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El gusano canasta, *Oiketicus kirbyi* Lands Guilding (Lepidoptera: Psychidae), plaga de la palma aceitera

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Introducción

El gusano canasta, *Oiketicus kirbyi* es un insecto polífago que se alimenta de varios cultivos y plantas silvestres: musáceas (*Musa*s pp.), cacao (*Theobroma cacao* L.), palma aceitera (*Elaeis guineensis* Jacquin), pejibaye (*Bactris gasipaes* Kunth), cocotero (*Cocos nucifera* L.), almendro (*Terminalia catappa* L), cítricos (*Citrus* spp.), teca (*Tectona grandis* L.), eucalipto (*Eucalyptus*spp.), níspero (*Eryobothria japonica*) y otras.

En las plantaciones de banano en la zona Atlántica de Costa Rica se produjeron explosiones poblacionales durante 1962 y 1964 como consecuencia de las aplicaciones de un insecticida de amplio espectro y gran poder residual (dieldrin) para controlar una infestación de un áfido, las cuales destruyeron las poblaciones de enemigos naturales (Lara 1970).

La presencia de *O. kirbyi* en palma aceitera era conocida desde hacía muchos años en Centro América (Chinchilla 1989), pero el primer incremento poblacional en este cultivo se observó en una plantación vecina a otra de plátano en Puerto Armuelles, Panamá en 1990. A inicios del año siguiente, se presentó un incremento poblacional en otra plantación de palma aceitera relativamente cercana a la primera, pero esta vez en Costa Rica. Al año siguiente, el foco de la plaga (inicialmente en solo dos lotes de cosecha), se extendió a varios centenares de hectáreas, y los incrementos poblacionales se repitieron en los siguientes años. Durante todo este periodo (hasta 1996), se realizaron varias aplicaciones aéreas de *Bacillus thuringiensis* (generalmente Dipel: 0.8 a 1.5 l/ha), hasta que la población declinó y fue sostenida por los enemigos naturales. En setiembre de 1998 se observó un nuevo pequeño brote poblacional (menos de 100 ha), el cual no prosperó. Posterior a esto, la plaga se ha mantenido controlada por sus enemigos naturales.

O. kirbyi también es conocido en otros países de América en donde también ha causado daño. En Colombia, la plaga se presentó en palma aceitera en el César, en donde entre 1973 y 1985 se produjeron tres incrementos importantes en Palmeras de la Costa S.A. La población alcanzó hasta 353 larvas /hoja. (Villanueva y Avila 1987). En el Valle del Cauca, Colombia también se produjo una explosión en plátano en 1975-1976 que afectó 150 ha (García 1987).

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El presente trabajo tiene como objetivo resumir parte del conocimiento escrito acerca de *O. kirbyi* en América tropical, y en particular lo aprendido en Costa Rica, durante los incrementos poblacionales a inicios de la década de los noventa.

La familia Psychidae

La larvas de la familia Psychidae se reconocen porque construyen canastas de seda y fragmentos de la planta o substrato. Las larvas son cilíndricas; la cabeza hipognata, pigmentada, patas torácicas bien desarrolladas; cuatro pares de propatas abdominales con crochetes uniordinales, dispuestos en una penelipse lateral. Poseen un par de propatas anales. Miden de 8 a 50 mm.

Los adultos presentan un dimorfismo sexual marcado; la hembra es neoténica de apariencia de larva y está dentro de una canasta protectora y el macho tiene apariencia de mariposa o polilla y es de vida libre.

La pupación ocurre dentro de la canasta y el último estado larval ata la canasta a algún soporte y luego invierte su posición con la cabeza hacia abajo. En los géneros más avanzados, la hembra no abandona la canasta, excepto cuando deposita los huevos, cae al suelo y muere. En el interior de la canasta las hembras colocan de 200 a 13000 huevos, dependiendo de la especie. Todas las especies son univoltinas, con un período larval largo y un período de adulto breve en el cual no se alimentan (Stehr 1987).

La familia se compone de cerca de 600 especies, de ellas 500 están en el Viejo Mundo; 26 especies se encuentran en Estados Unidos y Canadá. El género *Oiketicus* contiene tres especies en América tropical. *O. kirbyi* se encuentra distribuido en la tierras bajas desde Brasil a México e Islas del Caribe.

Morfología de *Oiketicus kirbyi*

Adulto: Las hembras son neoténicas, de apariencia larviforme, con la cabeza pequeña, sin antenas y con el aparato bucal atrofiado; no salen del cesto, sino que son fecundadas por el macho en el interior del mismo. Antes de la cópula el abdomen de la hembra está repleto de óvulos lo que le confiere un tamaño grande; después de la oviposición su volumen se reduce casi a la mitad, abandona la canasta y se deja caer para morir.

El macho es una polilla de color café, con zonas claras y oscuras; alas pequeñas de 42 mm de envergadura, cuerpo cubierto de escamas, tórax grueso, abdomen delgado y extensible, aparato bucal atrofiado y antenas bipectinadas. La longevidad promedio es de 3.9 días para las hembras y de 3.0 días para el macho.

Durante la cópula, el macho rompe el extremo de la canasta de la hembra con unos ganchos que tiene en el extremo del abdomen y penetra la abertura genital extendiendo su abdomen hasta 70 mm.

Huevo: es de forma cilíndrica con aristas redondeadas (0.34 x 0.53 mm). Al inicio son de color crema, luego anaranjados y próximos a la eclosión se tornan oscuros. Durante la oviposición son depositados dentro de la última exuvia pupal.

El período promedio de incubación es de 43 ± 1.4 días (27 a 47). La viabilidad es normalmente muy alta y el número de huevos varía de 3500 a 6000 unidades.

Larva: recién nacida es de color amarillo y en los últimos estados de desarrollo son de color ceniza; las hembras son más oscuras que los machos, con manchas negras y de tamaño irregular en el tórax y la cabeza. La cabeza es quitinosa, con mandíbulas fuertes; tórax con tres pares de patas fuertes; abdomen con 8 segmentos, cuatro pares de propatas. La parte anal es un segmento café oscuro, un poco quitinoso y también con un par de propatas.

Al nacer las larvas salen por una abertura en el extremo inferior de la canasta, secretan un hilo de seda y se dispersan con ayuda del viento (foresia). Al descender en la vegetación de inmediato inician el raspado de la epidermis del follaje usando los restos, los cuales pegan con secreciones salivares, para formar la canasta.

A medida que desarrolla la larva va ampliando la canasta (Cuadro 1) con pedazos de follaje, ramitas y nervaduras. Al nacer mide 1.5 mm y al finalizar la etapa larval mide 39 mm en el macho y 55 mm en la hembra. La canasta del macho es color café claro o gris, y mide de 40 a 65 mm, y en la hembra es de color café oscuro y mide de 58 a 85 mm (Campos et al. 1987).

La duración del período larval oscila entre 145 a 185 días, una duración promedio de 140 días en los machos y de 151 días en las hembras. Los machos y las hembras tienen 8 y 9 estados de desarrollo, respectivamente. Stephens (1962) menciona 15 a 20 estados larvales en el macho y de 12 a 15 en la hembra.

Cuadro 1. *Oiketicus kirbyi:* dimensiones de la larva y del cartucho protector (Coto, Costa Rica, 1992) *

Clase	Larva (mm)		Cartucho (mm)		
	Diámetro	Largo	Diámetro	Largo	Ámbito
I	3.97 ± 0.46	15.9 ± 0.90	8.72 ± 1.0	25.5 ± 1.6	10 - 20
II	5.83 ± 0.60	21.2 ± 3.30	11.4 ± 1.8	34.4 ± 1.3	21 - 40
III	7.15 ± 0.90	26.7 ± 3.60	14.0 ± 1.0	45.2 ± 1.8	41 - 63

* Muestra de 120 individuos

Pupa: la pupa hembra tiene ambos extremos redondeados, es de apariencia segmentada y sin señales externas de patas, antenas y otras estructuras. La pupa del macho tiene el extremo posterior puntiagudo y encorvado hacia la parte ventral y exhibe las placas que le van a dar origen a las estructuras externas. En las hembras es de coloración castaño oscura y en los machos de coloración gris. La duración promedio es de 38.2 ± 2.0 días; la pupa en el macho mide 6.7 ± 3.4 x 27.7 ± 1.4 mm y en la hembra mide 9.3 ± 0.8 x 35.7 ± 2.1 mm (diámetro x largo) (Campos et al. 1987).

El ciclo de vida ha sido estudiado por varios autores (Stephens (1962; Campos et al. 1987; García 1987), y existen diferencias en la duración de las etapas de desarrollo informadas por ellos. Esto se puede explicar por diferencias en el procedimiento de cría, condiciones climáticas, substrato de alimentación utilizado y sobre todo por la dificultad que representa estudiar a un

insecto que permanece encerrado en una canasta por un período de tiempo prolongado. Los datos del ciclo de vida se resumen en el Cuadro 2.

Fig.1. *Oiketicus kirbyi* larva

Fig.2. *Oiketicus kirbyi* macho

Fig.3. *Oiketicus* macho recién emergido del cartucyo (Foto M.Rhainds)

Fig.4. *Oiketicus* hembra mostrando las escamas impregnadas con feromonas

Comportamiento

Las hembras recién emergidas de la piel pupal, impregnan los pelos o escamas que producen en el extremo inferior de la canasta con una mezcla de feromonas para atraer a los machos. Se han identificado a menos cinco compuestos (ésteres) con actividad sexual, y de estos, el 1-metil-butil decanoato es el que se produce en mayor cantidad y muestra más actividad (Rhainds et al. 1994).

Cuadro 2. Ciclo de vida de *Oiketicus kirbyi* (25 ±3°C; R.H.=70%; foto fase: 13hr)*

Estado	Dias	Largo (mm)
Huevo	27-47	0.53
Larva 1	12	1.85
2	10	2.74
3	10	4.25
4	15	7.50
5	12	9.53
6	13	13.8
7	21	22.2
8 (macho)	47	37.0
8 (hembra)	45	39.2
9 (hembra)	13	56.5
	225-245	
Pupa (macho)	29-36	27.7
Pupa (hembra)	23-31	35.7
Adult (macho)	3.5	27.0
Adult (hembra)	3.9	30.0
Total	284.4-319.4	

* Campos et al. (1978); García (1987); Stephens (1962)

Las hembras que pupan en las puntas de las hojas más jóvenes de la palma (posición más erecta) son fecundadas en una mayor proporción que aquellas que están en las hojas más viejas (hojas más horizontales). De hecho ocurre que las hembras pupan en una mayor proporción que los machos en el estrato superior de las palmas. No obstante, los machos prefieren a las hembras más grandes que no pupan necesariamente en el estrato superior.

La mayor preferencia de los machos por las hembras más grandes, se debe posiblemente a que estas producen una mayor cantidad de feromonas y tienen también el potencial de producir una mayor masa de huevos. De esta forma, las hembras de menor tamaño podrían compensar estas desventajas pupando en las partes más altas de la planta para tener más oportunidades de atraer a los machos que tienden a volar en el estrato superior del follaje. Por otro lado, la pupación en las hojas más altas, ofrece la ventaja adicional de mejorar la dispersión de la feromona y de las larvas recién nacidas que producen seda para colgar y dejarse arrastrar por el viento.

No obstante este comportamiento, no se encuentra una mayor cantidad de hembras de pequeño tamaño en las puntas de las hojas más jóvenes (la mayoría de las hembras pupan en los estratos medios del follaje), por lo cual se puede asumir que la selección del sitio para pupar por parte de las hembras, depende de otros factores, además del tamaño. Un elemento que puede estar en juego, es la mayor exposición a algunos depredadores, de un individuo que se localice en el exterior de la palma (Rhainds et al. 1995a; 1995b).

El macho llega a la canasta de la hembra, se sujeta para introducir su abdomen extensible por la abertura inferior del mismo, y copula a la hembra por un período promedio de 30.7 ± 4.6 minutos (ámbito de 23 a 61). La hembra inicia la oviposición casi de inmediato en la cual dura 1.8 ±0.6 días (1 a 3) en la cual deposita varios miles de huevos (Campos et al. 1987).

Los machos son vespertinos y nocturnos y vuelan en forma activa. Se ha observado que son atraídos por la luz eléctrica. Las hembras empiezan a emerger unas tres semanas antes que la mayoría de los machos, por lo cual inicialmente se presenta una relación muy alta de hembras a machos (entre 10:1 y 2:1). De esta forma, la posibilidad de una hembra de aparearse, está limitada por el bajo número de machos disponibles. El resultado final, es que muchas hembras no obtienen un compañero, abandonan el canasto y mueren entre dos y cuatro días después de haber emergido como adultos (Rhainds et al. 1995b).

Daños

La larva puede alimentarse en una gran variedad de especies vegetales que incluye cultivos y malezas. Cuando la larva desciende sobre el follaje de la planta inicia su alimentación de inmediato; muchas veces las corrientes de aire, animales o vehículos trasladan a las larvas, a gran distancia. Las larvas pequeñas tienen poca capacidad de desplazamiento por sí mismas, sin embargo, las larvas grandes pueden movilizarse en el follaje de la misma planta o bien entre plantas.

Cuadro 3. Capacidad de alimentación de las larvas de *Oiketicus kirbyi* (laboratorio)*

Periodo (días)	Tejido consumido	Consumo diário	# de larvas permitidas/hoja **
1-20	1.08	0.05	580
21-40	4.31	0.22	132
41-60	10.87	0.54	54
61-80	20.64	1.03	28
81-100	79.15	3.96	7
101-125	188.46	7.56	4
Total	304.51		

* Villanueva y Avila

** Número de larvas por hoja que aún no causan daño económico

Las larvas desarrolladas pueden soportar ayunos prolongados, lo que sumado a la excelente protección que brinda la canasta, un ciclo de vida prolongado y gran fecundidad de las hembras, le confiere una gran capacidad de sobreviviencia.

Una larva consume aproximadamente 304.5 cm² de follaje de una palma. En el Cuadro 3 se resume la capacidad de consumo promedio y el número de larvas por hoja permitidas sin causar daño económico. A partir de este punto se considera un nivel crítico en el cual se debe intervenir para evitar mayores defoliaciones.

Muestreo

Dado que la mayoría de las larvas se localiza en las porciones apicales y subapicales de las hojas superiores de la palma, Rhainds et al. (1996) encontraron que la población de larvas en una hoja intermedia en el follaje, correlacionaba muy bien con la población total de larvas en toda la palma. El muestreo de 160 folíolos terminales en la hoja en posición 17 (80 a cada lado del raquis) representa un compromiso entre costos, eficiencia y confiabilidad del muestreo. Durante el aumento poblacional en Coto, Costa Rica, los autores mencionados obtuvieron un número promedio de 45.22 ± 4.21 larvas de primer estado de desarrollo cuando muestrearon 160 folíolos en la hoja 17. En este estudio, el muestreo de una palma por hectárea ofreció un buen estimado de la población en toda el área afectada.

Enemigos naturales

Durante los incrementos poblacionales del gusano canasta en América tropical se ha observado una amplia gama de organismos que participan en la regulación de las poblaciones (Cuadro 4), incluyendo lagartijas, pájaros y otros vertebrados.

Entre los enemigos naturales insectiles, las avispas parasitoides son las más importantes como reguladores: *Digonogastra diversus* (=*Iphiaulax* pos. *psychidosphagus*) (Braconidae), *Conura brethesi*, *Conura oiketicusi* (= *Psychidosmicra* sp.), *Brachymeria*sp. (Chalcididae), *Ateleute* sp. y *Filistina* sp (Ichneumonidae). El orden de mención podría decirse que también es el orden decreciente de abundancia de estas avispas, observado en explosiones de población. Entre los dípteros parasitoides destacan las familias Sarcophagidae y Tachinidae, y en varias oportunidades también se han observado grandes epizootias causadas por entomopatógenos, en algunos casos por la bacteria *Klebsiella oxitoca* (Stephens 1962; Lara 1970; Gravena y Almeida 1982; García 1987; Villanueva y Avila 1987)

Durante una explosión poblacional de *O. kirbyi* en las plantaciones de palma aceitera en Coto, Costa Rica en 1991-1992 se observó un fuerte parasitismo de las larvas por *D. diversus* y *Conura* spp. En una muestra de varios centenares de larvas recolectadas en el campo, se clasificaron las larvas en tres categorías de tamaño de la canasta (Cuadro 1) y de ellas se obtuvieron los parasitoides de una muestra de 379 larvas (Cuadro 5). Los parasitoides más abundantes fueron *D. diversus* (57.7%) y dos especies de *Conura* (posiblemente *C. oiketicusi*, 17.15% y *C. brethesi*, 17.15%).

Fig.5. Parasitoides en *Oiketicus*

Malezas huéspedes de los enemigos naturales. Las avispas parasitoides de *O. kirbyi* se han observado alimentándose de las flores y las glándulas extraflorales de varias especies vegetales. En las siembras jóvenes de palma, las larvas del insecto son fuertemente parasitadas (hasta un 95%) por las avispas, lo cual contrasta con el parasitismo observado en plantaciones adultas (menos de 10%), posiblemente debido a la escasez de enemigos naturales, los cuales están limitados por la carencia de vegetación melífera.

Cuadro 4. Enemigos naturales de *Oiketicus kirbyie* en América tropical *

Dermaptera: Forficulidae, *Doru lineare*

Neuroptera: Chrysopidae, *Chrysopa* sp.

Hymenoptera:
Braconidae, *Cotesia* sp, *Digonogastra diversus* (1-26 wasps/pupa)

Chalcididae, *Conura oiketicus* (1 wasp/pupa), *Conura brethesi* (1 wasp/pupa), *Conura* sp (1 wasp/pupa), *Brachymeria* sp. (1 wasp/pupa)

Eulophidae, *Elachertus* sp., *Tetrastichus pseudoceticola*

Ichneumonidae, *Filistina* sp. (1 wasp/pupa), *Ateleute* sp. (1 wasp/pupa), *Carinodes* sp., *Cristolia* sp.

Bethylidae, *Perisiorola* sp.

Diptera: Tachinidae, *Achaetoneura* sp.

Sarcophagidae, *Sarcophaga lambens* (1-5 flies/pupa)

Enterobacteriaceae, *Klebsiella oxitoca*,

Fungi imperfecti, *Beauveria bassiana*

* Stephens (1962); Ponce et al. (1970); Gravena and Almeida (1982); García (1987); Genty (1989); Villanueva (1987)

En siembras nuevas, y durante la floración de la vegetación en el sur de Costa Rica (diciembre a abril), es común observar a *D. diversus* en *Amarantus spinosus* (bledo), *Baltimora recta* (florecilla), *Cassia tora*, *Scleria melaleuca* (navajuela) y *Vitis sycioides* (uva cimarrona). Las avispas *Conura* spp. son comunes en *A. spinosus*, *C. tora*, *Melanthera aspera* (paira), *Solanum jamaicense* (tomatillo) y en otras especies. Así también, *Ateleute* sp. y *Filistina* sp. comparten las mismas especies vegetales que las avispas mencionadas (Cuadro 6).

Cuadro 5. Parasitismo en larvas de tres tamaños de *Oiketicus kirbyi* (Coto, Costa Rica, 1992)

Parasitoide	Tamaño de larva			Total	%
	I	II	III		
Conura pos. oiketicus	56	9	-	65	17.15
Conura pos. brethesi	61	4	-	65	17.15
Conura sp.	2	-	-	2	
Brachymeria sp.	4	1	-	5	1.31
Digonogastra diversus	48	159	12	219	57.70
Filistina sp.	7	1	-	8	2.11
Ateleute sp.	8	-	-	8	2.11
Eulophidae n.i.	3	-	-	7	1.84
Total	189	178	12	379	100.0
%	49.86	46.96	3.17		

Manejo integrado

El combate del insecto requiere de la combinación de varias medidas de manejo, las cuales se ejecutan apoyadas en un recuento de las larvas en la hoja 17, incluyendo las sanas y parasitadas o enfermas, y un muestreo de los enemigos naturales en la vegetación acompañante.

Combate químico. En el pasado, las aplicaciones aéreas de insecticidas de amplio espectro y residuales, agravó los problemas con el gusano canasta porque se afectaron las especies de enemigos naturales que son muy susceptibles a los insecticidas. La protección que le brinda la canasta al insecto y la capacidad de soportar ayunos prolongados, hace de este insecto un organismo difícil de combatir por medios químicos.

Las opciones de combate químico se deben limitar al uso de insecticidas selectivos que no tengan efecto sobre los enemigos naturales, o en su efecto que no estén en contacto con los mismos. El insecticida organofosforado monocrotofos (Azodrin) ha sido usado mediante la técnica de inyección al tronco (14 a 18 cc/palma), y se ha logrado hasta un 98% de mortalidad en 15 días. No obstante, esta técnica puede resultar muy laboriosa y difícil de ejecutar, y no está libre del riesgo de afectar a los enemigos naturales de la plaga.

Table 6. Relative abundance of 3 families of parasitoids of *Oiketicus kirbyi* on some plants*

Plant species	Braconidae	Chalcididae	Ichneumoinidae
Amarantus spinosus	C	A	E
Baltimora recta	C	C	-
Byttneria aculeata	E	C	P
Cassia reticulata	E	C	P
Cassia tora	C	C	C
Flemingia congesta	C	-	-
Melanthera aspera	P	C	C
Priva aspera	E	P	E
Scleria melaleuca	C	A	C
Senna stenocarpoides	E	P	P
Solanum jamaicense	-	A	P
Triunfetta semitriloba	P	E	P
Urena lobata	P	C	P
Vitis sycioides	C	A	C

Fom Mexzón (1997). Braconidae: *Digonogastra diversus;* Chalcididae: *Conura* sp. and *Brachymeria* sp. Ichneumonidae: *Ateleute* sp. and *Filistina* sp. Relative abundance: E= very few (1-9 individuals/10 plants of the same specie), P= few (1-4 individuals/plant), C= common (5-15 individuals/plant), A= abundant (more than 15 individuals/plant.

Las preparaciones comerciales o artesanales de *Bacillus thuringiensis* (Thuricide, Dipel y otros) da resultados variables, dependiendo de las condiciones climáticas. Un buen control del insecto se logra únicamente cuando se hacen al menos dos aplicaciones espaciadas 3-4 semanas para quebrar la estructura de la población. El Dipel se ha usado con cierto grado de éxito (2-3 kg/ha: 70 % de mortalidad), lo mismo que los inhibidores de síntesis de quitina como triflumuron (0.45 a 0.75 g de i.a./ha) y la nereistoxina (Padan).

Combate cultural. En palmas jóvenes se ha utilizado la recolección manual de las canastas del follaje, y en algunos casos, el corte con machete de las puntas de las hojas donde se concentra la mayor densidad de larvas. Esta última práctica no puede recomendarse pues, dependiendo de la población de larvas, el daño causado podría ser superior al beneficio obtenido. La recolección manual es costosa en términos de mano de obra, y tiene el inconveniente de que es muy probable que la mayoría de los individuos recolectados sean machos, ya que las hembras tienden a preferir las puntas de las hojas más jóvenes, las cuales no son fácilmente alcanzadas por los trabajadores.

Durante un incremento poblacional de esta o cualquier otra plaga, se debe realizar un control de malezas selectivo, e incluso suspender el control de malezas por algún tiempo, de ser esto posible. En forma complementaria se debe implementar un programa de siembra de malezas atractivas de las avispas parasitoides (Cuadro 6), o que sirvan de refugio a los depredadores.

El control de malezas sistemático y en grandes extensiones del cultivo deben substituirse por opciones como el trazado de carriles entre palmas, desmatonado, la chapia en franjas y otras prácticas similares.

Combate biológico. La recolección de las larvas y su colocación en jaulas de cedazo donde emerjan las avispas parasitoides es una medida que podría tener aplicación en situaciones de densidades altas del insecto. No obstante, es más eficiente el manejo de especies vegetales apropiadas.

Combate etológico. El uso de trampas cebadas con feromonas de atracción sexual puede utilizarse para dar seguimiento a la población, e incluso podría tener potencial para ser utilizadas en un esquema de rompimiento del acoplamiento sexual.

Las trampas de luz tienen el inconveniente de que se requiere de una fuente de luz que emita una longitud de onda apropiada para atraer al insecto; las lámparas de kerosene atraen pocos adultos. El uso de cebos envenenados es este caso no tiene sentido porque el adulto tiene piezas bucales atrofiadas y no se alimenta.

Comentarios finales

La emergencia de las hembras de *O. kirbyi* anterior a los machos, en un cultivo uniforme como la palma aceitera, combinado con el escaso traslape entre generaciones, puede llevar a una reducción en la base genética de la población, pues se está favoreciendo el desarrollo de una subpoblación generada por un mayor éxito en el apareamiento de machos que emergen primero (y encuentras más hembras disponibles), con las hembras que emergen tardíamente (y que tienen disponibles una mayor cantidad de machos) (Rhainds et al. 1995).

Posiblemente el deterioro en la variabilidad genética de la población, junto con un aumento en la población de enemigos naturales, sea lo que eventualmente lleve a este insecto a perder su capacidad de plaga en una plantación dada de palma aceitera.

Durante las defoliaciones ocurridas en el sur de Costa Rica, fue evidente con los años (aunque no cuantificada), una reducción en el tamaño promedio de los canastos, lo cual podría indicar un debilitamiento por autogamia.

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Insect pollination of oil palm - a comparison of the long term viability and sustainability of *Elaeidobious kamerunicus* in Papua New Guinea, Indonesia, Costa Rica, and Ghana

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Abstract

The African pollinating weevil *Elaeidobious kamerunicus* was introduced from Africa into the oil palm growing regions of Asia and the Pacific in the early 1980s. These introductions were very successful, dispensing with the need for assisted pollination, significantly improving fruitset, and hence increasing yields. The introductions therefore made a significant contribution to the economic viability of oil palm throughout the region. However there have recently been serious concerns expressed about the narrow genetic base of the weevil population, as well as the detrimental effects of parasitism of the weevils by nematodes. This paper describes work undertaken to evaluate the long-term viability and sustainability of insect pollination of oil palm by *E. kamerunicus*. The study had three main objectives: (1) To screen the existing *E. kamerunicus* populations within Papua New Guinea, Indonesia, Costa Rica and Ghana for evidence of infection by parasitic nematodes; (2) To determine the degree of genetic separation between weevil populations in Papua New Guinea and Ghana, and natural populations in West Africa, using the amplified fragment length polymorphism (AFLP) technique for genetic fingerprinting; and (3) To assess the potential to improve the genetic base of the existing population of *E. kamerunicus* within areas into which it has previously been introduced. Field work for the study was undertaken in Papua New Guinea, as well as Indonesia, Ghana, and Costa Rica. The results of the study are presented, and recommendations made for future work.

Resumen

El insecto polinizador de la palma aceitera, *Elaeidobious kamerunicus* fue introducido desde África hasta Asia continental y el Pacífico a comienzos de los años ochenta. Estas introducciones fueron muy exitosas en cuanto se eliminó la necesidad de la polinización asistida, se mejoró la conformación de los racimos (fruitset), y se incrementaron los rendimientos de fruta por hectárea; todo lo cual hizo una contribución significativa a la viabilidad económica de la actividad de la siembra de la palma aceitera en esta región del mundo. Recientemente, sin embargo, han existido dudas sobre la estrecha variabilidad genética de la población de insectos introducida, así como también sobre los efectos adversos del parasitismo de los insectos por parte de nematodos.

En este trabajo se evalúa la viabilidad y sostenibilidad en el largo plazo de la población de *E. kamerunicus* en algunos de los países en donde fue introducido. Los tres objetivos del trabajo

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fueron: (1) Analizar la población existente de *E. kamerunicus* en Papua Nueva Guinea, Indonesia, Costa Rica y Ghana, para buscar infección por nematodos parasíticos (2) Determinar el grado de separación genética entre las poblaciones de insectos provenientes de Papua Nueva Guinea y Ghana, y las poblaciones naturales en África del Oeste, utilizando la técnica AFLP (amplified fragment length polymorphism); y (3) Evaluar el potencial para mejorar la base genética de la población existente de *E. kamerunicus* dentro de las áreas donde ha sido introducido. El trabajo de campo se realizó en Papua Nueva Guinea, Indonesia, Ghana y Costa Rica.

Se encontraron nematodos parasíticos en varias muestras de *E. kamerunicus* procedentes de PNG: hembras maduras, huevos y varios estadios larvales, pero no machos del nematodo. El porcentaje de infestación varió entre 0% y 50% de los insectos analizados según su procedencia.

Prácticamente todas las muestras de *E. kamerunicus* procedentes de Ghana estaban libres de nematodos, con excepción de una hembra. En las muestras de Costa Rica no se encontraron nematodos. El nematodo que parasita los adultos de *E. kamerunicus* en PNG se localiza en el hemocelo y es una nueva especie en un nuevo género, *Elaeolenchus parthenonema* n.g., n.sp.

La comparación de las poblaciones de *E. kamerunicus* indicó que las muestras de insectos procedentes de PNG son genéticamente diferentes a las de Ghana, pero similares a las de Costa Rica, lo cual indicaría que los insectos ahora presentes en este último país, son una sub-población de la que fue introducida en PNG, y no una población traída de Africa.

Durante las visitas de campo en Africa, se determinó que posiblemente existen al menos dos nuevas especies de *Elaeidobious* que no han sido descritas aún, lo cual tiene implicaciones para definir qué nuevas especies podrían introducirse en una localidad particular para ampliar la base genética de la población existente de polinizadores.

Introduction

Background

Up until the late 1970s the oil palm was widely thought to be anemophilous rather than entomophilous (Turner & Gillbanks 1974; Hardon and Corley 1976). The belief that the oil palm was wind-pollinated stemmed from the supposed lack of evidence of any effective insect pollinator. This seemed to be substantiated by the high atmospheric pollen densities observed at considerable distances from male inflorescences (Hardon and Turner 1967). However, Hardon and Turner found that the onset of rains caused an immediate reduction in atmospheric pollen density and that pollen density was influenced more by the number of days on which rain falls than by total rainfall. Syed (1979) reported that this observation led some planters, especially those who had worked in Cameroon, to suspect the involvement of other agencies in pollen dispersal, because in the wet season rain occurs most days yet natural pollination during this period was adequate.

Prior to Syed's (1979) paper there was little published evidence to suggest that insects play any significant role in the pollination of oil palm. Syed began a project in the late 1970s to determine whether insects or any other organisms contributed to pollination in oil palm. Syed's initial studies were undertaken in Cameroon, in an area where oil palm is indigenous and assisted pollination was not required. In this area fruitset levels were considered to be adequate without human intervention, and it was therefore reasonable to assume that natural pollination was satisfactory. This was the case even during the wet season when wind dispersal of pollen is uncommon.

Syed's work (1979) involved studies of wind dispersal of pollen from male inflorescences. These studies indicated that some pollen, especially from taller palms, reached adjacent palms and thus were able to adequately pollinate female inflorescences. However, Syed concluded that in the absence of a large number of well placed male inflorescences, wind alone could not be expected to give adequate pollination, particularly during the rainy season.

Upon inspection of male and female inflorescences Syed found large numbers of insects. These insects were present on the male inflorescence during anthesis and on the female flowers during the first few days of receptivity. Of those insects present on male inflorescences, *Elaeidobious* spp. and *Atheta* spp. were the most abundant. These included *E. kamerunicus*, *E. plagiatus*, and *E. subvittatus*. Of those insects present in large numbers on the male inflorescences, only *E. subvittatus* and *Atheta* sp. were found in the female inflorescences, and these were only present in very small numbers. However, continuous observations of female inflorescences throughout the period of their receptivity revealed that a large number of insects visited then during the daytime, and that these insects tended to arrive in intermittent storms.

Syed (1979) found that all the species that were found on male inflorescences also visited female inflorescences. A large number of insects were found on the male inflorescence, and he reported that some of these continuously left the flowers and were usually laden with pollen grains. To investigate whether the pollen was actually carried to the female flowers, he captured insects on the female inflorescences and examined them under the microscope.

Syed found that species of *Elaeidobious* carried the largest number of pollen grains, but there was considerable variation in the number of grains that were carried. The viability of the pollen obtained from the bodies of the insects captured on female inflorescences was examined in germination tests. He reported that 68.5% of pollen was viable, and concluded that most of the pollen was fresh.

Introduction of *Elaeidobious kamerunicus* into Asia and Papua New Guinea

E. kamerunicus was selected for the introductions into Asia and Papua New Guinea because Syed (1979) reported that it was the most numerous in both dry and wet weather, and that it carried more pollen grains than the other *Elaedobious* species. He also reported that *E. kamerunicus* had a high reproductive rate, good searching ability, and it was host specific. It was concluded that this weevil would be safe to introduce into other oil palm growing areas.

Between July and December 1980 *E. kamerunicus* arrived into Malaysia under the quarantine care of the Department of Agriculture (Kang and Zam 1982). Having confirmed that the weevil was host specific to oil palm it was released into two Pamol estates in Johor and Sabah in February and March 1981. By April 1982, *E. kamerunicus* was present in virtually all the oil palm estates in Malaysia. Basri (1984) concluded that the introductions of *E. kamerunicus* into Malaysia had the following positive effects:

1. Dispensing with the need for assisted pollination.
2. Significant improvement in fruitset from an average of 52% to 71%.
3. Increase in fruit to bunch ration from an average of 57.7 to 64.7%.

4. Increase in mean bunch weight from 14.6 to 18.7kg.
5. Improvement in oil to bunch ration from a mean of 23.3 to 25.4%
6. Improvement in kernel to bunch ratio from 4.6 to 6.6%

E. kamerunicus was introduced into Papua New Guinea in 1982. This resulted in significant improvements to oil palm pollination, similar to those in Malaysia as described by Basri (1984). The introductions thereby improved fruitset levels and oil extraction ratios, and hence increased yields. The introductions of the pollinating weevil made a significant contribution to the economic viability of the oil palm industry in PNG, and was particularly helpful to the smallholder sector because yields were significantly increased with no direct cost to the farmers, and this continued in the medium to long term.

Introduction of *Elaeidobious kamerunicus* into Central and South America

Before the recent introduction of various species of *Elaeidobious*, oil palm pollination in South America was attributed to two species of pollinators, *Mystrops costaricensis* and *E. subvittatus*. The genus *Mystrops*, which is exclusive to South America, is found along the Pacific edge of America and throughout Central America up to Southern Mexico. It is also found along the Atlantic coast up as far as the extreme west of Venezuela, and has penetrated within the Andes along the Magdalena valley in Colombia. The species *costaricensis* has been sub-divided into three sub-species: *c. costaricensis* in Central America, *c. orientalis* in the Colombian interior, and *c. pacificus* on the coast. The genus *Elaeidobious* is only represented by a single species, *E. subvittatus*. This species probably came from West Africa via the east coast of Brazil, and then went on to colonize the whole of Neotropical America.

Mariau and Genty (1988) reported that in Colombia rain generally has a very depressive effect on both *Mystrops* and *Elaeidobious* populations. These fluctuations give rise to considerable variations in fruitset, which for pollinated inflorescences in the rainy season can fall to 40%, whereas in the dry season fruitset levels of more than 80% are possible. Low fruitset levels generated interest in the introductions of other pollinators into South America to improve oil palm yields. Other *Elaedobious* species were therefore introduced into South America in 1986: *E. kamerunicus* in Colombia, *E. singularis* in Brazil, and a mixture of four *Elaeidobious* species on other plantations in the Manaus region. In Ecuador the situation differs greatly between the Pacific zone where *Mystrops costaricensis* pacificus exists and the Amazonian sector where neither of the two insects had penetrated, and where *E. kamerunicus* has been introduced.

Observations carried out by Syed (1985) in Costa Rica and Honduras made him to recommend the introduction of a new pollinator for the oil palm to complement the work done by *M. costaricensis* and *E. subvittatus*. Despite low fruit set values observed during some months of the year, assisted pollination was never considered necessary in Central America, except in some localized areas and under very particular situations (Bulgarelli et al. 2002).

E. kamerunicus was introduced into Costa Rica in March 1986, and the changes observed in fruit set and the populations of the introduced and native pollinators were documented by Chinchilla and Richardson (1991).

The long term viability and sustainability of *Elaeidobious kamerunicus* in Papua New Guinea, Indonesia, Costa Rica, and Ghana

There have recently been concerns regarding the periodic occurrence of poor pollination and yield loss in certain locations in Malaysia (Ming 1999). Ming (1999) reported that low weevil populations were associated with the problem and suggested that this may be caused by the direct or indirect effects of weather, or due to parasitism by the nematodes enhanced by weather changes. He suggested that a second pollinator, e.g. *Elaeidobious subvittatus* could be able to overcome this, and hence complement *E. kamerunicus*.

Rao and Law (1998) reported on the problem of poor fruitset in parts of East Malaysia due to poor pollination. It was found that seasonally low fruitset was due to poor pollination because of insufficient weevils. Weevil numbers fell dramatically when their breeding sites, the male inflorescences, became less abundant, and this was coincident with extensive infection by parasitic nematodes and unfavorable weather. They suggested that the present weevil populations, derived from only a few pairs, may be suffering inbreeding depression, and hence more rapidly succumbed to nematode parasitism. Furthermore, it was suggested that these weevils lacked the features necessary for adaptation to wet conditions. Rao and Law (1998) considered that a complex of pollinating insects, some of whose niche is not the oil palm inflorescence may eventually be necessary.

Rao and Law (1998) highlighted that suggestions of importing fresh batches of *E. kamerunicus* or indeed other species of pollinating insects from Cameroon requires some priority research into some key issues. For instance, in their native Cameroon, weevil populations were also observed to decline in the rainy season but the reduction was less pronounced. It would therefore be useful to determine the cause of the decline in native Cameroon. A high proportion of the dead pupae and weevils of the original importation into Malaysia were nematode infected and hence destroyed. So it was suggested that the nematodes that are now causing the problems were probably brought in with the original populations.

These authors considered that the suggestion of inbreeding depression, or extreme homozygosity, raised the question of why the effects were not manifested sooner after the introduction given the weevil's short generation time. Furthermore, populations at other localities, some experiencing seasonal wet weather, also grew from a limited number of mating pairs. They suggest that it would therefore be interesting to find out how potentially serious the nematode parasitism was in these areas. They concluded that containing nematode parasitism requires an understanding of the manner of parasitism and the predisposing factors favoring the rise in nematode parasitism in the weevil population. They also highlighted that the introduction of new weevils into a given area requires the predetermination of levels of resistance, or at least heterozygosity, in sub groups if they exist and in different species on the weevil in the genera.

Although excellent levels of fruit set are being achieved in most project areas within PNG it is considered that urgent action is necessary to address the sustainability of the current levels of insect pollination, as well as to possibly make improvements for the future. The current population of pollinating weevils within PNG is derived from a relatively small number of weevil individuals introduced from West Africa in 1982. It is therefore apparent that the same problems are faced as in South East Asia, that possible nematode infection along with the narrow genetic base of the weevil population poses a very significant risk to viable and sustainable production.

A two-year research project, funded by the European Union in Papua New Guinea was undertaken between 2000 and 2002. The objectives of the project were:

1. To screen the existing *E. kamerunicus* populations within PNG, Indonesia, Costa Rica and Ghana for evidence of infection by parasitic nematodes.
2. To determine the degree of genetic separation between weevil populations in Papua New Guinea, Costa Rica and natural populations in West Africa, using the amplified fragment length polymorphism (AFLP) technique.
3. To assess the potential to improve the genetic base of the existing population of *E. kamerunicus* within PNG, either by the introduction of fresh batches of the same weevil species, or possibly by the introduction of one or a number of new species of pollinating insects from West Africa or South America.

Field sampling

During the project weevils were collected from PNG, Ghana, Costa Rica, and Indonesia. In PNG weevils were collected from all oil palm growing regions (Fig. 1):- New Ireland Province (Lamerika and Kabil plantations), Milne Bay Province (Baraga and Waigini plantations), Oro Province (Sangara and Embi plantations), and West New Britain Province (Dami, Kumbango, Kavugara, Kautu, and Hargy plantations). A total of nine samples were collected from a number of oil palm growing areas in Ghana:- Eastern region (Kade), Western Region (Ajumako, Twifo Oil Palm Plantation, Ankaako, Assin Dadieso, and Egyirkrom), and Western Region (Benso Oil Palm Plantation, Ewusiedjoe, and Sekondi School for the Deaf. Weevils were also collected from Bah Lias Research Station, near Medan, Indonesia, and from Palma Tica oil palms plantations in Costa Rica (Coto, Celajes, Mirador, and Quepos).The collections of weevils were either preserved in formaldehyde or kept alive and returned to the UK where live specimens were selected and frozen at -80°C until required for DNA analysis.

Nematode screening

Materials and methods

Dissections were carried out under the stereomicroscope at a magnification of ×25 and ×50. Individual beetles were placed in a small drop of fixative in a Petri-dish and, using fine watchmaker's forceps, mounted micro-pins and a fine syringe needle, the elytra were removed and examined for nematode dauer larvae and ectophoretic mites. The abdomen was then carefully dissected and examined for internal nematode parasites (mature females, eggs and/or juveniles). Instruments were rinsed between dissections to avoid cross-contamination. The original intention was to dissect 50 beetles of each sex, but this was not always possible due to

limited material of *E. kamerunicus* in some samples from Ghana. Early results indicated that there were no differences in nematode infection rate between males and females and so in most accessions only females were dissected.

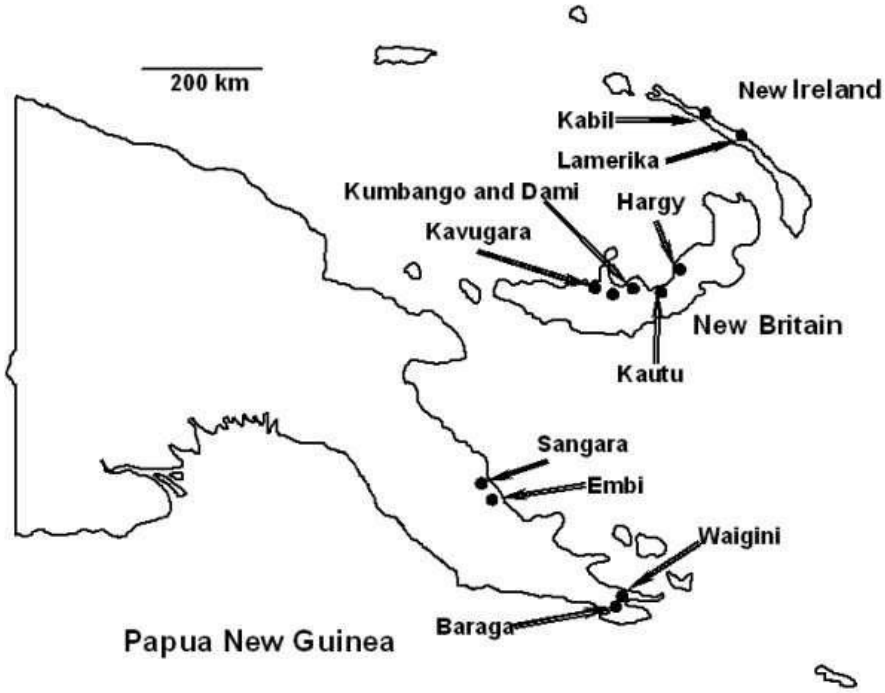

Fig.1. Map of Papua New Guinea showing the main sampling sites for the project.

Results

The main results of the nematode screening are presented in tables 1-3 and figure 3. During the study nematodes were found for the first time within the weevil populations in PNG. It was found that the number of parasitic females per weevil varied considerably, the lowest number being one and the highest 15. Most weevils infected by the mature female nematodes also carried a considerable burden of eggs and various juvenile stages of the nematode parasite. In heavily infected weevils, almost all the haemocoelic cavity appeared to be occupied by the various stages of the parasite.

The samples from PNG revealed that some weevil populations were apparently free of the nematode whereas in others the parasite was present in 50% of the weevils examined. Low infection rates seemed to be prevalent in samples from West New Britain where nematode parasites were found in three (Kumbango, Kavugara and Kautu) of the five accessions examined. Over the five sites from West New Britain the infection rate varied from 0% to 10% with a mean value of 4.8%, and the mean number of mature parasitic females per infected weevil ranged from 1.4 to 2.0. The accession from Baraga, Milne Bay showed a 50% infection rate with a mean of 1.6 female nematodes per beetle while Waigini, the other site from Milne Bay, had a much lower percentage infection (4%). The accession from Sangara, Oro Province, although only 8% parasitized, had a mean of 2.5 female nematodes per infected beetle whilst Embi, also from Oro Province, was 20% infected with a mean of 1.8 female parasites per infected weevil. Other

accessions from Lamerika (New Ireland), Dami and Hargy (both from West New Britain) revealed no parasitic nematodes.

Table 1. Occurrence of parasitic nematodes, dauer larvae and ectophoretic mites on female *Elaeidobious kamerunicus* from Papua New Guinea in 2000

Site		% infected with parasitic nematodes	Mean number mature female nematodes /parasited insect	% with nematode dauer larvae	% with ectophoretic mites
New Ireland Province	Lamericai	0	0	0	0
	Kabil	0	0	0	0
Milene Bay Province	Waigini	4	3.0	0	0
	Baraga	50	1.6	0	0
Oro Province	Sangara	8	2.5	2	0
	Embi	20	1.8	0	0
West New Britain	Dami	0	0	0	0
	Kumbango	8	2.0	0	0
	Kavugara	6	2.0	0	0
	Kautu	10	1.4	0	0
	Hargy	0	0	0	0

The samples from Ghana, on the other hand, proved to be free of nematode parasitism apart from a single female weevil from Kade, Eastern Region. This infection was represented by a solitary female nematode, a number of juveniles and eggs and several immature males. Weevils from Ghana often carried large burdens of ectophoretic mites, indeed the sub-elytral space was often crammed with such passengers.

The haemocoel parasitizing nematode found infecting *E. kamerunicus* in PNG is now known to be a new species and new genus, *Elaeolenchus parthenonema* n.g., n.sp. (Poinar et al. 2002), and is likely to be widespread in the oil palm growing areas of South East Asia. This type of parasitic nematode draws all its nutritional requirements from the host and therefore high parasitic burdens would be expected to have an effect on fecundity, perhaps even resulting in sterility due to depletion of the host's fat body. A reduction in energy reserves could also inhibit the ability of the weevils to fly and therefore impact on their ability to pollinate the oil palm flowers.

It is not yet clear whether the nematode parasite *E. parthenonema* was carried to South East Asia and PNG with the original weevil introductions from West Africa (this would have to have been at a very low infestation level as the weevils were screened before release), or may have been acquired subsequently from a local infection source. Of the nine weevil populations examined from Ghana, only one was recorded with a nematode parasite in the haemocoel and this record was restricted to a solitary female nematode and associated juvenile stages in a single weevil. This nematode was not particularly well preserved, but appeared to belong to a different genus to *Elaeolenchus parthenonema*. None of the six accessions from Costa Rica, another country into which *Elaeidobious kamerunicus* was introduced to improve pollination, were infected with the entomoparasitic nematode. It is always possible, of course, that the parasite is present, but in numbers too low to be detected by the current investigation.

Table 2. Occurrence of parasitic nematodes, dauer larvae and ectophoretic mites on female *Elaeidobious kamerunicus* from Costa Rica

	Site	% infected with parasitic nematodes	Mean number mature female nematodes /parasited insect	% with nematode dauer larvae	% with ectophoretic mites
Female	Coto1	0	0	16	0
	Coto2	0	0	0	0
	Celajes	0	0	18	0
	Mirador	0	0	22	0
	Quepos1	0	0	10	0
	Quepos2	0	0	12	0
Male	Coto1	0	0	12	0
	Coto2	0	0	2	0
	Celajes	0	0	20	0
	Mirador	0	0	18	0
	Quepos1			6	
	Quepos2	0	0	16	0

The life cycle of the nematode appears to be as follows:- The mature parasitic female is found in the haemocoel of the adult weevil (it is also likely to be found in late instar larvae). The sausage shaped female grows to a length of several millimeters, but smaller in multiple infections (Fig. 2). Almost the entire body is taken up by the genital system, the female producing hundreds of eggs. Reproduction is apparently parthenogenetic, no males having been found.

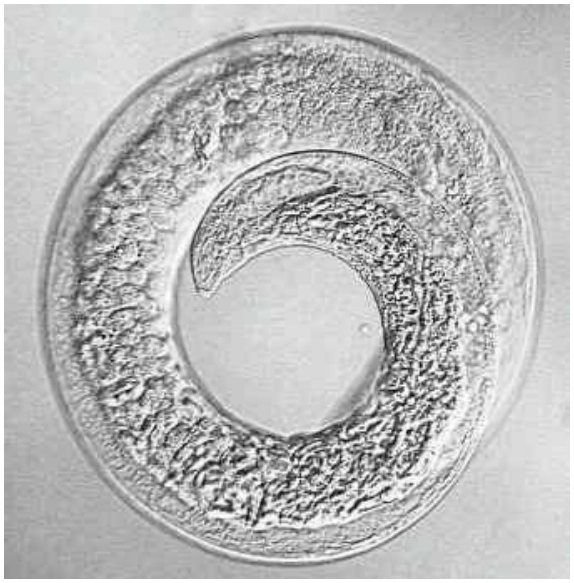

Fig. 2. Photograph of a nature parasitic female nematode from haemocoel of *Elaedobious kamerunicus*

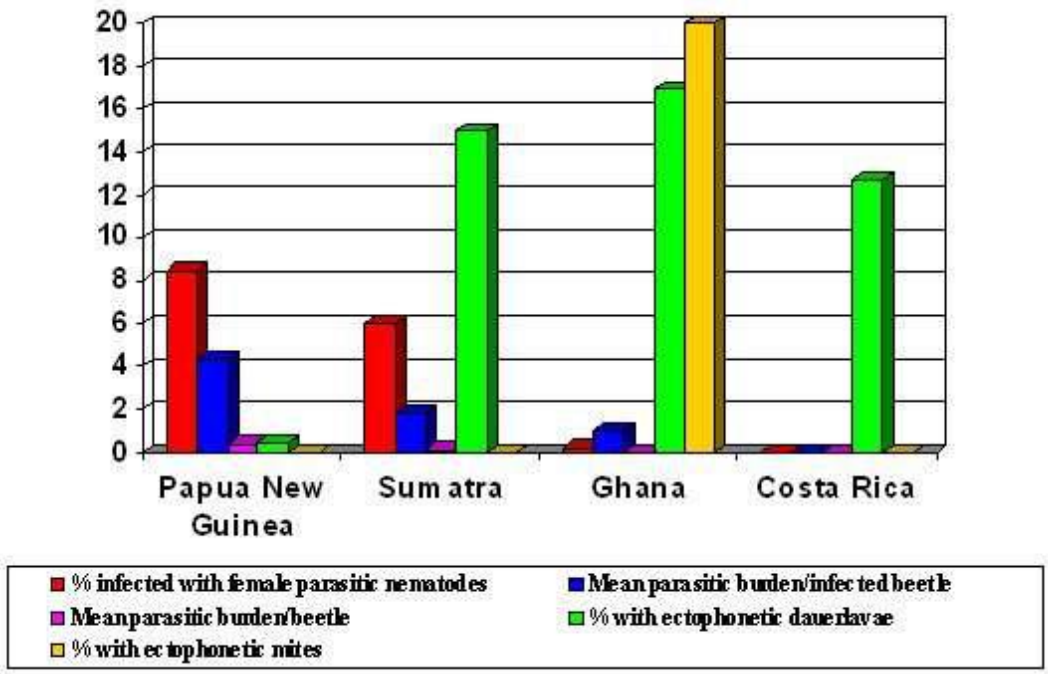

Fig 3. Contry comparisons of weevil burden (parasitic nematodes, dauer larvae and ectophoretic mites).

The eggs hatch within the host and the juveniles develop, probably to the immature vermiform female stage. Vermiform females (and possibly fourth stage juveniles) are oviposited by the female host along with her own eggs. The nematodes then exist in the environment for a while as a free-living stage, possibly feeding on fungal hyphae, before penetrating a weevil larva and migrating to the haemocoel. Here they absorb nourishment from the haemolymph (presumably depleting the fat reserves of the host as a result) and grow enormously as they mature.

Of course, the fact that the PNG nematode was not found in the Ghana samples does not unequivocally imply that the parasite did not originate in West Africa, but it is suggestive. If the nematode is of West African origin, then either it may be at a very low incidence (only one weevil, corresponding to 0.2% of the 450 insects examined, had any entomoparasite), or it may be of local or restricted occurrence. For instance, for logistical reasons no weevil material from Cameroon was examined, and it was from Cameroon that the founder colonies of the weevil were originally taken.

Table 3. Combined data from all sites (on a per country basis) for incidence of female parasitic nematode, ectophoretic nematode dauer larvae and ectophoretic mites

	Country of origin					
	PNG 2000	**PNG 2001**	**PNG (combined)**	**Sumatra**	**Ghana**	**Costa Rica**
Total number of weevils disected	500	450	950	100	450	600
Number of weevil with parasitic nematodes	53	28	81	6	1	0
% infected with parasitic nematode	10.6	6.2	8.5	6.0	0.2	0.0
Total female parasitic nematodes	190	161	350	11	1	0
Mean parasitic females/infected weevil	3.58	5.75	4.32	1.83	1.0	0.0
Mean parasitic female/weevil	0.38	0.36	0.37	0.11	0.002	0.0
Number of weevils with dauer larvae	1	3	4	15	76	76
% with dauer larvae	0.20	0.67	0.42	15.0	16.9	12.7
Number of weevils with ectophoretic mites	0	0	0	0	90	0
% with ectophoretic mites	0	0	0	0	20.0	0

Molecular genetics of *Elaeidobious kamerunicus*

Materials and methods

A number of live weevils from each site were selected from the total sample and frozen at -80ºC to await DNA extraction. Individual weevils were placed in a watchglass in 100 ml 1xTE buffer (10 mM Tris pH 8.0, 1 mM EDTA). Each insect was dissected under a dissecting microscope and checked for contamination with nematodes and/or mites. Where possible only weevils without either of these contaminants were used for DNA extraction. Where this was not possible the weevil material was picked out leaving as many of the nematodes/mites as possible behind. The dissected material from each individual weevil was placed in a sterile 1.5 ml microcentrifuge tube and stored at -20ºC until all individuals from a single site had been dissected. DNA extraction was carried out using a Phytopure DNA extraction kit (Nucleon Biosciences) as per the manufacturer's instructions. After extraction the DNA pellets were resuspended in 50 ml sterile water and stored at -20ºC.

Five individual weevils were analysed from each site (Table 4). AFLP reactions were performed using a protocol modified from the original of Vos et al. (1995). Restriction enzyme digestion of 20 ml of genomic DNA was performed using 5 units of EcoR I and 5 units of Hpa II in Multi-Core buffer (Promega) in volumes of 40 ml for 1 h at 37°C. Double stranded adapters were ligated onto the digested genomic DNA fragments in total volumes of 50 ml using 1 unit of T4 DNA Ligase (Promega) in 10x Multi-Core Buffer and 0.1 ml of ATP (100 mM). The reactions were incubated overnight at 37°C. Adapter strands were as follows ECO-AD1 5' CTC GTA GAC TGC GTA CC 3' and ECO-AD2 5' AAT TGG TAC GCA GTC TAC 3', HPA-AD1 5' GAC GAT GAG TCC TGA G 3' and HPA-AD2 5' CGC TCA GGA CTC ATC GT 3'.

All primers were synthesized by Amersham Pharmacia Biotech. The adapter strands were mixed together in equimolar amounts, heated at 95ºC for 5 min. and allowed to cool slowly to room temperature to form the double stranded adapters. The ECO-AD was used at a concentration of 5 pmols/ml and the HPA-AD at 50 pmols/ml. The adapter ligation mixes were diluted 1 in 10 with sterile water and stored at -20°C.

Preamplification reactions were carried out in a Hybaid PCR-Express using the ECO-AD1 and HPA-AD1 adapter strands as primers in volumes of 20 ml containing 2.0 ml of 10x Buffer, 2.0 ml MgCl2 (25 mM), 0.5 ml of each dNTP (20 mM), 0.5 ml of each primer (100 pmol/ml), 0.2 ml Taq polymerase (5 U/ml) (Sigma) and 1 ml of the diluted sample. Amplification conditions were, 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 56°C for 60 s, 72°C for 90 s and a final step of 72°C for 5 min. After amplification the reactions were diluted 1 in 10 with sterile water and stored at -20°C.

Selective PCR reactions were performed using three combinations of primers, ECO-AT and HPA-CAT, ECO-AT and HPA-CTC, ECO-GC and HPA-CTC. The core sequences of the primers were ECO-NN 5' GAC TGC GTA CCA AAT TC-NN 3' and HPA-NNN 5' GAT GAG TCC TGA GCG G-NNN 3'. The fluorescently labelled ECO primers were synthesized by MWG Biotech and the unlabelled HPA primers by Amersham Pharmacia Biotech. Selective amplifications were made up in the same manner as the preselective amplifications except that the ECO primer was used at a concentration of 2 pmols/ml and 5 ml of the preselective amplification was added to each reaction. Cycling conditions were, 94°C for 2 min followed by 13 touchdown cycles of 94°C for 30 s, 65°C for 30 s (reduced by 0.7°C each cycle), 72°C for 90 s, followed by 23 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 90 s and a final step of 72°C for 10 min. Prior to electrophoresis 10ml of loading buffer (Cambio) was added to each reaction which then were heated at 90°C for 3 min and immediately placed on ice. The reactions were run on 8.0% denaturing Long Ranger (Flowgen) acrylamide gels on a LiCor 4200 automated sequencer. A molecular weight marker ladder containing band sizes of 350, 325, 300, 255, 230, 204, 200, 175, 145, 120, 105, 100, 95 75 and 50 base pairs (MWG Biotech) was loaded between each ten sample lanes to allow for calibration of each gel in subsequent analysis.

The computer package GelComparII (Applied Maths) was used to analyse these AFLP data. A binary table was constructed from these AFLP data showing the presence/absence for each of 341 different bands yielded in total by the various primer combinations. The binary table was then analysed in PAUP v4b.10 using Neighbor Joining with the BioNJ option in effect, a 1000 round bootstrap calculation (also based on Neighbor Joining) as well as a further parsimony heuristic search also with 1000 rounds of bootstrapping.

Results

AFLP analysis using three different selective primer combinations yielded a total of 341 bands. These were scored as present/absent using GelCompar II for each of the 85 samples analysed and the binary table produced treated to three different forms of analysis using PAUP. All three tree building techniques yielded trees with similar topologies. Figure 4 shows the Neighbor joining phylogram, showing two major clades, one containing the Papua New Guinea and Costa Rica isolates and the other containing the samples from Ghana. Each of these major clades can be further sub-divided. Within the Ghana samples there are two clades. The first contains all of the isolates from sites B, D and G and two samples from site H and 1 from site E. The second

contains all of the isolates from site A, C and F as well as the remaining samples from sites E (4) and H (3).

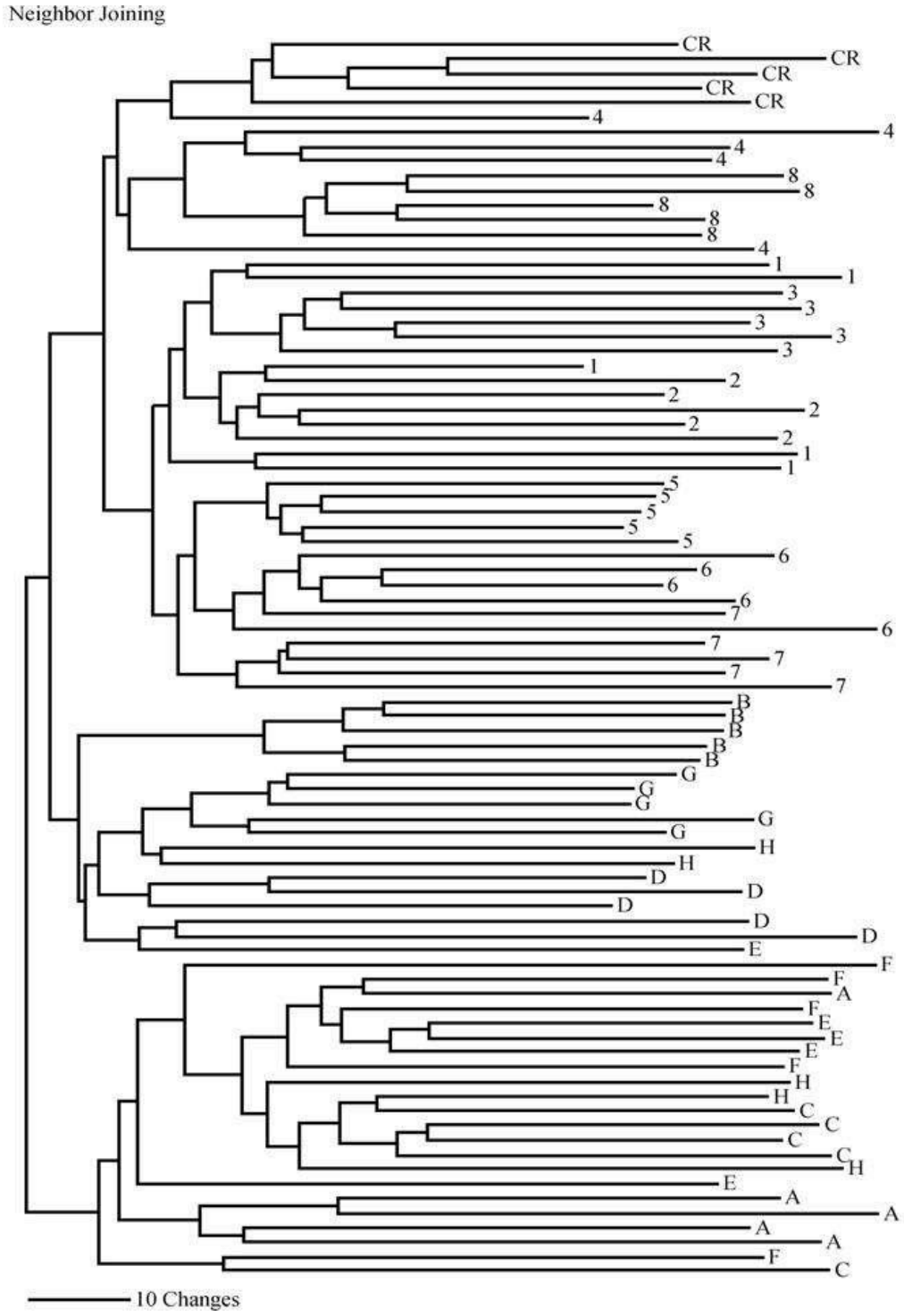

Fig. 4. Neighbor joining phylogram

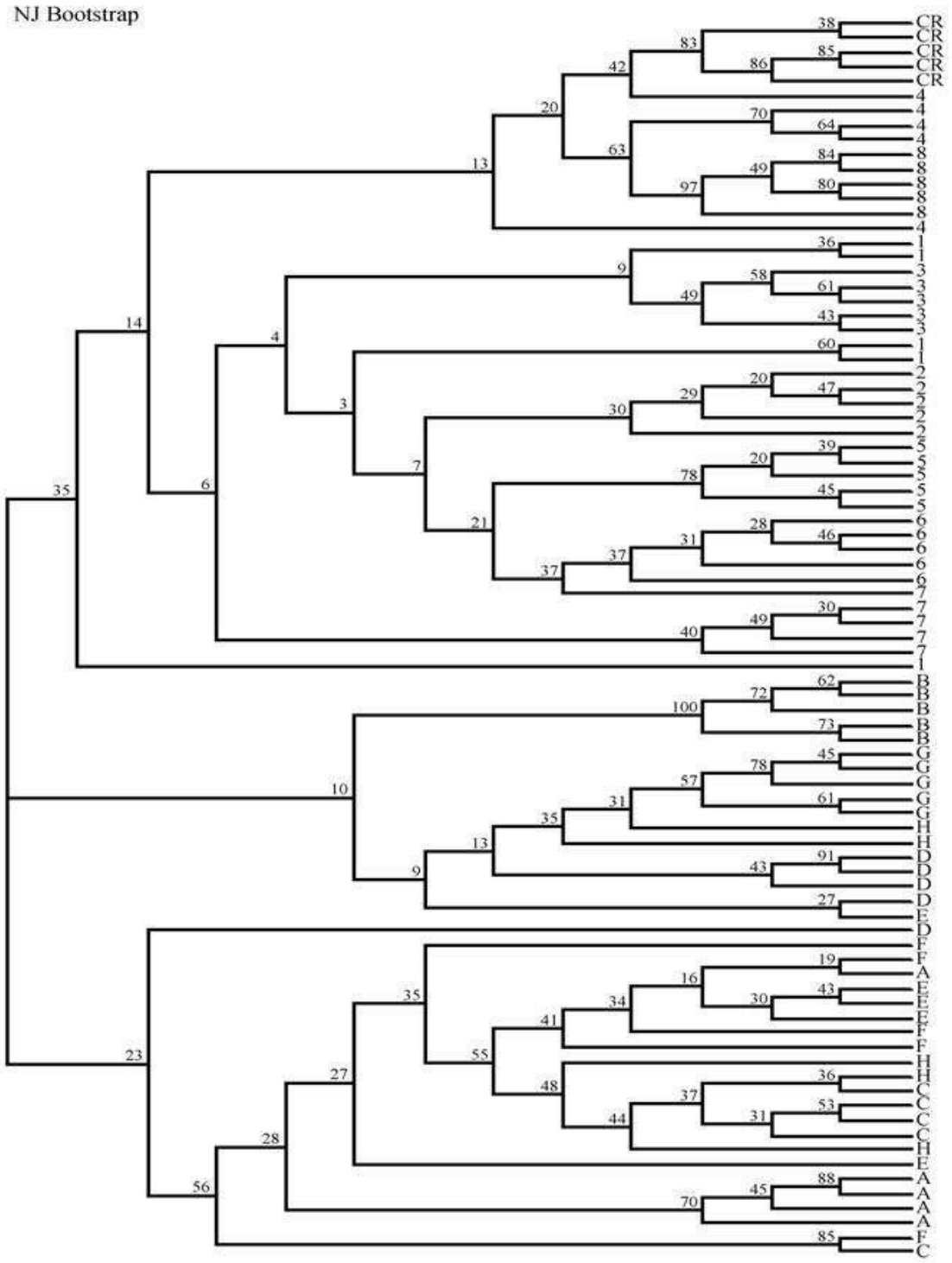

Fig. 5. Neighbor joining bootstrap cladogram. The numbers at the nodes are bootstraps values

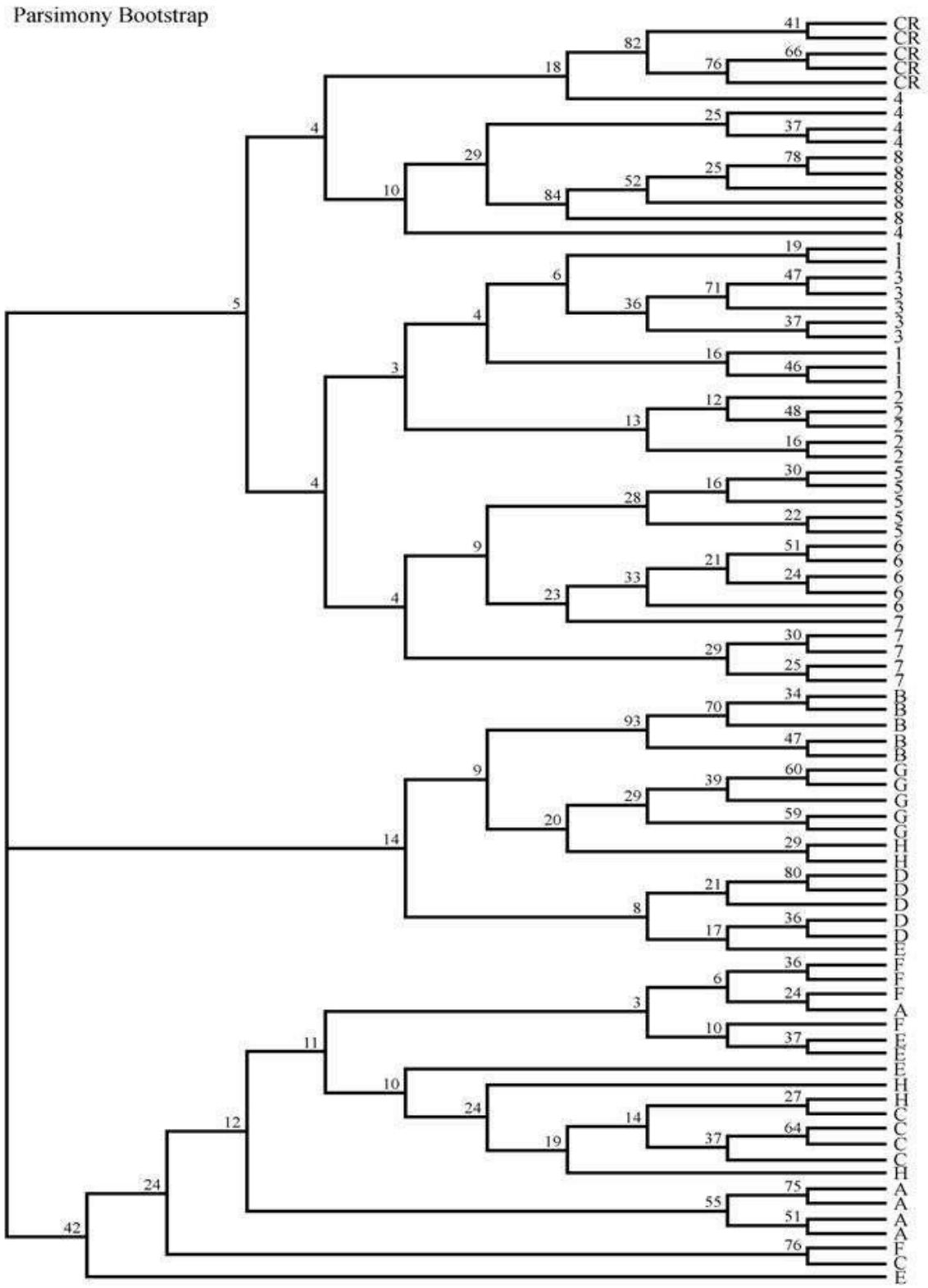

Fig.6. Parsimony analysis bootstrap cladogram. The numbers at the nodes are bootstrap values.

Within the Papua New Guinea clade there is one branch containing the Costa Rican samples as well as the samples from sites 4 and 8. The remainder of the Papua New Guinea samples cluster together on a separate branch within which it is possible to distinguish two further branches one containing samples from sites 1, 2 and 3 and the other sites 5, 6 and 7 (Table 4). When a bootstrap analysis is performed on these data using neighbour joining the cladogram (Fig. 5) shows a similar topography to the NJ tree (Fig. 4). The Ghana and Papua New Guinea samples remain separated and there are still two branches for the Ghana samples. These two branches mostly contain the same samples as for the NJ tree. The bootstrap tree still separates the Costa Rican and Papua New Guinea sites 4 and 8 sites from the remainder of the Papua New Guinea sites but in this tree these remaining samples are not so easily separated into two further branches.

Table 4. Origins of *Elaeidobious kamerunicus* oil palm weevil samples examined by AFLP and their indentifying codes used on trees

Country	Sample size	Code
PNG	Sangara, Oro Province	1
PNG	Hargy, West New Britain	2
PNG	Lamerika, New Ireland	3
PNG	Waigini, Milne Bay	4
PNG	Dami, West New Britain	5
PNG	Kavugara, West New Britain	6
PNG	Kumbango, West New Britain	7
PNG	Kautu, West New Britain	8
Costa Rica	Coto	CR
Ghana	Kade, Eastern Region	A
Ghana	Ajumato, Central Region	B
Ghana	Benso Oil Palm Plantation, Western Region	C
Ghana	Sekondi School for the Deaf, Western Region	D
Ghana	Twifo Oil Palm Plantation, Central Region	E
Ghana	Ankaako, Central Region	F
Ghana	Assin Dadieso, Central Region	G
Ghana	Ewusiedjoe, Western Region	H

The heuristic parsimony analysis coupled with bootstrapping yields a cladogram (Fig. 6) with exactly the same groupings as the NJ tree in Figure 4. In general the bootstrap values for the deeper rooted nodes are not well supported for either trees in figures 4 and 5 but as the general topographies for all three trees are broadly similar this is not a major concern.

All three methods for tree construction give a similar result, that is, the samples from Papua New Guinea are genetically distinct from those from Ghana. The samples from Costa Rica clusters

with the Papua New Guinea isolates, which strongly suggests that this population is a subset of those originally introduced to Papua New Guinea and was not a fresh isolation from Africa. The strongly supported separate branches for the Papua New Guinea and Ghana samples indicates that the former population is evolving separately from those in Africa. However, the branch lengths in the Neighbor Joining tree (Fig. 4) do not indicate any less genetic diversity within the Papua New Guinea isolates than within those from Ghana. This could either be a reflection that the founder population was large enough to prevent a genetic bottleneck occurring or, more likely, that not enough time has yet elapsed for this effect to be seen. Any drop in efficacy in the weevil population seen in some areas of South East Asia could therefore be largely due to the parasitism by the nematodes, although this susceptibility could, in turn, have been due to low levels of genetic diversity within the founder population.

A rapid method for the screening of weevil populations for nematode infection would be advantageous. This could be achieved by the cloning of a species specific fragment of DNA from the nematode. PCR primers could be designed to amplify this specific piece of DNA thus allowing the rapid screening of weevil populations to assess parasite burden.

Unfortunately it is not possible to determine the level of gene flow using AFLP data. This is due to the way that AFLP markers are dominantly inherited thus rendering it impossible to score heterozygotes. To obtain a true reflection of the levels of gene flow between sites in PNG it would be necessary to screen the populations using a marker that allows the scoring of heterozygotes. Microsatellite markers are ideally suited to such a study.

The advantage of the construction of a library of such markers are twofold. First, it would be possible to determine the level of integration of any subsequent introduction of new populations of the weevil from Africa. Microsatellite markers would also enable the spread of newly introduced isolates from their source to be measured.

The potential for new genetic material

During the project large numbers of insects were found on male and female oil palm inflorescences in Ghana. These insects were present on the male inflorescence during anthesis and on the female flowers during the first few days of receptivity. Of those insects present on male inflorescences, *Elaeidobious* spp. and *Atheta* spp. were the most abundant. These included *E. kamerunicus*, *E. plagiatus*, and *E. subvittatus*. Of those insects present in large numbers on the male inflorescences, only *E. subvittatus* and Atheta sp. were found in the female inflorescences, and these were only present in very small numbers. However, as Syed (1979) reported, continuous observations of female inflorescences throughout the period of their receptivity revealed that a large number of insects visited them during the daytime, and that these insects tended to arrive in intermittent storms.

During the project *E. kamerunicus*, *E. plagiatus*, and *E. subvittatus* were found to visit female inflorescences and were usually laden with pollen grains. Species of *Elaeidobious* were found to carry the largest number of pollen grains. However during the fieldwork it was found that the taxonomy of the *Elaedobious* genus is somewhat complicated, and that at least two previously undescribed species are present within the genus. Two new species of *Elaedobious* have been discovered and these are currently being described. In addition to this the *Elaedobious* genus has

also been revised. A taxonomic field guide, that is absolutely essential for any further field operations, is to be produced at the Natural History Museum in London.

In Costa Rica, where *M. costaricensis*, *E. subvittatus*, and *E. kamerunicus* are all present, *E. kamerunicus* was found to have out competed the other two species. *E. kamerunicus* is the most dominant pollinator for most of the year, and is particularly useful as it is numerous and active in both moderately dry and wet weather. It was also found to carry more pollen grains than the other two species, and also responds better to the scent of the female inflorescence (Chinchilla and Richardson 1991). *E. kamerunicus* also has the advantage of being host specific to oil palm, whereas *M. costaricensis* feeds from a number of other palms, including coconut. It was found that rain generally had a very depressive effect on both *M. costaricensis* and *E. subvittatus*, indeed it was this that generated interest in introducing *E. kamerunicus* from Africa in the first place.

During the project it has therefore been very difficult to identify any candidate insects that might be able to compete with *E. kamerunicus* to improve oil palm pollination in PNG. Hence the introduction of fresh batches of *E. kamerunicus* into PNG from Africa is recommended. However this cannot be done until the uncertainty surrounding the taxonomy of the *Elaedobious* genus is resolved, particularly regarding the status of the two previously undescribed species within the genus, and what impact they might be having on pollination. Other problems include the widespread occurrence of nematode parasitism in the populations of pollinating weevils within PNG, and the lack of basic biological information concerning these parasites, particularly with regard to the effect that they might be having on the host. As the origin of the nematodes is still uncertain, this has implications for the quarantining requirements of any new introductions.

General discussion

Considerable progress has been made in addressing each of the three original objectives of the project. However, further work is required in order to gain a full understanding of a number of scientific issues that emerged during the project. Once these have been dealt with, then the overall goal of the introduction of new genetic material could go ahead.

Future work should include:

1. Further studies on the ecology, biology and distribution of the entomoparasitic nematode are required to quantify the actual and potential impact of the parasite on its host and the knock-on effect on pollination and hence palm oil production. As the origin of the nematodes is still uncertain, this has implications for the quarantining requirements of any new introductions. In addition to this, the widespread occurrence of the parasite has made DNA extraction very difficult and time consuming.
2. Additional surveys in West Africa are required to establish whether the entomoparasite is present in other areas and, if so, at what level. Examination of related species of *Elaeidobious* (including as yet undescribed species now known, as a result of this project, to be present in Ghana) is essential if an enhanced pollinator suite is to be considered for introduction to non-indigenous oil palm areas.

3. A molecular analysis of new *Elaeidobious* species should be undertaken to determine the genetic variability between the various species. This could be achieved by analysis of sequence data generated from the internal transcribed spacer region of the ribosomal DNA cistron. Generation of such data would lead to a greater understanding of the relationships within the genus.
4. A rapid technique, preferably quantifiable and robust enough for field use, needs to be developed to detect the entomoparasitic nematode in weevil 'squashes', thus obviating the time consuming need to dissect numerous insects. Such an assay would also be useful for quarantine purposes.
5. A microsatellite based assay needs to be developed to enable the measurement of actual gene flow between different populations of the weevil in both Africa and PNG.
6. The uncertainty surrounding the taxonomy of the *Elaedobious* genus needs to be resolved, particularly the status of the two previously undescribed species within the genus. The role of these two species in oil palm pollination should also be investigated.

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