468	0.396	0.000			-0.500	1.000	0.003			-0.546	0.549	0.000	17
8	1.214	0.000	-0.468	0.396	0.000	1.0	0.01	-0.500	1.000	0.003	1.214	0.000	-0.596	0.666	0.003	16
9	1.214	0.000	-0.468	0.396	0.000	1.0	0.01	-0.700	0.250	0.010	1.216	0.000	-0.566	0.387	0.084	20
10	1.214	0.000	-0.468	0.396	0.000	1.0	0.01	-0.300	4.000	0.010	1.005	0.001	-0.458	1.680	0.000	20
11	1.214	0.000	-0.468	0.396	0.000	25.0	0.01	-0.300	0.250	0.010	1.215	0.000	-0.554	0.421	0.061	11
12	1.214	0.000	-0.468	0.396	0.000	1.0	0.01	-0.500	1.000	0.003	1.130	0.000	-0.600	0.770	0.000	20
13	1.214	0.000	-0.468	0.396	0.000	1.0	0.01	-0.700	0.250	0.010	1.238	0.016	-1.235	0.937	0.113	20
14	1.214	0.000	-0.468	0.396	0.000	1.0	0.01	-0.300	4.000	0.010	1.262	0.020	-0.888	0.555	0.077	20
15	1.214	0.000	-0.468	0.396	0.000	25.0	0.01	-0.300	0.250	0.010	1.295	0.021	-0.680	0.337	0.044	20

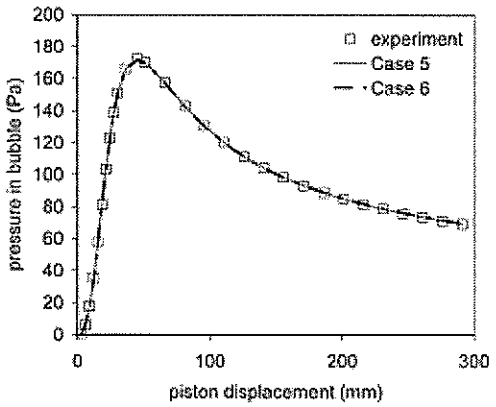

Figure 3. Comparison of bubble pressure-piston displacement relationships between the 'experimental' data and the results from the inverse method for Cases 5 and 6.

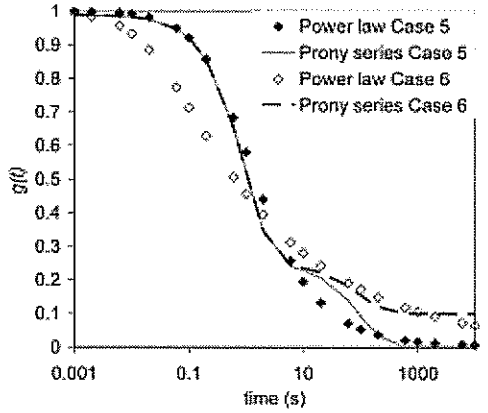

Figure 4. Comparison of $g(t)$ as described by the modified power law equation and the best-fit of the Prony series to the power law equations for Cases 5 and 6.

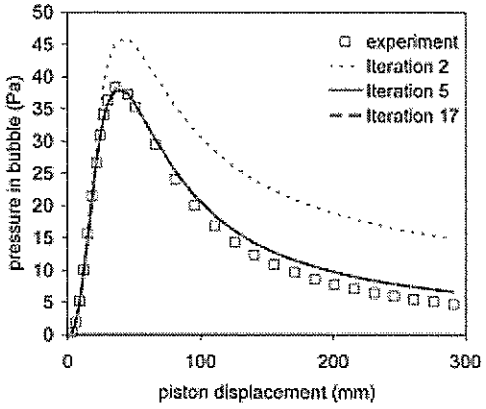

Figure 5. Changes in the bubble pressure-piston displacement relationship from the inverse method for Case 7.

series were inadequate to capture the differences in the modified power law equations. Comparison of the modified power law equation and the fitted Prony series for these two cases (Fig. 4) showed that although the power law equations displayed different time dependent characteristics, their characterisation by the Prony series was very similar except at larger time values. Secondly, the inverse identification of the time dependent parameters was sensitive only within the relevant times encountered in the experiments. Since the duration of the test was only 12s, the time dependent behaviour at larger times could not be characterised accurately. Similarly, in Case 7, when the inverse method was repeated on experimental data corresponding to a piston speed of 0.25 mm/min, the predicted bubble pressure-piston displacement relationships did not match the experimental data (Fig. 5). In this case, the values of τ_i were much lower than the test duration of 1200s. It can be concluded therefore that judicious selection of the number and the values of the time constants τ_i is required to obtain accurate results.

For Cases 8 to 11, the final predicted bubble pressure-piston displacement relationships matched the experimental data very well except for Case 10 (Fig. 6). The corresponding time dependent characterisation by the modified power law equations and the Prony series for these cases is shown in Figure 7. For Cases 8, 9 and 11, their time dependent characteristics were very similar and the problems observed for Cases 5 and 6 were applicable here as well. For Case 10, however, it appeared that the inverse method encountered a local minimum since the predicted bubble pressure-piston displacement relationships was different from the experimental data.

The final predicted bubble pressure-piston displacement relationships for Cases 12 to 15 are compared to experimental data in Figure 8. The conditions for these cases were the same as those for Cases 8 to 11, except that five exponential terms instead of two were used in the Prony series. For all cases except for Case 12, the final predicted bubble pressure-piston displacement relationships matched the experimental data very well. Figure 9 shows the corresponding time dependent characterisation by both the modified power law equations and the Prony series for these cases. For Cases 13, 14 and 15, their time dependent characteristics were very similar. In particular, the higher number of exponential terms

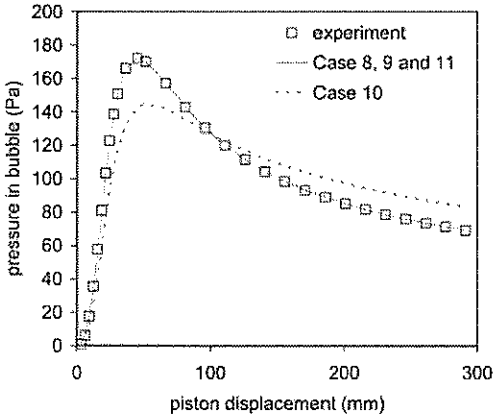

Figure 6. Comparison of bubble pressure-piston displacement relationships between the 'experimental' data and the results from the inverse method for Cases 8, 9, 10 and 11.

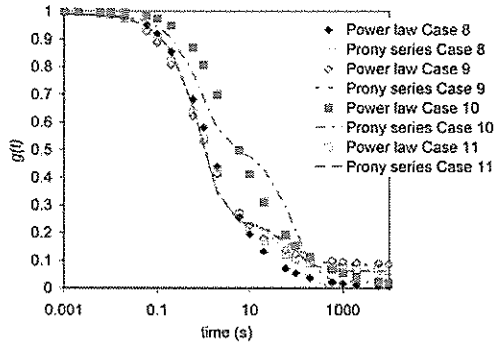

Figure 7. Comparison of $g(t)$ as described by the modified power law equation and the best-fit of the Prony series to the power law equations for Cases 8, 9, 10 and 11.

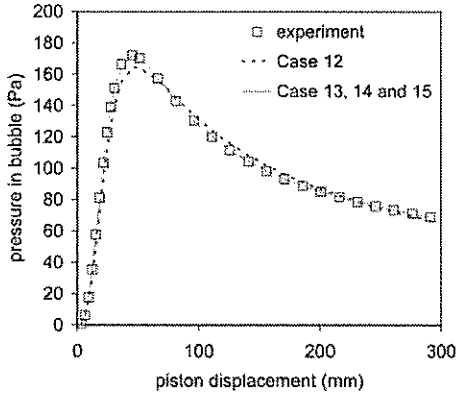

Figure 8. Comparison of bubble pressure-piston displacement relationships between the 'experimental' data and the results from the inverse method for Cases 12, 13, 14 and 15.

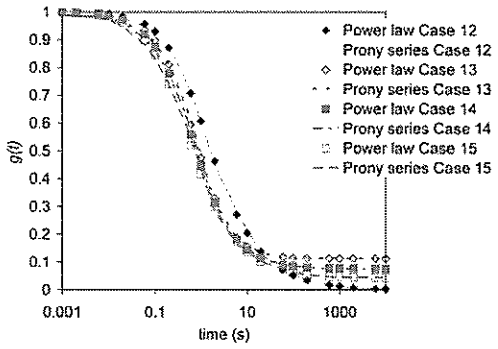

Figure 9. Comparison of $g(t)$ as described by the modified power law equation and the best-fit of the Prony series to the power law equations for Cases 12, 13, 14 and 15.

provided a much smoother characterisation of the time dependent behaviour. The kinks that were found in the Prony series for the earlier cases and that are unrealistic in practice have been removed. However, for these cases, different values of C_{10} and C_{01} were obtained although they tended to be within a 10% error of the solutions.

DISCUSSION

Inverse identification methods which combine optimisation technique and finite element analysis have been previously found to be useful for estimating material parameters from the bubble inflation test [e.g. 7]. However, previous inverse methods were limited to characterising only the strain dependent behaviour. For highly viscoelastic materials like dough, excluding the time dependent behaviour can lead to inaccurate material characterisation. This is evidenced by the differing values of C_{10} and C_{01} from [4] and [18] which were used for Cases 1 to 4 and Cases 5 to 15 respectively in the current work (see Table 1).

It was earlier thought that incorporating viscoelastic effects in the bubble inflation analysis would render the inverse identification analysis impractical [4]. However, the results obtained here suggest that it is possible to apply the inverse method to obtain both the strain and the time dependent material parameters. In particular, the viscoelastic parameters could be predicted without the need for information regarding the bubble geometry. Only data describing the pressure in the bubble and the piston displacement were necessary. The use of the piston

displacement instead of bubble volume or height implies that the pressurised air is incompressible. In the case of the material being soft, the volume of the air in the bubble could be assumed to be equal to the volume of air displaced by the piston [5]. For stiffer materials, the bubble pressure-piston displacement relationship would either need to be corrected for the compressibility of the air or be replaced by the bubble pressure-bubble height relationship [e.g. 7], or the air can be replaced by an incompressible fluid like oil [e.g. 20].

A somewhat surprising finding in the current work was that very reasonable identification of material parameters was achieved despite using data from one piston speed. This would most probably be due to the range of strain rates that were encountered at the pole of the bubble even though the piston speed was kept constant. To improve the accuracy of the inverse identification of the time dependent behaviour, it would be possible to use data corresponding to a number of piston speeds. However, this would increase the computational expense significantly since for the five parameters to be determined, there are six finite element simulations to be run at each iteration. If data from different speeds are used, then each speed would require six simulations in each iteration. It may instead be more practical to devise test conditions so that only one test is required. For example, the inflation could be stopped and the stress (pressure) relaxation of the bubble could be monitored.

Similar to other attempts to use inverse methods to identify both the strain and time dependent parameters simultaneously [e.g. 12], accurate identification of the material parameters has been observed to be dependent on the initial guesses of the parameters. However, the use of the modified power law equation was found to be useful to alleviate the difficulties in using the Prony series where a large number of exponential terms are required to give a smooth, broadband characterisation of the time dependent behaviour. Indeed, using the modified power law equation allows the flexibility of altering the Prony series without altering the number of material parameters to be identified in the inverse method.

This study has shown that both the strain and the time dependent behaviour can be characterised from the bubble inflation technique using an inverse methodology. The strain dependent behaviour can be characterised by a hyperelastic potential while the time dependent behaviour can be characterised by the Prony series. However, the Prony series can be difficult to implement in an inverse method due to its large number of variables. This can be overcome by pre-fitting the Prony series with a modified power law model and using the power law model in the inverse method instead.

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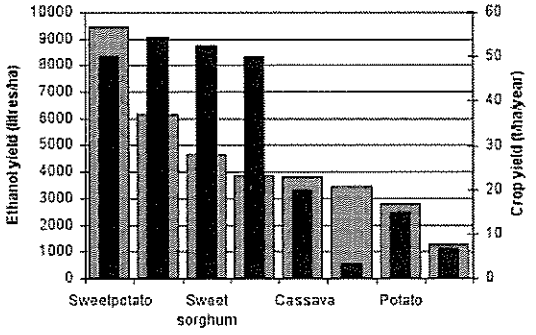

Figure 2. Comparative crop and ethanol yields of selected crops (with double-cropping of sweetpotato on the same piece of land). Modified from [4]. ▨ Ethanol ■ Crop yield

Table 1 compares the cropping of sweetpotato and corn for ethanol production. It may be seen that not only is our climate not particularly favourable for grain corn production, sweetpotato is a far more environment-friendly crop, using less chemical inputs (for weed, nutrient and pest management) and is less demanding of water.

In USA, the ethanol yield of corn has been boosted by converting the corn stovers into alcohol as well. Plant biomass can be converted into cellulosic ethanol using a combination of thermal, chemical and biochemical techniques. One tonne of fibre can yield more than 340 litres of alcohol. While there is as yet no commercializable technology for doing the same with sweetpotato crop wastes, this is the promise for the future. Wastes, made up of vines and leaves, can be as high as or even higher in biomass than sweetpotato root yields at the time of harvest. No doubt, the moisture content in these wastes is higher than in corn stovers which are almost dry at the time of harvest, but we are talking about volumes no less than 15 tonnes of dry biomass per hectare.

There is also the question of net energy ratios. Net energy ratio refers to the number of energy units produced for every unit of energy used in producing bio-fuel. Table 2 shows that of the three crops, the highest net energy ratio is for sugarcane, with sugarbeet and corn trailing behind. This is due in part to the use of bagasse (i.e. the fibre remaining after the extraction of the sugar-bearing juice from sugarcane) as a primary source of boiler fuel. However, recent research has shown the possibility of using cellulose from switchgrass (a naturally occurring dominant tallgrass species of the central North American prairies) resulting in a net energy ratio equivalent to that of sugarcane. Likewise, using the cellulose of corn stovers will boost the net energy ratio of corn substantially.

Data from Thailand [8] shows that in terms of net energy ratio, cassava is more efficient than corn in

Table 1. Comparison of the advantages of using sweetpotato instead of corn for bio-ethanol production in Malaysia.

Parameter	Sweetpotato	Corn
Suitability to agro-climatic conditions of Malaysia	Well suited for year-round planting, and even marginal soils.	Climate too wet for grain production. Requires fairly fertile soils.
Seed/Planting materials	Vegetative propagation. No seed cost after first crop.	Need to buy expensive hybrid seed every season.
Herbicide use/Erosion	Only pre-emergence herbicide used. Vines and leaves cover soil surface. Reduce erosion and weeds.	Causes more soil erosion, and uses more herbicides than any other crop.
Fertilizer use	Low requirement of N and P, but requires more K.	Uses more N than any other crop.
Pesticide use	Requires insecticide against weevil and stemborer. Can practise rotational cropping instead of chemical control.	Uses more insecticides than any other crop.
Water use	No irrigation after initial stage (except on sandy soils).	>1,700 litres of water to produce 1 litre of ethanol.

Table 2. Net energy ratios and greenhouse gas emission for selected materials used in bio-fuel production.

Material	Net energy ratio	Greenhouse gas emission	
		kg/L	Reduction
Sugarcane	8.3	1.08	56%
Sugarbeet	1.9	n/a	35-56%
Corn	1.35-1.5	1.94	22%
Cellulose from corn stover	4.39	n/a	n/a
Cellulose from switchgrass	8.3	n/a	n/a
Cellulosic ethanol	2-36	0.23	91%
Bio-diesel (from canola)	2.5	0.91	68%
Bio-diesel (from soybean)	2.5-3.0	n/a	41%
Bio-diesel (from palm oil)	6.0-9.5	n/a	>70%
Petrol	1.0	2.44	0%
Diesel	1.0	2.80	0%

Source: Adapted from [5], [6], [7], Worldwatch Institute (2006)

producing bio-ethanol, coming just after sugarcane (Fig. 3). We can probably expect the same with sweetpotato, another root crop.

There have also been claims that the sap of nipah palm (*Nypa fruticans*) can yield anything from 6,480-15,600 litres of ethanol per hectare [9]. These claims, however, have not yet been substantiated by actual research data, and are possibly based on extrapolations of sap yield from single palms. It must also be remembered that currently nipah stands are 'wild', i.e. not domesticated, and are located in swamps. The logistics of the labour-intensive tapping operation for the sap when manoeuvring a boat through a swamp are difficult to imagine. The sap is tapped from the peduncle of the young developing fruit, much the same way as coconut nectar is collected to make toddy. Moreover, the palms may not all be flowering and fruiting at the same time, and subsequent collection of the sap requires the tapper to 'hammer' the peduncle to encourage higher sap yield [10].

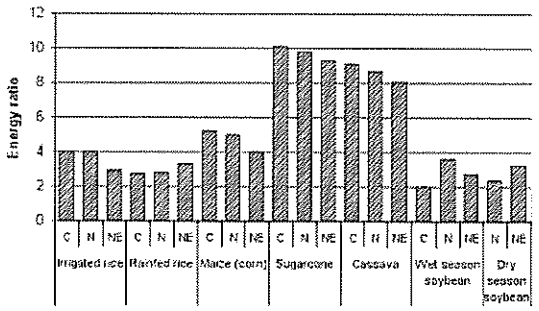

Figure 3. Energy ratios for different crops and regions in Thailand. C=Central plain, N=North, NE= Northeast. Source: [8]

PROS AND CONS OF BIO-FUELS

Bio-fuels may seem the natural solution for a world facing a future with ever-diminishing sources of fossil fuels. They are environmentally friendly as the combustion of bio-fuels produces very much reduced emissions of greenhouse gases. It has been estimated that using bio-diesel made from palm oil reduces CO_2 emissions by more than 80% compared to using petroleum diesel. Also, palm oil bio-diesel has a net energy ratio which surpasses even that of sugarcane (Table 2). Best of all, bio-fuels are renewable energy sources; hence, there will be no worry that their sources will run out.

Nevertheless, there are a number of issues which beg attention:

1. Bio-diesel has 86% of the energy content of diesel, while ethanol has only 67% of the energy content of petrol (Source: US Energy Information Administration).

2. Growing crops to produce bio-fuels is effectively reducing the amount of arable land available for food and feed crops.
3. Already, the effects of channelling corn into bio-ethanol production are apparent – the supply of corn for animal feed has been significantly reduced, leading to a world-wide shortage and to rising feed prices. Today, the price of grain corn imported into Malaysia has climbed to RM1.00 per kilogramme, up from RM0.37 in the late 1980s!
4. In the earlier part of 2008, the price of palm oil shot up tremendously because demand outstripped supply – probably as a result of palm oil being diverted from use as cooking oil to bio-diesel production.
5. It was estimated that if the palm oil price is US$500 (RM1,738) per tonne and crude mineral oil costs US$60 per barrel, the subsidy required to make bio-diesel competitive *vis-a-vis* petroleum diesel will be US$1.25/litre (RM4.39/litre)!

However, this scenario will change when the world eventually runs out of petroleum sources, and we still need to run our cars and lorries. It is a matter of supply and demand.

THE ROLE OF R&D

Currently, the viability of using Jatropha and nipah palm may be questionable. However, this does not mean that R&D will not be able to reverse the situation considerably. It must be remembered that the greatest advantage of both species is their ability to adapt to marginal land. This means their cultivation will not threaten or compete with food crops for arable land.

For example, shorter trees of Jatropha (either achieved through breeding and selection or through pruning practices) with the capability of uniform fruiting and fruit ripening, and thus amenable to mechanization, will vastly reduce the labour required for harvesting. Likewise, nipah palms planted in rows will provide easy access for tapping, while some form of floral induction will not only result in more reliable flowering but will also improve the overall productivity of the stand.

There are also other areas of R&D which merit attention. One is producing butanol instead of ethanol as bio-fuel. Butanol can be used directly in existing petrol cars without the need for modification of their engines. It produces more energy and is less corrosive and less water soluble than ethanol. The only reason it is not currently widely used is because it is too expensive to produce [11]. R&D can address this problem.

Another area for research focus is cellulosic alcohol. This type of production technology will be applicable to almost any type of biomass plant material, e.g. straw, grasses, leaves, stalks, wood or sawdust. Being able to produce cellulosic alcohol will not only supply bio-fuel, it will also help reduce the problem of crop waste disposal, of which burning – a particularly environmentally polluting operation – is the commonest solution, as well as cut down greenhouse gas emissions by as much as 91%. Producing cellulosic alcohol means there will be no conflict in using food and feed crops for fuel; thus, no competition for arable land. Again, there is a need to reduce the current high cost of production and to improve further the net energy ratio.

FINAL REMARKS

More seriously, in our race to produce bio-fuels an important matter has been overlooked: we are taking the heat off automobile manufacturers. The looming threat of impending shortage and eventual depletion of fossil fuels should have been used to pressure them into inventing more fuel-efficient cars or, better still, cars powered by other energy sources – such as solar power. Instead, by using bio-fuels to replace petrol or diesel, we are in fact 'feeding an undesirable habit' – almost an addiction, if you will – of driving gas-guzzlers. Besides we are probably helping the automotive industry to save millions of dollars which would otherwise have been used to fast-track R&D into designing more efficient cars, weaning us from the wasteful use of fuels – whether from non-renewable or renewable sources. Worse still, by tying up arable land in growing crops for bio-fuel production, we may unwittingly be depriving ourselves of access to cheap food and feed!

So, to answer the question posed by the title of this paper: Are bio-fuels a red herring or a sustainable option? No, they are not a red herring and can indeed be a very sustainable option with the backing of relevant R&D, as well as the government. Perhaps, we should concentrate on using the voluminous amounts