FRUIT FLIES IN ASIA (especially Southeast Asia)

Species, biology and management

Paul Ferrar

Consultant (formerly Research Program Manager in Crop Protection, Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia)

Summary

This paper attempts to list various aspects of the fruit flies that may be involved in the present project – the species, their significant biological characteristics, and a summary of methods of management.

In Section 1 a species list covers 11 major species likely to occur in the crops/geographical areas to be involved in the project, including the Oriental fruit fly complex (*Bactrocera dorsalis* and relatives) and the melon fly (*Bactrocera cucurbitae*).

Section 2 covers the life cycle of typical fruit flies, male lures (particularly methyl eugenol and Cuelure), protein baits, fruit fly damage and crop losses, and the host fruit preferences of the main fruit flies involved in the project.

Section 3 discusses various methods for control and management, including cover-spraying, fruit bagging, cultural controls, protein bait spraying, male annihilation and the sterile insect technique. The advantages and disadvantages of each method are listed.

Section 4 lists key websites and paper-based references for information on fruit flies generally, including keys to species, biological information, host fruit records and much more.

In Section 5 are some key institutions and contacts for people who may be able to help the project with specific expertise related to fruit flies, especially in Asia.

Finally, Appendix 1 describes both morphological and molecular methods that are used for identifying fruit flies, and contrasts the two. This is included because the fruit flies involved in this project are not all easy to identify. In particular, the species of the Oriental fruit fly complex (*Bactrocera dorsalis* and relatives) are in an active phase of evolution, and species are not yet absolutely clearly defined. Taxonomic problems may become an issue in the project, and the notes supplied may help.
1. Some important species of fruit flies in Asia

**Taxonomy**

Class *Insecta*: insects

Order *Diptera*: two-winged flies

Family *Tephritidae*: includes fruit flies, and also some subfamilies that attack flowerheads and are used as biological control agents for weeds

Sub-family *Dacinae*: true fruit flies of Asia/Pacific

[Note: *Drosophila* flies are sometimes also called fruit flies, but they do not attack intact fruits. Some breed in fermenting fruits, and should be called vinegar flies not fruit flies.]

**Genera:**

*Bactrocera*: a large genus, with about 440 described species, and including the majority of the economically important fruit flies. Among these are:

*Bactrocera dorsalis*. Oriental fruit fly. India, Sri Lanka, Nepal, Bhutan, southern China (Sichuan, Guizhou, Hunan, Fujian, Yunnan, Guangxi, Guangdong, Hong Kong, Hainan), Burma, Thailand, Laos, Vietnam, Cambodia, Taiwan.


*Bactrocera carambolae*. Carambola fruit fly. Southern Thailand, India (Andaman and Nicobar Is.), Singapore, Malaysia (Sabah), Brunei, Indonesia (Java, Lombok, Sumbawa).

*Bactrocera cucurbitae*. Melon fly. Probably originates from Asia; present in Afghanistan, Pakistan, India, Sri Lanka, Nepal, Bangladesh, Burma, southern China, Thailand, Laos, Cambodia, Vietnam, Malaysia, Singapore, Taiwan, Philippines, Brunei, Indonesia (Sumatra, Java, Kalimantan, Sulawesi, Sumba, Timor, Irian Jaya).

*Bactrocera latifrons*. Solanum fruit fly. Pakistan, India, Sri Lanka, Burma, China (Fujian, Yunnan, Hong Kong, Hainan), Thailand, Laos, Vietnam, western Malaysia, Singapore, Taiwan, Brunei.

*Bactrocera minax*. Chinese citrus fruit fly. East India (Sikkim, W. Bengal), Bhutan, China (Jiangsu, Sichuan, Hubei, Hunan, Guangxi, Guizhou, Yunnan).
**Bactrocera occipitalis.** Philippines, Brunei, Malaysia (Sabah).

**Bactrocera philippinensis.** Philippines (Luzon, Panay, Negros, Cebu, Mindanao).

**Bactrocera tau.** India (Andaman & Nicobar Is., northeast India), Bhutan, Burma, China (north to Xizang, Sichuan, Hubei and Zhejiang), Taiwan, Thailand, Laos, Cambodia, Vietnam, Malaysia (western Malaysia and Sarawak), Singapore, Brunei, Indonesia (Sumatra; Java, Sulawesi?).

**Bactrocera umbrosa.** Southern Thailand, Malaysia (western Malaysia and Sabah), Singapore, Philippines, Brunei, Indonesia (Sumatra, Java, Kalimantan, Sulawesi).

**Bactrocera zonata.** Peach fruit fly. Pakistan, India, Sri Lanka, Bangladesh, Burma, Thailand, Laos, Vietnam.

Other economically important genera of Dacinae include:

**Dacus** – species particularly in Africa and in some parts of Asia.

**Ceratitis** – mainly in Europe and Africa, though the most damaging species (the Mediterranean fruit fly or Medfly, *Ceratitis capitata*), has spread to some other areas including America and Australia.

**Anastrepha** – a genus with a number of economically important species attacking various fruits in North and South America.

Some other genera also have economic importance in temperate to cold areas of Europe, North America and North Asia.

**The Oriental fruit fly species complex**

The sub-family of fruit flies appears to be in a state of active evolution, in which some major species are splitting into several different species, but these new species are not yet fully differentiated. This causes big headaches for taxonomists who have to try to decide how many different species there really are, and there are arguments and disputes between different experts as to what is the true situation.

This uncertainty in turn leads to headaches for quarantine authorities. If they have one species of an evolving complex in their country, do the other closely related species count as new quarantine threats to their country, or is there really no difference?

Within the area in which the present project will operate, there are at least three different species of the *Bactrocera dorsalis* complex – the true *B. dorsalis* (Oriental fruit fly), *B.
*papayae* (papaya fruit fly) and *B. carambolae* (carambola fruit fly). However, they are not easy to tell apart, and from the point of view of the project it is probably appropriate to regard all the members of the complex as “*Bactrocera dorsalis*”. There are some behavioural and biological differences between the species, but for broad management purposes they can probably be regarded as the same. However, project staff should be aware that somewhat different species are involved, and if export crops are being contemplated by any project farmers, it will be essential to know exactly which species of the complex are involved, as quarantine authorities will definitely recognize each individual species as a separate quarantine problem.

Some notes on how taxonomists are attempting to use molecular methods to distinguish the different species of the various species complexes are given in Appendix 1 of this paper.
2. Fruit fly biology

Life cycle

The life cycle of fruit flies is typical of higher flies (Diptera: Cyclorrhapha) – the female lays eggs into host fruits, and these eggs hatch to larvae (or maggots). The larvae that hatch initially are small and delicate first instar (or first stage) larvae. They moult into slightly more robust second instar larvae, and these in turn moult into quite stout and tough third instar larvae.

When the third instars have finished feeding they leave the fruits, fall to the ground, and crawl away to a sheltered spot (usually in the soil) where they pupate. The larval skin becomes barrel-shaped, tanned brown and hard, and is known as the puparium. The true pupa is formed inside this puparium “shell”. The pupa turns into an adult fly, which escapes from the puparium by splitting open the anterior end and squeezing out.

Female flies do not develop eggs for several days after emergence, and need a protein feed to be able to do this. This is why protein baits mixed with insecticide are an effective control method – see next section on attractants, and Section 3 on management methods.

Most fruit flies are facultative breeders that will lay eggs whenever their host fruits are available, and so may have many generations per year depending on host fruit availability. One exception is the citrus fruit fly, *Bactrocera minax*, found in sub-tropical to temperate parts of Asia, where the fly has one generation per year after which it must go into an obligatory diapause or resting stage (probably an adaptation for survival in cold winters).

Attractants and trapping

Male lures

Males of many fruit flies are attracted by one or more of certain chemical compounds, called male lures. The best known and most used of these lures are:

Cuelure – attracts males of many *Bactrocera* and *Dacus* species – chemically it is 4-(p-acetoxyphenyl)-2-butanone.

Methyl eugenol (ME) – attracts males of many *Bactrocera* species, but not members of the subgenus *Bactrocera* (*Zeugodacus*) (which includes the melon fly, *B. cucurbitae*, and also *B. caudata* and *B. tau*). It is more correctly called methoxy eugenol, and chemically it is 4-allyl-1,2-dimethoxybenzene.

Cuelure will attract flies from up to 300 m away; methyl eugenol attracts up to 500 m.
The species listed in Section 1 have the following attractions to male lures:

Methyl eugenol (ME): *Bactrocera dorsalis, B. papayae, B. carambolae, B. occipitalis, B. philippinensis, B. umbrosa, B. zonata.*

Cuelure: *Bactrocera cucurbitae, B. tau.*

No male attractant known: *Bactrocera latifrons, B. minax.*

**Female lures**

Some preliminary research is showing that it may be possible to develop female lures, but none is commercially available at this stage.

**Protein baits**

In addition to these lures that only attract males, there are food baits and other attractants that draw both sexes, usually females somewhat more than males. These include yeasts, certain bacterial odours, and hydrolysed protein. This last includes yeast hydrolysates and brewery waste containing yeast residues.

Early protein hydrolysates were produced by hydrolyzing plant protein with hydrochloric acid. This produced a protein bait with a low pH, so the acid was neutralized with sodium hydroxide. This in turn produced sodium chloride, and salt-burn of vegetation could result when the protein bait was applied.

More recently a yeast autolysate has been used, produced by enzymatic autolysis of yeast. One of the best known and most widely used of these baits is the Australian Mauri’s Pinnacle Protein Insect Lure. In Malaysia a similar bait called Promar has been produced from waste yeast from stout; in Tonga the Royal Tongan Brewery produces Royal Tongalure (which is cheaper than Mauri’s Pinnacle and so is preferred in the South Pacific); in Vanuatu a similar product is produced by the Tusker Brewery. In Thailand a bait is produced by the Boonrod Brewery in Bangkok, and in Vietnam a protein bait is available from Foster’s Brewery at Tien Giang. Commercial arrangements for bait production in Indonesia are also now under way.

In high rainfall areas or at wet times of year, hydrolysed protein baits may be washed off vegetation too quickly to be effective. To stick the bait to vegetation for longer, it can be mixed with Bactrogel™, produced by Aventis, or similar products.

Lures and baits are exposed inside traps that protect the lure from the weather and provide a receptacle inside which the flies accumulate. Two types of traps are commonly used for dry baits – the Lynfield trap (particularly used in low rainfall areas) and the Steiner trap (better in high rainfall zones).
The Lynfield trap is a clear, 1-litre plastic container with a 100 mm base, a 90 mm diameter top and is 115 mm deep. It has a screw-top lid which may be white or yellow. There are four entry holes 25 mm in diameter, evenly spaced 15 mm below the lip of the trap. Two, three or four cotton dental wicks are held together with a wire clip and hung from a wire loop under the lid of the trap.

The Steiner trap is a horizontal plastic cylinder with a broad opening at each end. One end of the trap is removable. Cotton wicks with the lure impregnated on them are suspended inside the trap.

In both types of trap insecticide is usually mixed with the lure so that the flies die and remain within the trap.

There has been much speculation on why baits and attractants work, and this is an important aspect of behaviour because of the value of baits and attractants in sampling and control. Many fruits give off odours containing distinctive volatile chemicals, and some of these are similar to methyl eugenol (ME). It has been noted that flies attracted to ME are also attracted to the fruits giving off similar odours, and it may be that the males orient by these odours to fruits where females may be found.

With males for which lures have not yet been found, it is likely that chemicals exist that do attract these species but they have simply not been discovered yet.

The surfaces of many fruits are also covered with natural yeasts, and it is likely that fruit flies of both sexes recognize the yeast odours as indicators of possible feeding and breeding sites. The hydrolysed protein baits probably mimic the yeast odours and work similarly.

**Fruit fly damage and crop losses**

The damage starts when the female fruit fly punctures the fruit with its long and sharp ovipositor. The fruit skin is breached, and bacteria enter and the fruit starts to decay. The larvae that hatch from the eggs feed on the decaying fruit tissue, and on the yeasts and bacteria that multiply in it. It is believed that some (maybe all?) fruit fly females carry bacteria with them that they inject into the fruit at oviposition so that the fruit decays faster (making it more nutritious for the larvae).

Fruits with fruit fly larvae in them decay quickly. It is sometimes possible to cut out the damage for home consumption of the remaining part of the fruit, but infested fruits are generally unsaleable, and can certainly not be exported.

Crop losses can vary from a few per cent up to 100%, and losses of 90% or over are common. In some cases losses can be reduced by other treatments applied by the farmers in an area, e.g. against another orchard pest, or in another crop intercropped in the orchard.
Host fruit ranges and preferences for the species listed in Section 1 are broadly as follows. Much more detail is available from the Allwood et al. (1999) and White and Elson-Harris (1992) references listed in Section 4 below.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host fruit range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bactrocera dorsalis</em></td>
<td>A wide range of hosts from many different families</td>
</tr>
<tr>
<td><em>Bactrocera papayae</em></td>
<td>A very wide range of hosts from many different families</td>
</tr>
<tr>
<td><em>Bactrocera carambolae</em></td>
<td>A wide range of hosts from many different families</td>
</tr>
<tr>
<td><em>Bactrocera cucurbitae</em></td>
<td>Mostly Cucurbitaceae, but also eleven other families</td>
</tr>
<tr>
<td><em>Bactrocera latifrons</em></td>
<td>Mostly Solanaceae, but also nine other families</td>
</tr>
<tr>
<td><em>Bactrocera minax</em></td>
<td>Various citrus</td>
</tr>
<tr>
<td><em>Bactrocera occipitalis</em></td>
<td>Mango, guava, citrus recorded</td>
</tr>
<tr>
<td><em>Bactrocera philippinensis</em></td>
<td>Mango, papaya, jackfruit recorded</td>
</tr>
<tr>
<td><em>Bactrocera tau</em></td>
<td>Mostly Cucurbitaceae, but also eight other families</td>
</tr>
<tr>
<td><em>Bactrocera umbrosa</em></td>
<td>Several <em>Artocarpus</em> (jackfruit, breadfruit)</td>
</tr>
<tr>
<td><em>Bactrocera zonata</em></td>
<td>Rosaceae including peach, but also fourteen other families</td>
</tr>
</tbody>
</table>
3. Methods for management of fruit flies

**Cover spraying**
Every tree in an orchard is sprayed over its entire surface with pesticide.

**Advantage:**
- Generally effective in killing fruit flies.

**Disadvantages:**
- Very expensive in cost of pesticide
- Very time-consuming in labour
- Also kills beneficial organisms that keep other orchard pests in check – cover-spraying often results in increased damage by other pests, especially borers (that are hidden from the spray)
- Undesirable for the environment generally
- Can cause health problems for person applying the spray
- May also leave chemical residues in the fruits

**Bagging**
Fruit is covered with some sort of protective layer that prevents fruit flies laying eggs in the fruit. Various sorts of bags are used, and are applied at a stage of fruit development before the fruits have become attractive to fruit flies.

**Advantages:**
- Effective when applied properly.
- Often increases the fruit quality, which also increases the price.
- Materials are very cheap.

**Disadvantage:**
- Very laborious to apply.

**Cultural controls**
These include:
- Growing less susceptible varieties
- Early harvesting of the fruit, before fruit fly attack has occurred (OK with some species that do not attack until the fruit is almost ripe, but with others the attack occurs when the fruit is small, green and unharvestable)
- Crop hygiene and sanitation – preventing old infested fruit lying on the ground and acting as a reservoir of fruit flies to attack the next crop

**Protein bait spraying**
This involves diluting protein bait concentrate with water and mixing it with an insecticide. Malathion used to be used, but more recently chlorpyrifos, fipronil or Spinosad have been used. All have some advantages and some disadvantages, and each situation needs its own decision on what is best to use.
The mixture can be applied with a sprayer or even from a bucket with a brush. A small squint or splash is applied to a leaf or two on scattered trees in an orchard, preferably to the underside where fruit flies prefer to rest. The flies come to feed on the mixture, and are killed when they ingest the insecticide. The mixture is not applied to the fruits. Some farmers apply it to foliage on every tree in an orchard, but because the fruit flies are strongly attracted over quite a distance, it is only necessary to apply it to trees at set distances. The particular combination will vary from orchard to orchard. Similar scattered applications can be used in field crops (such as chilli and cucurbits) as well as tree orchards.

**Advantages:**
- Cheap in terms of materials because little is required to treat a large area
- Greatly reduced health impact on operators
- Less pesticide put into the environment
- Virtually no impact on non-target organisms because only fruit flies are attracted to the bait
- No risk of residues in the produce if applied correctly

**Disadvantages:**
- Still requires a certain amount of labour to apply, though much less than cover spraying or bagging
- May need to be repeated more than once during a crop cycle, especially if weather is very wet

**Male annihilation**
This method is mainly used for eradication of fruit flies from areas like islands where natural barriers minimize the chance of re-infestation. It involves placing absorbent blocks impregnated with a male attractant and pesticide at regular intervals through the target area. The male flies in the area come to feed on the blocks and are killed. The female flies in the area remain unfertilized, and so cannot breed and the population dies out. Not really suitable for general fruit fly control at the level of individual farmers.

**Sterile insect technique**
This method is also only used for eradication of fruit flies from areas like islands where natural barriers minimize the chance of re-infestation. It involves mass-rearing and sterilization of male fruit flies. The sterile males are released into the wild in the target area, and they compete with fertile males in mating with wild females. If there are many more sterile males than fertile ones, most of the females will remain unfertilized, and the population will eventually die out. It is a multi-million dollar operation requiring major facilities and much manpower, and is not suitable for general fruit fly control at the individual farmer level.
One interesting case study

In the mountains of northern Vietnam, the Government of Vietnam promoted the growing of peaches as an alternative to the opium poppies that were previously grown in the area. There was a market for the peaches in Hanoi, and the area was physically and climatically suited to peach-growing. However, 100% of the crop was destroyed each year by the fruit fly, *Bactrocera pyrifoliae*. The only fruits that could be obtained from the trees were picked very small, hard and green, and were of little commercial value.

After protein bait spraying was introduced (with the assistance of an Australian aid project – ACIAR), losses that had been around 100% were reduced to less than 5% loss, and peaches became a commercial reality for the region. And the young children of the area saw their first ripe peaches – in their lifetimes no crop had ever reached maturity before.
4. Some useful references

Important websites of fruit fly relevance

Global fruit fly keys and information


Background information on morphology of cyclorrhaphous Diptera

Australian National Insect Collection (ANIC) anatomical atlas of flies – great for illustrations of every feature of acalyptate flies (the group to which Tephritidae belong): http://www.csiro.au/resources/ps252.html

On the fly: interactive atlas and key to Australian fly families: http://www.csiro.au/resources/ps236.html

Website of International Centre for Management of Pest Fruit Flies (Griffith University and Malaysia): http://www.icmpff.org

Australian fruit flies


South Pacific fruit flies

South Pacific fruit fly website (Pacifly): http://www.pacifly.org (contains profiles of all species found in the South Pacific, including excellent photos of almost all species and much biological information where this is available)
Relevant or useful literature


5. Some key contacts

Griffith University

Address: International Centre for Management of Pest Fruit Flies, Griffith School of Environment, Nathan Campus, Griffith University, 170 Kessels Road, Nathan, Qld 4111, Australia

Principal contact for fruit fly identifications: Professor RAI (Dick) Drew AM FTSE
Phone: +61-7-3735-3696
Fax: +61-7-3735-3697
Email: d.drew@griffith.edu.au

Private consultant (previously with Griffith University – wide fruit fly experience in Asia and elsewhere): Dr Vijay Shanmugam, 25 Mabb Street, Kenmore, Qld 4069, Australia
Phone: +61-7-3701-5418
Mobile: +61-423-542261
Email: vijayseg77@gmail.com

Queensland University of Technology

Address: School of Natural Resource Sciences, Queensland University of Technology, GPO Box 2434, Brisbane, Qld 4001, Australia

Principal fruit fly contact: Dr Anthony (Tony) R Clarke, Senior Lecturer in Ecology
Phone: +61-7-3138-5023
Fax: +61-7-3138-1535
Email: a.clarke@qut.edu.au

Alternative fruit fly contact: Ms Amy Carmichael, Research Associate, Entomology
Phone: +61-7-3138-5050
Fax: +61-7-3138-2330
Email: ae.carmichael@qut.edu.au

Southeast Asia

Name: Ms Asna Booty Othman, Director
Address: Malaysian Regional Centre of International Centre for Management of Pest Fruit Flies, Jalan Sultan Salahuddin, 500480 Kuala Lumpur, Malaysia
Phone: +60-3-2694-0076 / +60-3-2691-6501
Fax: +60-3-2698-7312
Email: asna@icmpff.org or asnadoa@hotmail.com
Centre for Biological Information Technology, University of Queensland, Brisbane, Australia

Producer and seller of Lucid interactive key to *dorsalis* complex

Website:  [www.cbit.uq.edu.au](http://www.cbit.uq.edu.au)
Appendix 1

Methods used for identification of fruit flies (morphological and molecular)

There are two main methods currently in use for fruit fly identification – morphological techniques and molecular protocols. Additional information that can point towards the identity of a particular species can also be obtained by two other types of study – chemical and behavioural. However, neither alone is adequate for species identification.

Currently most identification is undertaken by morphological features, which for many species are clear-cut and provide unambiguous identifications. The problem areas are mostly with species complexes, in which the species can be very difficult to separate for all but experts. Most taxonomists consider that while molecular protocols are showing promise in separating these species, information is not yet complete for all species that need to be included, and so in most cases they do not stand alone for IDs. The extent of development of molecular protocols is discussed further below.

Morphological features used in fruit fly identification

Adult key features are a combination of:
- wing vein shape and detail
- presence or absence of various setae, and relative setal size
- overall colour and colour patterning

The characters are not always easy to determine, and morphological identification of fruit flies requires training and experience. The task has, however, been made easier in the last few years through the development of interactive keys. Lucid-based and other interactive keys are able to highlight (and magnify) on the screen the characters used in the key at each decision point, though some skill in interpreting the specimens is still required. Section 4 above lists an electronic key to the economically important fruit flies of the world, and some other electronic keys.

Interpretation of fly anatomical features has also been made easier by an excellent computer-based Anatomical Atlas of Flies available on the Australian National Insect Collection website (see Section 4 above for reference).

Amongst electronic keys available (references in Section 4 above) are:
White and Hancock (2003) (interactive key for dacine fruit flies)
White and Hancock (1997) (interactive CABIKEY for Indo-Australasian dacine fruit flies)
Lawson et al. (2003) (Lucid CD key to the Bactrocera dorsalis complex)

Drew and Raghu (2002) is also a useful paper-based key to Indian Bactrocera.
Larvae

Morphological identification of cyclorrhaphous fly larvae is quite difficult, and requires even more practice and experience than identification of adults. A further problem is that information on larval morphology is still incomplete, and only certain species can be reliably identified.

Characters used are larval size, presence or absence of preoral teeth on mouthhooks, number of lobes (tubules) on anterior spiracles, nature of oral ridges, presence or absence of accessory plates, hair bundles on posterior spiracles, and relative dimensions of posterior spiracular slits.

A key to known fruit fly larvae has been given by White and Elson-Harris (1992), and an interactive key is also available on the Fruit Flies of the World website (reference in Section 4 above). Some information on the larvae of four Asian species (Bactrocera cucurbitae, B. dorsalis, B. umbrosa, B. tau) has been given by Rohani (1987).

Molecular methods for taxonomic separation of “difficult” species complexes

Two advantages of molecular techniques over morphological ones are that the molecular can be used for larvae (which are very hard to identify reliably on morphological features) and eggs (virtually impossible morphologically), and they can also be used for incomplete adults that may be missing specific anatomical features required for morphological keys, or teneral specimens (recently hatched and still soft adults) that have not fully developed their features (especially their colour patterns).

In theory every separate taxon should have its own unique set of genes, and by looking at the DNA the species should be identifiable, and it should be possible to determine to which other species it is most closely related. In practice it is not so easy. One of the difficulties is to know which piece(s) or sequence(s) of the genetic material should be used for the comparison.

Two different attempts to produce usable molecular protocols have been made. The first is a draft molecular protocol developed by Christine McKenzie, Peter Gillespie and Deborah Hailstones of New South Wales Department of Primary Industries in Australia. In this protocol, a region of the fly genome is amplified using a polymerase chain reaction (PCR). It is an internal transcribed spacer region of the ribosomal RNA operon referred to as ITS1. This fragment is digested using each of up to six different restriction enzymes by restriction fragment length polymorphisms (RFLP). The flies are identified by the size of the ITS1 fragment. If the species is still not identified this way, a restriction digest on the ITS1 PCR product is performed to differentiate between species.

The information obtained is not complete for all fruit flies yet, and the protocol is still draft and is being evaluated. Significant reservations have been expressed about the numbers of specimens of each species analysed in the study, and the ability of the
protocol to identify species in blind tests. At present it does not conform to government requirements for a diagnostic protocol. It is possibly a step towards production of such a protocol, but it is not one yet.

The second molecular approach is DNA barcoding, now being developed as an alternative to PCR based techniques in the belief that it will provide more accurate and consistent results than PCR, with less confusing overlap between taxa. The methodology and the results with 60 species of fruit flies from many different areas are discussed in detail by Armstrong and Ball (2005). The method focuses on mitochondrial DNA and uses cytochrome oxidase subunit I ($\text{coxI}$). The results reported appear to be better than with the PCR method, though some inconsistencies and anomalies still arise. However, with more refinement this technique appears to be potentially valuable.

Mention should also be made of the Tephritid Barcoding Initiative (TBI) – chaired by Professor Bruce McPheron (Penn State University, USA), assisted by Dr Marc de Meyer (Royal Museum for Central Africa, Belgium) and Dr Allen Norrbom (USDA, Washington, USA). The TBI aims to barcode 10,000 specimens representing 2,000 species of fruit flies, including all taxa (about 350 species) of major and minor economic importance. Dr Karen Armstrong’s work will feed into this, and the overall study will give coverage of a wider range of species. Any reservations about practical usefulness will, however, also apply to this study.

As noted above, most fruit fly diagnosticians regard molecular methods as not yet developed enough to be of use for routine diagnostics, though most also regard the techniques as showing promise and worthy of further research and development. Exceptions are the New Zealand Ministry of Agriculture and Fisheries which uses molecular methods for its routine fruit fly IDs, and the New South Wales Department of Primary Industries which has developed the protocol noted above that it uses routinely.

It is logical that molecular methods ought to be the best placed to sort out these difficulties. The genome of each organism that is a valid taxon must differ from the genome of any other taxon no matter how closely related, if only the right part of the genome can be selected that will show these differences. The most difficult groups of organisms to which these techniques can be applied are those that have evolved recently and are still undergoing active evolution, where they have not accumulated fixed differences in their sequences, and where there may be inter-specific hybridization and incomplete lineage sorting. There is evidence that this is the situation with some of the fruit fly complexes.

As noted above, DNA barcoding may offer the best possibility for molecular distinguishing of fruit fly species, though this may need to be combined with a second set of molecular sequences to separate all species including those in species complexes. The New Zealand researcher Karen Armstrong has been among the most active researchers on DNA barcoding in the Australasian area, and she reported on the progress of her studies, and their limitations, in Armstrong and Ball (2005).
One of the most important species complexes from the biosecurity point of view of many countries is the *Bactrocera dorsalis* (Oriental fruit fly) complex, which has been the subject of morphological taxonomic research for many years and is still not fully resolved. A project to use molecular methods amongst other techniques to resolve this complex fully and accurately is currently being developed by the Cooperative Research Centre for National Plant Biosecurity in Australia. If successful, this work will be made available when complete to all countries that need it.

Another important project to establish the true identities of all species in the *Bactrocera dorsalis* complex – and some other problem areas in fruit fly taxonomy – has now been started by the International Atomic Energy Agency in Vienna, in cooperation with 15 key fruit fly experts around the world. This project offers great hope that the taxonomy of these difficult flies will finally be resolved.