BIOSYSTEMATIC STUDIES OF *PSYTTALIA* SPECIES (HYMENOPTERA: BRACONIDAE): PARASITOIDS ATTACKING FRUIT-INFESTING FLIES (DIPTERA: TEPHRITIDAE) IN AFRICA

BY

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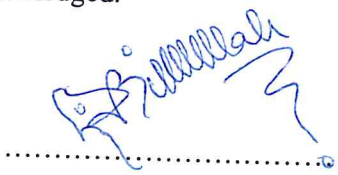
DECLARATION

This is to certify that this thesis has not been submitted to any other University for a Degree. It is entirely the product of my research work, which was conducted under the supervision of Dr. S. Kimani-Njogu and Dr. W. A. Overholt, both of the International Centre of Insect Physiology and Ecology (ICIPE), Prof. R. A. Wharton of the Texas A&M University, USA, Dr. D. D. Wilson and Ms. M. A. Cobblah, both of the Department of Zoology, University of Ghana, Legon, Accra. All help and assistance received have duly been acknowledged.



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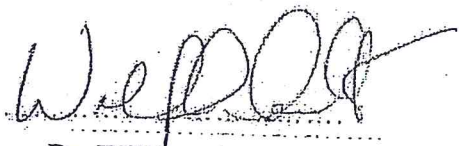

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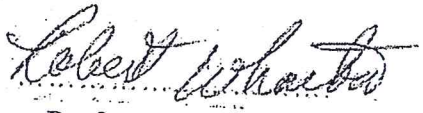

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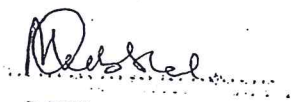

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DEDICATION

To the loving memory of my late parents. Though illiterate, they gave me the necessary basic education that has brought me this far. To my wife and kids - who were distanced from me and relegated to second place because of my studies. It was painful and "unfatherly" not to have been there to hear my kids say their first words, take their first steps or celebrate their first birthdays. That was the biggest sacrifice! For your wonderful love, patience and understanding, you will always be remembered, Jennifer – you are so amazing, and thank you for single-handedly playing the role of two parents.

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ABSTRACT

Biosystematic studies of *Psyttalia* species and populations (Hymenoptera: Braconidae), parasitoids of fruit-infesting Tephritids in Africa were conducted to try and resolve their taxonomy and develop species-specific diagnostic characters to facilitate their reliable use as biological control agents. These studies involved the use of morphology, morphometrics, cross mating and Amplified restriction fragment length polymorphism (AFLP). The species/populations studied included *P. concolor* (from Italy, but originally described from Tunisia), *Psyttalia cosyrae, P. phaeostigma, P. lounsburyi,* and *Psyttalia* samples from Rurima, Ruiru, Mrima Hill, Shimba Hills (locations in Kenya) and populations from Nkolbisson, Cameroon (*P. perproximus*), Tafo (Ghana) and Pietermaritzburg (South Africa). Additional samples included *P. concolor* reared from *C. cosyra* larvae and *P cosyrae* reared from *C. capitata* larvae.

Morphological comparison of measurements of ovipositor, ovipositor sheath and hind tibia, showed ratios of ovipositor/tibia (OTR) and ovipositor sheath/tibia (STR) distinctly differentiating *Psyttalia cosyrae* and *P. phaeostigma* from all other populations. The STR was further able to distinguish clearly between the species, while separation into natural groups was also achieved using flagellomere counts. Parasitoids reared on host larvae other than their natural hosts (with different body size and colour) showed significant changes in linear measurements as well as body colour within one generation of rearing. Morphometric studies on 25 wing measurements in all thirteen (13) populations, assigned populations of unknown identity to groups based on variances. Three clusters were formed when *P. cosyrae* and *P. phaeostigma* were used as checks (out-groups). Two populations from Kenya (Rurima and Ruiru) formed one cluster together with *P. concolor*. The second cluster consisted of one coastal Kenya population (Shimba Hills), the Ghana population (Tafo) and *P. perproximus* (from

Cameroon). The third cluster was formed by *P. cosyrae* and *P. phaeostigma*. Comparison of individual populations showed veins enclosing the submarginal cell together with their adjoining veins as the main variables accounting for differences in these populations. Cross mating studies were conducted between both morphologically distinct species (*P. concolor, P. cosyrae, P. phaeostigma,* Mrima Hill and Sabaki (Kenya), and morphologically similar species (*P. concolor,* Rurima and Ruiru). Once mating was successful, viable female offsprings were produced in all crosses up to F_2, indicating the absence of post-copulatory isolating mechanisms, and suggesting the inadequacy of the biological species concept (BSC) alone in separating species in this genus. Total genome comparison by AFLP in *P. concolor*, *P. cosyrae, P. perproximus*, *P. lounsburyi* and populations from Rurima, Ruiru, Shimba Hills and Tafo, detected variations (presence or absence) in deoxyribonucleic acid (DNA). *Psyttalia concolor*, Rurima and Ruiru shared the highest number of similar loci, followed by *P. perproximus*, Shimba Hills and Tafo, while *P. cosyrae* and *P. lounsburyi* stood out as separate entities. The separation also matched the species-groupings defined by morphometric analyses and thus, lent genomic support to the clusters.

The study was able to separate the populations into their natural groupings, which supports earlier conclusion from cross mating data that the populations from Rurima and Ruiru are morpho-species of *Psyttalia concolor sensu stricto* (i.e. *Psyttalia* cf. *concolor*), and *P. cosyrae* is a different species. The population from Shimba Hills was found to be similar to *P. perproximus* and that from Tafo in the morphological as well as DNA studies and they were clustered together by morphometric analysis. It is therefore suggested as belonging to *P. perproximus*, and the Shimba Hills population is hitherto a new occurrence record of *P. perproximus* from Kenya and East Africa. Body colour and size were found to be determined by host-larvae characteristics and it does not make

these characters alone reliable for identification of parasitoids. This is especially important in post-release sampling surveys to ascertain establishment of parasitoids in new environments where they may adapt to new host species. The populations are also characterized based on outcome of the different taxonomic tools used.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Introduction

Sub-Saharan Africa is the native home to several well-known fruit pests, such as the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Silvestri, 1914), the Natal fly, *C. rosa* Karsch, (Clausen, 1978), *C. anonae* Graham, the Mango fruit fly, *C. cosyra* (Walker) and several other species in the genera *Ceratitis* MacLeay, *Trirhithrum* Bezzi and *Dacus* Fabricius (Cogan & Munro, 1980; Munro, 1984; White & Elson-Harris, 1992; De Meyer, 1996, 1998, 2000). Due to the conducive climatic conditions and the potential for the cultivation of a wide range of horticultural crops (Purcell, 1998), Africa is also now home to one invasive genus *Bactrocera* Macquart (Silvestri, 1914) from the Indo-Australian region. Fruit-infesting flies constitute an enormous threat to fruit and vegetable production throughout the world (Clausen, 1978; Zalom, 1997). In the tropics, this problem is aggravated by the prevailing warm weather which is conducive to asynchronous fruiting patterns, resulting in overlapping generations and the potential of fruit fly infestation all year round (Purcell, 1998).

In addition to the local markets, there is also considerable interest in the export of fruits to Europe and other world markets, but quarantine regulations imposed by importing countries can either deny producing countries potential markets (Waterhouse, 1993a), or force them to carry out expensive disinfestation treatments (White & Elson-Harris, 1992).

Fruit producers must, therefore, meet demands for high quality, blemish-free fruits for both fresh and processed markets. In some cases, insect damage standards are

established which must be met by growers for them to market their products. If these standards are not met, growers must sort out damaged fruits, a typically expensive process, or face losing the ability to market with their fruits. Growers also risk losing an important source of income generation and foreign exchange (Oerke *et al.*, 1994; El Khoreiby, 1997), job-creating opportunities (Oerke *et al.*, 1994), and providing micronutrients and vitamins to improve their diet. In general, it is estimated that about 45 million people depend on the export of horticultural products for their livelihoods in the African, Caribbean and Pacific (ACP) countries (Chan, 2000; Chan & King, 2000).

Fruit flies cause enormous losses in tropical Africa through direct attack on the edible product and rendering the fruits unsuitable for local consumption. For example, small scale production of mango, *Mangifera indica* L. (Anacardiaceae) in Kenya is estimated to suffer losses of 30–80% (Lux *et al.*, 1998).

Strategies used to combat fruit-infesting pests include physical, cultural, behavioural, genetic, chemical, and biological control methods. Of these, chemical control has been the commonest and most widely used method, and it constitutes a significant proportion of all control measures in the tropics and subtropics because of the higher intensity of pests in these climatic conditions (Hutson & Roberts, 1985; Pimentel, 1997). Chemical insecticides have also been used in combination with other control methods such as food attractants, sterile male fly releases, fruit stripping and other sanitation measures, and post-harvest treatments employing elevated temperatures (McPheron & Steck, 1996).

The use of chemical insecticides has, however, become a subject of controversy due to environmental issues and the growing public concern over residue levels in farm produce (Gary & Mussen, 1984). Many classes of pesticides are directly toxic, in one

way or the other, to many categories of natural enemies (Van Driesche & Bellows, 1996) and, it is important to assume that any pesticide might affect a natural enemy until data are available to demonstrate that it does not (Hassan, 1989).

The issue of the effect of chemical pesticides on wildlife and the environment has long been debated, but it came into the political arena by Rachel Carson's book *Silent Spring* (Carson, 1962). The recent harmonisation of the Maximum Residue Level (MRL) by the European Union (EU) (Commission Directive 2000/24/EC in 2000) to restrict levels of pesticide residues in foodstuffs in Europe is an attestation to the nagging pesticide issue. The stringency of the EU regulation has attracted comments from various organizations and countries, and it is said to be higher or tougher than the current regulation set by the Codex Alimentarius Commission (which sets International food safety standards), the World Health Organization (WHO), the U.N. Food and Agriculture Organization (FAO), and the United States of America (Chan, 2000; Chan & King, 2000).

The situation also led the FAO to adopt the latest version of its "International Code of Conduct on the Distribution and Use of Pesticides" in late 2002 (FAO, 2002). The revised Code has recently received the "unqualified support" of the Global Federation of Plant Protection and Science Industry - CropLife International. The aim of the voluntary code "is to increase the responsible and judicious use of crop protection products and to provide a global standard, particularly in those regions where existing controls are inadequate". Moreover, CropLife International notes that the Code "underscores the increased responsibility of all stakeholders in further reducing health and environmental risks that may be associated with any inappropriate use of crop protection chemicals".

Apart from the direct costs involved, chemical control measures can have indirect costs that are often overlooked, and also difficult to quantify (Pimentel, 1997). These include the non-target effects on native or beneficial species (Perkins & Patterns, 1997). They can reduce natural enemy effectiveness either by directly causing mortality or by influencing the behaviour, foraging efficiency, movement or their relative rate of reproduction compared to that of the pest, or by causing imbalances between host and natural enemy populations such as catastrophic host synchronization (Jepson, 1989; Waage, 1989; Croft, 1990).

Among the lists of control chemicals implicated, are malathion bait-sprays, which have the capability of disrupting the natural control of some insects in trees and tree crops (Dahlsten, 1985; Dahlsten *et al.,* 1985). It is also reported that honey bees were seriously affected by the extended aerial application of malathion sprays in a medfly eradication programme in the U.S. between 1980 and 1982 (Gary & Mussen, 1984). The organophosphate insecticide diazinon, which is currently the most widely used soil insecticide against fruit fly larvae/puparia, has been associated with various ecological problems and the development of resistance (Croft, 1990). Additionally, the persistence of diazinon in the soil is known to decrease within 2 weeks and, thus, requires repeated applications (Roessler, 1989). Moreover, it has recently been found in nearby watersheds (away from the points of application) at levels that violate the 'Clean Water Act' for aquatic toxicity (Domagalski, 1999; Kratzer, 1999); and its future use in fruit fly eradication and suppression programmes has, therefore, been strongly questioned (Roessler, 1989; Zalom *et al.*, 1999). This and other organophosphates, in general, are also facing restrictions due to the Food Quality Protection Act, 1999 (Zalom *et al.*, 1999; Badenes-Perez *et al.*, 2002).

The need, therefore, exists to find more environmentally acceptable strategies for the management and control of fruit flies and other pests. One such approach is the Sterile Insect Technique (SIT). This is based on the premise that the release of sterile male flies will compete favourably with wild fertile males to mate with the females which will eventually produce sterile eggs. In this case, the number of insects released must be very large to overwhelm that of the wild pest population. They must also be released when the wild population has not reached its peak, and usually over very wide areas (Tan, 2000). These features make the SIT approach complex and expensive, and its efficacy may be compromised in situations where the population density of the pest flies are high (Knipling, 1992), and more so, when several species co-exist. In the face of the rising demand for fresh blemish-free fruits, the SIT may not be the first approach by growers, as sterile females still continue to oviposit and damage fruits (Sivinski *et al.*, 1996), despite the fact that the eggs they produce are not viable.

Despite the increasing sophistication of control tactics, the growing health and environmental concerns by the public (Penrose, 1996) have led to an intensified search for more environmentally acceptable methods. A resurgence of interest in biological control as a management tool for medfly and other tephritid pests has, therefore, been advocated by Knipling (1992), Wong *et al.* (1992), Waterhouse (1993b), Sivinski (1996), and Purcell (1998).

One of the non-chemical control strategies of tephritid fruit flies has been the biological control option (Clausen, 1978; Wharton, 1989a) which relies on the purposeful introduction of natural enemies for control. It may be employed either for the suppression of pests or for the restoration of natural systems affected by the adventive pests (Van Driesche & Bellows, 1996). It is considered environmentally acceptable,

economically feasible (Van Driesche & Bellows, 1996), cheaper in the long run when parasitoids are established, and can be used in combination with other compatible methods (Knipling, 1992; Wong *et al.*, 1992).

The known tephritid parasitoids used against fruit flies belong to the families Braconidae, Chalcididae, Diapriidae, Eulophidae, Eupelmidae, Eurytomidae, Figitidae (subfamily Eucoilinae), Ichneumonidae and Pteromalidae (Silvestri, 1914; Dresner, 1954; Clausen, 1978; Gilstrap & Hart, 1987; Wharton, *et al.*, 1981; LaSalle & Wharton, 2002). Nearly all the parasitoids that have been recorded from sub-Saharan fruit-infesting tephritids attack the egg or larval stage of their host and emerge from the puparium (Clausen, 1978; Lawrence, 1988).

The opiine Braconidae, has the largest diversity of parasitoids attacking tephritid fruit flies (Clausen, 1978), and are well known for their specificity (Christenson & Foote, 1960; Clausen *et al.*, 1965). These species have a long historical record of use as biological control agents against tephritid pests (Silvestri, 1914; Fullaway, 1915; Bianchi & Krauss, 1937; Clausen *et al.*, 1965; Wharton 1989a), and are among the few effective parasitoids that are presently receiving increased attention for biological control in the neotropics (Messing, 1996; Sivinski, 1996; Kimani-Njogu *et al.*, 2001). Sub-Saharan Africa is the aboriginal home of *C. capitata* and several other potentially important species and is thus expected to have the highest diversity of co-evolved parasitoids that attack these flies (Silvestri, 1914; Bianchi & Krauss, 1937). However, the search for indigenous natural enemies of these flies in Africa is severely hampered by the paucity of work done in their aboriginal home (Wharton, 1997a), and the lack of proper identification of the pests and the parasitic wasps that are their main natural

enemies (Gasparich *et. al.*, 1997; De Meyer 1996, 1998). This has resulted in misdirected efforts in several biological control projects (Wharton, 1997a).

1.2. Justification and rationale of study

With the increased demand for exotic fruits and vegetables, coupled with the increased capability of exporting fruits in relatively fresh condition to distant foreign markets, there is a potential for the accidental introduction of exotic tephritid pests; and this poses a threat to agricultural production in many economies and confirmed by the fact that half the total number of pests intercepted at quarantine checkpoints in Europe and other parts of the world originate from Africa (I. M. White, personal communication).

To reduce the risk of introducing these flies to new areas, stringent measures and standards have been put in place by importing countries. This makes it imperative for growers and exporters to control and manage the infestation levels of fruit flies. Since the management of fruit flies has mostly depended on blanket insecticide sprays, the recent European Union harmonisation regulation makes export even more difficult. In order not to lose the export market, a more integrated approach will have to be adopted to reduce the amount of insecticide sprays used. This approach must include biological control as a major component.

Since the number of fruit fly parasitoids on the continent is high and diverse (Silvestri, 1914), studies carried out to distinguish between the parasitoids and their association with the different fruit fly species will provide valuable information for their use as distinct entities in biological control programmes. However, with parasitic and predatory groups, data on strains or races, geographical distribution, host or prey relationships are often useful taxonomic tools, and obviously of utmost importance in

biological control (DeBach & Rosen, 1991). For example, there has been a long standing problem of synonymy between three African *Psyttalia* species (Hymenoptera: Braconidae) - *P. concolor* (Szépligeti), *P. humilis* Silvestri, and *P. perproximus* (Silvestri) which are closely related and have been separated from one another by subtle differences in the length of the ovipositor and size of the eye (Silvestri, 1914; Wharton & Gilstrap, 1983). The three species have been used extensively in both classical and augmentative biological control programmes against tephritid pests (Wharton, 1989a, b) and are often treated as synonyms of one another. According to Kimani-Njogu *et al.* (2001) and Wharton (personal communication), the matter is far from resolved.

Difficulties in determining the exact identity of biological control agents (Clausen, 1942; Davies, 1988; White & Elson-Harris, 1992; Wharton, 1997a) and host associations (DeBach & Rosen, 1991) have also been reported to result in misdirected efforts in several biological control projects. A typical example is the sequence of events that occurred as a result of the successive introduction of three braconid parasitoid species into Hawaii to control the Oriental fruit fly, *Bactrocera dorsalis* (Hendel). *Diachasmimorpha longicaudata* (Ashmead) was initially introduced and was largely replaced by *Fopius vandenboschi* (Fullawayi) which was also later superseded by *Fopius arisanus* (Sonan) (Davies, 1988; Vargas *et al.*, 2001) which has since remained the dominant parasitoid species of *B. dorsalis* (Haramoto & Bess, 1970). *Fopius arisanus* was originally introduced into Hawaii with other opiine parasitoids, but was initially misidentified as *Opius persulcatus* Silvestri (van den Bosch & Haramoto, 1953; Ramadan *et al.*, 1992). A similar situation was experienced in Kenya, where coffee mealy bugs were inadvertently introduced into Kenyan coffee plantations from Uganda during the World War I era. The mealy bug was initially identified as

Planococcus citri (Rossi) and later as *Planococcus lilacinus* (Cockerell), before its true identity was established as *Planococcus kenyae* (LePelley). The pest is known to be of limited distribution in East Africa. It was also reported in Nigeria in 1954, but that has not been confirmed. The pest established, flourished, and finally reached epidemic proportions, thus becoming the worst destructive pest ever experienced on Kenyan coffee between 1923 and 1951. When it was correctly identified, it was realized that the pest existed in neighbouring Uganda, where it was kept under natural control by the presence of a large complex of hymenopterous parasites. Subsequently, nine (9) primary parasites species were imported and bred in Kenya, from which 5 were released out of which two species, *Anagyrus kivuensis* Compere and *A. beneficians* Compere (Hymenoptera: Encyrtidae) became established (Abasa, 1983) and resulted in the successful control of the pest. This was after the wrong natural enemies and predators imported from many other places had failed to control the misidentified pest.

In order to utilize these native parasitoid species more effectively, a clear knowledge of their systematics and behaviour is needed to separate them from other species, and establish their true identities for their use in future biological control programmes. The present study was, therefore, carried out to establish the relationships between 13 African *Psyttalia* species/populations to facilitate their reliable use as biological control agents.

1.3. Objectives of the study

The general aim was to study features of African *Psyttalia* species and come up with more reliable, species- and host-specific data to characterize the different species/populations, and develop an appropriate concept to facilitate their recognition and use as biological control agents.

The specific objectives were;

- To test the validity of the selected species/populations using the biological species concept,
- To identify specific reliable diagnostic characters through the study of morphology and morphometrics of the adult populations,
- To study host-habitat effects on the species/populations and develop an appropriate concept for the genus, and
- To establish taxonomic relationships of the species by using molecular studies to complement the morphometric and cross mating studies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy of experimental flies

The experimental fruit flies, Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann), marula or mango fruit fly, *Ceratitis cosyra* (Walker) and *Dacus ciliatus* Loew, belong to the order Diptera, family Tephritidae and subfamily Dacinae. *Ceratitis capitata* and *C. cosyra* belong to the tribe Ceratitidini and subtribe Ceratitidina. Medfly belongs to the subgenus *Ceratitis sensu stricto* MacLeay, while the mango fruit fly belongs to the subgenus *Ceratalaspis* Hancock (De Meyer, 1996; 1998; 2000). *Dacus ciliatus* on the other hand, belongs to the genus *Dacus* Fabricius, subgenus *Didacus*, tribe Dacini and subtribe Dacina (White & Elson-Harris, 1992).

2.2 Origin and distribution

The Italian Dipterologist, Mario Bezzi was the first to suggest tropical Africa as the origin of *Ceratitis capitata* (Silvestri, 1914). He considered West Africa as the probable home, though notes on the original description by Wiedemann in 1824 indicated a dual type locality (see De Meyer *et al.*, 2002a for full taxonomic history). The medfly is the only species that readily colonizes tropical and mild temperate habitats far outside its home range (Gilstrap & Hart, 1987), and is now reported from five different continents. These include all the countries surrounding the Mediterranean Sea, Central and South America, Western Australia and Hawaii (USDA, 1983; Sheppard *et al.*, 1992). The rapid spread is thought to have occurred mainly by human activities such as fruit shipment, tourist travel, packing material, nursery stock (Back & Pemberton, 1918;

USDA, 1983) and, to a lesser extent, through wind currents and natural migration (Back & Pemberton, 1918).

To clarify the uncertainties surrounding the origin of *C. capitata*, recent work by De Meyer (2001a) and De Meyer *et al.* (2002a) based on phylogeny, biogeography, host plant range, and abundance of medfly and its congeners within the subgenus *Ceratitis*, support the view of an eastern African origin; possibly from the highlands, and subsequent dispersal from there. However, molecular evidence presented by Gasparich *et al.* (1997) and recently by Douglas and Haymer (2001) shows higher mitochondrial DNA diversity from West Africa. This supports the hypothesis of *C. capitata* originating from West Africa, as asserted earlier by M. Bezzi. *Ceratitis cosyra* is native to Africa and is more or less restricted to the African continent (De Meyer, 2001a). *Dacus ciliatus* is also native to Africa but has been introduced to the Middle East, southern Asia east to Burma and to Mauritius (prior to 1901) and Le Réunion (prior to 1972) (Norrbom, 2002).

2.3 Fruit fly biology

Adult female fruit flies lay their eggs (singly or in clusters) beneath the skin of suitable hosts by puncturing the host fruit skin with their long and sharp, extendible ovipositors. Physiologically mature, ripening or ripe fruits are usually preferred, depending on the fruit fly species and the host plant attacked. Oviposition deterrent pheromones are known to be used by some species such as *C. capitata*, and several species of *Anastrepha* Schiner and *Rhagoletis* Loew to signal conspecifics of already attacked fruits (Averill & Prokopy, 1989). The eggs take between 2-12 days to hatch and the numbers produced in a life time range from 50 in *R. cerasi* Loew to as many as 1500 in *Bactrocera dorsalis* (Hendel).

There are three larval instars that develop within the fruit by skin shedding as the larvae feed and grow. The larval stage lasts from 4-37 depending on species. The apple maggot fly *Rhagoletis pomonella* Walsh larvae are known to overwinter during cold seasons (Christenson & Foote, 1960). At the third instar, most fruit-feeding larvae drop to the ground and move into the soil by jumping along the ground to find suitable pupariation sites. At the end of the third instar, the larval skin hardens to form a puparium enclosing a dormant or inactive instar larva inside (Christenson & Foote, 1960). The pupal stage takes between 6 days (in *C. capitata*) to 30 days in *B. dorsalis*. Pupae of the genus *Rhagoletis* have been reported to hibernate during winter and resume activity when conditions become favourable (Christenson & Foote, 1960). The larva within the puparium sheds its skin to form a pupa, from which the adult fly eventually emerges. Emerging adults crawl upwards through the soil, making use of cracks and crevices to the surface (Christenson & Foote, 1960). The newly emerged adults require a carbohydrate energy source and water in order to survive, and a protein source for egg maturation. Adult life span ranges from one month to as long as eleven months in *A. ludens* (Loew) (White & Elson-Harris, 1992).

After mating and a pre-oviposition period, the females start laying eggs to begin new cycles (Plate 2.1). Tropical species of *Anastrepha*, *Bactrocera* Macquart, *Ceratitis* and *Dacus* are typically multivoltine i.e. they have several generations per year.

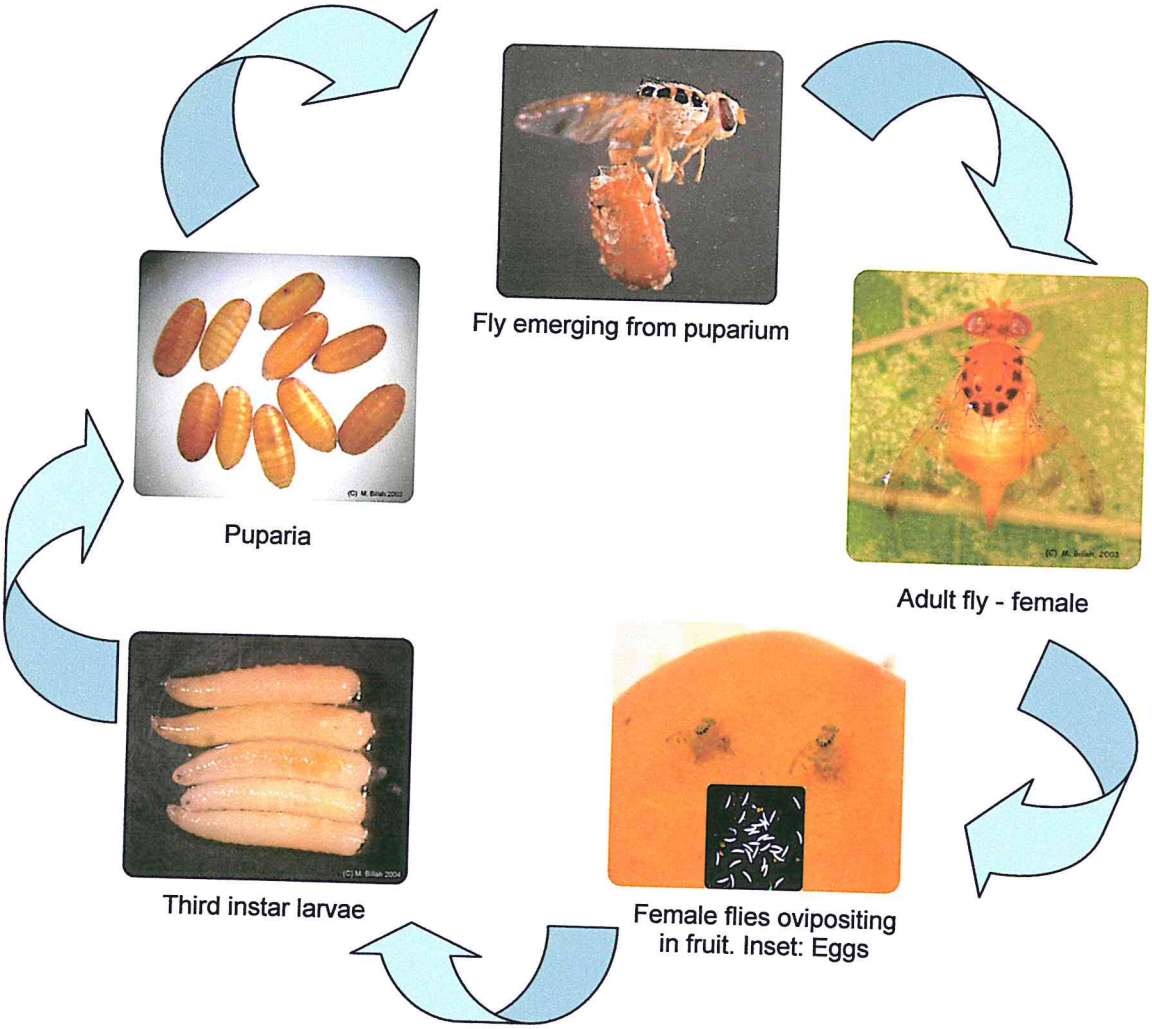


Plate 2.1 Generalized life cycle of a fruit fly (*Ceratitis cosyra* (Walker)). (Photos & Scheme: M. Billah).

2.4 Tephritids as pests

Globally, there are about 1400 species of tephritids that develop in fleshy fruits, with nearly 250 of these capable of achieving pest status by feeding on plants of economic importance (White & Elson-Harris, 1992; Drew & Hancock, 1994). The genus *Ceratitis* alone comprises close to 90 species (De Meyer *et al.*, 2002a, b), all of which have an afrotropical distribution. In addition to being the native home of economically important fruit flies, Africa has also been the recipient of at least three accidentally introduced exotic species of Asian origin (Silvestri, 1914). These are the melon fly, *Bactrocera cucurbitae* (Coquillet) which is established in most Eastern African countries and has recently been reported in the Gambia in West Africa (Norrbom, 2002). It is believed that if no immediate action is taken, it could probably spread throughout the whole West African sub-region. The other two species are *Bactrocera dorsalis* (Hendel) which was briefly established in the Indian Ocean island of Mauritius but has been eradicated (Seewooruthun *et al.*, 1998), while *Bactrocera zonata* (Saunders) was introduced in 2000 in Egypt, Mauritius and Le Réunion (Norrbom, 2002). Recently, a new fruit fly species of Asian origin was detected in Kenya, which was identified to be a member of the *Bactrocera dorsalis* species complex. The distribution and extent of spread suggest that the pest might also be present in neighbouring Uganda and Tanzania (and may still be spreading further) (Lux *et al.*, 2003b).

2.5 Tephritid host range

Fruit-infesting tephritids have a broad range of feeding habits. There are polyphagous, monophagous, as well as stenophagous species attacking a wide range of hosts. *Ceratitis capitata* or the Medfly has one of the broadest host ranges of all known tephritids. It is

considered the most devastating and widespread species of all fruit flies, attacking hosts in nearly 70 plant families (Mitchell *et al.*, 1977; Weems, 1981); 353 plant species are reported as hosts or potential hosts (Liquido *et al.*, 1991). According to De Meyer *et al.* (2002b), more than 150 host plants are reported to be attacked by *C. capitata* with certainty in Africa. The close relatives of *C. capitata* have restricted host spectrum - either confined to a single host genus or to a few genera at most. For example, apart from mango, *Mangifera indica* L (Anacardiaceae), *Ceratitis cosyra* develops in fruits of marula, *Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae), which is considered as its primary endemic host (Lux *et al.*, 2003a) and a few others like custard apple, *Annona senegalensis* Pers. (Annonaceae) and guava, *Psidium guajava* L. (Myrtaceae). The host range of *Dacus ciliatus* Loew (Diptera: Tephritidae) is primarily cucurbits, and can sometimes develop in their flowers. However, they have also been recorded from a few non-cucurbit hosts (White & Elson-Harris, 1992). Though a great number of fruit species have been documented to harbour fruit flies, numerous new host associations have recently been reported in Kenya (Copeland *et al.*, 2002) and Ghana (M. Billah, unpublished data), thus revealing current literature records as far from complete and indicating the ever widening fruit fly adaptation to and/or invasion of new plant hosts.

2.6 Damage and economic impact

Apart from the initial puncturing of fruits by adult females to lay eggs, the growing larvae in the fruit feed voraciously on the fruit pulp and inflict heavy losses. Larval feeding is the most destructive period of the flies and leads to either decay of fruits on the tree or to premature fruit drop. This results in both quantitative and qualitative losses, and thus makes fruit flies one of the major production concerns for fruit growers (Zalom, 1997). Plate 2.2 shows various damage levels of fruits due to fruit fly attack.

The economic effects of fruit fly pest species include not only direct loss of yield and increased control costs, but also the cost of constructing and maintaining fruit treatment and eradication facilities. Due to the rising demand for high quality, blemish-free fruits for both fresh and processed produce, control measures have to be applied for producers to meet market demands. Chemical control has always been the first line of action for most producers. In many countries, the exportation of commercial fruits is severely restricted by quarantine laws to prevent the spread of fruit fly species. These restrictions can either deny producing countries potential markets (Waterhouse, 1993a) or important sources of income generation and foreign exchange (Oerke *et al.*, 1994; El Khoreiby, 1997) and job-creating opportunities (Oerke *et al.*, 1994). The situation is further aggravated by the recent harmonization of the maximum residue level (MRL) by the European Union (EU), thus putting the resource-poor African producer in a "production dilemma". Damage levels of 100% have been attributed to the Mediterranean fruit fly in some stone fruits (Karpati, 1983) and losses of up to 50% in citrus (Weems, 1981). The Natal fruit fly has also been reported to cause 50-100% infestation in plums in one locality in South Africa (Weems, 1981). In Kenya, *Ceratitis rosa* and *C. capitata* have been reported to cause up to 40% premature dropping of coffee berries (Waikwa, 1979), while mango producers suffer losses of between 30-40%, and rising to 70% on small-holder farms as a result of *C. cosyra* infestation. Fruit flies would therefore be a potential threat to the growing horticulture industry in Kenya if no control measures are put in place, as they constitute an enormous threat to fruit and vegetable production throughout the world (Clausen, 1978).

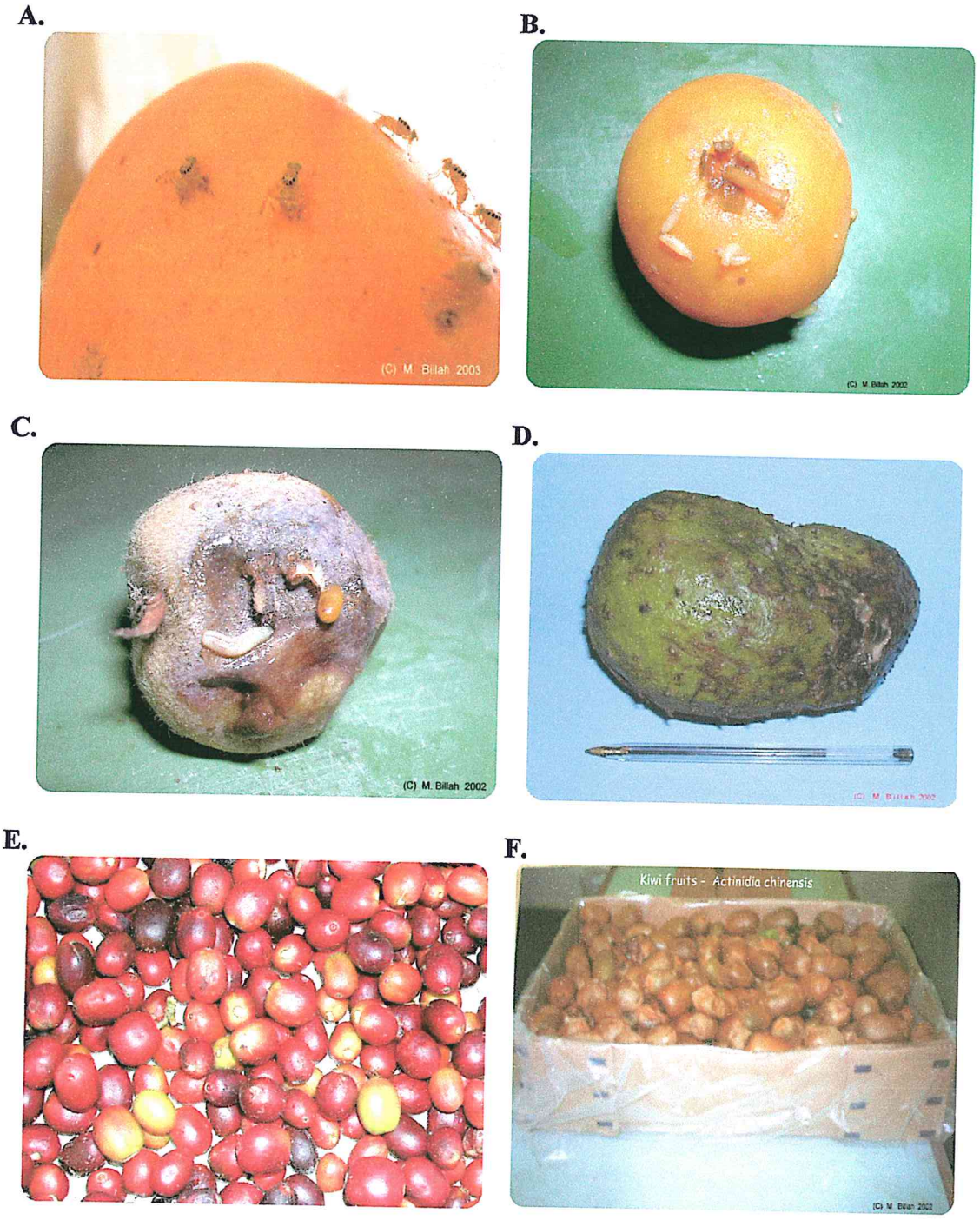


Plate 2.2 Fruits of different crops showing various levels of fruit fly damage. A. = *Ceratitis cosyra* ovipositing on mango, B. = Kei apple (with larvae crawling out of fruit), C. = Loquat (with larva and freshly formed puparium), D. Jack fruit (showing infestation point), E. = Coffee berries, and F. = Kiwi (both with numerous small black oviposition marks). (Photos: M. Billah).

2.7. Biological control of fruit-infesting flies

Biological control is a potentially useful approach in suppressing fruit fly densities (Wharton, 1989b; Knipling, 1992; Waterhouse, 1993a, Sivinski *et al.*, 1996), and some of the most common approaches used are augmentative and classical biological control techniques. Classical biological control involves the collection of natural enemies from the aboriginal home of the pest and the release of these natural enemies in the new area the pest has invaded. If the natural enemies are successfully established in the new area, they re-establish the host-natural enemy relationship that occurs in the aboriginal home, leading to a decreased density of the pest. Sometimes, not only are exotic natural enemies introduced against introduced pest species (i.e. classical biological control), but they are also introduced against endemic pests (Potting, 1996). This is referred to as the "new association" approach (Hokkanen & Pimentel, 1989). It augments the effects of the natural enemies by increasing the populations (Rabb *et al.*, 1976). A comprehensive review of augmentation in biological control is found in Ridgway & Vinson (1977). The technique involves periodic releases and environmental manipulation (as discussed above). Periodic releases may be labelled as inundative or inoculative, depending upon the numbers of natural enemies released and the interval during which they are expected to provide control. These releases are designed to control the pest by the actions of the released natural enemies, not by the actions of their progeny, and thus can be considered as "biotic insecticides". Augmentation biological control has been particularly successful in glasshouse crops (Hussey & Scopes, 1985).

Recently, there has been renewed interest in the neotropics in collecting parasitoids and utilizing them to reduce fruit fly populations, with much of the attention on medfly (Wong *et al.*, 1992; Headrick & Goeden, 1996). However, no classical biological

control introductions have been made in Africa against fruit flies, though Africa has been a recipient of such exotic species as the melon fly, *Bactrocera cucurbitae*, which is believed could be a suitable candidate for classical biological control.

2.7.1 Biological control agents other than parasitoids

2.7.1.1. Predators

Common larval and pupal predators include the families Carabidae, Chrysopidae Coccinellidae, Dermaptera, Formicidae, Pentatomidae, Staphylinidae, some crickets and myriapods and a few mites and predaceous wasps (Bateman, 1972; Boller & Prokopy, 1976; Fletcher, 1987; Sivinski, 1996; and review by Norrbom, 2002). However, most of these predators are not specific to fruit flies. In addition, Drew & Allwood (1985) described a species of Strepsiptera that parasitizes ten species of *Bactrocera*. However, not much has been done on their use against fruit flies.

The Argentine ant, *Iridomyrmex humilis* (Mayr) has been observed under laboratory conditions to cause mortality up to 50% in medfly pupae after a 10 minute period of attack (Wong *et al.* 1984). However, the authors contend that ant predation could be important only in localized areas and is not adequate to regulate medfly populations. Marucci (1955) also studied the efficiency of two earwigs as predators of *Bactrocera dorsalis* in Hawaii, while Neuenschwander *et al.* (1983) reported of a cecidomyiid egg predator of *Bactrocera oleae.* Spiders are also observed to trap and kill fruit flies in their webs (Plate 2.3).

2.7.1 2 Microbial agents

a) Fungi: Fungi of the genera *Penicillium*, *Serratia* and *Mucor* have been reported to cause considerable larval and pupal mortality to *Bactrocera dorsalis* (Newell & Haramoto,

1968). A study at ICIPE, Kenya, by Ekesi *et al.* (2002) also revealed that several isolates of *Metarhizium anisopliae* (Metsch.) Sorok and *Beauveria bassiana* (Balsamo) Vuillemin were found to be effective against adults, larvae and puparia of a number of African fruit fly species (*C. capitata, C. rosa, C. cosyra, C. fasciventris* and *C. anonae*). The isolates reduced adult emergence from treated soil by 6-68% in the laboratory and field cages, respectively. Goeden & Benjamin (1985) and Hedstrom (1994) reported records of *Stigmatomyces* (Ascomycota) fungus species that attack Tephritidae, and the order Laboulbeniales species have been found on abdomens of various fruit flies (see Norrbom, 2002). Mortality rates between 82% and 100% were achieved in the laboratory by De La Rosa *et al.* (2002) in Mexico by spraying adult *Anastrepha ludens* (Loew) with *Beauveria bassiana* (Hyphomycetes) at a concentration of 1×10^8 conidia ml^{-1}.

b) Bacteria: When mixed into a diet of protein and sugar, *Bacillus thuringiensis* (Berliner) (*Bt*) subspecies *darmstadiensis*, was found to kill *A. ludens* in Guatemala (Robacker *et al.*, 1996; Martinez *et al.*, 1997). Field application of a suspension of *B. thuringiensis* applied at OD_{600} spores and crystals in 4% yeast enzymatic hydrolysate has also been reported to significantly reduce damage to olive fruits by *Bactrocera oleae* in Greece (Navrozidis *et al.*, 2000).

c) Nematodes: These have also been used for control of several tephritid species (Sivinski, 1996). Medfly was susceptible to the Mexican strain of the entomopathogenic nematode *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae). Emerging adults and puparia were not susceptible to the nematode but the third instar larvae (prior to pupariating in the soil) suffered high mortalities (50-90%) when exposed to high nematode concentrations (150,000-500,000 nematodes/cup) (Lindegren & Vail, 1986).

Field exposure of mature larvae to a dose of 500 nematodes/cm^2 yielded high mortality rates of *C. capitata* (Lindegren *et al.*, 1990), but naturally occurring attacks have not been reported (Norrbom, 2002).

In a more recent study, Yee and Lacey (2003) exposed the larval, pupal, and adult stages of the western cherry fruit fly, *Rhagoletis indifferens* Curran to different doses (50 and 100 infective juveniles (IJ)/cm^2) of three species of *Steinernema* nematodes (*S. carpocapsae* Weiser, *S. feltiae*, and *S. intermedium* (Poinar)) both in the laboratory and in the field. It was found that the larval stages were the most susceptible, with mortality ranging from 62-100% in the three treatments. Larvae were also introduced into different soil types previously treated with *S. carpocapsae* and *S. feltiae* at 0, 2, 4, and 6 days, and were found to be persistent and effective. *Steinernema carpocapsae* was the most effective species at 100 IJs/cm^2 and infected 11-53% of adult flies that emerged. Pupae were not infected. It was, therefore, concluded that the high pathogenicity of *S carpocapsae* and *S. feltiae* against *R. indifferens* larvae, as well as their persistence and efficacy in different soil types, renders them prospective biological control agents of flies in isolated and abandoned fields.

d) Viruses:

Viruses are known to attack *Ceratitis capitata*, *Bactrocera oleae* (Gmelin) and the Queensland fruit fly, *B. tryoni* (Froggatt). These viruses have not yet been identified and they are only described as "*Ceratitis* V virus", *B. oleae* virus" and the "Queensland fruit fly virus (QFFV)", respectively (Plus *et al.*, 1984; Manousis *et al.*, 1987; Bashiruddin *et al.*, 1988). Sivinski (1996) also mentioned a virus that attacks *B. tryoni*, but it is not know whether it refers to the QFFV above.

Plate 2.3 Numerous web carpets of ground-dwelling spiders on the floor of a mango orchard (Muhaka, Kenya). Insert: A close shot of the entrance hole where the spiders rush out and capture fruit fly larvae that drop from fruits on trees to pupate in the soil. (Photos: M. Billah).

2.7.1.3 Other natural enemies

The lizard, *Anolis grahami* Gray (Sauria: Iguanidae) was introduced from Jamaica to Bermuda for the control of fruit flies, though its role in controlling the pest has not been evaluated (Clausen, 1978). Birds and rodents were reported to consume infested fruits resulting in a high level of larval mortality (Drew, 1987).

2.7.2 Biological control using parasitoids

Among the known families of parasitoids which attack fruit flies, the opiine Braconidae has the largest diversity of parasitoids (Clausen, 1978), with a long historical use as biological control agents against tephritid pests (Silvestri, 1914; Fullaway, 1915; Bianchi & Krauss, 1937; Clausen *et al.*, 1965; Wharton 1989a). In Africa, species of the genus *Psyttalia* are among the commonest parasitoids that are reared from fruit-infesting flies.

2.7.2.1 Biology of *Psyttalia* species

Parasitoids in the genus *Psyttalia* are solitary larval-pupal endoparasitoids. They allow their hosts to continue to feed and grow after parasitism and are, therefore, referred to as koinobionts (Askew & Shaw, 1986).

The genus *Psyttalia* is known to contain species which attack many fruit fly species (Wharton, 1997a). The female wasp pierces the skin of the fruit with its ovipositor and deposits an egg just beneath the skin of the fruit fly larva, usually in full-grown larvae although younger larvae may also be successfully parasitized. The first instar of opiine parasitoids feed and grow but the first larval moult is delayed until the host has pupated (Pemberton & Willard, 1918). The moult of the first larval instar and its development into subsequent stages has been reported to be triggered by the hormonal titer of its host

(Lawrence, 1982). The adult emerges through an opening from the host puparium (Clausen, 1978), with the males generally emerging a day or two before the females, and are ready to mate with newly emerged females (Greany *et al.*, 1976; Ramadan *et al.*, 1992). Females may start ovipositing on the first day of emergence and may continue laying eggs during the ensuing three weeks. The life cycle from egg to adult takes about 15 to 18 days, and adults can live for up to three months at optimum summer temperatures (Plate 2.4).

2.8 History of biological control of tephritid pests

Classical biological control has a long history which first began in Australia in 1902 when George Compere was hired by the government of Western Australia to search for natural enemies of medfly. Several parasitoid species were introduced against the medfly. Prominent among them was *Aceratoneuromyia indica* (Silvestri) (Hymenoptera: Eulophidae) but none of the species got established at the time (Compere, 1912). However, *A. indica* has now been established in many countries and it is now one of the more commonly encountered parasitoids of tephritid pests in Central America (Wharton *et al.*, 1981). These efforts have extensively been documented, while several detailed reviews have also been made available by Rao *et al.* (1971), Greathead (1971, 1976), Clausen (1978), Gilstrap & Hart (1987), and Wharton (1989a, b).

Filippo Silvestri, a consulting entomologist, conducted the first successful biological control programme for tephritids. In 1912, Silvestri was hired by the Hawaii Board of Agriculture and Forestry to search for natural enemies of the medfly. Based on information obtained from Mario Bezzi (another explorer scientist), Silvestri initiated his search in Africa, travelling along the West Coast of Africa, across to Australia and,

finally, to Hawaii. During his voyage, Silvestri collected fruits from many localities, and eventually reared 16 species of parasitic Hymenoptera from a variety of fruit-infesting tephritids (Silvestri, 1914). Unfortunately, most of this material was lost during the 10-month voyage and he arrived in Hawaii in 1913 with only five of the species still alive, of which four were successfully cultured and released. These included two pupal parasitoids, *Coptera silvestrii* (Kieffer) (Hymenoptera: Diapriidae) and *Dirhinus giffardii* Silvestri (Hymenoptera: Chalcididae), and two opiine braconids - *Psyttalia humilis* Silvestri (Hymenoptera: Braconidae), originally collected in South Africa, and *Diachasmimorpha tryoni* (Cameron) from New South Wales, Australia.

A second expedition to Africa in 1914 resulted in the establishment of two additional species, *Tetrastichus giffardianus* Silvestri (Hymenoptera: Eulophidae) and *Diachasmimorpha* (=*Biosteres*) *fullawayi* Silvestri (Hymenoptera: Braconidae), both from Olokemeji, Nigeria (Fullaway, 1915). Although the pupal parasitoids were largely ignored after their establishment, the efficacy of *P. humilis, D. tryoni, D. fullawayi*, and *T. giffardianus* was extensively documented over a 23-year period (Willard & Mason, 1937). All four species were still established in 1933 - the last year for which data are available, with *P. humilis* and *D. tryoni* exhibiting roughly equivalent levels of parasitism on medfly (Wharton, 1989b). The principal benefit of these introductions was the considerable reduction in infestation levels in coffee which had previously been so badly infested that the berries could not ripen (Willard & Mason, 1937). Infestation in other fruits was also reduced, though not as much as in large, fleshy, preferred fruits such as mango. Nevertheless, reduced infestations made it possible to integrate other control measures more successfully and thus eliminate medfly in some non-preferred fruits. The parasitoids brought to Hawaii by Silvestri and Fullaway were later sent to other parts of the world in attempts to control other tephritid pests. Though these

programmes were largely unsuccessful, they resulted in the establishment of *T. giffardianus* in Brazil, Fiji and Spain. In addition, *T. giffardianus* has been introduced to the Réunion Islands, Argentina, Puerto Rico, U.S.A. Australia, New Caledonia, Samoa, and Vanuatu (LaSalle & Wharton, 2002). However, establishment in these areas was not documented and its current status is unknown, except in Hawaii. The status of the other *Tetrastichus* species is largely unknown partly because of the lack of adequate information on how to differentiate the species (LaSalle & Wharton, 2002).

Further explorations for medfly natural enemies were conducted from 1935-36 in East Africa by the U. S. Department of Agriculture (Bianki & Krauss, 1937), and again in the late 1940s in East and West Africa (Clausen *et al.*, 1965). Among the species collected were large numbers of unidentified parasitoids (Gilstrap & Hart, 1987). As a result of these latter explorations, nine species were released in Hawaii (Wharton, 1989a) but none got established (Clausen *et al.*, 1965).

The discovery of the Oriental fruit fly (*Bactrocera dorsalis*) in Hawaii immediately after World War II led to the largest classical biological control programme ever undertaken against tephritids (Clausen *et al.*, 1965). Of the many species collected and shipped from all over the world during this programme, 24 were released, 11 were subsequently recovered, and 5 were established for the first time in Hawaii. Only 4 of these (all opiine braconids from the Indo-Pacific Region) were ever sufficiently abundant to cause reductions in the Oriental fruit fly populations (Bess *et al.*, 1961; Clausen *et al.*, 1965; Wong *et al.*, 1984). Other parasitoids routinely used in augmentative programmes include *Aceratoneuromyia indica* (Silvestri) in Central America (Wharton *et al.*, 1981) and *Psyttalia concolor* (Szépligeti) in the Mediterranean Region (Greathead, 1976).

Of all the parasitoid species thought to be established against tephritid pests (including those used in augmentative programmes), only four originated from Africa. Two of these are the pupal parasitoids in the genera *Coptera* (Diapriidae) and *Dirhinus* (Chalcididae) brought to Hawaii by Silvestri in 1913. The species of *Coptera* has rarely been found since its initial introduction and its status is thus largely unknown. Taxonomic problems with *Dirhinus* (Boucek & Narendran, 1981) have left the origin of the species, now established in Hawaii, in some doubt because similar species from other parts of the world were introduced during later programmes. The other two species indigenous to Africa are *P. concolor* and *T. giffardianus*. The former was originally collected from Tunisia on *Bactrocera oleae* (Gmelin). Thus, *T. giffardianus* collected from Nigeria in 1914 (Fullaway, 1915) may be the only native parasitoid of *Ceratitis* established outside of Africa. Most of the remaining parasitoids introduced for biological control of tephritids were originally obtained from fruit flies of the genera *Anastrepha* and *Bactrocera* which occur outside the native range of *Ceratitis*.

The paucity of introduced natural enemies from the aboriginal home of medfly led to renewed efforts to obtain medfly parasitoids from sub-Saharan Africa in the early 1980s (Steck *et al.*, 1986), but the introductions from this programme (from Cameroon, Togo, and Hawaii to Costa Rica) apparently did not result in any new establishments. However, a useful end-product was the careful documentation and enumeration of the parasitoids used in all previous tephritid biological control projects (Wharton *et al.*, 1981; Gilstrap & Hart, 1987; Wharton, 1989a), and the resolving of a number of taxonomic problems (Wharton & Gilstrap, 1983; Wharton, 1987, 1988). Based on these sources as well as examination of specimens from more recent collections, at least 24 species of parasitic Hymenoptera were reared from *Ceratitis* in Africa during various biological control programmes. Therefore, in the search for more effective natural

enemies to use in current biological control programmes, Africa is unquestionably an excellent source of material.

The role of parasitoids in the natural regulation of tephritid populations was reviewed by Debouzie (1989) and the parasitoids of *Bactrocera* and *Rhagoletis* species by Fletcher (1987) and Boller & Prokopy (1976), respectively. Rates of parasitism were very high (90%) in some of the examples discussed by those authors but Fletcher (1987) noted that low levels (0-30%) of parasitism are more typical. The history and effectiveness of biological control was reviewed by Wharton (1989a & b).

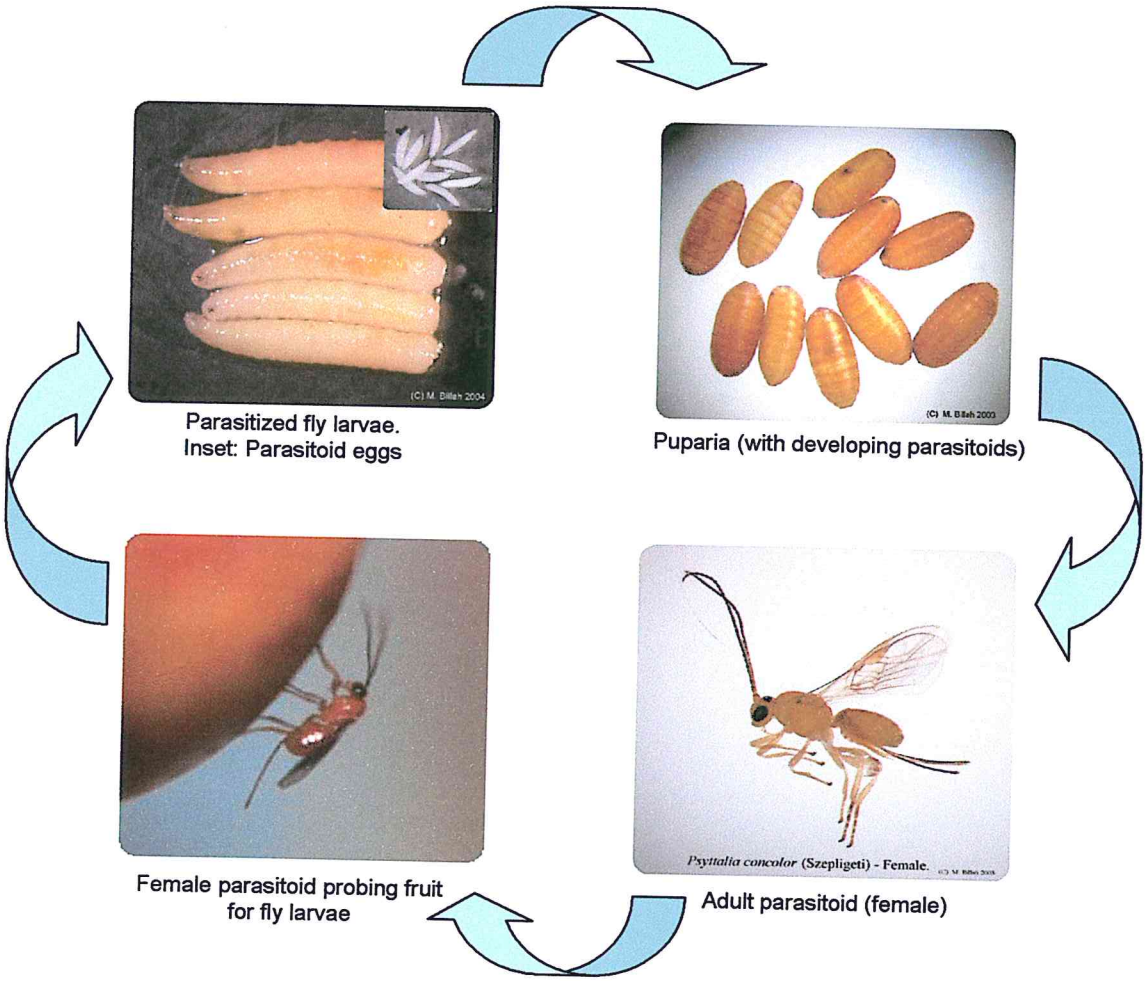


Plate 2.4 Generalized life cycle of a fruit fly parasitoid (*Psyttalia concolor* (Szépligeti)). (Photos & Scheme: M. Billah).

2.9 Systematics of tephritid parasitoids

Correct identification of a natural enemy is essential for its use in biological control. The taxonomy of fruit-infesting tephritid parasitoids in Africa has not been fully clarified, and there are a variety of systematics problems. The known tephritid parasitoids from sub-Saharan Africa belong to the families Braconidae, Chalcididae, Diapriidae, Eulophidae, Eucoilidae (subfamily Eucoilinae), Figitidae and Pteromalidae (Silvestri, 1914; Clausen *et al.*, 1965; Clausen, 1978; Gilstrap & Hart, 1987; Wharton, 1989a; LaSalle & Wharton, 2002). The chalcidids, diapriids and pteromalids are all pupal parasitoids and include the genera *Dirhinus, Coptera, Spalangia,* and *Pachycrepoideus*, each containing one or two described species previously reared from fruit-infesting tephritids in sub-Saharan Africa.

Nearly all of the remaining parasitoids that have been recorded from sub-Saharan fruit-infesting tephritids attack the egg or larval stage of their hosts and emerge from the puparium. These include members of the families Eucoilidae (subfamily Eucoilinae), Eulophidae, and Braconidae. The African eucoilids have never been successfully introduced. They were never determined to species level, and even their generic identity is in doubt (Gilstrap & Hart, 1987).

The Afrotropical eulophids attacking fruit-infesting tephritids all belong to the subfamily Tetrastichinae (LaSalle & Wharton, 2002) and include several species of *Tetrastichus* and at least, one species from the genus *Tachinobia*. *Aceratoneuromyia indica* (Hymenoptera: Eulophidae) which was shipped to many parts of the world for biological control during the past 90 years may also occur in the Afrotropical Region. There has long been confusion regarding the identities of the species of *Tetrastichus* imported to Hawaii for biological control (Back & Pemberton, 1918), though most of

the work in Hawaii has gone under the name *T. giffardianus* (Willard & Mason, 1937; Ramadan & Wong, 1990; Purcell *et al.*, 1996). Fullaway (1915) listed the species in question as *T. giffardii* Silvestri, while Clausen *et al.* (1965) reported recoveries of *T. dacicida* Silvestri during the Oriental fruit fly programme. A fourth species, *T. oxyurus* Silvestri, was described from specimens reared from a species of *Ceratitis* in Nigeria, and may also have been unknowingly introduced (Steck *et al.*, 1986). Though tetrastichines are notoriously difficult taxonomically (LaSalle, 1994), recent advances in their classification following the reclassification of the European Tetrastichinae provided by Graham (1987) include the break-up of the genus *Tetrastichus* into several smaller and more natural manageable groups (LaSalle, 1994; Wharton, 1997b). This has greatly facilitated small-scale revisions. The situation is further facilitated by the recent clarification of the African Tetrastichines by LaSalle and Wharton (2002). The species *Tetrastichus dacicida* which is found in sympatry, and often confused with *T. giffardii*, has now been synonymized with *T. giffardii*. The synonymization was based on the fact that none of the original characters used by Silvestri to separate the two species proved to be consistent and had many variations. Characters for the recognition and differentiation of *Tetrastichus giffardii*, *T. giffardianus*, and *T. oxyurus* have also been revised, and lectotypes designated for all four species (i.e. designation of one particular specimen as the name-bearing type from the original group of specimens from which the species was described. This is normally done after the group has been revised to clear any confusion for the species to be more closely defined).

Members of the family Braconidae are the most commonly used natural enemies for the biological control of tephritids (Clausen *et al.*, 1965; Clausen, 1978). More species of Braconidae have been introduced against tephritid pests than any other family of

parasitoid or predator. The species recorded from fruit-infesting tephritids in sub-Saharan Africa belong to the subfamilies Opiinae, Helconinae, and Braconinae, though the record for the single helconine, *Triaspis daci* (Szépligeti), has never been verified. The only described species of braconine attacking tephritids in the Afrotropical Region is *Bracon celer* Szépligeti which is a larval parasitoid, attacking the host larva and permanently paralyzing it (thus differing from the other parasitoids described here, all of which emerge from the host puparium). The genus *Bracon* is one of the largest in the family Braconidae, and the Afrotropical species of the genus are 'badly in need of revision' (Wharton, 1997b). Until 2000, it was not possible to readily determine whether species of *Bracon* reared from Afrotropical tephritids are *B. celer* or some other species. In a recent survey by Wharton *et al.* (2000), *B. celer* was reared for the first time from coffee in Kenya.

At least 17 species of opiine Braconidae have been reared from fruit-infesting tephritids in the Afrotropical Region (Silvestri, 1914; Clausen *et al.*, 1965; Gilstrap & Hart, 1987), but this number is likely to change with the recent collections by Copeland *et al.* (2002). For a 50-year period following the work of Gahan (1915), these and many other opiines were placed in the genus *Opius* which grew to contain nearly 1000 species largely due to the efforts of Max Fischer. Later works (Wharton & Gilstrap, 1983; Fischer, 1987; Wharton, 1987, 1988; van Achterberg & Maetô, 1990) have led to dramatic changes in the classification of the opiines, with several genera now recognized. Most of the opiines attacking tephritid pests have now been removed from *Opius*, and several nomenclatural problems and synonymies resolved for these species (Wharton & Marsh, 1978; Wharton & Gilstrap, 1983; Wharton, 1987). However, several other systematic problems still remain.

The genus *Psyttalia*, for example, appears to contain a number of very closely related species that are very difficult to distinguish (Wharton, 1987, 1997a). There are also several undescribed opiines that have been reared from Afrotropical tephritids during the past 15-20 years, and these may be the same species that were incompletely identified during explorations for medfly parasitoids in the 1930s (Gilstrap & Hart, 1987). A historical account of the *Psyttalia* species is presented in Chapter 4; 4.2.

2.10 Horticulture production and importance

Agricultural is a dominant sector in the Kenyan economy accounting for 24% of the Gross Domestic Product (GDP), and provides employment as well as livelihood for an estimated 75% of the population (IPC, 2002). The sector is the largest contributor of foreign exchange through export earnings from tea, coffee and horticulture products.

It is estimated that by the late 1990s, Kenya supplied some 75 different horticultural products to overseas markets not only as raw products, but also as pre-packed and pre-prepared vegetables (Jaffee, 1995; Dolan & Humphrey 2000).

The horticulture sub-sector has expanded rapidly in the last two decades to overtake coffee to become the second most important foreign exchange earner in the agricultural sub-sector (IPC, 2002). Presently, the horticulture sub-sector is the third highest foreign exchange earner for the economy after tea and tourism (Jaffee, 1995). These production levels rank Kenya as the third biggest horticultural producing country in the world (FAO, 2000), and by far the largest exporter of vegetables to the European Union (Dolan & Humphrey 2000).

Though the horticultural industry in Ghana is not as big as in Kenya, there are efforts being made recently to encourage growth in the sub-sector by "creating an environment for sustainable growth and development in the general agricultural sector". The

Ministry of Food and Agriculture and its Directorates have therefore developed policy instruments and guidelines that constitute the framework for effectively addressing such major socio-economic concerns (MOFA, 2004). If caution is not exercised in pesticide use, the stringent residue levels set by the EU could spell doom for the industry when it comes into force. Table 2.1A shows figures of some export and foreign exchange earnings from horticulture compared with those from Tea and Coffee for the period 1995-2000, while Table 2.1B gives a breakdown of the contribution of the various crop items under horticulture (1996-1998). Table 2.2 also shows some export and figures from horticulture in Ghana for the period 1997-2003.

Table 2.1 Performance of selected horticultural produce in Kenya.

A. Export and foreign exchange figures for the period 1995-2000.

B. Hectarage, production level and income from1996-1998.

A.

Year	Quantity Exported (000 tonnes)			Value of Exports (Kshs Billion)		
	Horticulture*	Tea	Coffee	Horticulture*	Tea	Coffee
1995	228.6	217.9	88.5	10.6	18.0	14.4
1996	304.5	262.1	116.7	13.6	22.7	16.6
1997	192.6	199.2	70.1	13.8	24.1	17.3
1998	232.2	263.8	51.6	14.9	33.0	13.0
1999	200.6	245.7	71.5	17.6	32.8	11.9
2000	206.0	220.0	86.1	17.2	35.5	11.8

*Horticulture includes cut flowers, fruits and vegetables (fresh and processed).
Source: FAO, 2000.

B.

Year	Crop	Hectarage (ha)	Production (metric tonnes)	Value (KShs)
1996	Fruits	95,112	1,397,515	1,184,972,171
	Vegetables	81,010	935,884	465,756,025
	Herbs and Spices	1,8709	6,926	9,044,430
	Cut flowers	1,357	39,124	218,315,928
	Year Total	**179,349**	**2,379,449**	**1,877,588,554**
1997	Fruits	128,876	1,713,021	635,896,813
	Vegetables	88,318	988,400	614,039,265
	Herbs and Spices	1,085	5,963	7,343,845
	Cut flowers	1,445	39,837	372,164,250
	Year Total	**219,724**	**2,747,221**	**1,629,444,173**
1998	Fruits	134,859	2,141,236	718,373,534
	Vegetables	91,297	1,043,006	596,698,319
	Herbs and Spices	833	4,779	4,421,800
	Cut flowers	1,500	33,579	242,846,692
	Year Total	**228,489**	**3,222,600**	**1,562,340,345**

Source: IPC, 2002.

Table 2.2 Export figures and per cent contribution of horticulture to total non-traditional agricultural exports in Ghana (1997-2003).

Year	Crop	Quantity Exported (metric tonnes)	Value (USD)	% contribution to total Agric exports
1997	Fruits	70,739.3	26,046,778.2	
	Vegetables	2,338.7	785,669.0	
	Herbs and spices	9,251.0	1,552,342.8	
	Cut flowers	0.9	4,384.0	
	Year Total	**82,330.0**	**28,389,173.9**	**33.38**
1998	Fruits	70,000.6	31,528,575.6	
	Vegetables	2,180.3	896,533.8	
	Herbs and spices	7,951.4	1,720,654.9	
	Cut flowers	5.4	7,263.3	
	Year Total	**80,137.7**	**34,153,027.5**	**25.41**
1999	Figures not available			
2000	Fruits	87,514.9	30,651,903.0	
	Vegetables	3,681.1	1,259,707.3	
	Herbs and spices	2,997.0	1,333,471.2	
	Cut flowers	54.9	113,520.1	
	Year Total	**94,248.0**	**33,358,601.5**	**37.67**
2001	Fruits	3,114,007.1	26,817,168.6	
	Vegetables	6,151.3	1,399,398.2	
	Herbs and spices	4,656.6	1,713,661.7	
	Cut flowers	52.5	52,842.2	
	Year Total	**3,124,867.4**	**29,983,070.6**	**39.90**
2002	Fruits	101,205.7	32,879,468.3	
	Vegetables	7,385.8	2,053,377.9	
	Herbs and spices	16,752.7	3,050,204.7	
	Cut flowers	13.3	14,824.4	
	Year Total	**125,357.5**	**37,997,875.3**	**39.21**
2003	Fruits	130,977.8	39,488,456.7	
	Vegetables	8,144.5	1,904,568.4	
	Herbs and spices	32,866.5	7,018,879.3	
	Cut flowers	78.2	68,272.8	
	Year Total	**172,067.0**	**48,480,177.1**	**21.15**

Source: Ghana Export Promotion Council (GEPC) Head Office, Accra.

2.11 Management/control strategies against fruit flies and how they affect parasitoids

Strategies for fruit fly management include physical, cultural, behavioral, genetic, chemical, and biological control (Aluja, 1996). No single strategy can be used alone but, usually, combinations of the above are used to achieve maximum effect (Aluja, 1996; Allwood, 1997). The management strategies involve two basic approaches; the Integrated Pest Management (IPM) approach, which seeks to control fruit fly populations in order to reduce yield losses, and the eradication approach, which aims at eliminating fruit flies to create "fruit fly-free" zones. If parasitoids are to be integrated in such strategies, then the proper management of the tactics and environmental conditions to ensure survival of the parasitoids will be a necessary pre-requisite.

2.11.1 Sterile Insect Technique (SIT)

The eradication approach involves strategies like the sterile insect technique (SIT), bait and full cover spraying. The sterile insect technique is a species-specific and environmentally non-polluting method of control. The Sterile Insect Technique depends on the ability to mass rear millions of sterile flies for release at the appropriate time (when the pest population has not reached its peak) in order to overwhelm the pest population, and over very wide areas (Tan, 2000). This leads to a decrease in the reproductive potential of the resident pest population and ultimately to its eradication. For example, almost 1.5 billion flies were released in 1991 against *C. capitata* in the Kauai Coffee Plantation in Hawaii, resulting in a population suppression of 56% compared with a control of no release (Vargas *et al.*, 1994). These features make the SIT approach complex and expensive, and its efficacy may be compromised in situations where the population density of the pest flies are very high (Knipling, 1992);

and particularly when several species co-exist. As a result of these attributes, the approach is said to be justifiable only when a highly productive industry is threatened or is to be protected (White & Elson-Harris, 1992; Lux *et al.*, 2003a).

The Integrated Pest Management (IPM) approach involves the combination of most of the above methods that are compatible, so as to achieve maximum benefit or control. The major components of such an integrated approach are discussed below.

2.11.2 Bait sprays

This is based on the principle that both sexes are strongly attracted to a protein source from which ammonia emanates (White & Elson-Harris, 1992). A protein source, acting as an attractant and feeding stimulant, is mixed with a killing agent to kill attracted flies on contact, by vapour action, or on consumption of the protein. Protein or food baits are mostly attractive to sexually immature female flies in need of a protein meal to produce viable eggs (Allwood, 1997), although some mature females as well as males are also attracted.

Due to the numerous and escalating effects of chemicals on natural enemies and the environment (Gary & Mussen, 1984; Dahlsten, 1985, Perkins & Patterns, 1997), there has been considerable demand for reducing chemical usage (Pimentel, 1997; Zalom *et al.*, 1999; Badenes-Perez *et al*, 2002) and seeking bait sprays with alternative active ingredients (Michaud, 2003). Consequently, a new alternative fruit fly bait, "GF-120", has been developed by Dow AgroSciences (USA) which utilizes Spinosad (a soil-dwelling bacterium) as the active ingredient (NOSB TAP Review, 2002). These improvements are aimed at minimizing pesticide effects on the environment and how such methods can be used in the presence of introduced parasitoids. For example, the

effects of bait sprays containing malathion and two novel insecticides, Spinosad and Phloxine B (a phototoxic dye, which kills on consumption), have recently been compared on *C. capitata* in coffee fields (Peck & McQuate, 2000). A similar study with a concurrent non-target component on the effects of the three baits on populations of the egg parasitoid, *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) was also carried out on Kauai Island, Hawaii (Vargas *et al.*, 2001). In the two studies, although malathion was found to be the most effective treatment, Spinosad and Phloxine B gave significant levels of control. The study by Vargas *et al.* (2001) suggested that all the three bait spray treatments significantly reduced numbers of *C. capitata* and *F. arisanus* in the treatment plots when compared with those in control plots. However, they could not ascertain whether the reductions in the parasitoid populations were as a result of decreases in the host populations (*C. capitata*) or as a direct result of the bait sprays on adult parasitoids. Field cage tests also indicated that leaves picked two weeks after spraying with the three baits (Malathion, Spinosad and Phloxine B) caused approximately 95, 25, and 25% mortality, respectively, when exposed to flies for six hours (Peck & McQuate, 2000). Due to the very low contact toxicity of Spinosad for both vertebrates and invertebrates, coupled with the fact that the active ingredient must be consumed in order to cause toxicity, it is considered as acceptable for use in "organic" agriculture (OMRI, 2002). In their opinion, Lux *et al.* (2003a) assert that baiting stations is the most promising and appropriate insecticide method for fruit fly management in small-scale, fragmented horticulture set ups (as found in Africa), especially when integrated with other tools such as pathogens, orchard sanitation and the conservation of natural enemies.

2.11.3 Male Annihilation Technique (MAT)

This strategy employs the use of high density bait stations consisting of male lures combined with insecticides to attract and kill male fruit flies in order to drive their populations to such low levels as to cause imbalances in the frequency of mating that occurs. The commonest male lures, sometimes called parapheromones, include Cue Lure, Methyl Eugenol, Trimedlure, Terpinyl Acetate, and Vert Lure. This tactic is useful if it is used with an "area-wide" suppression strategy. For eradication purposes, Bateman (1982) noted that male lures must be set at very high densities over entire ranges of the target population.

2.11.4 Other insecticides used

Chemical control methods target various life stages of fruit flies. Systemic organophosphates such as Fipronil, Fenthion, and Dimethoate are highly effective against most species. Dimethoate is reported to kill eggs, larvae and adults (Boller & Prokopy, 1976) but its environmental effects have been questioned. Diazinon, which is currently the most widely used soil insecticide against fruit fly larvae and puparia, has been associated with various ecological problems and development of resistance (Croft, 1990). Diazinon has also been found in watersheds at levels that exceed acceptable aquatic toxicity limits (Domagalski, 1999; Kratzer, 1999).

2.11.5 Physical control

This involves creating a physical barrier between the egg laying female flies and the host fruits (Allwood, 1997). The commonest method that has been used involves wrapping developing fruits with a protective covering or bags before they reach the susceptible stage of maturity where infestation can take place. This technique is considered too laborious and time consuming, and has been viewed as applicable where

relatively small areas of production are involved, such as villages or places where the cost of labour is cheap.

2.11.6 Cultural control

This reduces populations of fruit flies through the manipulation of agricultural or farming practices. It includes practices such as altered planting time whereby cultivation of crops is done during periods of relatively low fruit fly activity, use of host-plant resistant varieties, crop rotation to break any links between fly populations and crops, and good orchard sanitation. Collection and destruction of fallen, damaged and over-ripe fruits also reduces fruit fly populations. However, cultural control methods are considered to be limited in effectiveness on their own (Smith, 1999) but are most useful as complementary control methods.

2.11.7 Post-harvest treatment

This tactic aims at eliminating any hidden infestations in the final produce with fumigation as the most commonly used treatment. It usually involves the use of methyl bromide and ethylene dibromide, and is used as a regulatory control to kill fruit flies and allow movement of produce from within quarantine areas to locations outside the quarantine boundaries. Other techniques involve heat treatment (either with hot vapour or hot water), cold treatment, insecticidal dipping, and irradiation. These techniques target the non-adult life stages that might be inside the produce by either killing them directly (irradiation) or creating unfavourable/hostile conditions for their survival. A review and discussion of various post-harvest treatments of fruits are fully covered by Armstrong & Couey (1989) and Paull & Armstrong (1994).

2.11.8 Genetic manipulation/modification

To date it is said that only artificial selection of natural enemies has successfully been employed. The potential roles of heterosis or hybrid vigour (the condition of higher production of female proportions in inter-strain/population crosses relative to female proportions in intra-strain/population crosses) and the use of recombinant deoxyribose nucleic acid (rDNA) techniques remain to be documented (Beckendorf & Hoy, 1985; Hoy, 1985, 1986).

While the use of resistant natural enemies would have been useful under the harsh African conditions, genetically modified organisms (GMOs) have been the subject of scientific debate in recent times as to their effects on human, animal, as well as on environmental health. Prominent in the heat of the debate is the introduction of genetically modified crops into current agricultural systems. While proponents of the technology assert that laboratory tests have been conducted and there is no known evidence of side effects, the opponents have argued that the lack of evidence does not necessarily mean the absence of evidence, and it can only be proven after many years of careful and systematic field trials.

2.11.9 Conservation of natural enemies

One factor which may have contributed to the low level of success in some classical biological control programmes could be the sole focus on the candidate biological control agents and too little emphasis on the environment in which releases are made (Gurr *et al.,* 1998). According to Ehler (1998), conservation of natural enemies is probably the oldest form of biological control of insect pests. It attempts to use the indigenous natural enemies by manipulating the environment in such a way that their activity and effectiveness is enhanced (Powell, 1986; Goettel & Hajek, 2001).

Some species in a community are particularly important (Letourneau, 1998) because they interact with, and affect many other species. They process materials disproportionately to their numbers or because their functions cannot be substituted or compensated for by other species in the community. These species are referred to as "keystone species" in their communities (LaSalle, 1993). They play a major role in the balance of terrestrial ecosystems through their ability to regulate populations of phytophagous insects (LaSalle & Gauld, 1992). According to LaSalle & Gauld (1993), by limiting the population size of a species that would otherwise out-compete other species, parasitoids do not only help maintain high species diversity in herbivores, but also help prevent herbivores from completely destroying their plant hosts. Most parasitoids possess characteristics which make them very sensitive to changes in the community, and community interactions involving parasitoids appear to be more stable than those involving predators (Pimm & Lawton, 1978; LaSalle, 1993).

2.11.9.1 Factors affecting conservation of natural enemies

Potentially, habitat manipulation can enable natural enemiès operating at very low levels to flourish and exert very considerable impact on pests (Gurr *et al.*, 1998). The most significant component of the habitat of most arthropod species and their parasitoids is the plants on which they live, eat, and depend. Landis and Menalled (1998) noted that many of the factors that are known to limit parasitoid effectiveness in cropping systems can be viewed within the context of disturbance. Van Driesche and Bellows (1996) therefore group the environmental practices which may have potential effects on natural enemies into five main categories: 1) the use of agricultural pesticides, 2) cropping patterns or techniques, 3) manipulation of non-crop vegetation within or adjacent to crop fields, 4) direct provision of food or shelter to natural

enemies or control of their antagonists, and 5) the management of soil, water, and crop residues. These factors are discussed in the following sections.

a) Use of agricultural pesticides

High agrochemical input into crop production causes many unintended effects such as the killing or behavioural changes of natural enemies, resistance in pests, water pollution, and many others (Pimentel & Lehman, 1993; Van Driesche & Bellows, 1996; Pimentel, 1997). This implies that, apart from the immediate effects, there is a continued and sustained persistence of the chemicals in the environment on the structure of communities (DeBach & Rosen, 1991). Managing pesticide impact is therefore one of the most important conservation measures to preserve viable and effective parasitoid communities. For example, in Kenya it is been reported that the intensive and "irresponsible use" of insecticides such as parathion for the control of *Leucoptera* Hübner species (Lepidoptera: Lyonetiidae) (a leaf mining moth in coffee) resulted in the giant looper, *Ascotis selenaria reciprocaria* (Walker) and the green looper, *Epignyopteryx coffeae* (Lepidoptera: Geometridae) becoming major pests (due to the elimination of their natural enemies by the insecticides). A fresh attempt therefore, had to be made to control these as secondary pests (Abasa, 1983). From that experience, the release of the parasitic wasp *Anagyrus* Howard (Hymenoptera: Encyrtidae) against the common coffee mealybug, *Planococcus kenyae* (LePelley) (Hemiptera: Pseudococcidae) had to be complemented with reduced spraying of insecticides in coffee plantations in order to achieve successful control (Abasa, 1983; Masaba *et al.*, 1986).

b) Cropping patterns or techniques

The clearance of diversified vegetation for the large scale cultivation of monoculture crops, referred to as "ecological monotony" (Nentwig *et al.*, 1998), is believed to reduce the diversity of natural enemies in the crop fields. Altieri (1991) argues that pest problems arise largely as a result of the expansion of these crop monocultures at the expense of biodiversity and natural vegetation. Any group of insects that gets used to this field or uses it as alternate crops, suddenly find large hectarages of unlimited source of food (with no need of searching). Consequently, their numbers grow and soon attain pest status. For example, natural enemies have been found to be more numerous in intercrop systems than in monocultures (Tonhasca, 1993).

c) Direct provision of food and shelter to natural enemies

The hunger state of searching parasitoids may determine whether they spend time searching for hosts or for food (Lewis & Takasu, 1990). Fecundity, longevity, survival, levels of parasitism (Idris & Grafius, 1995, 1997), foraging behaviour (Wäckers & Swaans, 1993; Wäckers, 1994) are all known to be affected by the availability of food. The provision of food in the environment in terms of diversity (abundance and types of plants) will ensure both the production of nectar and pollen, as well as the requisite shelter from harsh weather conditions to sustain parasitoid populations throughout the year.

d) Manipulation of non-crop vegetation within or adjacent to crop fields

Forest clearance is known to have a profound negative impact on insect diversity (Watt *et al.*, 1997), and the principal processes shaping living (biotic) communities and non-living (abiotic) conditions within forest fragments are, area effects, edge effects, shape

of fragment, degree of spatio-temporal isolation and the degree of habitat connectivity in the landscape (Didham, 1997).

e) Forest fragmentation, removal and bushfires

Forest fragmentation commonly occurs by heavy logging especially in the tropics. This has been estimated to be about 1% of annual tropical deforestations (WCMC, 1992) with the highest regional rate in West Africa (WRI, 1992). For a country with total land area of 238,540 km^2 and 37 % forest and woodland cover, Ghana has 477 registered firms in the Timber industry alone, out of which 250 are logging firms, 100 saw mills, and the remaining 127 dealing in furniture, furniture parts, mouldings, and flooring (Anon, 2003b). These figures can only mirror the extent of economic pressure on the environment. In the 1980s when the rate of deforestation was estimated to be about 12,000 km^2 annually (FAO, 1991), only 360 km^2 of plantation forests were established each year (Lawson, 1994), and with fast-growing species. Unfortunately, most of the fast-growing trees are exotic species such as Teak, Eucalyptus and Pine (Lawson, 1994; Watt *et al.*, 1997) and they do not necessarily support the same original indigenous diversity of arthropods in the forests. When forests are fragmented, they are affected by climatic conditions and thus, on the natural enemies that live in them. For example, hotter, drier and windier conditions (with higher light intensity) have been reported to occur at the edges of fragmented forests (Didham, 1997). Furthermore, natural enemies living at relatively low population densities are known to be more susceptible to habitat fragmentation than their preys (Kruess & Tscharntke, 1994).

Control burns are also used to manage natural habitats prior to the onset of rains in most small-holder African farms. This practice normally takes place during the drier period

and more often than not, they result in wild bush fires. These conditions do not augur well for parasitoid establishments, if they are to survive and effect control.

f) Management of soil, water, and crop residues

The management of soil, water sources (construction of water catchment areas and irrigation) and crop residues are known to keep pest levels low and maintain habitats where natural enemies can get food and take refuge. There is also the overall effect of climate and weather that limits the success of biological control agents, but these changes are difficult to predict.

g) Economic and political factors

The economic gains derived from the conservation of natural resources and the environment by national Governments are undoubtedly rewarding. There are, however, a "conflicts of interests" between the badly needed revenue from trading on these resources and the unpleasant repercussion of forest cover depletion. In 1994, 983,000 cubic meters of wood worth USD 222 million was exported from Ghana to various EU markets (Anon, 2003a). The complete stoppage of timber exports will therefore, mean looking elsewhere to secure the much needed foreign exchange for the economy. There is also the lack of adequate legislation to protect forests and natural resources.

In summary, before selecting the most appropriate strategy for pest control, the agro-ecosystem and the diverse ecological factors (food, shelter, hosts resources, temperature, light and relative humidity) must be understood (Pimentel, 1977). This underscores the importance of forests and insects (and their interaction) in the overall field of conservation (Forey *et al.,* 1994; Samways, 1994). The manipulation of plants in agro-ecosystems to favour parasitoids is essential if conservation biological control is

to be successful. It is therefore not surprising that changes in agricultural practices such as organic farming, crop rotation, small scale fields, maintenance of natural areas between agro-ecosystems (acting as refuge and/or dispersal centers) are on the increase (Nentwig *et al.*, 1998). LaSalle (1993) therefore concludes that the effect of the loss or decline of a large number of parasitic Hymenoptera cannot be predicted, but "the changing agricultural and forestry practices mean that we do not know which species might become the next pests. If it so happens that the parasitoids or natural enemies of these species have already been removed, then not only will it facilitate their achieving pest status, but we will also be denying future generations the option of biological control".

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS

3.1 Host Flies

Colonies of selected fruit fly species were initiated and maintained at the International Centre of Insect Physiology and Ecology (ICIPE) by the African Fruit Fly Initiative (AFFI) Project in Nairobi, Kenya. The selected colonies included the Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann), *Ceratitis fasciventris* Bezzi, the marula or mango fruit fly, *Ceratitis cosyra* (Walker), the Natal fly, *Ceratitis rosa* Karsch, *Ceratitis anonae* Graham, the melon fly, *Bactrocera cucurbitae* (Coquillett) and *Dacus ciliatus* Loew (Diptera: Tephritidae).

3.1.1. Experimental host flies

The experimental host species were *C. capitata, C. cosyra,* and *Dacus ciliatus* Loew (Plate 3.1). These were selected based on their status either as the most suitable laboratory hosts (in the case of *C. capitata*), or as the preferred natural hosts (*C. cosyra* and *D. ciliatus*) of the experimental parasitoids.

The initial cohort of *C. capitata* (~200 flies) was obtained from arabica coffee, *Coffea arabica* L. (Rubiaceae), from farms in the central highlands of Kenya at Ruiru (01° 5' 72S, 36° 54' 22E and elevation of 1609 m) and Rurima (00° 38' 39S, 37° 29' 69E and elevation of 1228 m). The initial cohort of *C. cosyra* (~100 flies) was obtained from mango, *Mangifera indica* L (Anacardiaceae) from Rurima, with additional materials from mango and marula, *Sclerocarya birrea* (A. Rich) (Anacardiaceae) from Nguruman, Kenya (01° 48' 39S, 036° 03' 28E and elevation of 817 m). The *D. ciliatus* founder colony (31 flies) was obtained from zucchini squash, *Cucurbita pepo* L. (Cucurbitaceae) from two cultivated gardens at Kasarani (ICIPE), Kenya (Garden 1:

01° 13' 14S, 036° 53' 44E and elevation of 1626 m; Garden 2: 01° 13' 29S, 036° 53' 51E and elevation of 1619 m). The cultures of *C. capitata* and *C. cosyra* were started in mid 1997, while that of *D. ciliatus* was initiated in early 2001.

The flies were kept in perspex cages (60 x 35 x 70 cm) with a round opening (14 cm diameter) in the front, to which a sleeve of fine net was fixed. One side of the cage (opposite to the sleeve side), was replaced with fine net material for ventilation. The flies were kept at 27-28° C and a photoperiod of 12L:12D and provided with water and a diet of hydrolysate yeast powder and sugar in a ratio of 1:4. Oviposition medium of artificial diet (a modification of Hooper's (1987) method), made into balls of 2-3 cm diameter by covering with parafilm, were provided for *C. capitata*. The diet consisted of carrot powder (24.2 g), brewer's yeast (8.1 g), citric acid (0.6 g), methyl 4-hydroxybenzoate (0.2 g), and water (50.7 ml). These were made into a smooth consistent paste. *Ceratitis cosyra* and *D. ciliatus* were provided with fresh fruits of mango (variety apple mango) and squash, respectively for oviposition.

A.

B.

C.

Plate 3.1 Experimental flies used in the study. **A.** = *Ceratitis capitata* (Wiedemann), **B.** = *C. cosyra* (Walker), and **C.** = *Dacus ciliatus* Loew. (Photos: M. Billah).

3.2 Parasitoid colonies

3.2.1 *Psyttalia concolor* (Szépligeti)

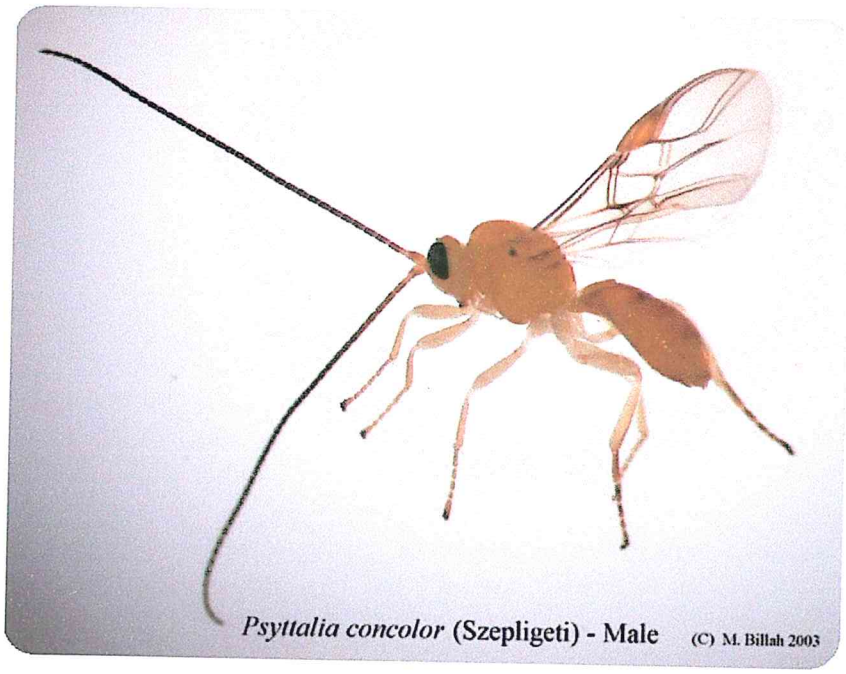
Psyttalia concolor was originally described from Tunisia by Silvestri (Silvestri, 1913), and has been mass cultured since the 1930s (Wharton, 1989a) (Plate 3.2). Fruit fly puparia (parasitized by *Psyttalia concolor*) were imported in 1999 (Permit No. BIP/1/99) from a culture maintained in Pisa, Italy (Raspi & Loni, 1994) to initiate a colony at ICIPE in Nairobi, Kenya. A second consignment was imported from a culture maintained in Hawaii (R. Messing, Kauai Agriculture Research Center, Hawaii) in 2001 (Permit No. 5. 5/2001) to revamp the colony. Mass cultures of *P. concolor* are maintained in Pisa, Italy, and Hawaii for basic research and for augmentative releases against the olive fly, *Bactrocera oleae* (Gmelin) and *C. capitata*, respectively.

All experiments involving *P. concolor* were undertaken in the quarantine facility at ICIPE, where the colony was maintained using *C. capitata* larvae as the preferred laboratory host. The parasitoids were reared in perspex cages (40 x 30 x 15 cm), with two openings (10 cm diameter) on the front side of the cage to which sleeves of fine net were fixed (Plate 3.3). The same material was fixed to the opposite side of the cage for ventilation. Four round openings (10 cm. diameter) were also made on the topside of the cage, to which pieces of organza material were fixed. Parasitoids were reared on *C. capitata* larvae, which were placed in a thin layer of their larval diet in a modified Petri dish. The Petri dish, originally measuring 0.5 cm-deep and 5.7 cm diameter, was reduced to a depth of 1 or 2 mm. This modification was to enable the parasitoids to reach all the larvae in the diet. The Petri dish (oviposition unit) was then inverted and placed on the organza-covered opening on the top of the cage for 4-6 hours. A weight of about 15-20 g was placed on the oviposition unit to prevent the larvae from escaping.

Psyttalia concolor oviposits in third instar larvae and emerge from the host puparia, so all host larvae in oviposition units were third instars.

After oviposition, the host larvae were transferred to bigger petri dishes (8.6 cm diameter) and provided with fresh larval diet. The Petri dishes were then placed in a plastic container (10.3 cm diameter x 6 cm depth) with a layer of sand on the bottom to serve as a pupation medium. The sand was kept moist to prevent pupal desiccation. An opening of about 8 cm diameter was cut in the lid of the container and replaced with fine net. The sand was periodically sieved to recover puparia, which were held in fresh petri dishes (8.6 cm diameter) till flies and/or parasitoids emerged The emerging parasitoids were added to the parasitoid colony while the flies were returned to the *C. capitata* rearing cages. Parasitoids were maintained at a photoperiod of 12L: 12D and provided with water in wet cotton wool and fine drops of pure honey streaked on the topside of the rearing cages.

A.



B.

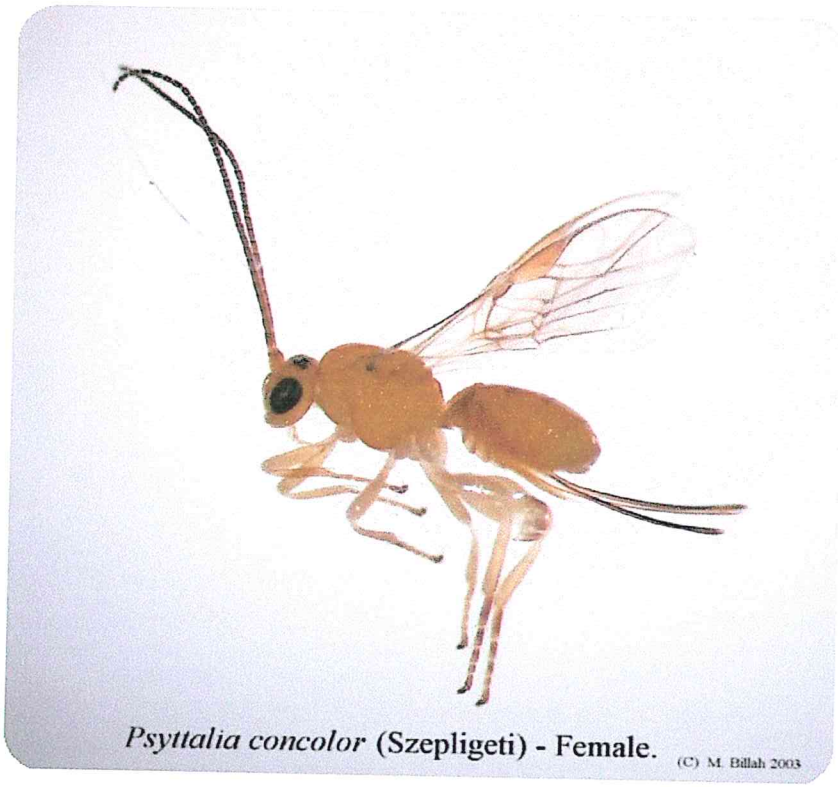
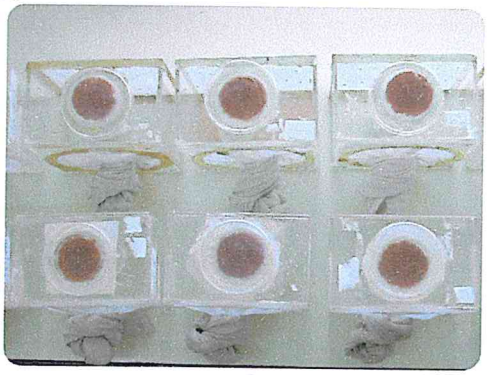


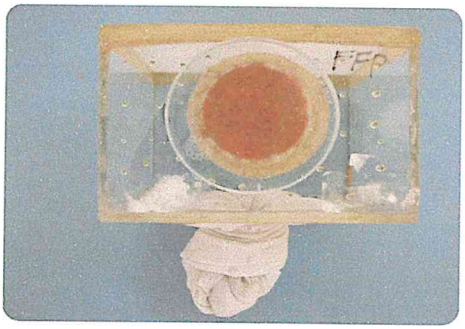
Plate 3.2 *Psyttalia concolor* (Szépligeti): A. = Male, B. = Female. (Photos: M. Billah).

A.

B.



C.



D.

E.

Plate 3.3 Various cages used in the study.
A.= Main parasitoid rearing cage (40 x 30 x 15 cm) (showing 2 front openings with sleeves and 4 top openings fixed with organza material from where fruit fly larvae are exposed to parasitoids). B.= Medium-sized cage (12 x 12 x 12 cm) with a parasitoid colony. C.= Small-sized cages (11.5 x 7.5 x 11.5 cm) showing the placement of oviposition units (inverted). D.= A closer view of a small cage showing fine drops of pure honey streaked on the topside (from inside) for parasitoid feeding. E.= A line-up of oviposition units (modified petri dishes) with thin layers of fresh larval diet into which fly larvae are placed and exposed to parasitoids as seen in C and D. (Photos: M. Billah).

3.2.2. *Psyttalia cosyrae* (Wilkinson)

The initial cohort was obtained from tephritid puparia collected from marula at Muhaka in the southern coast (Coast Province) of Kenya (04° 18' 27S, 039° 29' 98E and elevation of 74 m). Fruit flies obtained from that sample were exclusively *C. cosyra*. The rearing procedure was the same as described for the rearing of *P. concolor* above, but with *C. cosyra* as the host larvae and a modification in the exposure method. Instead of placing oviposition units on top, they were covered with tightly fitting organza lids and placed inside the rearing cages for 4-6 hours, as *P. cosyrae* colonies show higher oviposition levels when units are placed inside cages than on top. This procedure is similar to that described by Wong and Ramadan (1992) for other opiine braconid parasitoids of tephritids. Upon emergence, the parasitoids were added to the parasitoid colony while the flies were returned to *C. cosyra* rearing cages. The same food and lighting regime applications were provided for the parasitoids as in the case of *P. concolor*.

3.2.3. Field populations of *Psyttalia*

Field collections of *Psyttalia* species were made from both cultivated and wild fruits from Kenya and three other countries (Cameroon, Ghana and South Africa). With the exception of samples of *Psyttalia concolor* which were imported live from a laboratory culture in Pisa, Italy, parasitoids from all other countries were collected from the field and preserved in alcohol. Some field materials from Kenya that yielded both male and female parasitoids were exposed to fruit fly larvae and colonies initiated for other experiments.

3.2.3.1. Parasitoid collections from Kenya

a) Rurima

Coffee berries were collected from plantations from Rurima Farm, near Embu (Eastern Province). The fruit flies reared from this sample were mostly *Ceratitis capitata*, some *C. fasciventris* and a very small number of *Trirhithrum coffeae* Bezzi (Diptera: Tephritidae).

b) Ruiru

Coffee collections from Ruiru were from the Coffee Research Foundation (CRF) (Central Province). The fruit flies reared from this locality were, like those from Rurima, mostly *C. capitata* with some *C. fasciventris* and a small number of *T. coffeae.* Rurima and Ruiru localities are both in the central highlands of Kenya to the east of the Great Rift-Valley, and they are among the principal coffee-growing areas in Kenya (Masaba *et al.*, 1986).

c) Shimba Hills

Coffee berries collected from this locality were from an experimental robusta coffee plantation at the foot of the Shimba Hills (Coast Province) (04° 15' 64S, 039° 23' 25E and elevation of 59 m). This locality is part of a chain of coastal hills (Shimba Hills) that lies in the broadly coastal lowlands, with a few forested areas. The flies from this locality were all *Trirhithrum* species, mostly *T. nigerrimum* (Bezzi) and *T. coffeae* (Bezzi). The parasitoids from these flies were slightly smaller in size than those from the central highlands, and had dark markings on the abdomen.

d) Mrima Hill

Parasitoids from this locality were collected from wild fruits of *Lettowianthus stellatus* Diels (Annonaceae) as part of the ICIPE/Texas A&M University field collections by R. S. Copeland. The fruits, collected in the from Mrima Hills in the coastal lowlands of Kenya (Coast Province) (04° 29' 32 S, 39° 15' 27 E and elevation of 146 m), were infested with *C. rosa* flies. Parasitoids from this locality were unidentified and looked paler in colour than those from the other localities.

e) Sabaki

The parasitoids from this locality were collected from wild cucurbits, *Corallocarpus elliptical* Chiov. (Cucurbitaceae) and were supplied by R. S. Copeland (ICIPE/Texas A&M Project). Sabaki also lies in the coastal lowlands of Kenya (Coast Province) (03° 9' 481S, 40° 8' 82E and elevation of 15 m). The fruits were infested with *C. capitata*, but are also known to be attacked by *Dacus ciliatus* (Copeland *et al.*, 2002).

f) Burguret Forest

Burguret Forest is located in the central highlands (Central Province) of Kenya (00° 06' 720 S, 37° 02' 342E and elevation of 1961 m). Parasitoids from this locality were *Psyttalia lounsburyi* (Silvestri) from wild olives, *Olea europaea* subspecies *africana* (Miller) P. Green (Oleaceae). All larvae reared from the fruits were *Bactrocera oleae* (Gmelin), though Copeland *et al.* (2002) recorded a few medflies on a single occasion. The parasitoids from these fruits had a conspicuously dark body colour (both thorax and abdomen). This population was also part of the ICIPE/Texas A&M collections.

g) Kasarani -

The parasitoids, *Psyttalia phaeostigma* (Wilkinson), were obtained from the second of the two cultivated gardens of zucchini squash, *Cucurbita pepo* L. (Cucurbitaceae) at ICIPE, Kasarani (01° 13' 29S, 036° 53' 51E and elevation of 1619 m). The cucurbits were mostly infested by the fruit flies *D. ciliatus* and *D. bivittatus* (Bigot), and a small number of *B. cucurbitae* (Coquillett) (Diptera: Tephritidae). In October-March *D. bivittatus* was more abundant, followed by *D. ciliatus* and a few *B. cucurbitae*.

The *D. ciliatus* flies were fed on fresh squash fruits for oviposition in the cages. After four days, the fruits were transferred into ventilated plastic containers in which the fly eggs hatched into larvae. While the larvae were developing in the rotting fruits, slices of fresh fruits were added for them to feed on. Third instar mature larvae were exposed to the parasitoids in the same manner as described for *P. concolor* and *P. cosyrae* above.

3.2.3.2. Collections from Cameroon

a) Akonolinga

Samples from Akonolinga (elevation of 670m) came from a commercial plantation in South-central Cameroon where only robusta coffee is grown.

b) Nkolbisson

Collections from Nkolbisson (elevation of 700 m) came from an experimental plantation at the Institut de Recherché Agricole (IRA) consisted of several types of coffee hybrids. Nkolbisson is also located in South-central Cameroon.

The climate of Akonolinga and Nkolbisson is equatorial-guinean, with secondary semi-deciduous forest vegetation with an annual rainfall of 1.5-2.0 m per year (Steck *et al.*, 1986). Parasitoids from these two localities were tentatively identified by Steck *et al.* (1986) as *Psyttalia perproximus* (Silvestri) (R. A. Wharton, personal communication),

and were part of IRA 1982 collections from Togo and Cameroon. *Coffea canephora* (robusta coffee) is the main coffee species grown in West Africa (Oerke *et al.*, 1994) and the most commonly reared tephritid flies from the berries were *Trirhithrum* species and a few *Ceratitis anonae* (Graham) (Steck *et al.*, 1986; Billah, unpublished data).

Voucher specimens of this population are deposited in the collections of the Department of Entomology, Texas A&M University, and in the US National Museum, Washington, D.C. (Steck *et al.*, 1986).

3.2.3.3. Collections from Ghana

a) Bowire

Bowire (07° 24' 0N, 00° 28' 0E and elevation of 220 m) is a small farming town in the sparsely forested lowlands of the Volta Region. The coffee from this locality was all of the robusta variety, which was mostly grown on small scale private holdings.

b) Tafo (Cocoa Research Institute of Ghana, CRIG)

The Cocoa Research Institute of Ghana (CRIG), Tafo is located at (06° 13' 20N, 00° 21' 29W; 220 m) in the Eastern Region of Ghana, and is the research organization mandated to research on cocoa (*Theobroma cacao* L.), and more recently, coffee, cola and shea. CRIG is a subsidiary of the Ghana Cocoa Board. The main variety of coffee on CRIG experimental plots was robusta, with a few plots of arabica coffee and several other hybrids. Samples of coffee berries were taken mainly from the control experimental plots, where there was minimal or no insecticide application. Tephritid flies reared from these berries were *Trirhithrum coffeae* and *T. nigerrimum*. Of the other crops sampled (cocoa, citrus and cola), only cola yielded some tephritid flies.

Bowire and Tafo both lie in the southern half of Ghana in a tropi rain forest area which is broken by heavily forested hills, many streams, rivers, an vast stretches of sparsely forested lands.

3.2.3.4 Collections from South Africa

a) Nelspruit

Samples from this locality came from Burgershall farm, near Hazyvie (25° 1' 60S, 31° 7' 0E and elevation of 524 m) in the Mpumalanga Province. The samp s were obtained through the Institute for Tropical and Subtropical Crops (ITSC). Onl rabica coffee is grown in the farm, and tephritid flies that emerged from the berri were *Ceratitis capitata* and *C. rosa*.

b) Pietermaritzburg

Sampling at Pietermaritzburg was done from the Ukulinga Research F rm (29° 37' 0S, 30° 22' 60E and elevation of 721 m) of the University of Natal (now e University of KwaZulu Natal) in the KwaZulu Natal Province. All samples were ke t in the rearing room of the University of Natal (Pietermaritzburg campus) which w s fitted with a thermo-regulator to control the room temperature. Tephritid flies that em erged from this sample were *Ceratitis capitata* and *C. rosa*.

CHAPTER FOUR

4.0 MORPHOLOGICAL STUDIES

4.1 Introduction

The largest diversity of parasitoids that attack fruit-infesting flies is found in the opiine Braconidae family (Clausen, 1978), and they have a long historical record of use as biological control agents against tephritid pests (Silvestri, 1914; Fullaway, 1915; Bianchi & Krauss, 1937; Clausen *et al.,* 1965; Wharton 1989a). Species of the genus *Psyttalia* are among the commonest parasitoids reared from fruit-infesting flies in Africa. Appendix I shows a list of all species names known to have been used in association with *Psyttalia* parasitoids, while Table 4.1 shows species names and their origin in Africa. Due to the synonymization of many species names, loss of original descriptions, lack of biological and/or unknown host information (Wharton *et al.,* 1997a), coupled with limited morphological description or inadequate characterization of specimens, only eleven (11) species names are currently recognized from mainland Africa (Table 4.2). There are, however, a few undescribed species that have been reared from various sampled fruits from Kenya.

4.2 Characters states in the genus *Psyttalia* Walker

The genus *Psyttalia* belongs to the family Braconidae, subfamily Opiinae, tribe Opiini and subtribe Opiina. These opiines are defined as endoparasitoids of cyclorrhaphous Diptera. They lay their eggs in the host eggs or larvae and emerge from the host puparium (Pemberton & Willard, 1918; van den Bosch & Haramoto, 1951).

Features used in identifying this group include the basal displacement of the radial cross-vein r in the fore wing, loss of the epicnemial carina and part of the occipital

carina, and the tergal gland morphology. According to Wharton (1997b), these features are homoplasmic (i.e. either they are not restricted to the Opiinae, or not true for all members of the subfamily) and thus, the Opiinae is one of the most difficult subfamilies to define as a monophyletic group on the basis of either external or internal morphology. Other features used include:

Head - The mandibles, clypeus, size of compound eyes and antenna (number and the relative lengths of first two flagellomeres).

Mesosoma (thorax + first abdominal segment (= propodeum)) - Pronotum sculpture, size and shape of median pit, mesonotum sculpture (notauli), sternaulus, frons, propleuron, pronotum, mesoscutum and hind tibia.

Metasoma (abdomen excluding first segment) – Hypopygium, ovipositor, ovipositor sheath (after careful dissection), and the metasomal glands (Hagan's and the poison glands).

Variations in surface features, though important, are very variable and are mostly used as supplemental features. However, there have been cases where they are essential for distinguishing between species of other parasitoids. For example, female specimens of the stem borer parasitoids *Cotesia flavipes* Cameron and *C. sesamiae* (Cameron) (Hymenoptera: Braconidae) are separated by use of the roughness of the propodeal sculpture (Kimani & Overholt, 1995). The arrangement of veins in the fore and hind wings is also used as one of the most important character systems in identification of the Braconidae because they are properly separated out (Wharton, 1997b). Wings are, therefore, illustrated for as many taxa as possible, even when features of the venation are not used in the keys, since the venation pattern is often diagnostic for genera or groups of genera (Wharton, 1997a).

In the genus *Psyttalia*, many of the species have the veins of the fore wing variously thickened at the junction of m-cu and RS+M, and the curvature, position, and thickening of the veins in these regions are among the most useful characters for separation of the species. These include the position of the fore wing m-cu vein relative to vein 2RS, thickening or partial thickening of vein (RS+M)b, and the extent of separation of vein 1-1A from the wing margin (Figure 4.1).

Most of these characters and character states are more applicable at the genus level and useful in defining monophyletic groups (Wharton, 1988; 1997a, b).

4.2.1 Distinguishing characters of the genus *Psyttalia*

Members of the genus *Psyttalia* are distinguished from other species by a combination of features which include a relatively shorter second metasomal tergum than the third, presence of a short clypeus which is widely separated from the mandibles, and a large hypopygium that is strongly attenuate or tapers, with the apex drawn out to a sharp point. Other features include the absence of vein m-cu in the hind wing, loss of the basal half of vein RS, presence of a large second submarginal cell, and the absence of a midpit on the mesoscutum. Species of the *P. concolor* group are characterized by shagreened sculpture of the basal abdominal terga, though it is conceded that this feature is difficult to see in some preserved specimens (Wharton & Gilstrap, 1983). Nevertheless, this character serves to separate the *P. concolor* group from other groups. Extensive intra-specific variation has limited the use of characters other than the ovipositor length for distinguishing between species of the *P. concolor* group.

Table 4.1 List of *Psyttalia* species originally identified from Africa (Mainland and Islands).

Species	Year	Original Description
advenator (Fischer)	1963	Mujenje, Uganda.
africanus (Szépligeti)	1910	Wellington, Stellenbosch, S. Africa
agreutretae (Wilkinson)	1927	East London, S. Africa.
alleni (Fischer)	1964	Mweru, Brit. E. Africa (Kenya)
bisulcata (Szépligeti)	1914	Bismarck-burg, Togo
colombina (Fischer)	1972	Pietermaritzburg, S. Africa
concolor (Szépligeti)	1910	Souse, Tunisia
cosyrae (Wilkinson)	1927	Morogoro, Tanzania
dacicida (Silvestri)	1911	Eritrea, Ethiopia.
dexter (Silvestri)	1913	Dakar, Senegal
distinguenda (Granger)	1949	Rogez, Madagascar
efoveolatus (Szépligeti)	1913	Arusha-Ju, Tanzania
fuscitarsis Szépligeti	1913	Voi, Tanzania
gigantura (Fischer)	1972	Central uplands, Kenya
hemicauda (Fischer)	1972	Rutshuru Territory, Congo
humilis (Silvestri)	1913	Constantia, (Cape Colony) S. Africa
hypopygialis (Szépligeti)	1913	Ste. Marie, Madagascar
inconsueta (Silvestri)	1913	Olokemeji, Nigeria
infuscate (Granger)	1949	Ankaratra, Madagascar
insignipennis (Granger)	1949	Diego-Suares, Madagascar
inquirendus (Silvestri)	1914	Victoria, Cameroon
kirstenboschensis (Fischer)	1972	Kirstenbosch near Cape Town, S. Africa
kolomani Fischer	1996	Santiago, Cabo Verde
lindbergiana (Fischer)	1971	Tarrafal; S. Antao; Cape Verde Islands
lounsburyi (Silvestri)	1913	Transvaal, S. Africa
ngomeensis (Fischer)	1972	South Ngome forest, S. Africa
niloticus (Fischer)	1972	Egypt
palpalis (Szépligeti)	1902	Aschanti, Nigeria
papagena (Fischer)	1972	Mahembe, Rwanda
paralleni (Fischer)	1972	Rutshuru Territory, Congo
perproximus (Silvestri)	1913	Segboroué, Benin.
perproximus var. *modestior* (Silvestri)	1913	Aburi, Ghana
phaeostigma (Wilkinson)	1927	Natal, Durban, S. Africa
phorelliae (Wilkinson)	1929	Petronella, Transvaal, S. Africa
prothoracalis (Fischer)	1972	Gillits near Durban, S. Africa
puncticranium (Fischer)	1972	Port St. Johns, S. Africa
pusilla (Szépligeti)	1913	Arusha-Ju, Tanzania
sanctamariana (Fischer)	1978	Ste.-Marie, Réunion.
scleroticus (Fischer)	1972	N. Lake Kivu, Rwankwi, Congo
somereni (Fischer)	1972	C. Karura, Kenya
subsulcata (Granger)	1949	Bekily, Madagascar
tshuapana (Fischer)	1972	Tshuapa, Bokuma, Congo
urogramma (Fischer)	1972	Rutshuru Territory, Congo
vittator (Brues)	1926	Umbilo, Durban, Natal, S. Africa.
yangambiana (Fischer)	1972	Yangambi, Congo.

Compiled from: Fischer (1972), Wharton & Gilstrap (1983), Wharton (1987, 1997a), Koponen (1989) and Fischer & Koponen (1999a, b).

Table 4.2 List of fruit-infesting *Psyttalia* species currently recognized from mainland Africa.

Species	Year	Original Description
P. concolor (Szépligeti)	1910	Souse, Tunisia
P. cosyrae (Wilkinson)	1927	Morogoro, Tanzania
P. dacicida (Silvestri)	1911	Eritrea, Ethiopia
P. dexter (Silvestri)	1913	Dakar, Senegal
P. fuscitarsis Szépligeti	1913	Voi, Tanzania
P. humilis (Silvestri)	1913	Constantia, S. Africa
P. inconsueta (Silvestri)	1913	Olokemeji, Nigeria
P. inquirendus (Silvestri)	1914	Victoria, Cameroon
P. lounsburyi (Silvestri)	1913	Transvaal, S. Africa
P. perproximus (Silvestri)	1913	Segboroué, Benin.
P. phaeostigma (Wilkinson)	1927	Natal, Durban, S. Africa

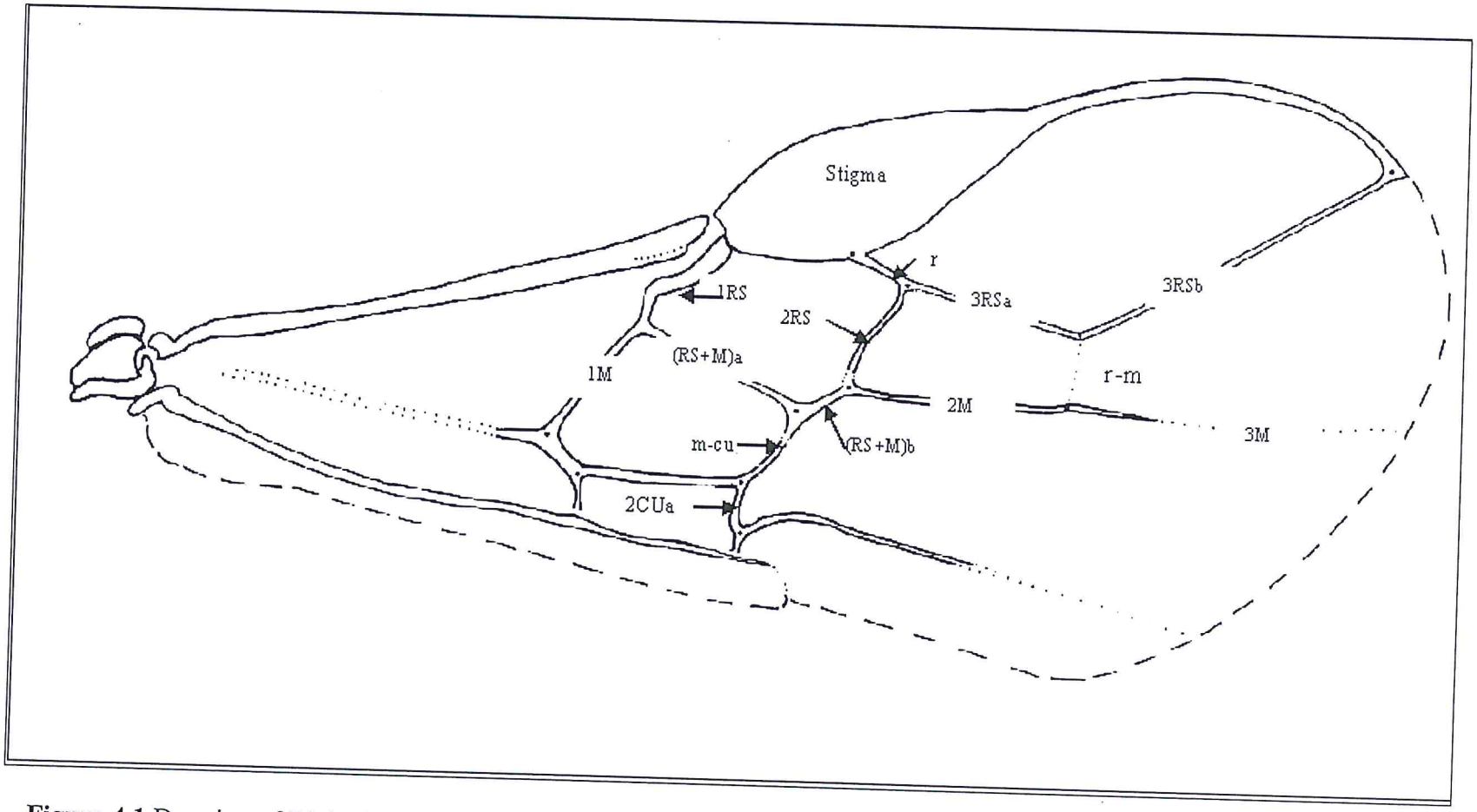


Figure 4.1 Drawing of Right fore wing of *Psyttalia concolor* (Szépligeti) showing details of veins.

4.3 Systematic status of the genus *Psyttalia* Walker 1860

The genus *Psyttalia* was described by Walker (1860), and then forgotten until Muesebeck (1931) treated it as a synonym of *Opius* Wesmeal. Fischer (1972) resurrected it as a subgenus of *Opius*, and Wharton (1987) elevated it to generic rank. *Psyttalia* contains approximately 50 described species which are all endemic to the Old World (Fischer, 1972, 1987; Wharton, 1987). They are all koinobiont endoparasitoids of Tephritidae i.e. they oviposit in the host larva and emerge from the puparium.

There are several distinct groups identified within *Psyttalia* (Fischer, 1972, 1987; Wharton, 1987, 1988, 1997a), and of particular interest is a series of closely-related species from Africa which are very difficult to distinguish (Silvestri, 1914; Wharton & Gilstrap, 1983). This group of *P. concolor* (Szépligeti) species, includes (among others) *P. concolor sensu stricto* which was described (in *Opius*) from material reared from olives in Tunisia (Machal, 1910; Szépligeti, 1910), while *P. perproximus* (Silvestri) and *P. humilis* (Silvestri) were described three years later from specimens reared from fruits infested by tephritids in Benin and South Africa, respectively (Silvestri, 1913). Resolution of this particular synonymy (between *P. concolor*, *P. humilis* and *P. perproximus*) is of interest and importance because members of the *P. concolor* species complex have been used extensively in both classical and augmentative biological control programmes directed against tephritid pests (Wharton, 1989a, b).

Historically, the two most important species are *P. concolor* and *P. humilis*. *Psyttalia concolor;* and they have been used in the Mediterranean region for augmentative releases against the olive fly and to some extent against medfly (Greathead, 1976). *Psyttalia humilis* was introduced to Hawaii in 1913 (together with other species) and was able to suppress medfly populations to an extent that was described as a substantial

biological control success (Willard & Mason, 1937; Clausen *et al.*, 1965). The two historical species are virtually identical and there have been problems distinguishing between them since the 1930s (when *P. humilis* disappeared from Hawaii for no known reasons). They have frequently been treated as synonyms, and just as frequently as separate species. According to Kimani-Njogu *et al.* (2001) and Wharton (personal communication), the matter is far from resolved. Having been described from the two ends of the continent (Tunisia and South Africa), there has been the temptation of referring to materials collected in Kenya or other areas in-between these two places in the historical literature as either *P. concolor* or *P. humilis* (Bianchi and Krauss, 1937; Clausen *et al.*, 1965). However, the true *P. humilis* has never been collected again from South Africa since the time of its original collection in 1913 by Silvestri. According to Wharton (personal communication), Kenyan material should be referred to as *P. concolor* or as *P.* cf. *concolor*, unless it can sufficiently and conclusively be demonstrated that it is a different species. This assertion was proven to be so in the work of Kimani-Njogu *et al.* (2001), where morphological, cross mating, and preliminary morphometric data showed that the *Psyttalia* populations in coffee from the central highlands of Kenya could not be completely separated out from individuals of *P. concolor*.

Two decades after the introduction of *P. concolor* to Italy to control the olive fly, *Bactrocera oleae* (Gmelin), Monastero (1931) described *Psyttalia siculus* from Sicily as a parasitoid of *B. oleae*. This generated considerable debate as to whether *P. siculus* was actually distinct from *P. concolor* (Monastero, 1934; Delucchi, 1957). Fischer (1958) compared several hundred specimens from across northern Africa, half of which were reared from the Olive fly and half from the medfly. He compared the number of

flagellomeres, venation, ovipositor length, shape of metasomal tergites, and body colour, but found no consistent differences. Fischer (1958), therefore, placed *P. humilis, P. perproximus,* and *P. siculus* as synonyms of *P. concolor*. Later, however, Fischer (1963, 1971 & 1972) treated *P. siculus* as a subspecies *of P. concolor* (with a slightly longer ovipositor), and retained *P. humilis* as distinct from *P. concolor* 'because of differences in morphology of developmental stages'.

In addition to the above considerations, Szépligeti (1913) described *Psyttalia fuscitarsis* from a single male specimen collected in Tanzania, which was treated as a synonym of *P. concolor* by Fischer (1972), but without females, it is difficult to determine the true identity of this species. Two other members of the *P. concolor* species complex, *P. ponerophaga* (Silvestri) and *P. dacicida* (Silvestri) (originally from Pakistan and Eritrea, respectively) were reared from olive fly but these two members have never been adequately characterized. Figure 4.2 shows the diagrammatic presentation of the history of the *Psyttalia concolor* species complex.

4.4 Morphological studies

A major factor, if not the most important one, is the relationship of parasitoids with their hosts which provide not only a secure source of food but also, frequently, the bulk of their environment during development (Godfray, 1994). The study of parasitoids must therefore be in close relationship with the host larvae on which they develop. This is because as the development of the parasitoid egg or larva proceeds, host tissue is converted into the required morphological structures of the following stage of the developing parasitoid. It is therefore important that host effects or factors are considered in morphological studies of parasitoids as these effects consequently play a vital role in understanding and interpreting many aspects of parasitoid morphology as

well as the fitness status of the parasitoids in their host-finding behaviour (Godfray, 1994).

Of the eleven *Psyttalia* species names currently recognized from Africa, the populations reared from mangoes and marula (*P. cosyrae*) and those from cucurbits (*P. phaeostigma*) have relatively big individuals with longer ovipositors. It is not known whether individuals of the two populations are just larger (size factor) or it is as a result of the host larvae from which they are reared (host factor). Occasionally hosts may vary in size for many reasons e.g. either they belong to different species or are different instars of the same species, and host flies of members of the genus *Psyttalia* have been observed to exhibit a two- to three-fold difference in body size and the wasps too vary tremendously in size. The positive correlation between host size and parasitoid adult size has long been established since the 1940s (Salt, 1940), but experimental evidence in support of this observation, especially for Kenyan populations, was found to be lacking (Kimani-Njogu *et al.*, 2001).

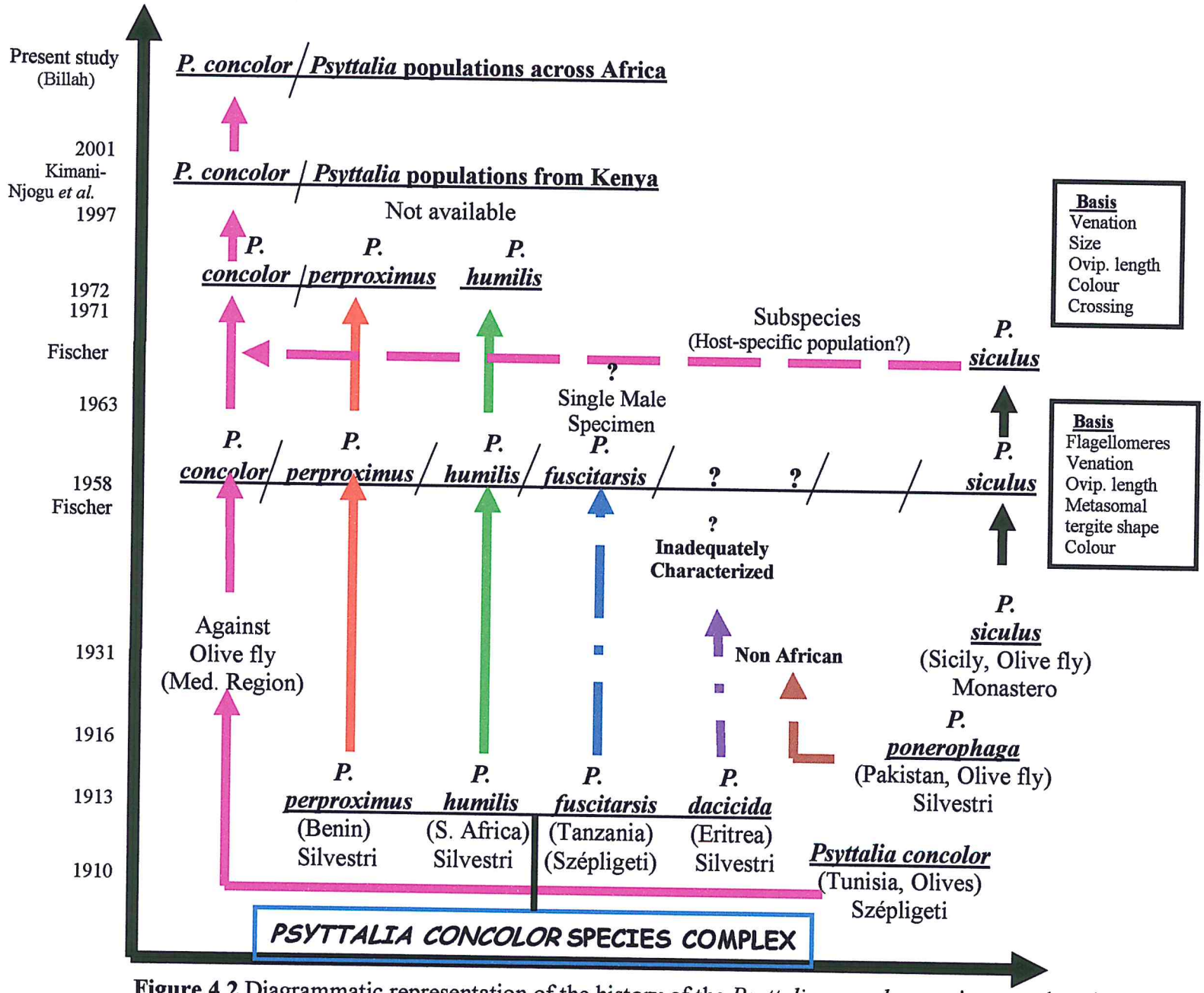


Figure 4.2 Diagrammatic representation of the history of the *Psyttalia concolor* species complex. (Scheme: M. Billah).

4.5 Materials and Methods

4.5.1 Effect of different host species on parasitoids

To determine the effect of different host species on parasitoids, individual parasitoids from the same colony and reared under the same laboratory conditions were used.

A total of forty (40) (2-4 day old) parasitoids (20 males and 20 females) of both *P. concolor* and *P cosyrae* were randomly selected for this experiment. Each species was further divided into two groups (10 males and 10 females each) and put in two different cages (20 x 15 x 15 cm). Early third instar *Ceratitis capitata* and *C. cosyra* larvae were exposed to each set of parasitoids for 4-6 hours. After oviposition, host fly larvae were transferred to fresh larval diet in petri dishes (8.6 cm diameter x 1.5 cm height) and placed in a ventilated plastic container to develop. Puparia were held till flies and/or parasitoids emerged. After seven days, parasitoids were killed by freezing at -20° C and preserved in 70 % ethanol. Parasitoids were then dissected and mounted on glass slides for measurement and comparison of various body parts (see section 5.2.2 of Chapter 5). Prior to the rearing of the wasps on the different host larvae, the length, width and weight of 30 freshly formed puparia (2-3 days after formation) were measured using a calibrated Leica WILD M3Z microscope to serve as an indicator of host size for each of the four experimental host flies (*C. capitata, C. cosyra, Dacus ciliatus* and *Trirhithrum nigerrimum*). This was done to determine the host sizes of the original preferred larvae, and how those sizes are related to the sizes of the wasps.

4.5.1.1 Ovipositor, sheath and tibia measurements

Slide mounted specimens were measured using video microscopy - a Leica MZ APO dissecting microscope fitted with a scanning camera ProgRes® 3008 (Laser Optiks Systeme, GmbH, Germany) (Plate 4.1). Images were captured at 25X magnification

based on the size of the biggest parts of the specimens (wings and ovipositors), to fit to the measuring window screen on a twenty-one (21) inch monitor. Measurements were automatically recorded by tracing the lengths of the parts on the monitor screen using a combination of Adobe PhotoShop 6.0 (Educational Version) and Optimas (version 6.5) software programmes (see sections 5.2.3-5 of Chapter 5 for detailed description).

4.5.1.2 Pupal measurements

The four fly species served as the host larvae from which *P. concolor*, *P. cosyrae*, *P. phaeostigma*, and the *Psyttalia* population from Shimba Hills were reared. The pupal lengths and widths were measured using a calibrated Leica WILD M3Z microscope. The pupal area was then calculated by multiplying the length and the width (Area (mm^2) = length x width) (Ode & Heinz, 2002) (Table 4.3). The weights of puparia were also taken individually using a Mettler AT 261 (DeltaRange®) digital precision balance.

The dissecting microscope (Leica WILD M3Z) used for the measurement of puparia was calibrated using a 2 mm microscope slide scale divided into 100 parts at 0.01 mm intervals, and a graduated eye-piece graticule. Under each magnification and proper focusing adjustment, the graduations on the microscope slide were matched with those on the eye-piece graticule. Perfectly coinciding graduation marks on the two scales were noted and the conversion factor calculated. For example, at magnification 6.5X, the full length of the 2 mm slide scale matched perfectly with twenty-six (26) graduated marks on the eye-piece graticule, and the calculation was worked out as follows;

26 graduated units = 2.0 mm

1 unit = 1/26 x 2.0 mm

= 0.07692 mm

Converting to micrometers (μm),

0.07692 x 1000 = 76.92 μm,

Therefore, 1 graduated unit (at 6.5X magnification) = 76.92 μm (Appendix 3).

4.5.2 Effect of host colour on parasitoids

Psyttalia lounsburyi (Silvestri) parasitoids collected from the African olive, *Olea europaea* subspecies *africana* (Miller) P. Green (Oleaceae) were noted to have a dark body colour, especially on the thorax and abdomen. On the thorax, the markings were conspicuously displayed on the lateral sides and the mid apical portion of the mesoscutum, while the abdominal markings were concentrated on the second and third tergites (tergite 2+3) through to the sixth tergite in both males and females (Plate 4.2).

The host fly, *Bactrocera oleae* (Gmelin) of *Psyttalia lounsburyi* also has a scutum which is predominantly black without any yellow stripes (lateral or median). According to White and Elson-Harris (1992), the black areas sometimes do not extend to the lateral margins, thereby leaving the lateral areas red-brown (especially in Africa). The abdomen has an orange/brown colour medially and black laterally, and the wings have reduced pattern. The larvae have a dark brown to black colour and are about the same size as those of *Ceratitis capitata*. *Ceratitis capitata* larvae are pale or creamy white in colour. These larvae were exposed to the dark-bodied parasitoids (*P. lounsburyi*) and the progenies compared. Magnified photographs (40X–100X) of the parasitoids were taken using a Leica WILD M3Z microscope and a Nikon CoolPix 4300 digital camera. The camera was hooked to a high power eyepiece (MaxView 40 Plus, from ScopeTronix®, USA) by means of a step-down ring adapter (UR-E4, Nikon®) which was then attached to the microscope via a threaded universal adapter (UNI-T, ScopeTronix®, USA) to show body colour changes in the parasitoids.

The objective of this experiment was to determine whether host colour has any influence on adult parasitoids. In which case if it did, would not be a reliable character for distinguishing species in this genus. *Psyttalia concolor* was originally described from *B. oleae* (Silvestri, 1913). It was therefore necessary to check the parasitoids from that host to ascertain whether or not they were the same *P. concolor* which had re-established host association with *B. oleae* (its original host), and changed in body colour relative to the usual yellowish-brown colour.

4.6. Data analysis

All measurements, pupal sizes and proportional data for ovipositor to tibia as well as ovipositor sheath to tibia ratios were compared using the general linear model (PROC GLM, SAS Institute, 2001) in the analysis of variance (ANOVA). Proportions were arcsine root transformed to normalize the distribution and preserve the independence of the variance and the mean before analysis (Sokal & Rohlf, 1995). When ANOVAs showed significance, means were separated using Student-Newman-Keuls (SNK) test. Relationships between host size, host type and tibia lengths were also examined using the general linear model (PROC GLM, SAS Institute, 2001).

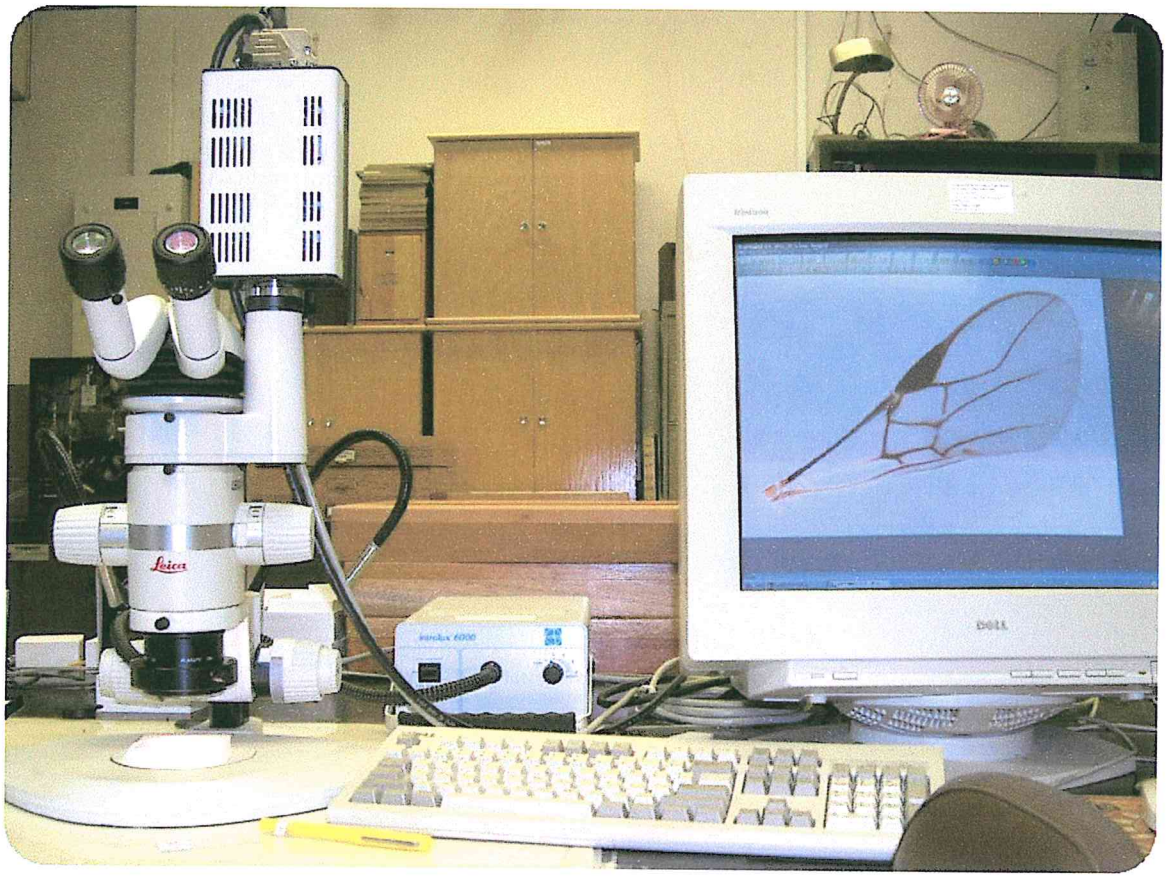


Plate 4.1 Video microscopy equipment used in measurement of parasitoid body parts. Courtesy: Dr. J. B. Woolley, Texas A&M University, USA. (Photo: M. Billah).

Table 4.3 Pupal characteristics of the four experimental hosts (Mean measurement ± SE, [range], and (number of specimens)).

Host	Mean pupal measurements (± SE)				Hind tibia (± SE)
	Length (mm)	Width (mm)	Weight (mg)	Size (mm^2)	Length (mm)
Ceratitis capitata	3.81 ± 0.02 c [3.54-4.20] (30)	1.92 ± 0.01 c [1.80-2.10] (30)	6.10 ± 0.13 c [5.0-6.6] (30)	7.31 ± 0.08 c [6.55-8.51] (30)	1.04 ± 0.01 c [0.95-1.17] (30)
Ceratitis cosyra	4.69 ± 0.05 b [4.36-5.23] (30)	2.03 ± 0.02 b [1.79-2.36] (30)	7.20 ± 0.25 b [4.5-9.7] (30)	9.55 ± 0.18 b [7.80-11.87] (30)	1.37 ± 0.01 b [1.17-1.49] (30)
Dacus ciliatus	5.42 ± 0.05 a [4.92-6.15] (30)	2.38 ± 0.03 a [2.08-2.62] (30)	13.93 ± 0.38 a [10.3-17.8] (30)	12.94 ± 0.25 a [10.23-15.51] (30)	1.46 ± 0.02 a [1.26-1.71] (30)
Trirhithrum nigerrimum	3.20 ± 0.03 d [2.87-3.59] (30)	1.54 ± 0.01 d [1.44-1.69] (30)	2.87 ± 0.13 d [1.3-4.2] (30)	4.94 ± 0.07 d [4.13-5.54] (30)	0.91 ± 0.01 d [0.76-1.01] (30)
F	603.15	255.16	361.68	435.82	333.59
df	3, 116	3, 116	3, 116	3, 116	3, 116
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means in the same column followed by different letters are significantly different (P = 0.05), using Student-Newman-Keuls (SNK) test. Figures in square brackets are ranges of measurements, while those in parentheses are numbers of replicates.

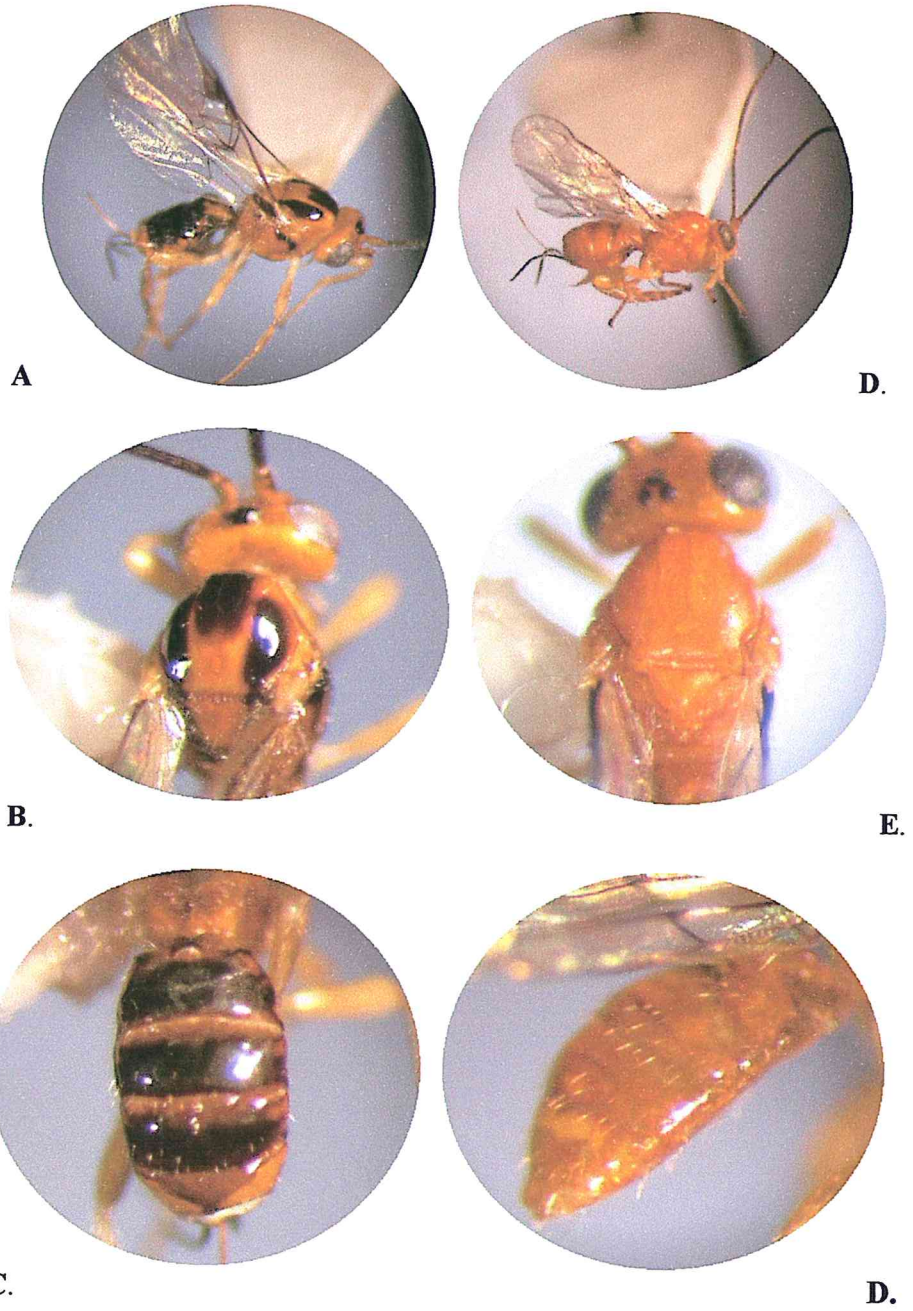


Plate 4.2 Body colour changes of *Psyttalia lounsburyi* (Silvestri) reared on different host larvae.
A-C. Body colour of parasitoid reared from *Bactrocera oleae* larvae.
A = Whole parasitoid, **B** = Thorax, **C** = Abdomen.
D-F. Body colour of parasitoid reared from *Ceratitis capitata* larvae.
D = Whole parasitoid, **E** = Thorax, **F** = Abdomen. (Photos: M. Billah).

4.7. Results

4.7.1 Effect of host size

Analyses from both the pupal and slide measurements showed that body size of parasitoids was dependent, to a greater extent, on host size. Significant differences were observed between tibia lengths of parasitoids from the four different host species ($F = 333.59$; $df = 3, 116$; $P < 0.0001$), stemming from the fact that the different host species were differently sized (*C. capitata* = 7.31 ± 0.08 mm^2; *C. cosyra* = 9.55 ± 0.18 mm^2; *D. ciliatus* = 12.94 ± 0.25 mm^2; *Trirhithrum nigerrimum* = 4.94 ± 0.07 mm^2). Parasitoid size also corresponded with the weight of puparia (Table 4.3), which is an indication that the size of the parasitoid produced depended on the quantity of food available in the host larva for the developing parasitoid.

Results from the slide measurements indicated that there were significant differences in the overall body sizes (mean linear measurements) of the four different offspring populations (*P. concolor* and *P. cosyrae* reared on *C. capitata* and *C. cosyra*, respectively, and vice versa) (Ovipositor: $F = 280.12$; $df = 3, 92$; $P < 0.0001$; Ovipositor sheath: $F = 290.82$; $df = 3, 92$; $P < 0.0001$; Tibia: $F = 165.70$; $df = 3, 92$; $P < 0.0001$). Comparison of the ratio values showed significant differences between the two *P. concolor* populations on the one hand, and the two *P. cosyrae* populations on the other hand (ovipositor/tibia: $F = 98.93$; $df = 3, 92$; $P < 0.0001$; ovipositor sheath/tibia: $F = 100.88$, $df = 3, 92$, $P < 0.0001$). However, the ratio values between the two *P. concolor* populations alone showed no differences (Table 4.4).

Table 4.4 Mean linear and ratio measurements (± SE) and number of specimens of *Psyttalia* species reared on different host larvae.

Species/ Population	Mean ratio (± SE)		Mean linear measurement (±SE)		
	Ovipositor/tibia	Ovip. sheath/tibia	Ovipositor	Ovipositor sheath	Hind tibia
P. concolor (reared on *C. capitata*)	2.53 ± 0.004 c (34)	1.67 ± 0.007 c (34)	2.60 ± 0.038 d (34)	1.72 ± 0.026 d (34)	1.02 ± 0.012 d (34)
P. concolor (reared on *C. cosyra*)	2.51 ± 0.002 c (17)	1.66 ± 0.003 c (17)	3.02 ± 0.038 c (17)	2.00 ± 0.023 c (17)	1.20 ± 0.013 b (17)
P. cosyrae (reared on *C. cosyra*)	3.53 ± 0.002 a (30)	2.46 ± 0.003 a (30)	4.83 ± 0.054 a (30)	3.36 ± 0.038 a (30)	1.37 ± 0.013 a (30)
P. cosyrae (reared on *C. capitata*)	3.01 ± 0.016 b (15)	2.03 ± 0.027 b (15)	3.36 ± 0.149 b (15)	2.29 ± 0.115 b (15)	1.08 ± 0.012 c (15)
F	98.93	100.88	280.12	290.82	165.70
df	3, 92	3, 92	3, 92	3, 92	3, 92
P	<0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001

Means in the same column followed by different letters are significantly different (P = 0.05), using Student-Newman-Keuls (SNK) test. ANOVA performed on arcsine transformed proportion values. Figures in parentheses are numbers of replicates.

4.7.2 Effect of host type/colour

When *P. lounsburyi* was reared on *C. capitata* larvae, it was observed that the first generation offspring had a slight change in body colour. Between the third and the sixth generations, there was a complete change in body colour, with the loss of all the dark markings on the mesonotum and metasoma. However, the more extensively rugulose or minutely wrinkled propodeum and the distinctly shagreened second abdominal tergum of *P. lounsburyi* (Wharton & Gilstrap, 1983) remained the same (Plate 4.2).

4.8 Discussion

Host size: Though parasitoids that emerge from their hosts are known to have overcome the host immune defense system, there are other host characteristics that affect the parasitoid fitness. These include host size, host age, host quality or condition and the host type. Results from the present study indicate that rearing *P. concolor* on bigger host larvae (*C. cosyra* = 9.55 ± 0.18 mm^2) results in the attainment of relatively larger offspring (e.g. hind tibia increased from 1.02 ± 0.012 to 1.20 ± 0.013 mm) (Table 4.4) and an increase in the number of flagellomeres. However, the changes resulted in intermediate body sizes, and not complete changes to the normal average sizes of *P. cosyrae* individuals (i.e. hind tibia: from 1.02 ± 0.012 mm (*P. concolor*) to 1.20 ± 0.013 mm (intermediate size), and not to1.37 ± 0.013 mm (*P. cosyrae*)). In a similar study, Eben *et al.* (2000) also reported results for another opiine parasitoid *Diachasmimorpha longicaudata* (Ashmead), in which the parasitoid attained a larger size when it was reared on *Anastrepha ludens* (Loew) (Diptera: Tephritidae) than when it was reared on a relatively smaller host, *A. obliqua* Macquart.

The results here show that the increase in body size was uniform across the measured parts for *P. concolor* (i.e. non-allometric increase) thus, resulting in no significant

difference in the ratio measurements (Table 4.4). However, the same cannot be said of *P. cosyrae*, which showed significant difference when it was reared on a relatively smaller host larva (*C. capitata* = 7.31 ± 0.08 mm^2). This could probably be the result of insufficient host food resource contained in the smaller larva for the developing parasitoid, which may require a minimum larval resource, below which the developing parasitoid develops differently in size. This may account for why *P. cosyrae* has been noted to be more host-habitat specific (Mohamed *et al.*, 2003) and restricted to *C. cosyra* larvae. If this is true, then it is a positive attribute of *P. cosyrae*, as host-specificity in biological control agents is an important attribute.

In a similar comparison by Mohamed *et al.* (2003), both *P. concolor* and *P. cosyrae* showed significant changes in wing and hind tibia lengths when they were reared on *C. cosyra* and *C. capitata*, respectively, but they were not compared as ratio expressions. These studies provide evidence that host size plays an important role in body size determinations of parasitoids, especially in the case of idiobiont parasitoids which kill or immobilize their hosts at parasitism. Hosts of koinobiont parasitoids continue to grow but their final size may be related to host size at oviposition (Godfray, 1994).

Most fruit fly parasitoid identifications have been based on different parameters, such as host-larvae, host habitats, and the relative length of the ovipositor. In a study of the ovipositor lengths in a guild of Braconid parasitoids attacking *Anastrepha* fruit fly species (Diptera: Tephritidae) in Southern Mexico, Sivinski *et al.* (2001) advanced various hypotheses that might account for differences in ovipositor lengths in different species. Notable among them were: 1) The *Host-instar Hypothesis* - that different parasitoid species attack different host instars, and species that specialize in less accessible hosts require longer ovipositors. This is mainly based on findings by Volterra

(1926) which concluded that multiple species cannot coexist and simultaneously exploit an identical resource, 2) the *Costly ovipositor Hypothesis* – that long ovipositors are expensive to produce and maintain, and they direct resources away from fecundity, and that long ovipositors may represent different investment strategies; and 3) the *Specialization Hypothesis* – that despite overlap in host ranges, each species has specialized foraging areas and, if fruits within these areas differ in size or penetrability, then the difference in host accessibility leads to different ovipositor lengths.

Sivinski *et al.* (2001) concluded that the data did not support the first hypothesis because all species attacked similar host stages. There was no relation between ovipositor length and the developmental stage of the host attacked. Neither was there any correlation between wing size or potential fecundity and ovipositor length. It was therefore suggested that species may typically occupy niches that are substantially different from one another in terms of fruit-host and fly-host, and/or altitude and season. There was also no general relationship between differences in ovipositor lengths and species co-occurrence. The most consistent explanation from their findings was that "ovipositor lengths have evolved to meet special, presently unspecified needs within niches that originally diverged on the basis of different phenomena that are unrelated to host accessibility and perhaps to factors such as temperature, humidity and/or host-fruit abundance and diversity".

Colour effects The results on body colour effects show that the parasitoids obtained from the olive fly, *B. oleae* are different from *P. concolor* (though *P. concolor* was originally described from the same host larvae from Tunisia). The results also indicate that the same host larvae can be attacked by different parasitoid species in different places. Plate 4.2 compares the differences in body colour of *P. lounsburyi* when it was

reared on its original host, *B. oleae,* and on *C. capitata*. This finding is consistent with the observations of Clausen *et al* (1965) who reported that variation in colour pattern of reared material of *P. lounsburyi* caused some difficulty in distinguishing the species during introductions from Africa to Hawaii for control of *Bactrocera* (=*Dacus*) *dorsalis*. This shows that *P. lounsburyi* is a distinct species from *P. concolor* and body colour changes are known to be associated with it when reared from different host larvae.

According to Godfray (1994), the observed host range of a parasitoid is influenced by factors acting on an evolutionary time scale that determine the set of hosts which can, at least potentially, support the parasitoid, and by behaviour factors that determine whether a particular host is acceptable for oviposition. This is especially important in post-release sampling surveys to ascertain establishment of parasitoids in new environments where they may adapt to new host species. Therefore, the use of body colour descriptions in taxonomy or separation of *Psyttalia* species should be in conjunction with other stable non-subjective characters.

These present findings may serve as the needed long-awaited experimental evidence expressed by Kimani-Njogu *et al.* (2001) that differences in size and body colour of *Psyttalia* species appear to be governed largely by the host on which the parasitoids develop.

CHAPTER FIVE

5.0 MORPHOMETRIC ANALYSIS

5.1 Introduction

Morphometrics is the quantitative description or the analysis of measurements (or ratios of measurements) of form, shape and structure (morphology) in organisms. These measurements produce continuous variables (Sokal & Rohlf, 1995) and are usually taken from well-defined landmarks which must be repeatable, homologous, and carefully described (Bookstein *et al.*, 1985).

Morphometric methods are frequently used for identification of populations within species and it is often necessary to use more than one character to discriminate between groups. According to Richtsmeier *et al.* (2002), the first approach involves the application of quantitative methods to discover new information within the data and then looking for patterns within the data that suggest any underlying biological processes or effects. To analyze such large data sets, computer programmes of multivariate methods are needed (Sneath & Sokal, 1973), and some of the commonest methods include (among others) principal components and canonical variates analyses.

5.1.1 Principal components and canonical variates analyses

Principal components analysis (PCA) and Canonical variates analysis (CVA) are techniques used in multivariate statistics and systematics to examine the information in complex multidimensional data sets (Woolley *et al.*, 1994). Principal components analysis also examines relationships among several quantitative variables and has been viewed as an attempt to uncover approximate linear dependencies among variables (SAS Institute Inc., 2001). It can be used for exploring polynomial relationships and for multivariate outlier detection (Gnanadesikan, 1977) with the plots as valuable tools in

exploratory data analysis (SAS Institute Inc., 2001). Often a small number of principal components can be used in place of the original variables for plotting, regression, clustering, and so on.

The purpose of principal component analysis is to derive a small number of linear combinations (known as principal components) of a set of variables that retain as much of the information in the original variables as possible (Rao, 1964). The principal components are variables whose values, called principal component scores, represent linear or weighted combinations of the original variables (Marcus, 1990). The weights or coefficients represent the cosines of the angles by which the axes are rotated (Jolliffe, 1986; Neff & Marcus, 1980). Wiley (1981) put it as a multivariate data reduction technique used to sort out or examine linear relationships among several quantitative variables contributing to the size and shape of organisms. It partitions the total variance into a limited number of uncorrelated new variables, with the relative contributions of the original characters to the variance of each principal component, yielding information concerning the value of those characters with respect to size and shape (Wiley, 1981).

Principal components (PCs) have a variety of properties that make them appropriate or useful for morphometry. The components are sorted by descending order of the eigenvalues, which are equal to the variances of the components (Rao, 1964; Kshirsager, 1972). The first principal component (PC 1) has the largest variance of any unit-length linear combination of the observed variables; the second principal component (PC 2) has the next largest variance of what variance is left in the data, and so on to the last principal component (Marcus, 1990; Rolf & Bookstein, 1990), which is the most invariant combination of the data, i.e. has the least variance. Each principal

component is a linear combination of the original variables, with coefficients equal to the eigenvectors of the correlation or covariance matrix (SAS Institute Inc., 2001). Eigenvectors are customarily taken with unit-norm, with the elements of each eigenvector being scaled so that the sum of squares of all the elements in each vector becomes one (unity). Therefore, the value of each element squared represents the proportion of variance that a variable contributes to the respective principal component (Neff & Marcus, 1980; Woolley *et al.,* 1994). The eigenvector values are orthogonal and so the principal components represent jointly perpendicular directions through the space of the original variables.

Canonical variates analysis represents new coordinate system but, in this case, the new dimensions are constructed so as to maximally discriminate between pre-assigned groups. It is the multigroup extension of linear discriminant analysis in which a single axis (or function) is constructed to maximally discriminate between two groups (Rohlf & Bookstein, 1990; SAS Institute, Inc., 2001). As in linear discriminant analysis, the first canonical variate (CV 1) is constructed to maximize between-group covariance and minimize within-group covariance. In the simple two-group case, the best axis for discriminating between the groups is the line connecting their centroids but, with more than two groups, the best discrimination tool must attempt to maximize the distance between the centroids of all the groups along the first canonical variate axis (Woolley *et al.,* 1994). The second canonical variate (CV 2) provides maximal discrimination, subject to the constraint that it is orthogonal to the first, and so on. The distance between any two centroids in canonical variates space is known as the Mahalanobis distance, and usually presented in the squared form (D^2). According to Woolley *et al.* (1994), it provides the best multivariate measure of the relative distance between

groups, taking into account the variation within each one of them. In canonical variates analysis, a clear separation of the populations is expected because computations involve minimizing the within-group variance and maximizing the between-group variance to provide the most distinct separation of classes (Albrecht, 1980; Campbell & Atchley, 1981). In practice, the two most different groups will generally be separated on the first canonical variate; the next most different group will be separated from the first two groups on the second canonical variate, and so forth.

Canonical variates, like principal components, are linear functions of the original variables weighted by coefficients. There is always a strong temptation to interpret these coefficients as done in principal component coefficients. A large absolute standardized canonical coefficient value can be used to determine the contribution of a variable to a canonical variate (Umphrey, 1996), and can generally be useful in discrimination (J. B. Woolley, personal communication). However, the interpretation is more difficult and more complicated (Marcus, 1990; Reyment, 1990) than interpreting principal component coefficients, as the points are negatively correlated with the position on the canonical variate plot (J. B. Woolley, personal communication). Woolley *et al.* (1994) add that the interpretations are tricky and must be done with great caution and in close relation with the principal components analysis results (if any are to be made at all). Bargman (1970), however, is of the view that the purpose of canonical variates analysis is only for separation of populations in terms of the within-group variability, and not for interpretation of the coefficients in a causal, structural or path framework.

5.1.2 Univariate and bivariate analyses

Univariate analysis produces simple descriptive statistics for numeric variables, with greater detail on the distribution of a variable (SAS Institute Inc., 2001). It provides details on the extreme values of variables, gives several plots to picture the distribution of values, and most importantly, tests whether the data are normally distributed. If polymorphisms are present, there may be several regions of dense concentrations of points (SAS Institute Inc., 2001).

Bivariate plots on the other hand, display the relationship between two variables. They provide means for characterizing pair-wise relationships between the variables such as the form, strength, and dependence of the relationship on external circumstances (to the two variables being examined) (SAS Institute Inc., 2001). They can also tell if the use of ratios may be appropriate, and are useful for locating outliers in a dataset. According to Marcus (1990), bivariate plots should be included in any data analysis for a quick overview of trends.

The test of normality produces a test statistic for the null hypothesis that the input data values are a random sample from a normal distribution (and it is representative of the group under study) (SAS Institute Inc., 2001). If the sample size is less than fifty-one (51), the Shapiro-Wilk (W) statistic is computed, which is the ratio;

$$\text{“W”} = \frac{\text{The best estimator of the variance (Based on the square of a linear combination of the order statistics)}}{\text{The usual corrected sum of squares estimator of the variance.}}$$

“W” must be greater than zero and less or equal to one ($0 < W \leq 1$), with small values of “W” leading to rejection of the null hypothesis (SAS Institute Inc., 2001).

5.2 Materials and Methods

5.2.1 Source of materials

A total of 442 specimens were measured for this study. This consisted of 210 males and 232 females, grouped into thirteen (13) populations from both laboratory-reared cultures and field-collected samples (Table 5.1).

5.2.2 Slide preparation

Specimens of the various *Psyttalia* populations preserved in 70 % ethanol were used for the slide preparations.

The procedure followed the general processes of slide preparation with modifications according to the needs or state of the specimen. For example, bleaching was avoided in order for the specimens to retain their original body colour forms for comparison. The specimens were dissected using a Leica Wild M3Z Microscope, and as follows:

- Wings and legs on one side (right) were carefully removed, and placed in absolute ethanol for at least 15 minutes (without heating) to dehydrate.
- The rest of the bodies were left in 10 % potassium hydroxide (KOH) for 3-4 hours at room temperature (to facilitate maceration and teasing out of muscles).
- They were then neutralized in concentrated acetic acid for 15 minutes at room temperature, and dehydrated together with the other parts (i.e. body, wings & legs) in absolute ethanol for 10 minutes.
- They were transferred directly into clove oil for at least 15 minutes.
- The bodies were then dissected as appropriate under a stereo microscope using dissecting needles and fine forceps (gently removing trapped air bubbles, if any).

- The various parts were placed on glass slides, properly aligned, and mounted in Canada balsam thinned-out in xylene.

Arrangements of body parts on slides were as follows;

Slide 1 (Two- and three-cover slip layout):

- Right fore and hind wings (under one cover slip),
- Right hind leg, and
- Ovipositor with sheath (for females).

Slide 2 (One-cover slip layout):

- Head with antennae (attached to head).

Gelatine Capsule:

- Thorax (in a drop of clove oil).

To prevent compression of the head, it was allowed to stand for 1-3 days in the oven (after placement in Canada balsam) for proper positioning, and two more layers of mountant added before cover slips were placed on them.

All other body parts not mounted were stored in absolute ethanol (except the thoracic segments, which were stored in clove oil in gelatine capsules). Slides and gelatine capsules were coded in a unique manner to facilitate easy reconstruction of individual insects for future reference. The slides were then oven-dried continuously for 3-5 weeks at 35°C.

Voucher specimens are maintained at the Biosystematics Unit of ICIPE, and the Zoology Department of the University of Ghana, Legon-Accra.

Table 5.1 Collection details, codes, and number of specimens of populations used in slide preparation.

Population	Fly host	Collection locality	Collection/ rearing date	No. of specimens	
				Males	Females
Psyttalia concolor	Reared on *C. capitata*	Culture maintained at ICIPE. Initial culture from Pisa, Italy.	26. v. 1998	37	35
Psyttalia concolor	Reared on *C. cosyra*	ICIPE Campus, Kasarani, Nairobi, Kenya. 01° 13 14'S 36° 53' 48E 1609 m	15. iv. 2001	7	20
Rurima *Psyttalia*	Reared on *C. capitata*	Rurima, Eastern Prov., Kenya 00°38 39'S, 37° 29 69'E, 1228 m	04. iv. 1997	26	25
Ruiru *Psyttalia*	Reared on *C. capitata*	Coffee Res. Foundation, Ruiru, Central Prov., Kenya 01° 05 72'S, 36° 54 22'E 1609 m	02. v. 2001	22	24
Psyttalia cosyrae	Reared on *C. cosyra*	Nguruman, Rift-Valley Prov., Kenya 01° 47'S, 36° 05'E 700 m	23. vii. 1997	25	24
Psyttalia cosyrae	Reared on *C. capitata*	ICIPE Campus, Kasarani, Nairobi, Kenya. 01° 13 14'S 36° 53' 48E 1609 m	04. ii. 2001	9	13
Psyttalia phaeostigma	Reared on *Dacus ciliatus*	ICIPE, Kasarani, Central Province, Kenya. 01° 13' 29S, 036° 53' 51 E 1619 m	13. vii. 2000	23	21
Mrima Hill *Psyttalia*	Reared on *C. cosyra*	Mrima Hill, Coast Prov., Kenya 04° 29.323'S, 39° 15.27'E, 146 m	16. x. 2000	13	7
Psyttalia perproximus	Reared from *Trirhithrum* spp.	Cameroon from Steck *et al.*, (1986) collection.	23. iv. 1982	10	5
Psyttalia lounsburyi	Reared from *Bactrocera oleae*	Burguret Forest, Central Prov., Kenya. 00° 06.720' S, 37° 02.342' E 1961 m	15.viii.2002	9	18
Shimba Hills *Psyttalia*	Reared from *Trirhithrum* spp.	Shimba Hills, Coast Prov. Kenya 04° 15' 64S, 039° 23' 25E 59 m	7. vi. 2001	16	23
Ghana *Psyttalia*	Reared from *Trirhithrum* spp.	Cocoa Res. Inst. Of Ghana, Tafo. 06° 13' 20 N, 00° 21' 29 W 220 m	14 .x. 2001	2	5
S. Africa *Psyttalia*	Reared from *C. capitata, C. rosa*	ITSC, Burgershall farm, near Hazyview, Mpumalanga Prov. 25° 1' 60 S, 31° 7' 0 E 524 m	11. vii. 2001	0	3
Total number of specimens		---	---	**210**	**232**

5.2.3 Equipment and software

Imaging of slide mounted specimens was done using video microscopy - Leica MZ APO dissecting Microscope, fitted with a scanning camera ProgRes® 3008 (Laser Optiks Systeme, GmbH, Germany). The computer was loaded with Adobe PhotoShop 6.0 (Educational Version) and Optimas (version 6.5) software programmes. Images were captured at 25X magnification, based on the size of the biggest parts of the specimens (wings and ovipositors) for them to fit to the measuring window screen on a twenty-one (21) inch colour monitor.

5.2.4 Calibration of microscope and measuring screen

The microscope and the Photoshop image window were calibrated to record the measurements in millimeters (mm). Several trial screen measurements were compared with manual measurements of the specimens on slide (using a ruler) to confirm the calibration and the true lengths of the parts before recording of the data. The calibration mode was then stored and selected as the active mode of the measuring screen at the beginning of each day's measurement process.

5.2.5 Image capture and measurements

Focused images were captured and scanned with the ProgRes® 3008 camera onto the computer in a PhotoShop window and saved as a standard window Bitmap (BMP) file format. The images were then recalled in the Optimas window and the measurements taken.

The Optimas software programme has the advantage and capability of taking both straight line and curved measurements (by tracing along the length of the curve). This was especially useful in the measurement of the ovipositor and ovipositor sheath lengths which were always curved. Measurements were automatically recorded at the

end of the tracing with a click of the mouse, and saved with the respective slide numbers.

5.3 Characters used

In the genus *Psyttalia*, fore wing characteristics used in separation of species include shape and size of the second submarginal cell, length, thickening and curvature of various vein segments (Wharton, 1988; 1997a). The lengths of the vein segments that enclose the second submarginal cell tend to determine the shape and size of the cell, and therefore, changes in those lengths may have some causal effects on the shape and size of the cell, and the lengths of adjoining veins that are directly attached to the cell (Plate 5.1). Characters were selected on the basis of their historical use in discriminating among species of *Psyttalia* (Fischer, 1958; Wharton & Gilstrap, 1983, Kimani-Njogu *et al.*, 2001).

5.3.1 Forewing measurements

Nineteen (19) landmarks were selected to represent homologous locations and points that would be unambiguously observed on all specimens as the measurements of biological forms are expected to be based on well-defined, landmark locations (Bookstein, 1982; Bookstein *et al.*, 1985).

The x- and y-coordinates of each landmark point were then automatically captured. To minimize the level of error arising from the measurement process, slides with signs of ambiguity or doubt about the location and/or clarity of a point were left out. Twenty-five (25) distances between the nineteen (19) landmarks constructed were computed to characterize the shape of the wing, as estimations of the size and shape differentiation in the specimens (Plate 5.1).

5.3.2. Ovipositor and tibia measurements

The lengths of the ovipositor, ovipositor sheath, and the right hind tibia were measured (Plates 5.2 and 5.3). To minimize the effect of size in the analyses of these measurements, the values were expressed as ratios to represent a standard measure of size (Sneath & Sokal, 1973). The hind tibia was chosen based on its correlation with many fitness parameters (Waage & Ng, 1984; van den Assem *et al.*, 1989; Godfray, 1994).

5.3.3. Flagellomere Count

The number of flagellomeres per antenna (excluding scape and pedicel) were counted for all specimens in each population (males and females). Ranges of these numbers are summarized in Table 5.15 (see Appendix 4 for details). This was used as an additional characteristic (Fischer, 1958, 1963, 1972) to determine the status of the species.

5.4 Data analysis

All morphometric analyses were performed using the Statistical Analysis System software (SAS version 8.2) (SAS Institute Inc., 2001). Multivariate and univariate normality were tested for the 25 wing variables. Measurements of the different body parts (wing, ovipositor, and tibia) were analyzed separately to allow covariances between the distances to interact in a more meaningful way (Woolley *et al.*, 1994).

Principal component analysis (PCA, PROC PRINCOMP) was performed on the variance-covariance matrix for the 25 wing variables computed from the logarithms (base 10) transformation of the raw data (Sokal & Rohlf, 1995). The transformation was to equalize the standard deviations of the measurements across differently-sized variables and to help ensure multivariate normality of the data (Woolley & Browning, 1987). This gives greater homogeneity of coefficients of variation compared to

variances for the differently-sized characters in the same taxon, and over the whole taxa for the same character (Marcus, 1990). Principal component analysis was performed, in part, to determine the effects of size and shape on the distribution of scores along the first two principal component axes and also to observe the distribution of observations without the *a priori* constraints of assigning them to a particular population or class.

The data matrix was also analyzed using PROC CANDISC in SAS (release version 8.2) with the canonical option to visualize shape differences more clearly, and to evaluate the value of the 25 variables for discrimination among the *Psyttalia* populations. The populations were treated as class variables in the analyses under the expectation that there would be differences between species or geographical populations. Both the ovipositor and ovipositor sheath lengths were divided by the hind tibia length to adjust for body size (Sneath & Sokal, 1973; Kimani-Njogu *et al.*, 2001) and the means for the ratios calculated for the thirteen populations. Data were analyzed with a general linear model (PROC GLM; SAS Institute Inc., 2001) and Student-Newman-Keuls (SNK) test used to separate means when significant ($P< 0.05$).

All populations were initially analyzed together to observe patterns in the data followed by various subset analyses.

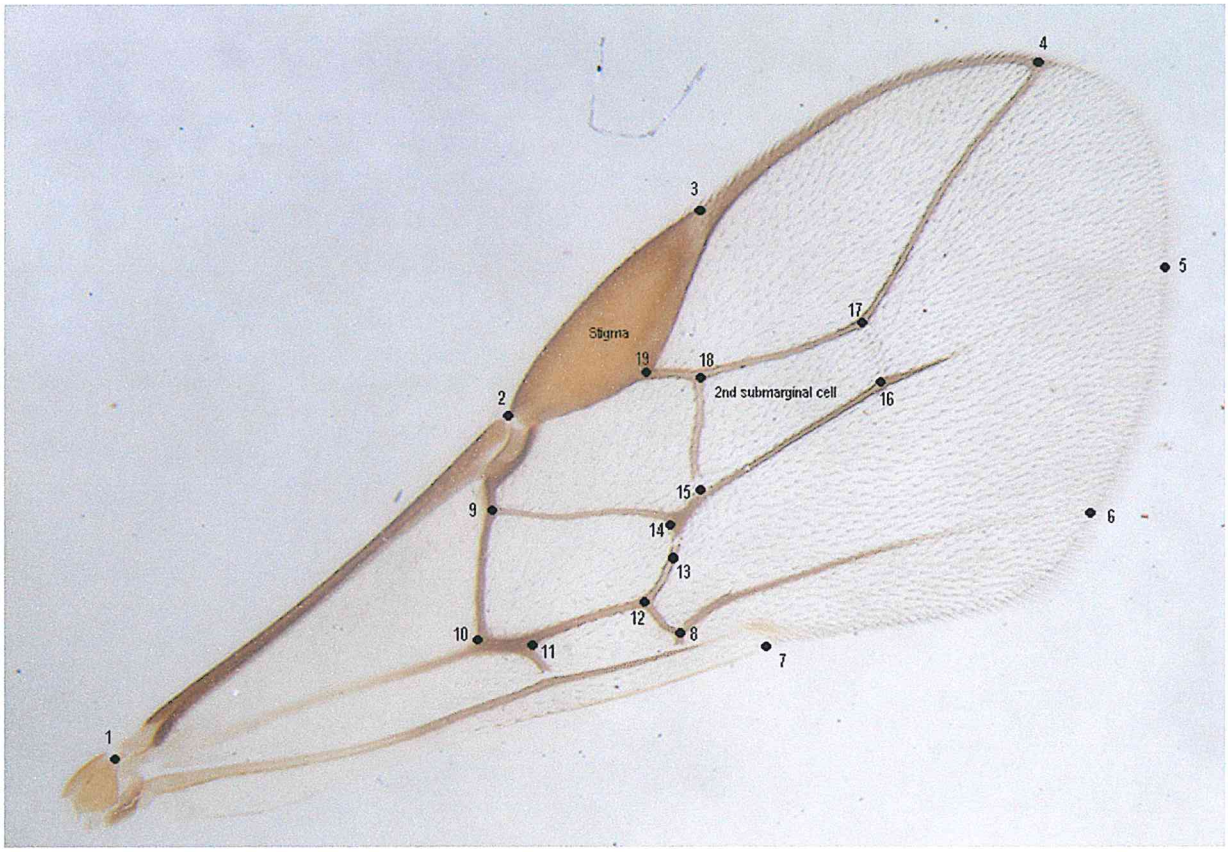


Plate 5.1 Right forewing of *Psyttalia concolor* (Szépligeti) showing landmarks (1 to 19) used in morphometric analyses. Variables consisted of straight-line distances between points. (Photo: M. Billah).

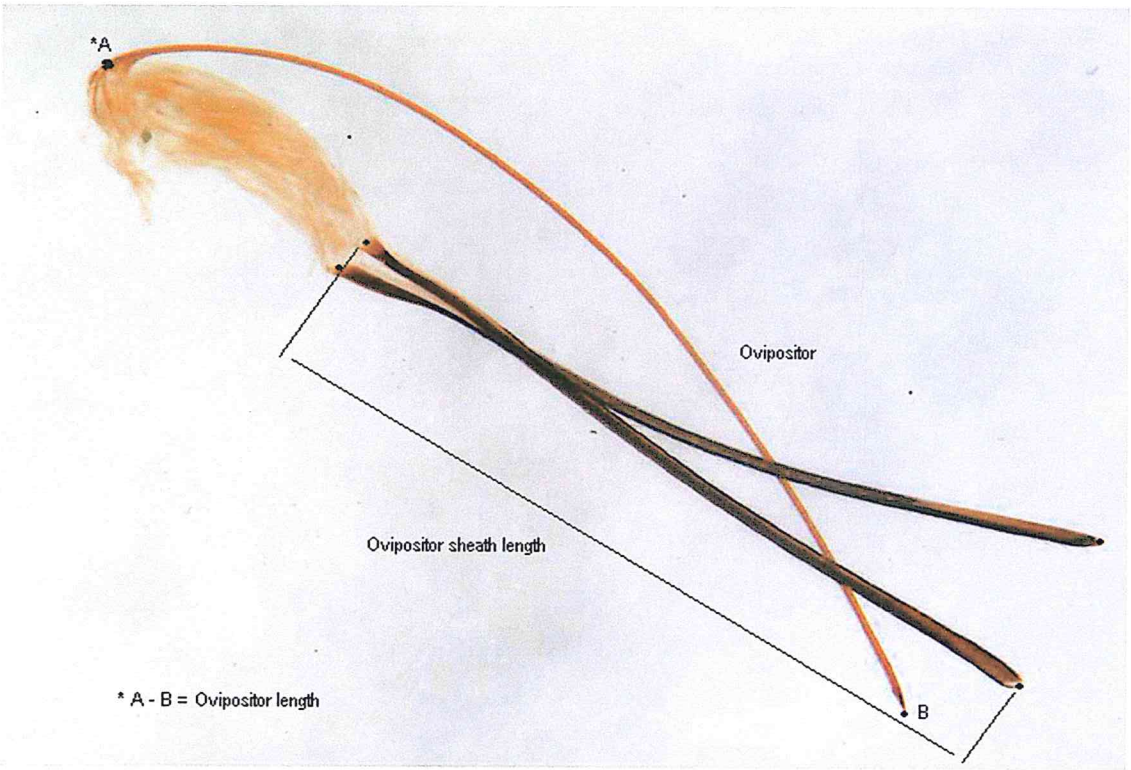


Plate 5.2 Dissected ovipositor and sheath showing points of measurement. (Photo: M. Billah).

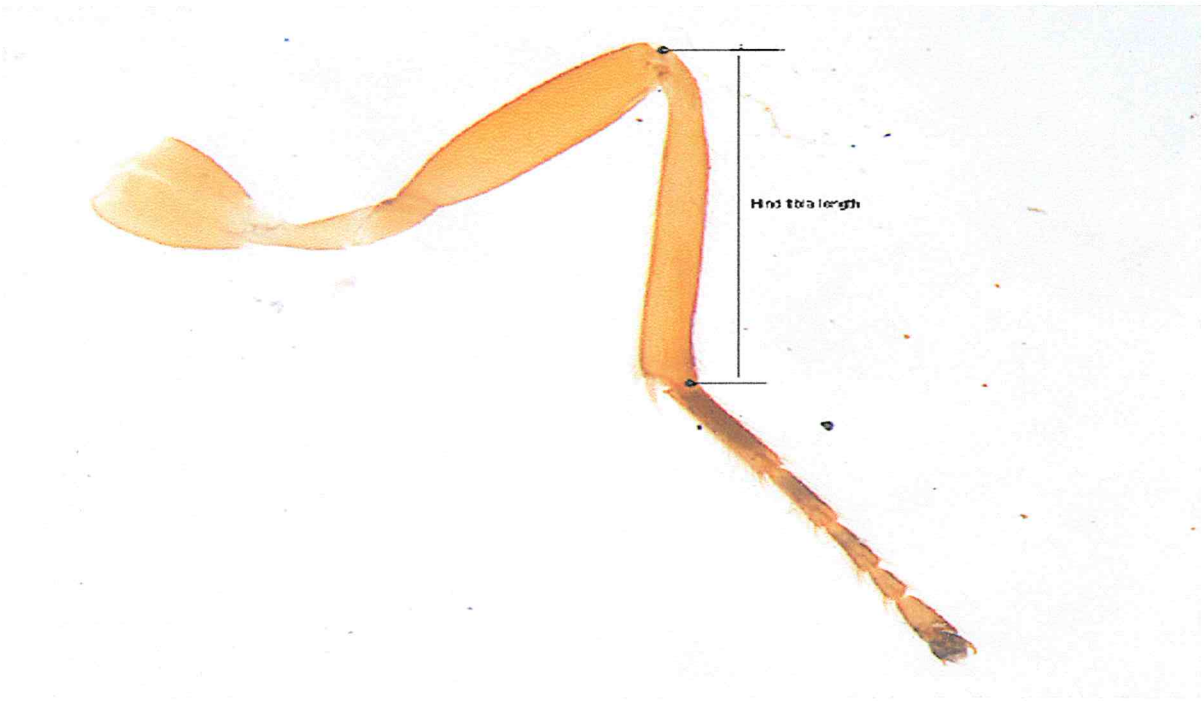


Plate 5.3 Right hind leg showing points of measurement of tibia. Variable in hind tibia consisted of straight-line distances between points. (Photo: M. Billah).

5.5 Results

5.5.1 Univariate and bivariate analyses

Univariate statistics and results of the normality tests of the variables for all the *Psyttalia* populations are summarized in Table 5.2. Figure 5.1 shows some bivariate scatter plots and the relationships between some variables. Strong positive correlations were observed in most of the bivariate scatter plots. However, separation of populations was not clear due to the large number of populations. For better separation, subsets of the populations were selected to include the main populations of interest which included *Psyttalia concolor* (from Pisa, Italy, but originally described from Tunisia). This population has been used in several biological control programmes since the early 1900s, and is maintained on *Ceratitis capitata* larvae (Raspi & Loni, 1994). Ruiru and Rurima populations (from coffee in the central highlands of Kenya) are morphologically similar to *P. concolor*. *Psyttalia cosyrae* and *P. phaeostigma* (with relatively longer ovipositors) are used as a check for ovipositor and ovipositor sheath comparisons. *P. cosyrae* mostly attacks *C. cosyra* larvae in mango and marula fruits, while *P. phaeostigma* attacks *Bactrocera* and *Dacus* larvae mainly from cucurbits. The sixth population, from Shimba Hills (in the coastal lowlands of Kenya), also comes from coffee and attacks fruit fly larvae of a different genus (*Trirhithrum* species).

Apart from *Psyttalia concolor* which is from a laboratory culture, the other five populations are readily available from the field in Kenya, and are found in relatively large numbers. The selection reflects comparison of populations across different adult sizes, different host-larvae, and different geographic distribution zones. Comparison of individual populations was also performed to find out variables that contributed most or accounted for any differences between populations.

5.5.2 Principal components and canonical variates analyses

a) All populations

Figure 5.2A shows the output of the wing dataset from all the 13 *Psyttalia* populations plotted on the first two principal components. In this case, the first two principal components account for 86.4% (PC 1 = 78.2% and PC 2 = 8.2%) of the variance in the data. Very little separation was observed between the populations. However, a general pattern of the formation of clusters was indicated. This was more pronounced when the data were subjected to canonical variate analysis (Figure 5.2B), with the formation of some clear and partial clusters. These include;

- the Shimba Hills-*P. perproximus* cluster (on the right, along the first canonical axis),
- the Mrima Hills-*P. phaeostigma* cluster (along the second canonical axis, and lying between the first and second quadrants),
- the *P. cosyrae-P. cosyrae* ex *capitata* cluster (in the second quadrant),
- the *P. concolor-P. concolor* ex *cosyra* cluster (in the third quadrant),
- the Ruiru-Rurima cluster (lying just to the right of the *P. concolor-P. concolor* ex cosyra cluster, with most of their points spreading into that cluster and blurring the separation between the two clusters), and
- the *P. lounsburyi* cluster (which is not a very tight cluster), but lies between the Shimba Hills-*P. perproximus* cluster and the other clusters, to the left.

When the number of populations was reduced to six, projection of the points on the first two principal components and canonical axes showed a much better separation (Figure 5.3).

Here, *P. cosyrae* together with *P. phaeostigma* are separated from the remaining populations along the first principal axis, while the others are separated more or less along the second principal axis.

In general, large specimens tend to have larger dimensions (in the absence of allometry) and have a great deal of variance associated with overall size (Woolley *et al.*, 1994). Therefore, separations along the first principal axis are usually associated with overall size, while those along the second principal axis are associated with shape. This is especially so when the weights of the variables for the first principal components are all positive and similar in magnitude (Jolicoeur & Mosimann, 1960). The separation of *P. cosyrae* and *P. phaeostigma* from the other populations along the first principal axis is therefore not unexpected, as the specimens of these two populations have large body sizes. The weights of all the first principal components are positive (Table 5.3), and are similar in magnitude (0.11-0.25). Separation of the other populations, which is along the second principal axis, suggests that the differences between *P. concolor* and especially the populations from Ruiru and Rurima are more due to shape factors than size factors (Figure 5.3A). However, there are situations where separations along the first and second principal axes are more than the simplistic association with overall size and shape (Bookstein *et al.*, 1985; Rohlf & Bookstein, 1987, 1990).

The standardized canonical coefficients (Table 5.4) are the products of the pooled within-class standard deviations and the canonical vector coefficients for each variable (Heraty & Woolley, 1993). These represent the amount of change in the canonical variate source for every change in the original variable by one standard deviation (Neff & Marcus, 1980). The total canonical structure values on the other hand indicate the total-sample correlations between the original variables and the canonical structure scores. The Mahalanobis squared distances between the clusters are shown in Table 5.5.

Table 5.2 Univariate statistics and results of normality tests of the variables of all the *Psyttalia* populations. Mean (Standard deviation), range, Shapiro-Wilk (W) test statistic and probability values.

Population	Variable (mm)		
	Ovipositor length	Ovipositor sheath length	Hind tibia length
P. concolor	2.60 (± 0.224) 2.10-3.01 (W =0.97, Pr < W 0.4892)	1.72 (± 0.149) 1.36-1.97 (W =0.96, Pr < W 0.2985)	1.02 (± 0.069) 0.87-1.17 (W =0.98, Pr < W 0.6893)
P. cosyrae	4.83 (± 0.294) 3.72-5.30 (W =0.86, Pr < W 0.0012)	3.36 (± 0.210) 2.56-3.70 (W =0.86, Pr < W 0.0010)	1.37 (± 0.070) 1.17-1.49 (W =0.96, Pr < W 0.3115)
Ruiru	2.28 (± 0.266) 1.62-2.71 (W =0.97, Pr < W 0.4603)	1.50 (± 0.165) 1.09-1.78 (W =0.97, Pr < W 0.4994)	1.01 (± 0.091) 0.80-1.15 (W =0.94, Pr < W 0.0883)
Rurima	2.48 (± 0.189) 2.20-2.90 (W =0.95, Pr < W 0.2015)	1.64 (± 0.127) 1.42-1.88 (W =0.96, Pr < W 0.3787)	1.05 (± 0.061) 0.93-1.21 (W =0.95, Pr < W 0.2634)
Shimba Hills	2.26 (± 0.206) 1.81-2.58 (W =0.96, Pr < W 0.3202)	1.60 (± 0.151) 1.28-1.84 (W =0.96, Pr < W 0.3136)	0.91 (± 0.072) 0.76-1.01 (W =0.94, Pr < W 0.0958)
P. phaeostigma	4.03 (± 0.267) 3.51-4.50 (W =0.96, Pr < W 0.3362)	2.99 (± 0.218) 2.55-3.38 (W =0.96, Pr < W 0.2703)	1.46 (± 0.106) 1.26-1.71 (W =0.99, Pr < W 0.9920)
P. lounsburyi	1.81 (± 0.069) 1.67-1.91 (W =0.96, Pr < W 0.5395)	1.24 (± 0.055) 1.14-1.32 (W =0.95, Pr < W 0.3991)	1.05 (± 0.068) 0.91-1.14 (W =0.91, Pr < W 0.0625)
P. concolor ex cos.	3.02 (± 0.156) 2.64-3.25 (W =0.95, Pr < W 0.3902)	2.00 (± 0.093) 1.76-2.16 (W =0.94, Pr < W 0.3368)	1.20 (± 0.052) 1.06-1.28 (W =0.92, Pr < W 0.1484)
P. cosyrae ex cap.	3.36 (± 0.581) 2.60-4.30 (W =0.89, Pr < W 0.0592)	2.29 (± 0.443) 1.68-2.93 (W =0.86, Pr < W 0.0284)	1.08 (± 0.046) 1.01-1.20 (W =0.91, Pr < W 0.1497)
Mrima Hills	3.15 (± 0.197) 2.28-3.44 (W =0.96, Pr < W 0.8150)	2.20 (± 0.138) 1.97-2.39 (W =0.96, Pr < W 0.7877)	1.27 (± 0.089) 1.14-1.38 (W =0.91, Pr < W 0.2677)
P. perproximus	2.17 (± 0.163) 1.99-2.38 (W =0.92, Pr < W 0.5089)	1.53 (± 0.106) 1.39-1.66 (W =0.99, Pr < W 0.9639)	0.84 (± 0.057) 0.79-0.92 (W =0.85, Pr < W 0.2001)
Ghana	2.52 (± 0.346) 2.21-2.90 (W =0.77, Pr < W 0.0460)	1.79 (± 0.239) 1.57-2.06 (W =0.79, Pr < W 0.0637)	0.94 (± 0.121) 0.85-1.09 (W =0.73, Pr < W 0.0207)
S. Africa	2.58 (± 0.015) 2.56-2.59 (W =0.63, Pr < W 0.0012)	1.69 (± 0.034) 1.65-1.72 (W =0.86, Pr < W 0.2710)	1.10 (± 0.044) 1.06-1.15 (W =0.88, Pr < W 0.3315)

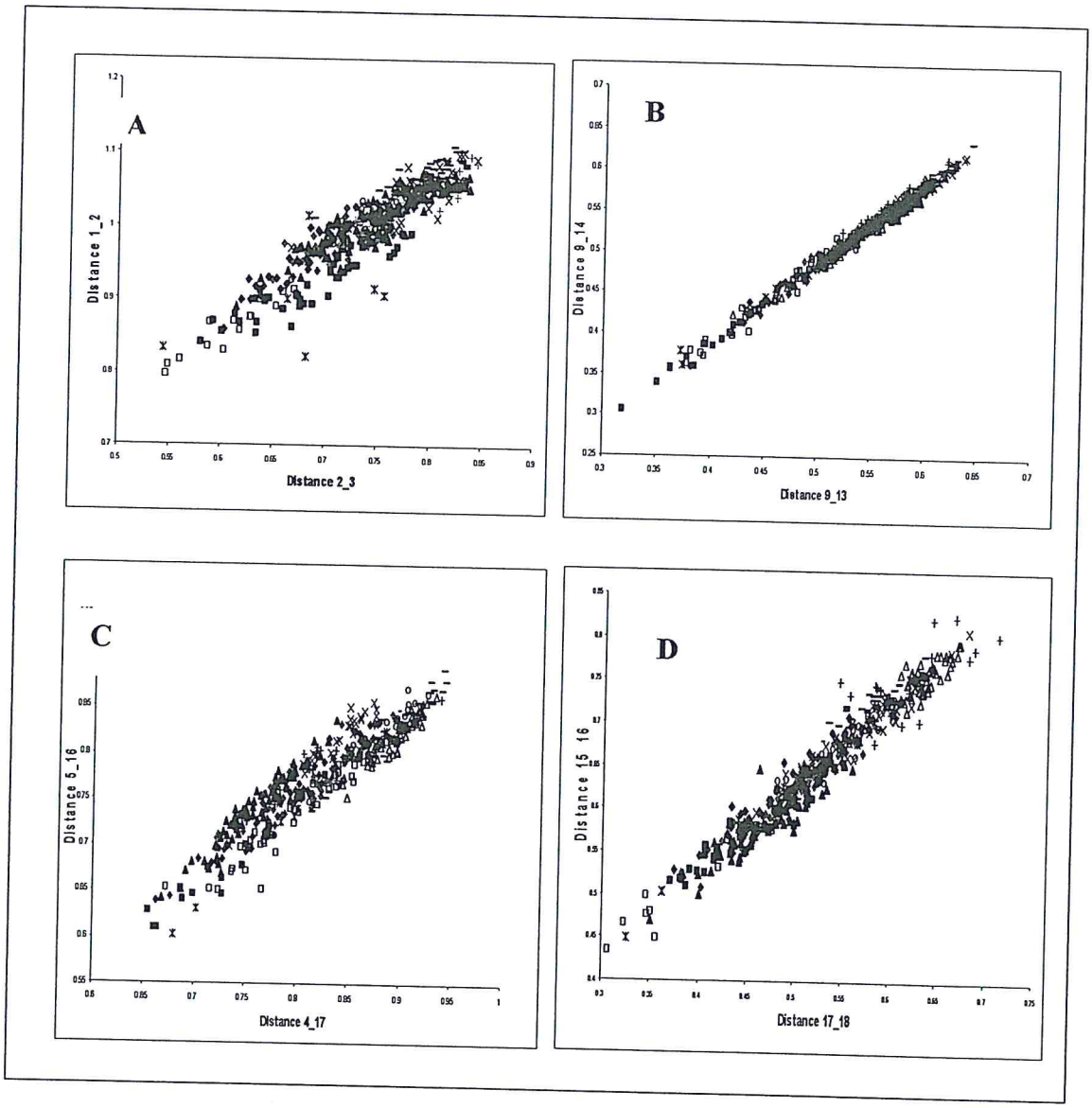
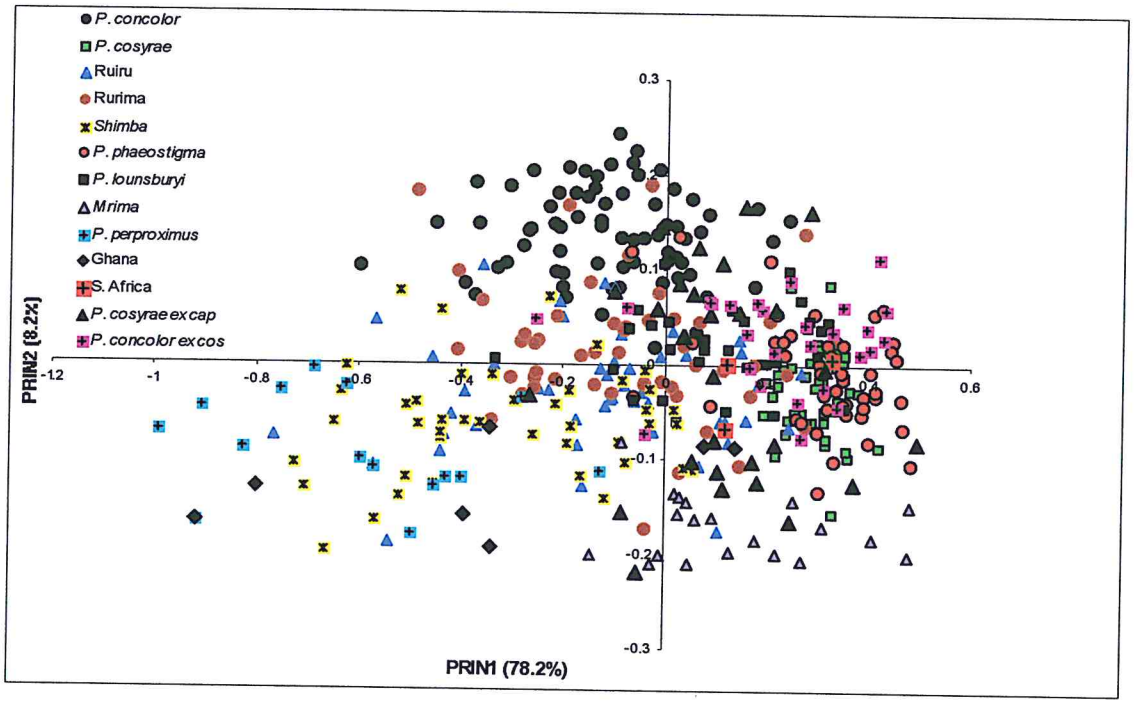


Figure 5.1 Bivariate scatter plots showing the relationships between some wing vein segments. A. = Distances 2_3 (length of stigma) vs. 1_2. B. = Distances 9_13 vs. 9_14. C. = Distances 4_17 vs. 5_16 (lengths of veins between submarginal cell and apical end of wing). D. = Distances 17_18 vs. 15_16 (long sides of the second submarginal cell).

A.



B.

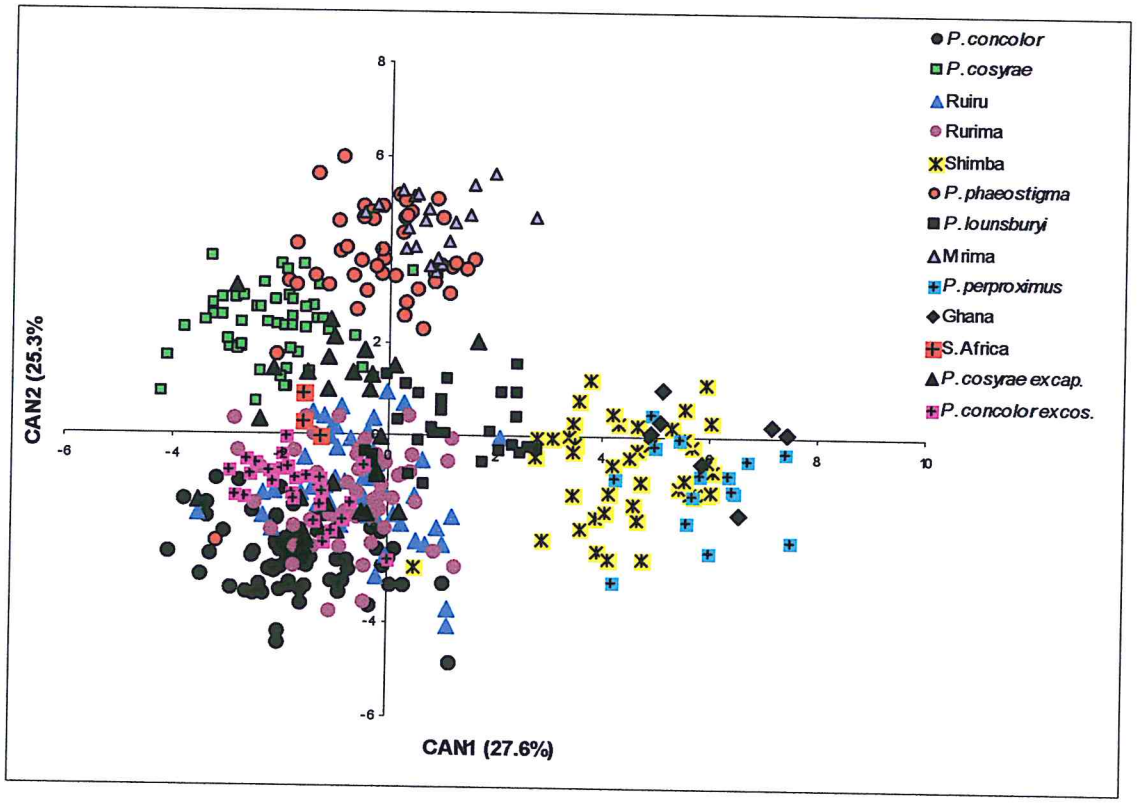
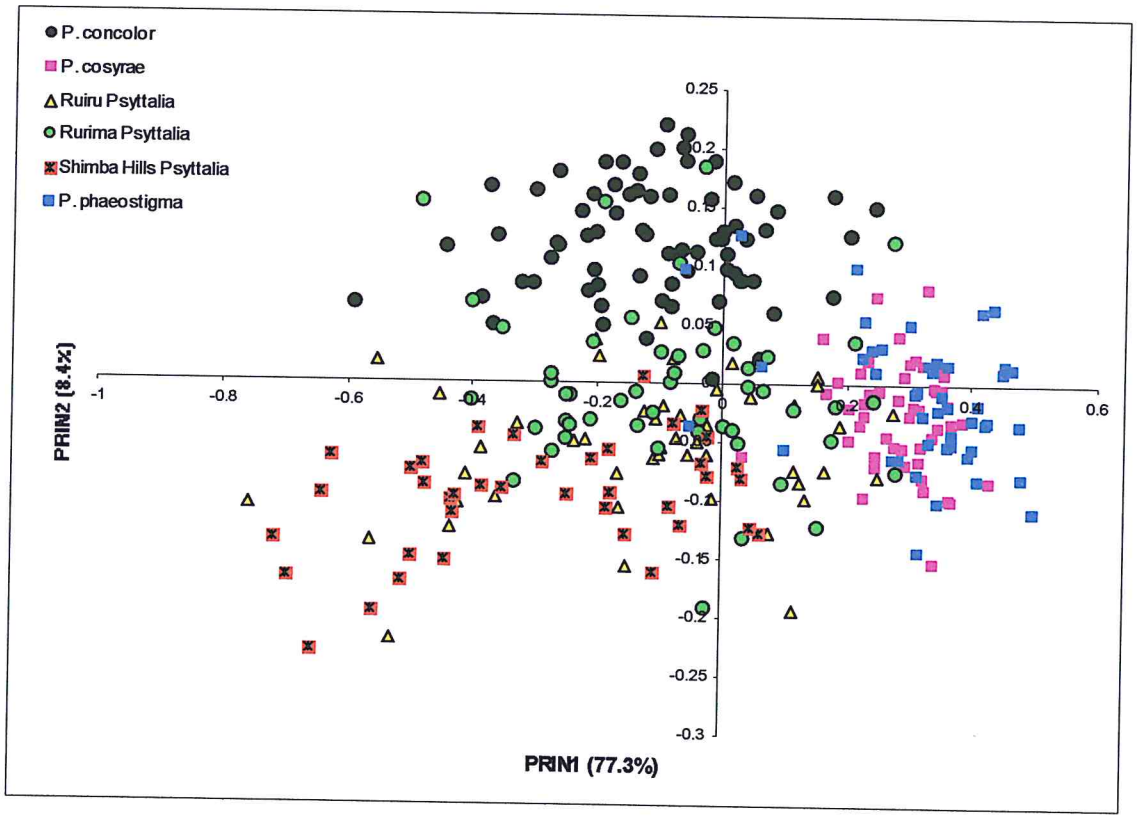


Figure 5.2 Projection of wing dataset from all the *Psyttalia* populations. A. = First two principal components. B. = First two canonical variates. (Proportions of sample variances are in parentheses).

A.



B.

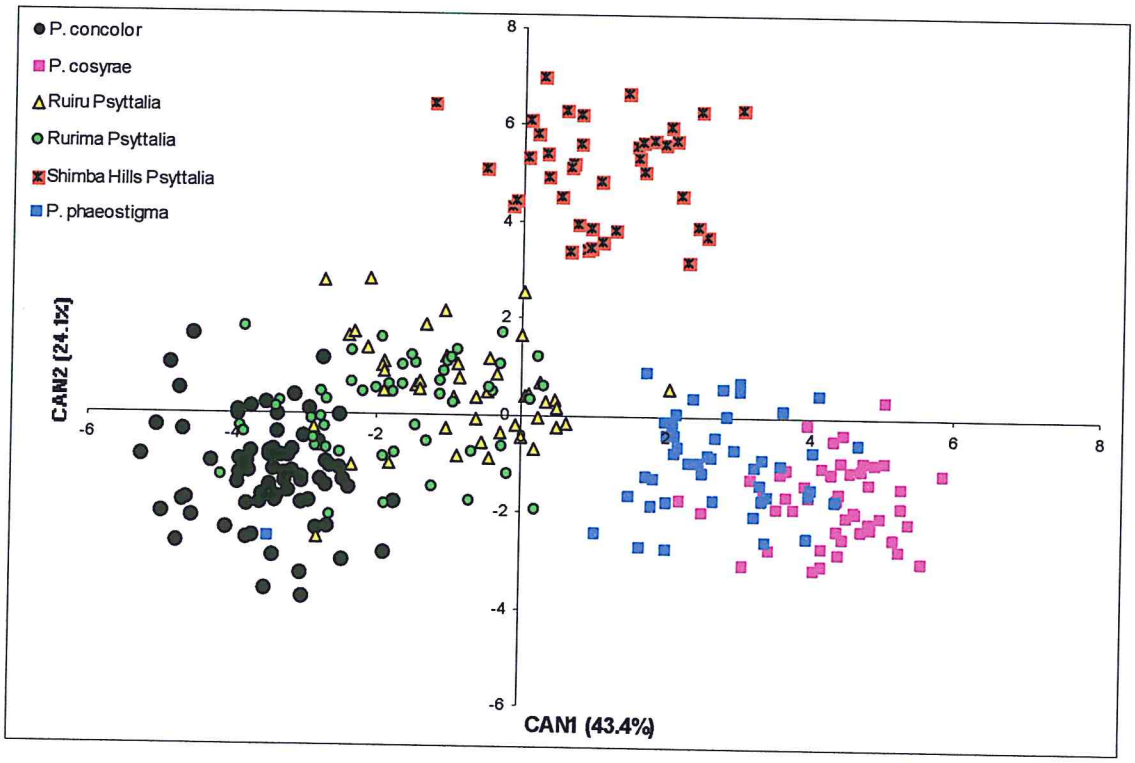


Figure 5.3 Projection of wing data for six *Psyttalia* populations. A. = First two principal components. B. = First two canonical variates.

Table 5.3 Eigenvalues and weights for the first two principal components, computed from the log-transformed wing data of the six *Psyttalia* populations. [Each weight squared represents the proportion of variance that the original variable contributes to that principal component].

	Weight	
Variable	Prin1	Prin2
Proportion of variance (%)	77.3	8.4
Eigenvalues	0.076	0.008
1_2	0.19	0.07
2_3	0.19	0.02
3_4	0.23	-0.29
4_5	0.18	0.02
5_6	0.19	-0.07
6_7	0.20	-0.08
7_8	0.20	0.03
8_12	0.23	-0.04
11_12	0.21	-0.02
10_11	0.19	0.18
9_10	0.19	0.11
9_14	0.18	0.07
12_14	0.17	0.14
10_13	0.19	0.06
9_13	0.19	0.08
14_15	0.11	0.66
15_16	0.25	-0.27
16_17	0.22	0.05
17_18	0.25	-0.20
18_19	0.25	0.41
15_18	0.20	-0.16
9_19	0.15	0.01
4_17	0.21	-0.25
5_16	0.18	-0.09
8_16	0.20	-0.05

Table 5.4 Raw, standardized, and total canonical structure coefficients for canonical variates analysis on the log-transformed wing data of six *Psyttalia* populations.

Variable	CV1 coefficients			CV2 coefficients		
	Raw	Standardized	Total CS	Raw	Standardized	Total CS
1_2*	7.66	0.42	0.47	-61.57	-3.40	-0.70
2_3	5.40	0.30	0.55	2.39	0.13	-0.52
3_4	19.48	1.38	0.82	10.19	0.72	-0.24
4_5	-8.26	-0.44	0.51	-7.45	-0.39	-0.5
5_6	-2.87	-0.17	0.50	-4.40	-0.25	-0.36
6_7	-0.33	-0.02	0.66	-12.96	-0.74	-0.52
7_8	-0.97	-0.06	0.48	6.75	0.41	-0.49
8_12	-5.04	-0.36	0.54	4.76	0.34	-0.41
11_12	-12.80	-0.79	0.50	-13.46	-0.83	-0.56
10_11	5.09	0.32	0.44	-12.50	-0.77	-0.64
9_10	-13.48	-0.75	0.39	11.92	0.67	-0.61
9_14	-28.96	-1.51	0.38	-12.08	-0.63	-0.68
12_14	18.14	1.00	0.37	9.15	0.51	-0.57
10_13	15.09	0.81	0.49	47.90	2.58	-0.62
9_13	10.06	0.54	0.40	-27.32	-1.46	-0.69
14_15	2.42	0.19	-0.06	3.13	0.25	-0.52
15_16	45.09	3.37	0.80	15.05	1.12	-0.39
16_17	-2.29	-0.17	0.36	3.73	0.27	-0.49
17_18	-2.76	-0.20	0.76	0.90	0.07	-0.42
18_19	0.17	0.02	0.23	-5.21	-0.47	-0.78
15_18	-8.91	-0.53	0.52	8.19	0.49	-0.43
9_19	-20.23	-0.91	0.45	26.59	1.20	-0.53
4_17	37.41	2.40	0.80	18.09	1.16	-0.22
5_16	-28.13	-1.46	0.58	7.57	0.39	-0.36
8_16	-18.66	-1.07	0.67	-41.36	-2.38	-0.55

*1_2 = distance between landmark 1 and 2, and so on.

Table 5.5 Mahalanobis squared distances (D^2) between the clusters representing the six populations of *Psyttalia*.

Population	*P. concolor*	*P. cosyrae*	*P. phaeostigma*	Ruiru	Rurima	Shimba
P. concolor	-					
P. cosyrae	69.5	-				
P. phaeostigma	58.0	51.7	-			
Ruiru	23.0	43.4	45.7	-		
Rurima	12.6	49.9	42.6	4.8	-	
Shimba	60.7	64.1	59.5	36.7	38.7	-

5.5.2.1 Subset analyses

For more detailed comparison of populations, subset analyses were performed.

a). Subset 1

This consisted of *P. concolor* and *P. cosyrae*. These are two different valid species whose identities are well known and certain. This comparison was performed to see the difference between them, and the variables responsible for those differences.

Projection of individual specimens on the first and second principal components resulted in the complete separation of the two populations with respect to the first principal component (with *P. concolor* lying to the left side of *P. cosyrae*) (Figure 5.4). The weights for the variables of the first principal component are all positive (Table 5.6), with the first two components accounting for 88.1% (PC 1 = 82.4% and PC 2 = 5.7%) of the total variance. The third, fourth and fifth PCs (2.4%, 2.0% and 1.6%) did not contribute much to a clear segregation of the specimens. The separation is along the first principal axis, thus indicating that the location of points is strongly affected by the overall larger size of the *P. cosyrae* specimens. The wide distribution range of the components (0.03-0.35) also suggests some shape influence in the separation process. The distances with the highest weights are 15_16 (vein 2M), 17_18 (vein 3RSa), and 4_17 (vein 3RSb) (0.35, 0.34 and 0.28), and these contribute the most to the separation of the two populations. The distances 15_16 and 17_18, however, are the two lengths of the second submarginal cell, and are directly attached to the vein segments 14_15 and 18_19, respectively at the anterior end. Segment 12_14 is also attached to 14_15. The decreased lengths of vein segments 12 through 15 (i.e. 12_14 + 14_15) and 18_19, are compensated for by an increase in the lengths of veins 15-16 and 17-18. There is also an increase in the lengths of segments 4_17 and 5_16, which are the segments attached

to the submarginal cell at the posterior end, to the tip of the wing (Table 5.6). These vein segments are those that principally account for the differences between the two populations.

b). Subset 2

This consisted of *P. concolor* and the population from Rurima. This is the first pair of the populations that were in close contact when all the populations were put together (Figure 5.2). These were compared to see the variables responsible for their closeness. Here, projection of the specimens on the first two components resulted in a partial separation of the two populations with respect to the second principal axis (Figure 5.5A). The distribution of individuals in both clusters is similar, with the spread stretching along the first principal axis. This means the two populations have almost the same size range, and cannot be separated on the basis of size. However, their centroids lie on either side of the first principal axis along the second principal component, which suggests a differentiation on the basis of shape. The situation is further complicated by the overlap of the two populations, thus allowing only partial separation on the basis of shape.

The very high negative PC 2 weights observed in variables 3_4 (vein R1a), 15_18 (vein 2RS), 15_16 (vein 2M), and 4_17 (vein 3RSb) (Table 5.7) indicate that these lengths tend to be shorter in *P. concolor*. The same variables in the first principal component have high positive weights and make those lengths longer in the Rurima specimens. For example, for vein 2RS (15_18) which is joined at both ends by veins (RS+M)b (14_15) and vein r (18_19), the shortening of vein 2RS results in a corresponding increase in the lengths of the two attached veins, thereby accounting for the shape differences between the two populations. A similar trend was observed in the comparison of *P. concolor*

with the Ruiru population, which is a member of the first cluster in the all-population plot (Figure 5.5B).

Interestingly, similar trends were observed in the second principal component values when *P. concolor* was compared with populations from Ruiru (Figure 5.5B) and those from Burguret Forest (*P. lounsburyi*) (Figure 5.8A and Table 5.8). These involved the same set of vein variables 3_4, 15_16, 15_18, and 4_17 (with the highest negative weights), and 14_15, 18_19, and 10_11 (with the highest positive weights). However, in the comparison of *P. concolor* with specimens from Shimba Hills, the same two sets of variables were involved, but with the first set (3_4, 15_16, 15_18 and 4_17) having the highest positive weights, and the second set (14_15, 18_19 and 10_11) having the highest negative weights. This means vein segments 3_4, 15_16, 15_18 and 4_17 tend to be longer in *P. concolor* and make the adjoining vein segments (14_15, 18_19 and 10_11) shorter.

c). Subset 3

This consisted of *P. concolor,* populations from *P. concolor* reared on *C. cosyra*, *P. cosyrae* and *P. cosyrae* reared on *C. capitata*. *Ceratitis capitata* and *C. cosyra* larvae are the preferred host larvae used in the laboratory to maintain cultures of *P. concolor* and *P. cosyrae*, respectively. Thus offspring from these larvae are considered as the natural populations of *P. concolor* and *P. cosyrae*. This subset comparison was performed to see the effect of host larvae (habitat) on parasitoid adult size. Projection of the specimens on the first two principal components (Figure 5.6A), showed the *P. concolor* and *P. cosyrae* populations lying at the extreme ends, with the *P. concolor* reared from *C. cosyra* and *P. cosyrae* reared from *C. capitata* populations lying between them. This means that when bigger host larvae (*C. cosyra* = 9.55 ± 0.13 mm^2)

are exposed to *P. concolor*, the resulting progeny attains larger body sizes than members of the original population. The converse is also true for the *P. cosyrae* population, which produced smaller-sized individuals when they were exposed to smaller host larvae (*C. capitata* = 7.31 ± 0.08 mm^2). The picture was clearer when the specimens were projected on the first two canonical variates (Figure 5.6B), with the two "intermediate" populations (from the "unusual" hosts) approaching each other from the ends of their original populations, to an intermediate position.

Results of the Mahalanobis squared distances calculated between their centroids (Table 5.9) showed the longest distance to occur between *P concolor* and *P. cosyrae* (65.3), while comparisons of *P. concolor* reared from *C. cosyra* larvae and *P. cosyrae* reared from *C. capitata* larvae with their respective original populations, showed distance values 31.2 and 30.4, respectively, from the two original populations (and lying in-between the original populations).

d). Subset 4

This consisted of *P. concolor, P. cosyrae,* populations from Rurima, Ruiru, Shimba Hills (all in Kenya), *P. perproximus* (Nkolbisson, Cameroon) and that from Tafo (Ghana). This was performed to compare all populations from coffee (Rurima, Ruiru, Shimba Hills, *P. perproximus* and Tafo) with *P. concolor* and *P. cosyrae*, which are two distinct and valid species, and to assign populations of unknown affinity to groups. According to Marcus (1990), this can be done by computing the Mahalanobis squared distance from the unknown populations to each of the known or pre-determined populations' centroids, and assigning the unknown population to the group with the smallest distance.

Projection of the specimens on the first two principal axes showed partial separation of the populations into three main clusters: (a) the *P. concolor* cluster, (b) the *P. cosyrae* cluster, and (c) the Shimba Hills-Ghana-*P. perproximus* cluster, with populations from Rurima and Ruiru spread between them (Figure 5.7A). The first two components contributed to 87.8 % of the total variance (PC 1 = 78.9 % and PC 2 = 8.9 %). The third, fourth and fifth components contributed 3.0 %, 1.7 % and 1.6 %, respectively which did not improve separation of the populations.

From Figure 5.7A, the clusters were separated as follows: Cluster A (*P. concolor*) was separated along both the first and second principal axes relative to cluster B (*P. cosyrae*). Here *P. cosyrae* (first quadrant) lies above and to the right of *P. concolor* (between the third and fourth quadrants). This means a straight line joining their centroids will be at an angle cutting across both axes, indicating that variance between the two clusters is as a result of both size and shape factors. Cluster A (*P. concolor*) was also separated along the second principal axis relative to cluster C (Shimba Hills-Ghana-*P. perproximus;* hereafter referred to as the *P. perproximus* cluster). The *P. concolor* specimens were spread in both the third and fourth quadrants, while the *P. perproximus* cluster was spread in the first and second quadrants. This means the difference or cause of separation between the two clusters, was mostly based on shape rather than on size factors. The long axes of the two clusters lying on either side of the second axis suggest that the *P. perproximus* cluster has many more individuals with shape influence than individuals of *P. concolor*. Cluster B (*P. cosyrae*) on the other hand, was completely separated from cluster C (*P. perproximus*) along the first principal axis. *Psyttalia cosyrae* was clustered in the first quadrant while specimens of the *P. perproximus* cluster were clustered mainly in the second quadrant, with a slight extension into the first quadrant. Here, size factors are indicated as the cause of

separation of the two clusters. Weights of the first two principal components, canonical structure coefficients and the Mahalanobis squared distances between the clusters are shown in Tables 5.10, 5.11 and 5.12, respectively.

Since the Shimba Hills population was also obtained from coffee and did not cluster with *P. concolor* as the other coffee populations from Kenya (Ruiru and Rurima), they were compared alone with *P. concolor*. *Psyttalia concolor* was also compared alone with *P. lounsburyi*. This comparison was necessitated by the fact that *P. lounsburyi* was obtained from *B. oleae* flies - the very host from which *P. concolor* was originally described. They were, therefore, compared to investigate whether the parasitoids from *B. oleae* were *P. concolor* that had re-established association with its original host. In both comparisons the Shimba Hills population and *P. lounsburyi* separated out from *P. concolor* as different species (Figure 5.8).

5.5.3. Analyses of variance

This was performed using the general linear model (PROC GLM, SAS Institute, Inc., 2001) on the measurements of the ovipositor/hind tibia ratio (OTR) and ovipositor sheath/hind tibia ratio (STR) for two groups: 1) the Shimba Hills (Kenya) population which clustered with the populations from Tafo (Ghana) and *P. perproximus* (Cameroon) (Table 5.13) and 2) the six main representative populations used in the principal component and canonical variate analyses (Table 5.14).

Table 5.14A shows that the means of the OTR were different for each of the populations, except for *P. concolor* and those from Shimba Hills which did not show any significant difference ($F = 136.93$; $df = 5, 172$; $P < 0.0001$). The results showed that *P. cosyrae* had the highest value (3.53) followed by *P. phaeostigma* (2.76), *P. concolor*

(2.53), Shimba Hills (2.47), Rurima (2.35) and Ruiru (2.24) with the smallest value. The STR and OTR for all six populations were significantly different (Table 5.14).

5.5.4. Flagellomere count – In a summarized table of the counts, it was observed that the number of flagellomeres for all the populations ranged from 26-50 (Table 5.15). When plotted against the number of specimens in each population, the specimens separated out into two broad categories; 1) the *P. concolor*-group (consisting of *P. concolor*, *P. perproximus*, *P. lounsburyi*, Rurima, Ruiru, Ghana, Shimba Hills and *P. concolor* reared from *C. cosyra* larvae), and 2) the *P. cosyrae*-group (consisting of *P. cosyrae*, *P. phaeostigma*, Mrima Hill and *P. cosyrae* reared from *C. capitata* larvae) (Figure 5.9).

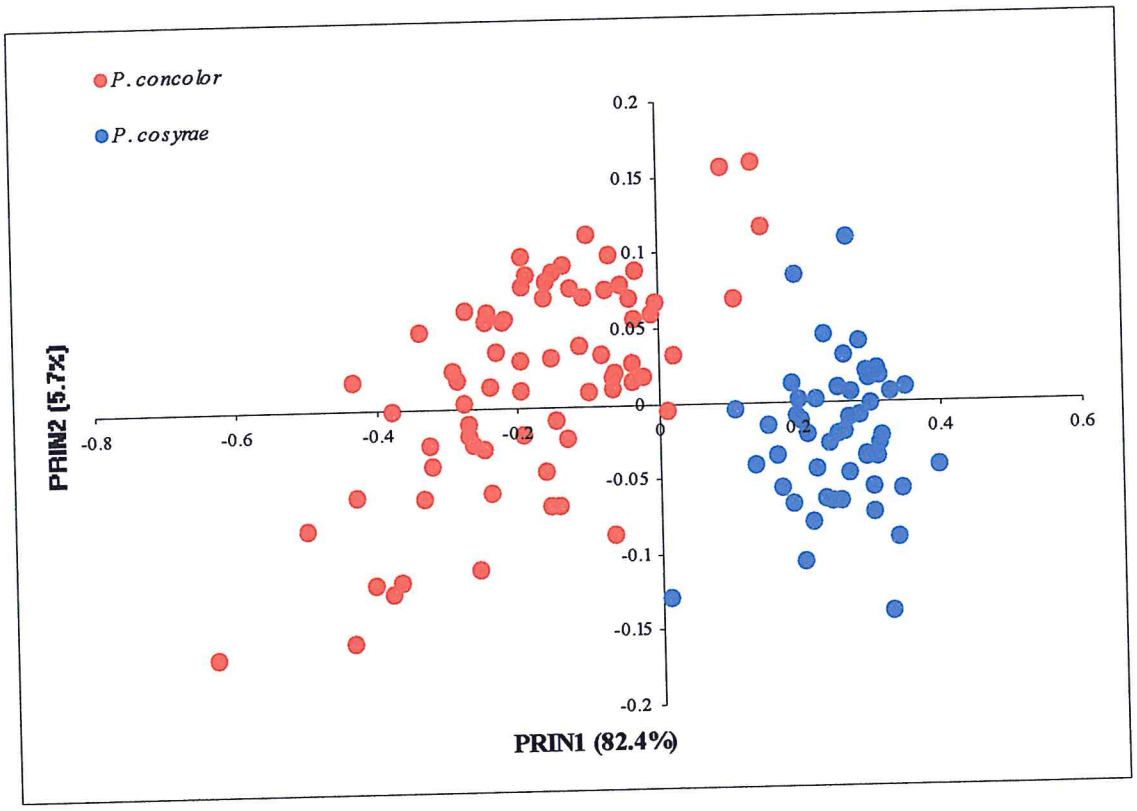


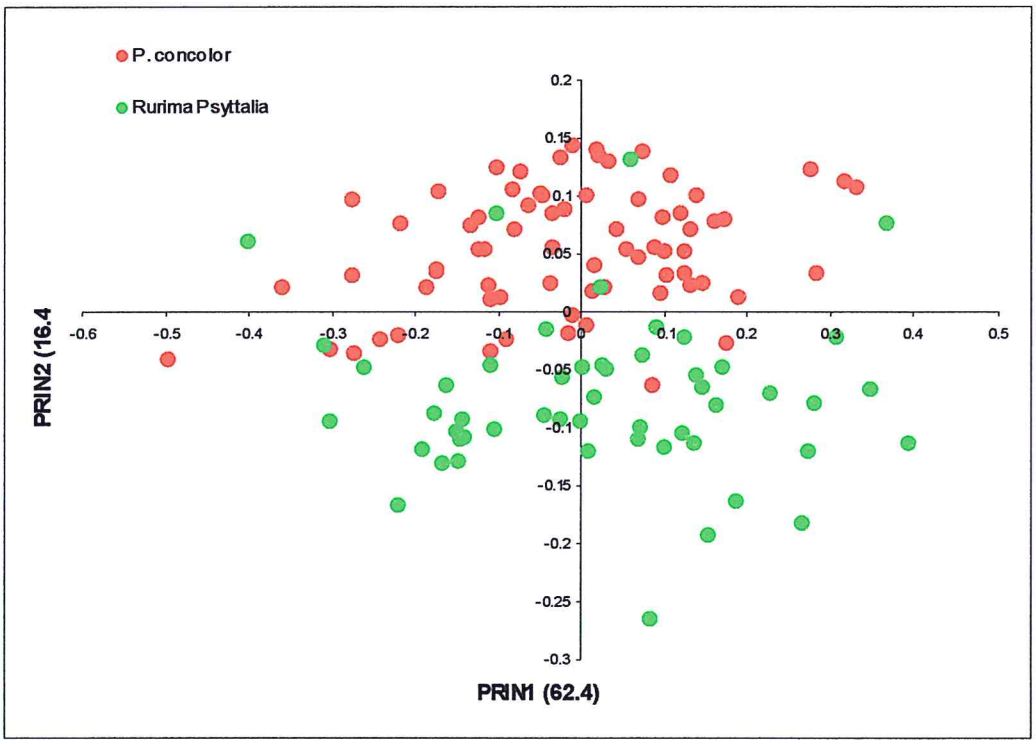
Figure 5.4 Projection of wing data of *Psyttalia concolor* and *P. cosyrae* populations on the first two principal components.

Table 5.6 Weights for the first and second principal components of wing dataset for *Psyttalia concolor* and *P. cosyrae* populations.

Variable*	Weight	
	PC 1	PC 2
Proportion of variance (%)	82.4	5.7
Eigenvalues	0.061	0.004
1_2	0.16	0.04
2_3	0.19	0.05
3_4	0.31	-0.14
4_5	0.17	0.09
5_6	0.18	0.07
6_7	0.23	-0.03
7_8	0.21	0.13
8_12	0.17	0.05
11_12	0.19	0.02
10_11	0.15	0.19
9_10	0.11	0.12
9_14	0.13	0.09
12_14	0.09	0.04
10_13	0.15	0.08
9_13	0.14	0.09
14_15	0.03	0.84
15_16	0.35	-0.26
16_17	0.13	0.18
17_18	0.34	-0.14
18_19	0.09	0.09
15_18	0.22	-0.08
9_19	0.15	0.09
4_17	0.28	-0.09
5_16	0.19	0.08
8_16	0.24	-0.06

* Numbers refer to landmarks; e.g. 1_2 is distance between landmarks 1 and 2.

A.



B.

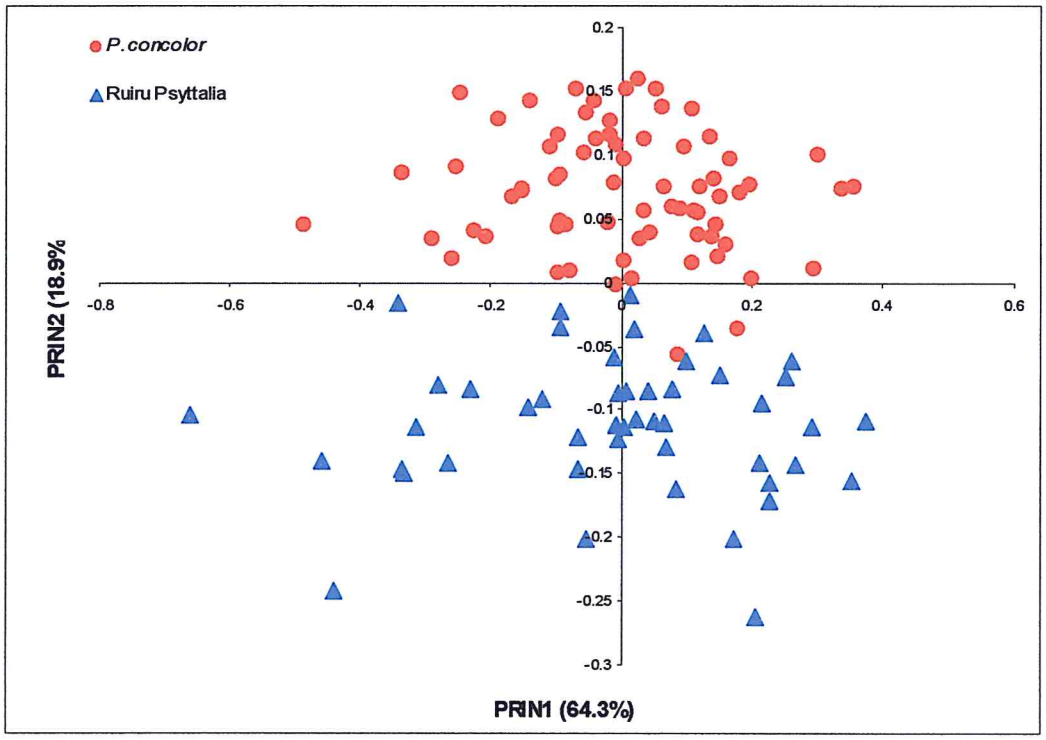
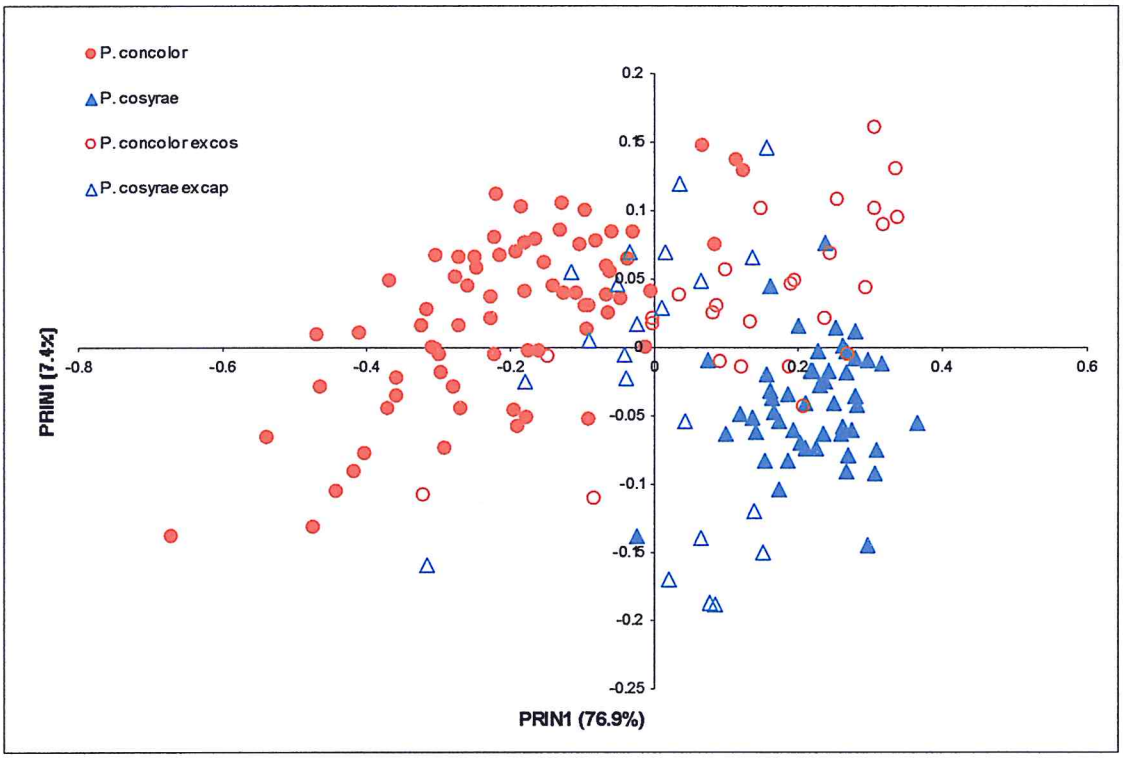


Figure 5.5 Projection of wing data on the first two principal components.
A. = *Psyttalia concolor* and Rurima. B. = *Psyttalia concolor* and Ruiru.

A.



B.

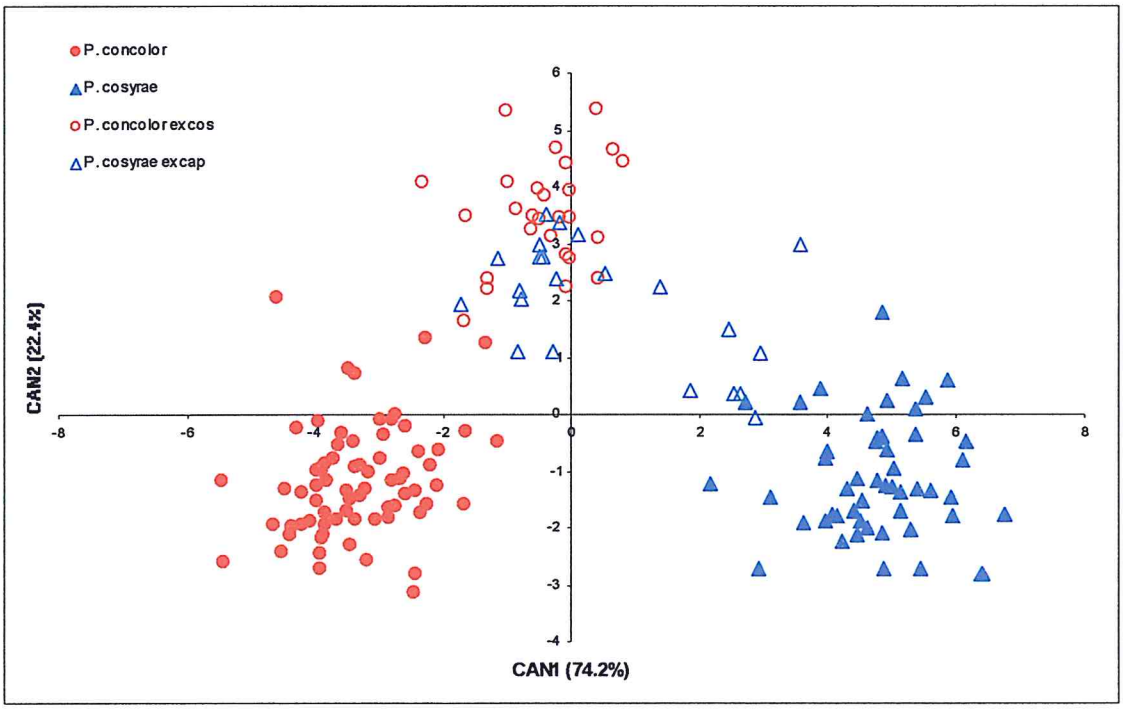
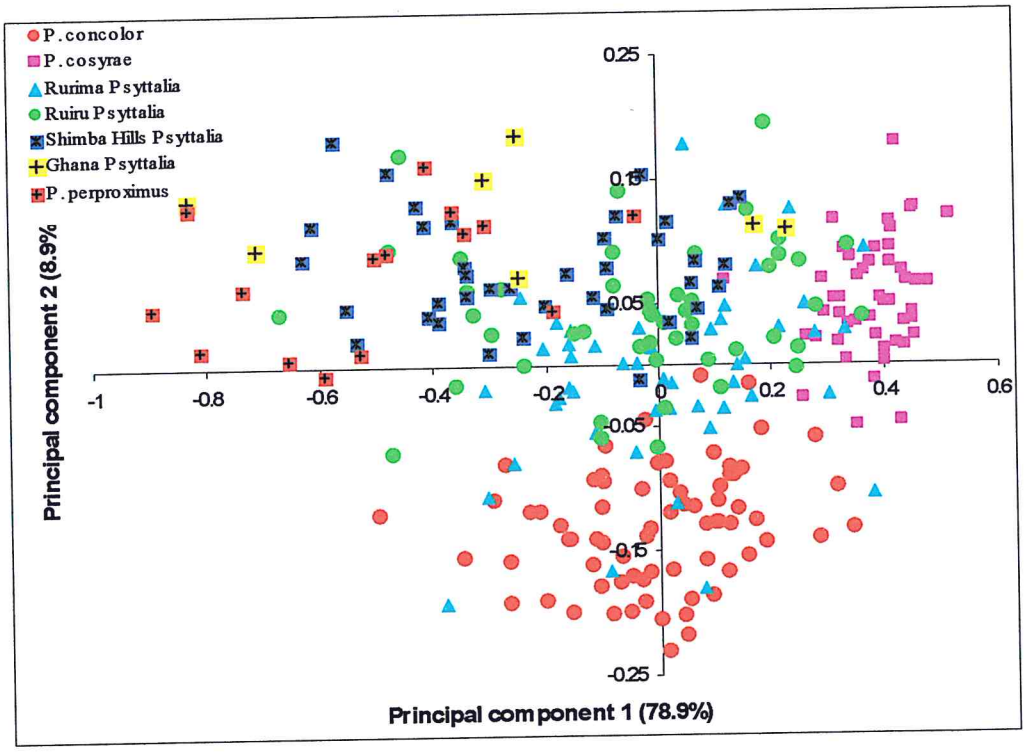


Figure 5.6 Projection of wing data for *Psyttalia* populations reared on different larval hosts (*P. concolor* ex *C. capitata*, *P. concolor* ex *C. cosyra*, *P. cosyrae* ex *C. cosyra*, and *P. cosyrae* ex *C. capitata*). A. = First two principal components. B. = First two canonical variates.

A.



B.

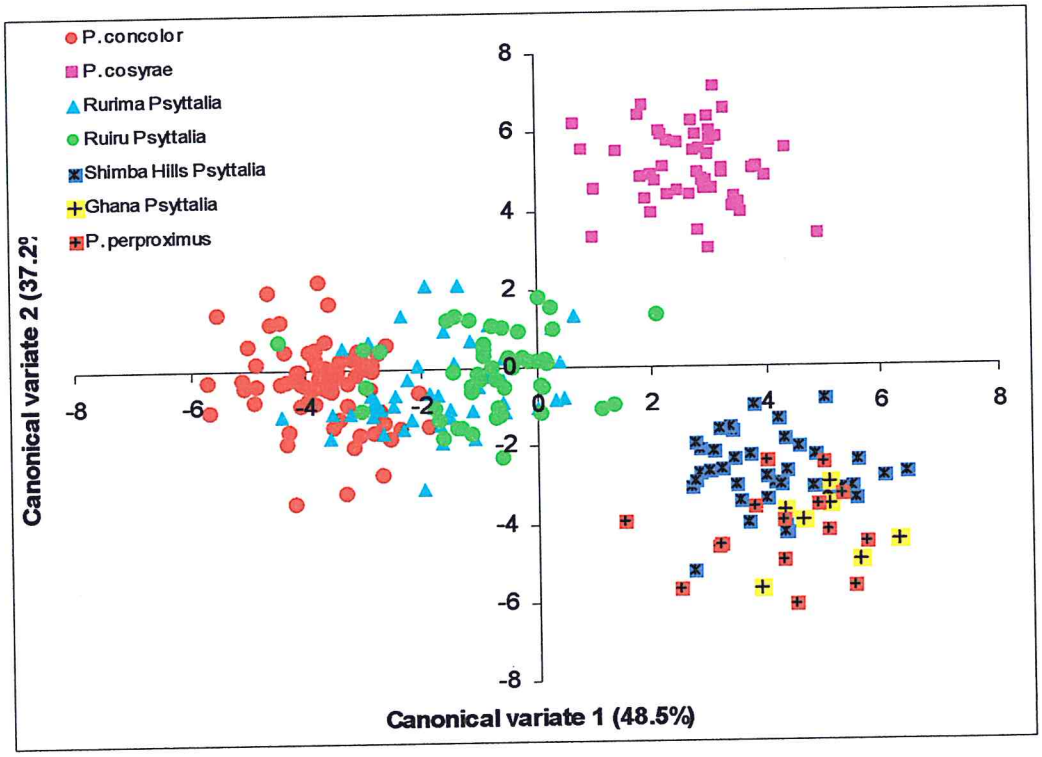
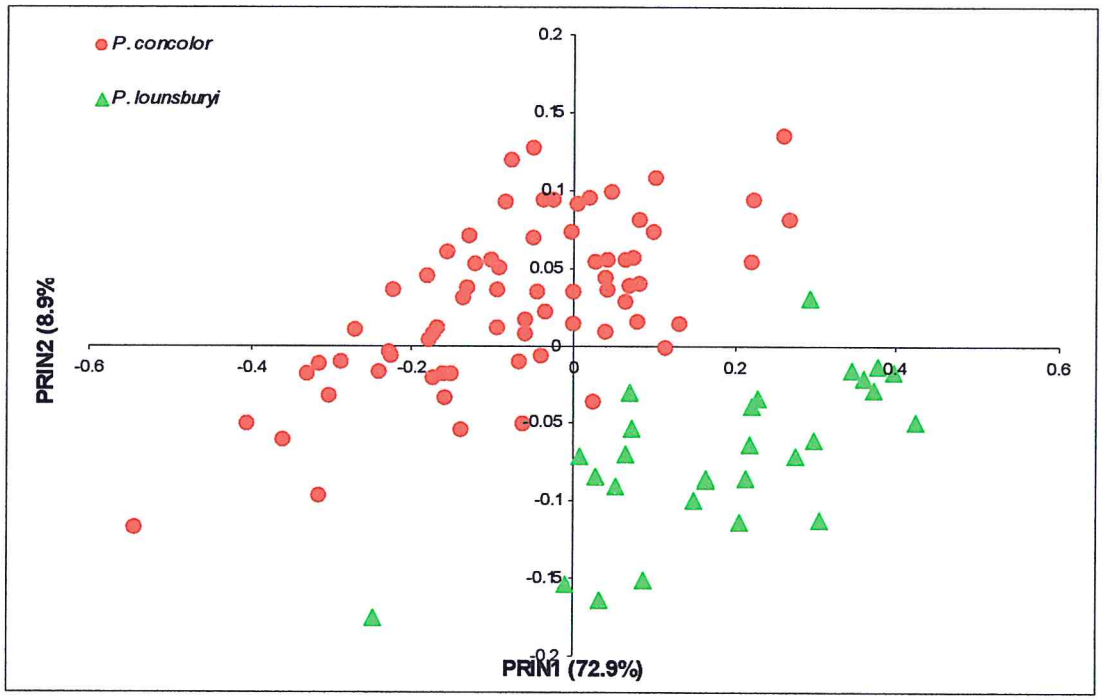


Figure 5.7 Projection of wing data of the five populations of *Psyttalia* from coffee (*P. perproximus* (Cameroon), Ruiru, Rurima, Shimba Hills (Kenya) and Tafo (Ghana)), compared with *Psyttalia concolor* and *P. cosyrae*. A. = First two principal components. B. = First two canonical variates.

A.



B.

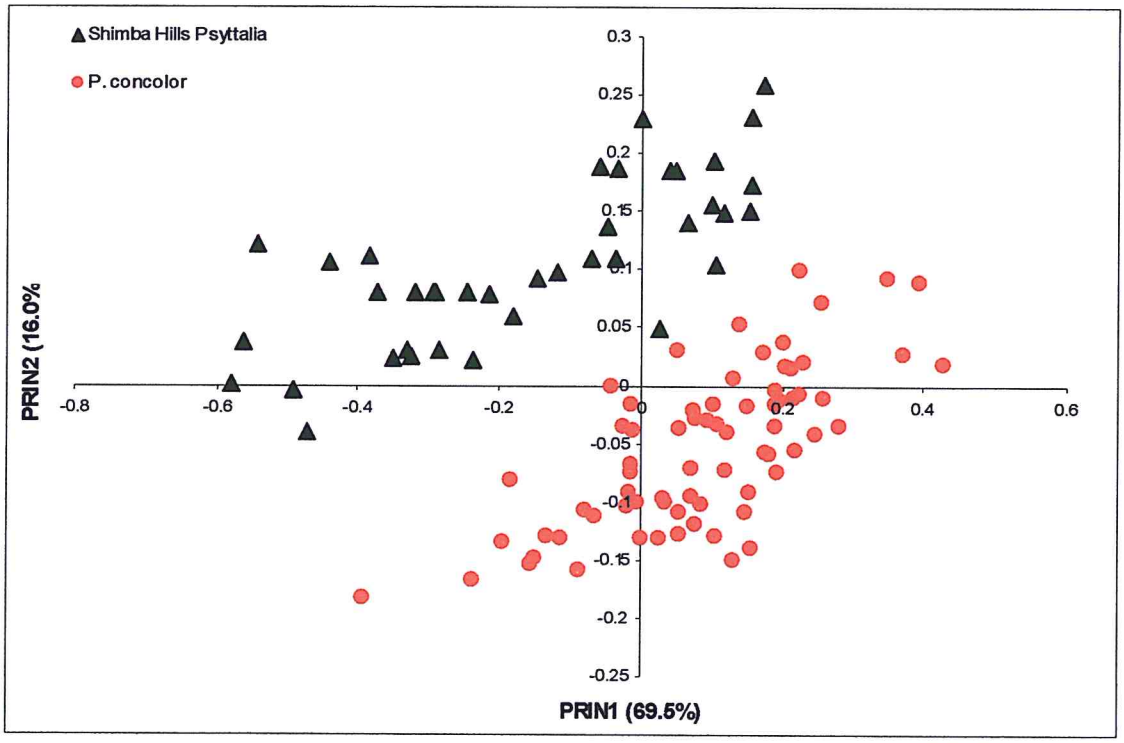


Figure 5.8 Projection of wing data on the first two principal components.
A. = *Psyttalia concolor* and *P. lounsburyi.* B. = *Psyttalia concolor* and Shimba Hills.

Table 5.7 Weights for the first and second principal components of wing dataset for *Psyttalia concolor* and Rurima populations.

Variable*	Weight	
	PC 1	PC 2
Proportion of variance (%)	62.4	16.4
Eigenvalues	0.029	0.008
1_2	0.17	0.03
2_3	0.20	0.03
3_4	0.22	-0.22
4_5	0.20	-0.01
5_6	0.23	0.00
6_7	0.19	-0.06
7_8	0.23	0.12
8_12	0.21	-0.02
11_12	0.23	-0.11
10_11	0.17	0.26
9_10	0.17	0.14
9_14	0.20	0.00
12_14	0.14	0.18
10_13	0.20	0.03
9_13	0.20	0.02
14_15	0.11	0.76
15_16	0.23	-0.21
16_17	0.20	0.04
17_18	0.25	-0.12
18_19	0.12	0.28
15_18	0.24	-0.22
9_19	0.19	0.00
4_17	0.22	-0.18
5_16	0.22	-0.04
8_16	0.19	0.00

Table 5.8 Weights for the first and second principal components of wing dataset for populations from Ruiru, Burguret Forest (*Psyttalia lounsburyi*), and Shimba Hills compared with *Psyttalia concolor*.

Variable	Weight					
	Ruiru		*P. lounsburyi*		Shimba Hills	
	PC1	PC2	PC1	PC2	PC1	PC2
Proportion of variance (%)	64.3	18.9	72.9	8.9	69.5	16.0
Eigenvalues	0.036	0.011	0.038	0.005	0.047	0.011
1_2	0.18	0.02	0.15	0.11	0.21	-0.06
2_3	0.20	0.05	0.16	0.15	0.19	0.09
3_4	0.20	-0.27	0.31	-0.33	0.08	0.34
4_5	0.19	0.00	0.20	-0.04	0.18	0.06
5_6	0.22	-0.09	0.26	-0.12	0.19	0.21
6_7	0.19	-0.11	0.21	-0.04	0.16	0.10
7_8	0.25	0.04	0.22	0.17	0.20	0.06
8_12	0.21	0.02	0.24	-0.12	0.19	0.15
11_12	0.21	-0.07	0.17	0.11	0.21	0.07
10_11	0.19	0.22	0.16	0.18	0.21	-0.10
9_10	0.19	0.12	0.14	0.17	0.22	-0.03
9_14	0.19	0.01	0.13	0.21	0.22	-0.04
12_14	0.16	0.19	0.10	0.20	0.20	-0.05
10_13	0.19	0.04	0.15	0.16	0.20	0.01
9_13	0.19	0.02	0.13	0.22	0.22	-0.06
14_15	0.22	0.69	0.16	0.43	0.32	-0.35
15_16	0.19	-0.29	0.25	-0.18	0.13	0.27
16_17	0.23	0.00	0.16	0.19	0.23	-0.01
17_18	0.21	-0.19	0.26	-0.08	0.16	0.25
18_19	0.18	0.28	0.04	0.42	0.33	-0.55
15_18	0.21	-0.26	0.22	-0.08	0.17	0.16
9_19	0.19	-0.03	0.17	0.08	0.18	0.07
4_17	0.20	-0.20	0.30	-0.32	0.09	0.35
5_16	0.21	-0.11	0.26	-0.11	0.16	0.21
8_16	0.19	-0.06	0.19	0.00	0.17	0.08

Table 5.9 Mahalanobis squared distances (D^2) between clusters of *Psyttalia concolor* and *P. cosyrae*, and their progenies from different host larvae.

Population	*P. concolor*	*P. concolor* ex *C. cosyra*	*P. cosyrae*	*P. cosyrae* ex *C. capitata*
P. concolor	-			
P. concolor ex *C. cosyra*	31.2	-		
P. cosyrae	65.3	49.3	-	
P. cosyrae ex *C. capitata*	28.3	10.6	30.4	-

Table 5.10 Eigenvalues and weights for the first two principal components, computed from the log-transformed wing data of the five populations of *Psyttalia* from coffee (*P. perproximus* (Cameroon), Ruiru, Rurima, Shimba Hills (Kenya) and Tafo (Ghana)), compared with *Psyttalia concolor* and *P. cosyrae*.

Variable	Weight	
	Prin1	Prin2
Proportion of variance (%)	78.9	8.9
Eigenvalues	0.084	0.009
1_2	0.20	-0.07
2_3	0.20	0.00
3_4	0.19	0.32
4_5	0.19	0.00
5_6	0.19	0.09
6_7	0.20	0.10
7_8	0.22	0.00
8_12	0.19	0.04
11_12	0.21	0.02
10_11	0.18	-0.13
9_10	0.18	-0.10
9_14	0.19	-0.07
12_14	0.15	-0.10
10_13	0.19	-0.04
9_13	0.19	-0.08
14_15	0.21	-0.61
15_16	0.24	0.30
16_17	0.20	-0.06
17_18	0.25	0.24
18_19	0.25	-0.44
15_18	0.21	0.15
9_19	0.17	0.01
4_17	0.18	0.27
5_16	0.17	0.11
8_16	0.21	0.08

Table 5.11 Raw, standardized, and total canonical structure coefficients for canonical variates analysis on the log-transformed wing data for the five populations of *Psyttalia* from coffee (*P. perproximus* (Cameroon), Ruiru, Rurima, Shimba Hills (Kenya) and Tafo (Ghana)) together with *Psyttalia concolor* and *P. cosyrae*.

Variable	CV1 coefficients			CV2 coefficients		
	Raw	Standardized	Total CS	Raw	Standardized	Total CS
1_2	-34.14	-2.03	-0.24	55.43	3.29	0.80
2_3	1.59	0.10	-0.06	-7.47	-0.45	0.75
3_4	18.82	1.24	0.40	-26.32	-1.73	0.67
4_5	-15.66	-0.92	-0.10	0.91	0.05	0.73
5_6	0.20	0.01	0.01	-4.92	-0.30	0.56
6_7	-4.48	-0.27	0.05	-2.03	-0.12	0.81
7_8	4.82	0.33	-0.05	-6.08	-0.41	0.70
8_12	-1.43	-0.09	-0.02	-11.99	-0.74	0.60
11_12	-6.93	-0.45	-0.16	4.09	0.27	0.72
10_11	1.51	0.10	-0.16	12.22	0.78	0.72
9_10	0.64	0.04	-0.26	-28.76	-1.58	0.66
9_14	-41.92	-2.37	-0.30	2.13	0.12	0.72
12_14	21.43	1.13	-0.16	-1.44	-0.08	0.58
10_13	28.63	1.59	-0.18	-27.89	-1.55	0.74
9_13	-5.65	-0.33	-0.29	19.49	1.14	0.75
14_15	0.23	0.02	-0.41	2.11	0.20	0.51
15_16	33.60	2.59	0.26	19.01	1.47	0.82
16_17	2.94	0.20	-0.21	-3.49	-0.24	0.56
17_18	4.52	0.36	0.22	9.52	0.75	0.82
18_19	-1.69	-0.16	-0.47	3.68	0.34	0.70
15_18	-11.93	-0.77	-0.04	-11.86	-0.77	0.66
9_19	7.05	0.37	-0.13	-28.15	-1.46	0.69
4_17	52.84	3.25	0.37	27.04	1.67	0.66
5_16	-30.78	-1.62	0.07	-15.89	-0.84	0.63
8_16	-28.47	-1.79	0.05	40.27	2.53	0.84

Table 5.12 Mahalanobis squared distances (D^2) between the clusters representing the five populations of *Psyttalia* from coffee compared with *P. concolor* and *P. cosyrae*.

Population	*P. concolor*	*P. cosyrae*	*P. perproximus*	Ruiru	Rurima	Shimba Hills	Ghana
P. concolor (Italy)	-						
P. cosyrae (Kenya)	71.9	-					
P. perproximus (Cameroon)	80.2	91.6	-				
Ruiru (Kenya)	24.1	48.2	54.4	-			
Rurima (Kenya)	13.3	53.8	56.9	5.1	-		
Shimba Hills (Kenya)	66.9	63.3	9.0	40.5	42.3	-	
Tafo (Ghana)	97.6	96.7	13.1	64.2	65.7	12.3	-

Table 5.13 Comparison of body sizes of individuals of *Psyttalia* populations from Shimba Hills (Kenya), Tafo (Ghana) and *P. perproximus* (Nkolbisson, Cameroon). A. = Mean linear measurements. B. = Mean ovipositor/hind tibia and ovipositor sheath/hind tibia ratio values.

A.

Population	Statistical parameter	Mean length (mm)		
		Ovipositor	Ovipositor sheath	Hind tibia
Shimba Hills		2.26 ± 0.038 b*	1.60 ± 0.028 b	0.91 ± 0.013 a
P. perproximus		2.17 ± 0.073 b	1.53 ± 0.047 b	0.84 ± 0.026 a
Ghana *Psyttalia*		2.52 ± 0.15 a	1.79 ± 0.11 a	0.94 ± 0.05 a
	F	3.68	3.84	2.52
	df	2, 37	2, 37	2, 37
	P	0.0349	0.0306	0.0938

B.

Population	Statistical parameter	Mean ratio (± SE)	
		Ovipositor/tibia	Ovipositor sheath/tibia
Shimba Hills		2.47 ± 0.002 b*	1.75 ± 0.004 b
P. perproximus		2.59 ± 0.003 a	1.83 ± 0.007 a
Ghana *Psyttalia*		2.68 ± 0.004 a	1.91 ± 0.005 a
	F	17.35	12.82
	df	2, 37	2, 37
	P	< 0.0001	< 0.0001

*Means in the same column followed by the same letters are not significantly different (P> 0.05), Student-Newman-Keuls (SNK) test. ANOVA performed on arcsine-transformed proportion values.

Table 5.14 Means for ovipositor/hind tibia and ovipositor sheath/hind tibia ratios, and linear measurements of the six populations used in the principal component and canonical variate analyses.

A.

Population	N	Statistical parameter	Mean ratio (± SE)	
			Ovipositor/Tibia	Ovip.sheath/Tibia
P. concolor	34		2.53 ± 0.004 c*	1.67 ± 0.007 d
P. cosyrae	30		3.53 ± 0.002 a	2.46 ± 0.003 a
P. phaeostigma	30		2.76 ± 0.002 b	2.04 ± 0.003 b
Rurima	26		2.35 ± 0.006 d	1.55 ± 0.009 e
Ruiru	28		2.24 ± 0.009 e	1.48 ± 0.013 f
Shimba	30		2.47 ± 0.002 c	1.75 ± 0.004 c
		F	136.93	138.57
		df	5, 172	5, 172
		P	<0.0001	<0.0001

B.

Population	N	Statistical parameter	Mean length (mm)		
			Ovipositor	Sheath	Tibia
P. concolor	34		2.60 ± 0.038 c	1.72 ± 0.026 c	1.02 ± 0.012 c
P. cosyrae	30		4.83 ± 0.054 a	3.36 ± 0.038 a	1.37 ± 0.013 b
P. phaeostigma	30		4.03 ± 0.049 b	2.99 ± 0.040 b	1.46 ± 0.019 a
Rurima	26		2.48 ± 0.037 c	1.64 ± 0.025 cd	1.05 ± 0.012 c
Ruiru	28		2.28 ± 0.050 d	1.50 ± 0.031 e	1.01 ±0.017 c
Shimba	30		2.26 ± 0.038 d	1.60 ± 0.028 d	0.91 ± 0.013 d
		F	583.83	656.26	230.53
		df	5, 172	5, 172	5, 172
		P	<0.0001	<0.0001	<0.0001

*Means in the same column followed by the same letters are not significantly different (P> 0.05), Student-Newman-Keuls (SNK) test. ANOVA performed on arcsine-transformed proportion values.

Table 5.15 Comparison of the range values of flagellomeres and mean tibia lengths in the *Psyttalia* species/populations.

Population	Range of flagellomeres		Mean tibia length (mm)	Tibia length ranges
	M	F		
P. concolor	31-36	26-33	1.02	0.87-1.17
P. concolor* ex *C. cosyra	**36-38**	**31-34**	**1.20**	**1.06-1.28**
Rurima *Psyttalia*	31-39	28-38	1.05	0.93-1.21
Ruiru *Psyttalia*	31-38	29-37	1.01	0.80-1.15
P. perproximus	30-34	29-*	0.84	0.79-0.92
Ghana *Psyttalia*	32-33	34-38	0.94	0.85-1.09
P. lounsburyi	33-37	32-36	1.05	0.91-1.14
Shimba Hills *Psyttalia*	30-37	31-38	0.91	0.76-1.01
South Africa *Psyttalia*	Lost	Lost	1.10	1.06-1.15
***P. concolor* group values**	**30-39**	**26-38**	**0.84-1.10****	**0.76-1.21****
P. cosyrae	42-47	42-46	1.37	1.17-1.49
P. cosyrae* ex *C. capitata	**42-46**	**40-45**	**1.08**	**1.01-1.20**
Mrima Hill *Psyttalia*	41-47	46-50	1.27	1.14-1.38
P. phaeostigma	40-49	43-50	1.46	1.26-1.71
***P. cosyrae* group values**	**40-49**	**40-50**	**1.27-1.46****	**1.14-1.71****

* Antennae with some lost segments.

** Species-group values (excluding populations from different host larvae).

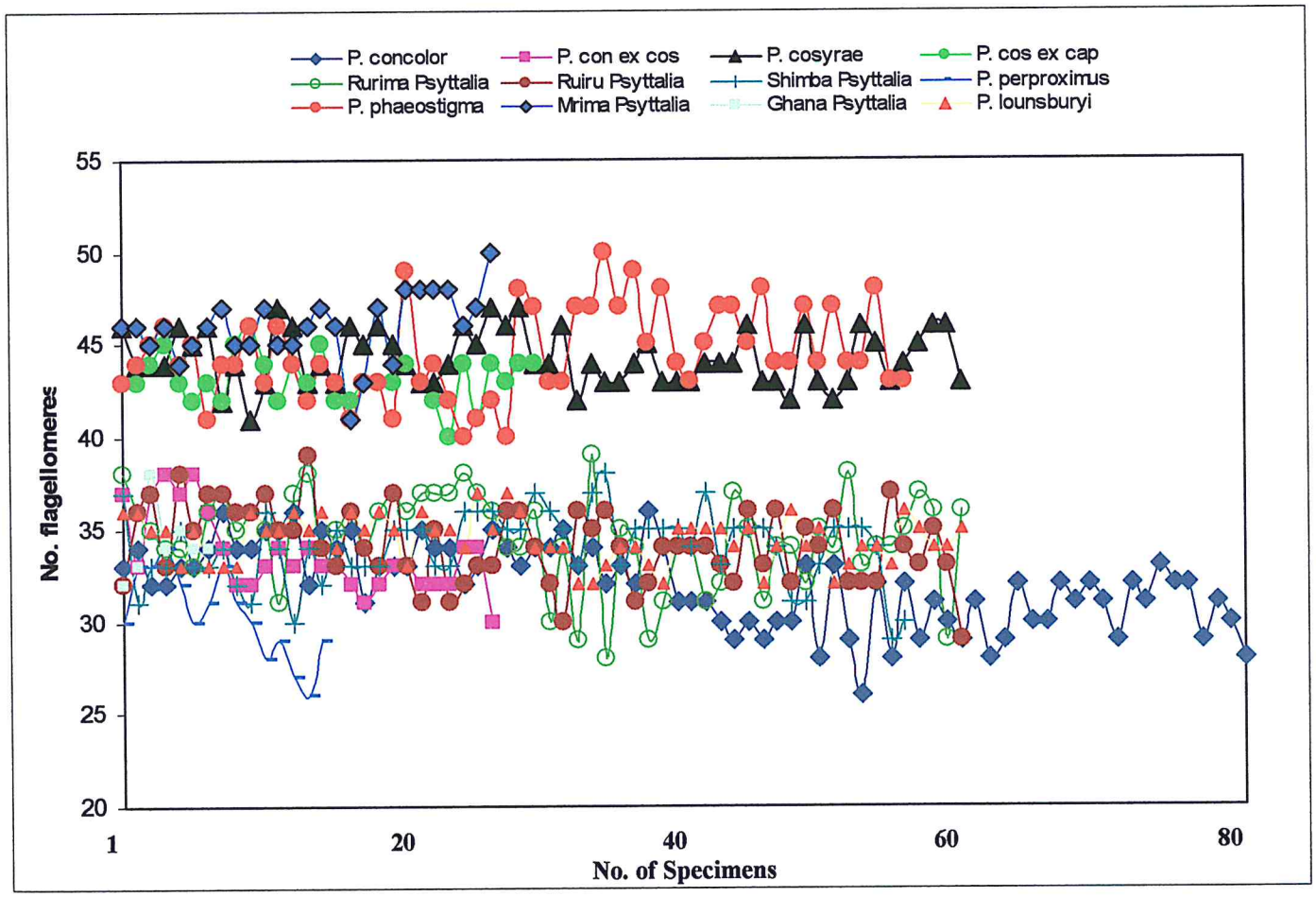


Figure 5.9 A plot of the number of flagellomeres of all *Psyttalia* populations used in the study.

5.6 Discussion

All Populations - The largest Mahalanobis squared distance of 69.5 was recorded between *P. concolor* and *P. cosyrae* followed by *P. concolor* and Shimba Hills (60.7). The smallest distance (4.8) was recorded between the Rurima and Ruiru populations. This is not surprising as both populations come from coffee from the central highlands of Kenya, and they utilize the same type of fly host-larvae resources (*Ceratitis* species). Interestingly, the Shimba Hills population which is also from coffee (but from the coastal lowlands of Kenya and utilizes a different host-larva (*Trirhithrum* species) differs from the Rurima and Ruiru populations by almost the same distance (38.7 and 36.7, respectively). The Rurima and Ruiru populations are also the closest to *P. concolor* (with distances 12.6 and 23.0, respectively), with a slight overlap of the points in both the principal components and canonical variates plots (Figure 5.7). The closeness of the points, which reflects the morphological similarity between the populations, was also noted by Wharton *et al.* (2000) in earlier collections from Kenya. The populations from coffee in central Kenya were subsequently referred to as *P.* cf. *concolor or* the Kenyan *P. concolor* population.

In the work of Kimani-Njogu *et al.* (2001) which aimed at establishing the identity of *Psyttalia* populations attacking *Ceratitis capitata* on coffee in Kenya, morphometric and hybridization studies were conducted to compare populations from Rurima and Ruiru (*P.* cf. *concolor*) with *P. concolor* (Szépligeti) *sensu stricto.* It was concluded from that study that the populations were very close to *P. concolor* and that further studies would be necessary to identify their true identity. The present study which included specimens/populations from both cultivated and wild fruits from the different regions of Kenya, as well as representative samples from the rest of Africa (North,

South, East and West) was, therefore, a follow up or a logical extension of the earlier study. Molecular methods were used in the present study as complementary tool.

Potential problems associated with interpretation of canonical variate coefficients have been discussed by Campbell and Atchley (1981). For the reasons, detailed interpretations of canonical variate coefficients have not been attempted in this study.

Subset 1 - As the relative magnitude of the weights provide an indication of the relative contribution of a variable to the location of a point on the principal component, the results in this case indicate that the distances between landmarks 14_15 (vein {RS+M}b), 18_19 (vein r) and 12_14 (vein m-cu) have the smallest weights (0.03, 0.09, 0.09), and hence contribute the least to the separation of *P. cosyrae* from *P. concolor.*

Among the *P. cosyrae* specimens, the spread or location of points along the horizontal axis (first principal component) is much more restricted than the spread of points for *P. concolor* (Figure 5.4). This means that individuals in the *P. cosyrae* population have a narrower size range (than those in the *P. concolor* population), and changes in shape have very little effect on the overall size of the wing. The oblique distribution pattern of specimens in the *P. concolor* population (across the first three quadrants) is an indication of an interaction between overall size and shape (Humphries *et al.,* 1981; Woolley & Browning, 1987; Heraty & Woolley, 1993). This makes it difficult to separate one factor from the other, but the long axis of the spread is more along the first principal component than along the second; which suggests a higher component of size effects than shape effects in the interaction.

Subset 2 – The projection of points on the first two principal axes showed the Shimba Hills cluster lying a little above, and slightly to the left of the *P. concolor* cluster whilst

P. concolor lies to the left of the Rurima, Ruiru and *P. lounsburyi* populations. There was also a noticeable positive gradient in the distribution of specimens of the two populations (*P. concolor* against Shimba Hills). The oblique orientation of the two clusters to the first and second principal components makes complete isolation of variables associated with either size or shape difficult (see section 5.5.2.1a above). However, some variables were strongly weighted on the first principal component (Table 5.8), suggesting that *P. concolor* does indeed have longer vein segments than specimens from Shimba Hills. This observation is in line with other field and laboratory information gathered on specimens from Shimba Hills (Billah, unpublished data).

Subset 3 – Another interesting observation was that the distance between the two populations from the different host-larvae was also the shortest (10.6), which means that as the parasitoids were exposed to relatively bigger or smaller host larvae, the sizes of their resulting offspring tended to change toward an intermediate size (but not completely changing to sizes of the two original populations). This observation is indirectly in line with the host size/quality hypothesis for solitary parasitoids by Charnov *et al.* (1981) and Charnov (1982), which predicts that as host sizes change, there is a corresponding change in parasitoid sex ratio (and adult fitness - which is normally measured by parasitoid adult size), until a certain critical threshold size is reached, below which only male eggs are laid and above which only female eggs are laid. This is based on the fact that the fitness of the parasitoid, to a large extent, depends on the amount of resource provided by the host larvae (among other biotic and abiotic factors) (Jervis & Kidd, 1996). Though, for various reasons, this relationship may not always be linear (Mackauer, 1986; Sequeira & Mackauer, 1993; Harvey *et al.*, 1994). For reviews of relationships between host size and parasitoid adult size, see Jervis &

Copland, (1996) and Roitberg *et al.* (2001), and also Greenberg *et al.* (1998), Joyce *et al.* (2002) and Ode & Heinz (2002) for relationships between host size and sex ratio.

Subset 4 - Table 5.10 shows that the weights of the first principal component were all positive and they fell within the range 0.15-0.25. This is an indication of the role played by overall size (to various extents) in the separation of the populations. The major contributing variables were vein segments 15_16, 17_18 and 18_19 (with the highest positive values of 0.24, 0.25 and 0.25, respectively), and segments 12_14, 9_19 and 5-16 (with the lowest positive values of 0.15, 0.17 and 0.17, respectively). Segments 15_16 (vein 2M) and 17_18 (vein 3RSa) are the two lengths of the second submarginal cell, while 18_19 is the cross vein r, which joins the second submarginal cell to the stigma at the anterior end.

Segment 12_14 (vein m-cu) is attached to a short inter-connecting vein (RS+M)b, which links it to the lower anterior part of the second submarginal cell. Segment 9_19 is the straight line distance from the point of attachment of the cross vein r on the stigma to the meeting point of veins1RS and 1 M, while 5-16 (vein 3M) is the distance from the lower apical point of the second submarginal cell to the tip of the forewing.

This observation is explained by the fact that as the cross vein r (18_19) becomes longer, it attaches further down onto the stigma and subsequently decreases the distance between it and landmark 9 (i.e. 9_19). The same effect is observed for segment 15_16 (vein 2M), which increases in length and results in the shortening of segment 5_16 (vein 3M). From the foregone, it is again realized that most of the changes accounting for the separation of the populations were centered on the second submarginal cell and the veins attached to it.

On projection of the points onto the first two canonical axes, the three clusters were distinctly separated with no overlaps (Figure 5.7B). Populations from Rurima and Ruiru which were spread between the other clusters in the principal component plot (Figure 5.7A), are now joined with *P. concolor* to form one big cluster. The first two canonical variates contributed a total of 85.7 % (CV 1 = 48.5 % and CV 2 = 37.2 %) to the total variance, with the third, fourth and fifth variates contributing 10.5 %, 1.8 % and 1.2 %, respectively. Table 5.11 shows the raw, standardized, and total canonical structure coefficients for the canonical variate analysis of the seven populations under study.

The strong positive standardized coefficient scores for the first canonical variate for variables 12_14, 3_4, 10_13, 15_16 and 4_17 (1.13, 1.24, 1.59, 2.59 and 3.25, respectively) indicate that members of the *P. cosyrae* and *P. perproximus* clusters tend to have relatively longer forewing segments in the following variables; 12_14 (medio-cubital cross-vein), 3_4, 10_13, 15_16 (vein 2M) and 4_17 (vein 3RSb). Length 10_13 measures the diagonal distance of the first discal cell, from landmark 10 to the mid point of the medio-cubital cross-vein, m-cu (12_14). The increment in length is an indication of the extent of curvature of vein m-cu. A strong curvature of m-cu to the right (convex) means the vein bulges outwards and increases in length, and subsequently increases the diagonal distance of the discal cell.

On the other hand, the strong negative score for variables 5_16, 6_18, 1_2 and 9_14 (-1.62, -1.79, -2.03 and -2.37, respectively) on the first canonical variate indicate that members of the *P. concolor*-Rurima-Ruiru cluster, especially individuals of *P. concolor* (which lie furthest in the negative range of the canonical variate plot), have longer segments of variables 9_14 {vein (RS+M)a}, 1_2 (vein C+Sc+R), 8_16 and 5_16 (vein 3M).

Results of the Mahalanobis squared distances calculated between the centroids of the clusters (Table 5.12) showed the largest distance of $D^2 = 97.6$ between the centroids of populations from Ghana and *P. concolor*. This was followed by *P. perproximus* ($D^2 = 80.2$), *P. cosyrae* ($D^2 = 71.9$), and Shimba Hills ($D^2 = 66.9$) from *P. concolor*, respectively. However, on the general pattern of the projections, the populations from Ghana, Shimba Hills and *P. perproximus* were projected together as a single cluster, while *P. cosyrae* formed a cluster of its own. The distances between the centroids of the populations of the *P. perproximus*-cluster were as follows; *P. perproximus sensu stricto*-Shimba Hills, 9.0 < Shimba Hills-Ghana, 12.3 < *P. perproximus sensu stricto*-Ghana, 13.1. On the other hand, the distances between the populations comprising the *P. concolor*-cluster were as follows; Ruiru-Rurima, 5.1 < *P. concolor s. s.*-Rurima, 13.3 < *P. concolor s. s.*-Ruiru, 24.1. Relative to *P. concolor*, the distances of the populations in the other clusters were as follows; Shimba, 66.9 < *P. cosyrae*, 71.9 < *P. perproximus*, 80.2 < Ghana, 97.6.

From the foregone comparison, the population from Shimba Hills (of unknown identity) has the shortest Mahalanobis distance (D^2) of 9.0, and it is the closest to *P. perproximus,* which can therefore be assigned to that population group (Marcus, 1990).

Identity of the Shimba Hills population - Individuals of this population and those of *P. perproximus* have similar dark markings on the abdominal tergites (males and females), and the patterns of these markings are almost the same (bearing in mind that body colourations are subjective characters). Both populations are found in coffee, and their preferred host-larvae are *Trirhithrum* species (Steck *et al.*, 1986; Billah, unpublished data). Their body sizes are also about the same, though some differences are observed in their ratio values.

A closer look at Table 5.13A shows no difference in the linear body measurements between the Shimba Hills population and *P. perproximus*. Even with the population from Ghana, the two differ only in the ovipositor and sheath lengths while their hind tibia measurements show no differences ($F = 2.52$; $df = 2, 37$; $P = 0.0938$). Comparison of the ratio values showed no difference between *P. perproximus* and those from Ghana, but the two on one hand (i.e. *P. perproximus* and Ghana), differed from Shimba Hills in both OTR and STR values (OTR: $F = 17.35$; $df = 2, 37$; $P < 0.0001$, STR: $F = 12.82$; $df = 2, 37$; $P < 0.0001$). The above presents a relationship in which *P. perproximus* seems to lie in the middle of the cluster, and shares commonalities with both the Shimba Hills and Ghana populations.

The two populations from Cameroon and Ghana have on different occasions been identified as *P. perproximus* by Steck *et al.* (1986) and by R. A. Wharton (from samples sent by M. K. Billah for identification). There is, therefore, a strong inclination that the population from Shimba Hills belongs to *P. perproximus* or is very closely related to it, but further studies are needed to confirm this.

The results also eliminate the possibility that the Shimba Hills population might be part of the *P. concolor* group which had changed morphologically as a result of its use of a different host-larva (*Trirhithrum* species). Moreover, the Mahalanobis squared distance between it and the *P. concolor* cluster is one of the largest values ($D^2 = 60.7$) and lies in the range of the other species/populations that are known to be completely different from *P. concolor*.

Up to date, the literature of fruit fly parasitoids shows no valid assertion of *P. perproximus* from Kenya. It was, however, mentioned among the collections of early explorers to Africa (Bianchi & Krauss, 1937) which were shipped to Hawaii for

biological control purposes. In their review of the 42 species of opiine braconids previously collected (up to 1980) in biological control programmes against *Ceratitis* and *Dacus sensu latum* species, Wharton and Gilstrap (1983) mentioned that some of the material introduced to Hawaii from East Africa under the names of *P. perproximus* and *P. phaeostigma* in Bianchi and Krauss (1937) and Clausen *et al.* (1965), were actually *P. cosyrae,* and indeed, most of it may have been.

The results in this study show that the Shimba Hills population belongs to *P. perproximus*, and it is suggested as the first true occurrence record of the species in Kenya and East Africa.

Analyses of variance - These analyses showed that *P. cosyrae* had the highest mean ovipositor/tibia ratio (OTR) value (3.53) > *P. phaeostigma* (2.76) > *P. concolor* (2.53) > Shimba Hills (2.47) > Rurima (2.35) > Ruiru (2.24).

The same trend was found in the work of Kimani-Njogu *et al.* (2001) in which the comparison was between four populations (*P. concolor, P. phaeostigma*, Rurima and Ruiru). *Psyttalia phaeostigma* had the highest ovipositor/hind tibia ratio (2.79) > *P. concolor* (2.46) > Rurima (2.43), and Ruiru (2.31) which has the shortest OTR. In that work, no significant difference was found between the OTRs of the *P. concolor* and Rurima populations. The apparent differences found in the OTRs in these two studies could probably be due to the techniques used; while a series of small straight lines were used to measure the ovipositor curvature in the previous study, measurement in this study used a technique that allowed tracing the curvature of the ovipositor and the length automatically recorded.

Flagellomere count – The categorization of the parasitoid populations into two groups, the *P. concolor* and *P. cosyrae* groups, based on the number of flagellomeres

coincidentally supports the initial grouping of the species/populations (based on size) before the study where *Psyttalia cosyrae, P. phaeostigma* and the population from Mrima Hill, were added to the study to be used as a check for ovipositor and ovipositor sheath length comparisons.

All members of the *P. concolor*-group had a cut-off point of thirty-nine flagellomeres (≤ 39), while those in the *P. cosyrae*-group had over forty flagellomeres (≥ 40). When *P. concolor* and *P. cosyrae* individuals were reared on relatively bigger and smaller host larvae respectively, the offspring from both species attained larger and smaller body sizes, respectively. Interestingly, the number flagellomeres in these two offspring groups fell consistently within the range values of their parental categories. Ratio values of the body parts, however, showed significant differences in *P. cosyrae* and not in *P. concolor*, indicating a consistent gain in the overall body size of *P. concolor*. This somehow confirms the use of ratios as representative standard measures of size (Sneath & Sokal, 1973).

Generally, separation of the populations based on morphometric analyses of the wing measurements was possible. When all the populations were analyzed together, the separations were not distinct; however when the number of populations was reduced, the clustering became clearer and even better when individual populations were compared. The reduced number of populations resulted in three main clusters: 1) the *P. concolor*-cluster (*P. concolor*, Rurima and Ruiru populations), with populations from Rurima and Ruiru very close to each other and always in close association with *P. concolor*, 2) the *P. perproximus*-cluster (Shimba Hills, Ghana and *P. perproximus*), and 3) the *P. cosyrae*-cluster (*P. cosyrae* and *P. phaeostigma*).

The lack of complete separation between *P. concolor* and the Kenyan central highland populations, even with canonical variate analyses, indicates that the observed morphological similarity between the three populations is a reality. This was one of the main reasons why the populations from the central highlands of Kenya have been referred to as *Psyttalia* cf. *concolor* (Kimani-Njogu *et al.*, 2001). The clusters also indirectly reflect separation of the populations based on host-larvae utilized – members of the *P. concolor* cluster were all reared from *Ceratitis* species (*C. capitata* = 7.31 ± 0.08 mm^2), while members of the *P. perproximus* cluster utilize hosts from *Trirhithrum* species (4.94 ± 0.07 mm^2). The members of the *P. cosyrae* cluster were either from the *Ceratitis* group of a different size and species (i.e. *C. cosyra* = 9.55 ± 0.18 mm^2) or from the *Dacus* species (*D. ciliatus*: = 12.94 ± 0.25 mm^2).

In this study, the ovipositor sheath/hind tibia ratio (STR) was also used, and the means were found to be significantly different for all six populations ($F = 138.57$; $df = 5, 172$; $P < 0.0001$). This means that it was better able to discriminate between the six populations, compared to the ovipositor/hind tibia ratio (OTR) which was able to distinguish between four of the six populations. The STR was therefore, a better discriminatory feature in this case, and can be used with/or in place of the OTR for the following reasons; 1) the ovipositor sheath is relatively easy to dissect out (compared with dissecting out the ovipositor); 2) it is usually distinctly coloured from the rest of the abdomen, and has the added advantage of offering no ambiguity about its length, and therefore, minimizes the chances of cutting it off at the wrong joint; 3) retraction upon death is minimal as compared to the ovipositor; and 4) can easily be straightened on slide for mounting more than the ovipositor. This further confirms the suggestion by Wharton and Gilstrap (1983) that the ovipositor sheath length may be an important

species level discriminant in this group. In their study, Wharton and Gilstrap (1983) divided the sheath length with the thoracic length to obtain ratios for interspecific comparison, which was able to distinguish three groups as follows; 1) *P. concolor-P. humilis* - with a ratio of 1.2-1.4, 2) *P. perproximus-P. phaeostigma* – with a ratio of 1.6-1.9, and 3) *P. cosyrae* – with a ratio of 2.0-2.2.

While use of flagellomeres, wing measurements and ovipositor ratios were all able to separate the populations to various extents, the careful use of these morphological tools together with host-habitat association, cross mating studies and the use of other taxonomic tools (such as molecular studies) would add up valuable information; and serve as the requisite taxonomic cocktail for a more reliable and accurate separation of species in this genus.

The major question is whether the difference between *P. concolor* and the central Kenya populations is one of species rank. The results here do not answer this question, but provide more information that can be used together with results from the other studies (cross mating and molecular) to help clarify their status.

CHAPTER SIX

6.0 CROSS MATING AND MATING BEHAVIOUR STUDIES

6.1 Introduction

Cross mating experiments have been particularly common in the parasitic Hymenoptera group because laboratory cultures of these wasps are often maintained during biological control projects. These experiments serve as a frequent source of data for taxonomic decisions at the species level (Rosen, 1986; Pinto & Stouthamer, 1994), and also for making important biological control decisions (Pinto *et al.*, 1986; Stouthamer *et al.*, 2000). For example, decisions on species in groups such as the Eulophidae, Aphelinidae, Trichogrammatidae, and aphidiine Braconidae have often utilized cross mating data (Grissell & Schauff, 1997; Stouthamer *et al.*, 2000).

Many factors contribute to variation within species. These include the use of different host species (Janssen, 1989), host size (Salt, 1941; Charnov *et al.*, 1981; Charnov, 1982), host age or quality (King, 1987; Godfray, 1994), host condition and diet (Vinson & Iwantsch, 1980; Wajnberg *et al.*, 1990), environmental (Phillips *et al.*, 1993) as well as genetic factors (Diehl & Bush, 1984). According to Grissell and Schauff (1997), these factors are further complicated by the limited extent to which the degree of morphological and biological plasticity inherent in single species is understood. When an insect has a wide geographic distribution, intraspecific variability might be expected (Diehl & Bush, 1984), and the importance of intraspecific variability of hymenopterous parasitoids in the biological control of insect pests has long been recognized (Hopper *et al.*, 1993). Since some species of *Psyttalia* are widely distributed on the African continent and elsewhere, comparative studies between these populations would help to reveal intraspecific variations which, if any, could be considered during the introduction

or conservation of parasitoids for biological control. Furthermore, the utility of any species as a biological control agent is limited by the difficulty in ascertaining their clear taxonomic identity (Stouthamer *et al.*, 2000) as well as a sound knowledge of their ecology, behaviour and genetics (Claridge, 1991; Hopper *et al.*, 1993). Experience in classical biological control has shown that selection of the right strains/populations for introductions is often of crucial importance for the successes of such programmes (van den Bosch *et al.*, 1979; Debach & Rosen, 1991). For these reasons, comparisons within or between populations of parasitoid species collected from different geographic regions or host-habitats have often been made for sources of variations (Hopper *et al.*, 1993). For example, strain-specific variations in parasitization success between populations of *Cotesia congregata* (Say) (Hymenoptera: Braconidae), and *Asobara tabida* (Nees) (Hymenoptera: Braconidae) have been considered in the utilization of these parasitoids (Potting *et al.*, 1997), though *A. tabida* has never been used in biological control because it does not attack pest insects (R. A. Wharton, personal communication).

Psyttalia species have been used in several classical and augmentative biological control programmes (Willard & Mason, 1937; Clausen *et al.*, 1965; Greathead, 1976; Wharton, 1989a, b). However, many of them continue to receive attention as a result of the taxonomic problems associated with them. Dominant among the species are a series of closely related species from Africa that have been distinguished by subtle differences in the length of the ovipositor and the size of the eye (Silvestri, 1914; Wharton & Gilstrap, 1983). These species appear identical to the common Mediterranean species *Psyttalia concolor* (Szépligeti) and the South African species *P. humilis* Silvestri. Additionally, several undescribed species of opiine parasitoids have been reared from Afrotropical tephritids during the past 15 years (Wharton *et al.*, 2000; Kimani-Njogu *et*

al., 2001), some of which may be the same species that were incompletely identified during explorations for medfly parasitoids in the 1930s (Gilstrap & Hart, 1987). The paucity of character states of use to taxonomists, frequent occurrence of convergent and parallel evolution as well as character reversal, have been mentioned as some of the common reasons for the difficulty in Hymenoptera taxonomy (Gauld, 1986).

This work, therefore, attempts to address the confusing identity problems of morphologically similar members of the genus *Psyttalia*, beginning with an assessment of mating compatibility using the biological species concept (BSC).

6.2 Materials and methods

6.2.1 Parasitoid populations

Fruit fly puparia (parasitized by *Psyttalia concolor* (Szépligeti)) were imported from a culture maintained in Pisa, Italy (Raspi & Loni, 1994) and used to initiate a colony at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. The colony was maintained using *Ceratitis capitata* larvae as the preferred laboratory host. A mass culture of *P. concolor* is maintained in Pisa, Italy for basic research and for augmentative releases against the olive fly, *Bactrocera oleae* (Gmelin).

Field collections of *Psyttalia* species were made from coffee berries - commonly infested with *Ceratitis capitata, C. rosa* Karsch and *Trirhithrum coffeae* Bezzi and *T. nigerrimum* Bezzi. Collections were also made from mango - mostly infested with *C. cosyra*; from the wild fruit *Lettowianthus stellatus* Diels (Annonaceae) - mostly infested by *C. rosa;* and from both wild and cultivated cucurbits which were mostly infested by species of *Dacus* Fabricius and *Bactrocera* Macquart. Fruits were held in cages in the insectary until tephritid larvae exited the host. Puparia were then collected and held until fly or parasitoid emergence.

Coffee berries were collected from a coffee plantation from Rurima, near Embu (Eastern Province). Mango samples were collected from Nguruman (Rift-Valley Province), while wild cucurbits (*Corallocarpus ellipticus* Chiov.) came from Sabaki (03° 09' 28S, 40° 08' 05E and elevation of 10 m), and *L. stellatus* collected from Mrima Hills (04° 29' 32S, 39° 15' 27E and elevation of 146 m), both in the Coast Province. The cultivated cucurbits (*Cucurbita pepo* L.) were obtained from two gardens at ICIPE, Nairobi (Garden 1: 01° 13' 14S, 36° 53' 44E and elevation of 1626 m; Garden 2: 01° 13' 29S, 36° 53' 51E and elevation of 1619 m). Each population was reared in the laboratory for at least three generations before experiments begun; with the exception of those from wild cucurbits which were only males.

Samples of the laboratory-reared parasitoid colonies were also sent to Stephane Dupas (Institut de Recherché pour le Développement (IRD), Paris, France) where they were checked and found to be free of viruses that could affect their reproductive capabilities.

6.2.2 Sources of host larvae

Third instar *Ceratitis* larvae (*C. capitata* and *C. cosyra* (Walker)) used for experiments were obtained from fruit fly colonies maintained at ICIPE by the African fruit Fly Initiative (AFFI). These were exposed to mated female parasitoids in all intra- and inter-population crosses for parasitization, while *Dacus ciliatus* Loew larvae were exposed to *Psyttalia* species/populations from squash. The *D. ciliatus* larvae were obtained from a laboratory colony established and maintained at ICIPE.

6.2.3 Identification of biological material

Parasitoids reared from fruits were identified, mostly using available literature and keys to the genus (Wharton & Gilstrap,1983; Wharton, 1997a,b), or by R. A. Wharton (Texas A&M University, USA), while the flies were identified using literature and keys

of White and Elson-Harris (1992), De Meyer (1996, 1998, 2000). Where there was doubt, flies were sent to I. W. White (Natural History Museum, London) or to M. De Meyer (Africa Museum, Belgium) for confirmation. Routine identifications of the common fruit fly and parasitoid species were performed by me.

Parasitoids from mango were identified as *Psyttalia cosyrae* by R. A. Wharton, while those from cultivated squash have been tentatively identified as *Psyttalia phaeostigma* (Wilkinson) (Steck *et al.*, 1986; Kimani-Njogu *et al.*, 2001). Parasitoids from coffee and the wild fruit *L. stellatus* are referred to as *Psyttalia* species with the source names attached to them; hereafter referred to as '*Psyttalia* from Rurima' (Prm), '*Psyttalia* from Ruiru' (Pru), '*Psyttalia* from Mrima Hill' (Pmh) and '*Psyttalia* from Sabaki' (Psb), respectively. *Psyttalia concolor, P. cosyrae* and *P. phaeostigma* are also referred to as 'Pcn', 'Pcs' and 'Ppt', respectively in the crossing experiments. Voucher specimens are deposited in the Biosystematics Support Unit of ICIPE, Nairobi, Kenya.

6.2.4 Crossing experiments and mating behaviour

Individuals of local *Psyttalia* species were crossed with those of *P. concolor* to test the mating compatibility. Two methods of crossing were used: single-pair and group mating (Tables 6.1 & 6.2). These were used for both crosses within (intra-population) and between populations (inter-population). According to Liu *et al.* (2002), single-pair mating provides a 'no choice' situation for mate selection, but would show any incompatibility between individuals under such restricted environment, while group mating provides some choice for mate selection and would be more likely to reveal any incompatibilities between populations.

Single-pair mating was achieved by putting one virgin male and one virgin female (2-4 day-old) in a clean glass vial (2.5 cm diameter x 7.5 cm height) covered at the top with

a piece of fine netting material and held in place with a rubber band. Group mating involved a group of virgin males and virgin females (4-25 pairs) confined in perspex cages (12 x 12 x 12 cm). Group mating was conducted for all seven populations while single-pair mating was concentrated on *P. concolor* and *P. cosyrae* (two well known valid species), which are morphologically distinct (smaller size with shorter ovipositor against bigger size and longer ovipositor, respectively). Virgin insects were obtained by individually isolating puparia (containing developing parasitoids) in flat-bottom cell culture wells (1.6 cm diameter x 1.8 cm height, Costar®, USA and 0.8 cm diameter x 1.0 cm height, Dynatech Immulon™, USA). These were covered with sheets of perforated Parafilm® "M" for ventilation to prevent mating upon emergence.

Each experiment consisted of the main cross (inter-population) and two control crosses (intra-population). The controls consisted of pairs of virgin males and females from each of the 2 parental populations, e.g., for Kenya x *P. concolor* experiment, controls were Kenya males x Kenya females and *P. concolor* males x *P. concolor* females, as done by Kimani and Overholt (1995). All trials were conducted between 11.00 h and 15.00 h, 25-27 $^{\circ}$C, 60-65 % R. H, and under partial natural light. Preliminary studies had indicated parasitoids to be most active during those hours.

After four hours of mating observation, single-pair females were individually transferred from the glass vials to small perspex cages (11.5 x 7.5 x 11.5 cm), while group mating females remained in their original cages (12 x 12 x 12 cm). Each cage had a 9.7 cm diameter opening on one side covered with fine netting material in the form of a sleeve for getting access into the cage. Additionally, the group mating and single-pair cages had 6.5 cm and 5 cm openings respectively at the top, which were fixed with organza material. These were used for the exposure of larvae to the parasitoids. Parasitoids were provided with a 10% honey solution soaked in cotton wool, water

soaked in cotton wool, and tiny droplets of pure honey streaked on the top side of the cage.

Females from all crosses were used to parasitize *C. capitata, C. cosyra* or *D. ciliatus* larvae which are the preferred hosts of the parasitoids. It was important to rear the parasitoids on their preferred hosts to get them as close to their natural performance as possible, as well as reduce the number of external factors that may influence their performance (Hall, 1993). Host larvae were aggregated in a thin layer of artificial larval diet on a modified petri dish (serving as oviposition unit) and placed (inverted) on the top organza material for 4-6 hours. A 15-20 g weight was placed on the oviposition unit to keep it in place. Larvae were placed in the diet for about 20-30 min. prior to exposure, to avoid the initial reaction of larvae jumping away from the unit and also to ensure some level of feeding and motion in the diet for easy attraction to parasitoids.

After oviposition, the larvae were transferred to bigger petri dishes (8.6 cm diameter) and provided with fresh larval diet. The petri dishes were then placed in a plastic container (13 cm diameter, 6 cm depth) with a layer of sand on the bottom to serve as a pupation medium. The sand was kept moist to prevent pupal desiccation. An opening of about 10 cm diameter was cut in the lid of the container and replaced with fine net. The sand was periodically sieved to recover puparia, which were held individually till flies and/or parasitoids emerged. Emerging parasitoids were counted, sexed and held separately (by sex) till ready for subsequent crosses. Parasitoids older than five days were added to the original colonies.

Upon successful production of males and females in the experimental crosses, F_1 females were crossed with parental males (as in Kimani-Njogu *et al.,* 2001 and Liu, *et al.,* 2002). As the parasitoids are arrhenotokous, the production of both sexes from the various crosses was used to confirm successful egg fertilization and indication of

reproductive compatibility between populations. Reproductive compatibility was further assessed by the ability of the inter-crosses to produce viable female offspring through at least two generations (Tables 6.1 & 6.2). Reproductive compatibility was expressed as the relative value of the proportion of females in progeny of an inter-population cross to that of the corresponding intra-population crosses (Pinto *et al.*, 1991). Mating behaviours of wasps were observed for three hours. These included wing fanning, male approach to females, female response to males, attempted mounting, and behaviour during and after copulation.

6.3 Data analysis

Mating data were analyzed with a general linear model (Proc GLM; SAS Institute, 2001). When ANOVAs were significant ($P< 0.05$), means were separated using Student-Newman-Keuls (SNK) test. Percent emergence and percent females were calculated from the number of parasitoids eclosed from the puparia. These proportions were then arcsine root transformed and subjected to ANOVA (Sokal & Rohlf, 1995). Relative compatibility (RC) for each inter-population cross was calculated as in Pinto *et al.* (1991);

$$RC = \frac{\text{Proportion of females in inter-population cross}}{\text{Proportion of females in intra-population cross}}$$

Two RC values were then calculated (i.e. the inter-population cross in relation to each of the two controls (intra-population crosses)), and a mean value determined.

6.4 Results

In the single-pair mating trials, there were a few replicates that failed to parasitize host larvae or produce progeny. This is reflected in the apparent differences in number of females used in Tables 6.1 and 6.3 (as those females were discounted from the analyses). There were also females that produced only males. The mean relative reproductive compatibility of the inter-population crosses fluctuated between 0.21 and 1.15 (Tables 6.3 and 6.4).

For females that produced both sexes, the mean proportion of females did not differ significantly between the crosses (inter- and intra-, single-pair or group mating; (Single-pair: $F = 1.03$; $df = 3$; 35; $P = 0.3918$; Group mating: $F = 1.49$; $df = 3$, 44; $P = 0.2306$) (Table 6.3 and Figure 6.1). However, proportion of parasitoids produced differed in various crosses (Table 6.3). Under single-pair mating, the highest proportions occurred in the two intra-population crosses (Pcn x Pcn {0.61} and Pcs x Pcs {0.51}) which did not differ from each other, but differed significantly from the two inter-population crosses (Pcn x Pcs {0.43} and Pcs x Pcn {0.34}) ($F = 6.46$; $df = 3$, 39; $P = 0.0012$). The two inter-population values too did not differ from each other (Table 6.3 and Figure 6.2).

Under group mating, the proportion of parasitoids produced was highest in the intra-population crosses (Pcn x Pcn {0.91} and Pcs x Pcs {0.51}), but differed from each other ($F = 43.62$; $df = 3$, 49; $P < 0.0001$). Pcn x Pcn also differed from the two inter-population crosses (Pcn x Pcs {0.24} and Pcs x Pcn {0.47}), while Pcs x Pcs differed only from Pcn x Pcs, and not the reciprocal cross Pcs x Pcn.

The progeny from the first filial generation (i.e. F_2 generation) had higher proportions of females compared with those from both the inter- and intra-population crosses under

group mating. Exceptions to this were observed in the F_1 x F_1 value for Pcn x Ppt, the intra-population values for Psb x Psb and Pmh x Pmh, which could not be compared, and the F_1 x F_1 value for Ppt x Pcn (58.8) which fell between the inter- and intra-population values) (Table 6.5). Table 6.6 shows a summary of the crosses and how the proportions of females compare in the crosses.

6.4.1 Mating behaviour

6.4.1.1 Intra-population observations

Wing fanning by males was usually in the form of continuous and sustained movement. Wings were spread out almost perpendicular to the long axis of the body, with flapping not as hard as in flying. This was observed when males were introduced into the vials or cages with females. When males were within distances of 3-5 cm from females, fanning became intensified with approach toward females becoming faster. If females moved, the approach too intensified and males seemed to be half-flying and half-running after females, with little and occasional limb contact with the floor of the cage - a movement that looked like male "gliding". As fanning intensified, *Psyttalia cosyrae* males were observed to stop momentarily at about 2-3 cm from females and then approached more cautiously, gently, and slowly. On reaching females, the males stroke bodies of female with the antennae especially on the middle section of the wings, and gradually upwards to the head and antennae. There was a short period of female quiescence and then males attempted mounting. On successful copulation, male wing fanning was intensified with a rhythmic flapping while the two antennae moved up and down in opposite directions (sort of drumming softly on the head and antennae of the female). After some time, the fanning slowed down to a stop with the pair still in copula with the male antennae moving slowly. This "grooming" period was generally more pronounced in the *P.*

cosyrae intra-population cross and least observable (if at all) in *P. concolor* males. *Psyttalia concolor* males at this stage did not stay with females for long, but pulled off, waited for about 4-8 seconds and started fanning gain. After separation or "grooming", *P. cosyrae* pairs usually stayed motionless for a while and then spent the next 20-45 seconds cleaning their bodies before walking away.

6.4.1.2 Inter-population observations

On putting the pairs together, *P. concolor* males started fanning almost immediately and pursued *P. cosyrae* females. On catching up, they quickly jumped onto the females for mating. There was no observation of that cautious approach when a female was within reach. No antennal stroking by *P. concolor* males (except for the normal movement observed during fanning); and they did not seem to solicit female response. Mounting of females by *P. concolor* males was attempted from all angles, even when facing the females. They always seemed to force themselves or "rape" the females, irrespective of the population. As a result, *P. concolor* males tended to make more mounting attempts.

After successful copulation, *P. concolor* males took advantage of the stationary females and tried to mount again. Females then tried to ward off the males by moving away. In the case of the bigger females, they lifted their bodies up by outstretching their limbs and arching the abdomen downwards with the wings carried straight on the back. This resulted in *P. concolor* males not getting easy access to the genitalia area, and most often with the tip of the abdomen hanging in the space between the wings of the females and the arched abdomen.

In the reciprocal cross of *P. cosyrae* males with *P. concolor* females, the first few minutes was spent by the *P. cosyrae* males trying to find a way out of the confinement. After a while, fanning began and subsequent pursuit of females. *P. cosyrae* males

tended to follow the trail of females, and escaping females occasionally collided with males who were still following trails which had crossed their paths. These resulted in the males changing course to follow the females directly or the two moving in opposite directions. Occasionally, *P. concolor* females were also observed to be fanning their wings like the males during such collisions.

Table 6.1 Number of parasitoids used in main, reciprocal and back crosses between individuals of *Psyttalia concolor* and *P. cosyrae*.

Crosses/Backcrosses (♂ x ♀)	No. of parasitoids		Remarks
	Single-pair mating	Group mating	
Cross and Reciprocal cross			
Pcn* x Pcs**	11	11♂ x 11 ♀	Inter-population cross
Pcs x Pcn	13	10 ♂x 10 ♀	Inter-population cross
Pcn x Pcn	12	25 ♂x 25♀	Intra-population cross
Pcs x Pcs	12	10 ♂x 10 ♀	Intra-population cross
Backcrosses			
Pcn x (Pcn x Pcs)	10	7 ♂ x 7 ♀	F_1 females back crossed to Pcn males
Pcs x (Pcn x Pcs)	10	4 ♂ x 4 ♀	F_1 females back crossed to Pcs males
Pcs x (Pcs x Pcn)	10	6 ♂ x 6 ♀	F_1 females back crossed to Pcs males
Pcn x (Pcs x Pcn)	10	5 ♂ x 5 ♀	F_1 females back crossed to Pcn males
F_1 Crosses			
(Pcn x Pcs) x (Pcn x Pcs)	10	5 ♂ x 5 ♀	F_1 males crossed with F_1 females
(Pcs x Pcn) x (Pcs x Pcn)	10	5 ♂ x 5 ♀	F_1 males crossed with F_1 females

*Pcn = *Psyttalia concolor*
**Pcs = *Psyttalia cosyrae*

Table 6.2 Results of other group mating crosses between *Psyttalia* parasitoids from five populations in Kenya and individuals of *P. concolor* from Italy.

Crosses/Back crosses (♂ x ♀)	No. of wasps (Group mating)	Remarks
Cross and reciprocal cross		
Pcn x Prm	24 ♂x 24 ♀	Inter-population cross
Prm x Pcn	25 ♂ x 25♀	Inter-population cross
Pmh x Pcn	7 ♂x 7 ♀	Inter-population cross
Pcn x Ppt	5 ♂x 5 ♀	Inter-population cross
Ppt x Pcn	4 ♂x 4 ♀	Inter-population cross
Pru x Pcn	2 ♂ x 2 ♀	Inter-population cross
Psb x Pcn	2 ♂x 2 ♀	Inter-population cross
Prm x Prm	25 ♂ x 25♀	Intra-population cross
Pru x Pru	9 ♂ x 9 ♀	Intra-population cross
Ppt x Ppt	5 ♂x 5 ♀	Intra-population cross
Pmh x Pmh	2 ♂x 2 ♀	Intra-population cross
Psb x Psb	-	Only males were available
Backcrosses		
(Pcn x Prm) x Pcn	5 ♂ x 5 ♀	F_1 females back crossed to Pcn males
(Pcn x Prm) x Prm	7 ♂x 7 ♀	F_1 females back crossed to Prm males
(Prm x Pcn) x Prm	8 ♂ x 8 ♀	F_1 females back crossed to Prm males
(Prm x Pcn) x Pcn	10 ♂ x 10 ♀	F_1 females back crossed to Pcn males
(Pmh x Pcn) x Pmh	5 ♂ x 5 ♀	F_1 females back crossed to Pmh males
(Pmh x Pcn) x Pcn	4 ♂ x 4 ♀	F_1 females back crossed to Pcn males
(Pcn x Ppt) x Pcn	4 ♂ x 4 ♀	F_1 females back crossed to Pcn males
(Pcn x Ppt) x Ppt	-	-
(Ppt x Pcn) x Ppt	2 ♂ x 2 ♀	F_1 females back crossed to Ppt males
(Ppt x Pcn) x Pcn	5 ♂ x 5 ♀	F_1 females back crossed to Pcn males
(Pru x Pcn) x Pru	2 ♂ x 2 ♀	F_1 females back crossed to Pru males
(Pru x Pcn) x Pcn	-	-
(Psb x Pcn) x Psb	5 ♂ x 5 ♀	F_1 females back crossed to Psb males
(Psb x Pcn) x Pcn	-	-
F_1 Crosses		
(Pcn x Prm) x (Pcn x Prm)	5 ♂ x 5 ♀	F_1 males crossed with F_1 females
(Prm x Pcn) x (Prm x Pcn)	4 ♂ x 4 ♀	F_1 males crossed with F_1 females
(Pmh x Pcn) x (Pmh x Pcn)	6 ♂ x 6 ♀	F_1 males crossed with F_1 females
(Pcn x Ppt) x (Pcn x Ppt)	1 ♂ x 1 ♀	F_1 males crossed with F_1 females
(Ppt x Pcn) x (Ppt x Pcn)	3 ♂ x 3 ♀	F_1 males crossed with F_1 females
(Pru x Pcn) x (Pru x Pcn)	4 ♂ x 4 ♀	F_1 males crossed with F_1 females
(Psb x Pcn) x (Psb x Pcn)	7 ♂ x 7 ♀	F_1 males crossed with F_1 females

- = Cross not done or died out
Pcn = *Psyttalia concolor,* from Pisa, Italy
Prm = *Psyttalia* from Rurima, Eastern Province
Pru = *Psyttalia* from Ruiru, Central Province
Psb = *Psyttalia* from Sabaki, Coast Province
Ppt = *Psyttalia phaeostigma*, Kasarani, Nairobi Province
Pmh = *Psyttalia* from Mrima Hill, Coast Province.

Table 6.3 Proportion of female progeny and parasitoids produced by *Psyttalia concolor* and *P. cosyrae* in single-pair and group mating crosses in the laboratory.

Biological parameters	Inter-population cross		Intra-population cross	
	Pcn x Pcs (♂ x ♀)	Pcs x Pcn (♂ x ♀)	Pcn x Pcn (♂ x ♀)	Pcs x Pcs (♂ x ♀)
	Single-pair mating			
Total number of females used	9	11	11	10
Proportion of female progeny	0.50 ± 0.06 a	0.63 ± 0.06 a	0.52 ± 0.05 a	0.57 ± 0.06 a
Proportion of parasitoids produced	0.43 ± 0.06 b	0.34 ± 0.04 b	0.61 ± 0.05 a	0.51 ± 0.04 ab
Relative compatibility	0.93	1.15	-	-
	Group mating			
Total number of females used	8	10	25	10
Proportion of female progeny	0.50 ± 0.01 a	0.49 ± 0.08 a	0.58 ± 0.02 a	0.54 ± 0.04 a
Proportion of parasitoids produced	0.24 ± 0.02 c	0.47 ± 0.07 b	0.91 ± 0.03 a	0.51 ± 0.04 b
Relative compatibility	0.78	0.79	-	-

Means in the same row followed by same letters are not significantly different (P = 0.05) using Student-Newman-Keuls (SNK) test. ANOVA performed on arcsine transformed proportion values.
Proportion of females: (Single-pair: $F = 1.03$, $df = 3, 35$, $P = 0.3918$; Group: $F = 1.49$, $df = 3, 44$, $P = 0.2306$)
Proportion of parasitoids (Single-pair: $F = 6.46$, $df = 3, 39$, $P = 0.0012$; Group: $F = 43.62$ $df = 3, 49$, $P < 0.0001$)

Table 6.4 Results of the relative compatibility levels calculated for group mating crosses and reciprocal crosses between individuals of six populations of *Psyttalia* from Kenya and those of *P. concolor* from Italy.

Inter-population cross	Proportion of females (a)	Intra-population cross	Proportion of females (b)	Relative Compatibility (a/b)	Mean RC
Pcn x Pcs	42.9	Pcn x Pcn	57.1	0.75	0.78
		Pcs x Pcs	52.6	0.82	
Pcs x Pcn	43.2	Pcn x Pcn	57.1	0.76	0.79
		Pcs x Pcs	52.6	0.82	
Pcn x Prm	43.6	Pcn x Pcn	57.1	0.76	0.78
		Prm x Prm	54.2	0.80	
Prm x Pcn	44.2	Pcn x Pcn	57.1	0.77	0.80
		Prm x Prm	54.2	0.82	
Pcn x Ppt	12.5	Pcn x Pcn	57.1	0.22	0.21
		Ppt x Ppt	61.9	0.20	
Ppt x Pcn	61.3	Pcn x Pcn	57.1	1.07	1.03
		Ppt x Ppt	61.9	0.99	
Pru x Pcn	38.1	Pcn x Pcn	57.1	0.67	0.92
		Pru x Pru	32.4	1.18	
Psb x Pcn	58.1	Pcn x Pcn	57.1	1.02	0.51*
		Psb x Psb	-	-	
Pmh x Pcn	38.6	Pcn x Pcn	57.1	0.68	0.34*
		Pmh x Pmh	-	-	

* Second control (intra-population cross) not done.
Pcn = *Psyttalia concolor,* from Pisa, Italy
Pcs = *P. cosyrae,* Nguruman, Rift-Valley Province
Pmh = *Psyttalia* from Mrima Hill, Coast Province
Ppt = *P. phaeostigma*, Kasarani, Nairobi Province
Prm = *Psyttalia* from Rurima, Eastern Province
Pru = *Psyttalia* from Ruiru, Central Province
Psb = *Psyttalia* from Sabaki, Coast Province

Table 6.5 Results of backcrosses and F_1 crosses using offspring from experimental crosses shown in Tables 6.1 and 6.2.

Cross (♂ x ♀)	Backcross I (F_1♀ x ♂Parent I)	Backcross II (F_1♀ x ♂Parent II)	F_1 Cross (F_1♂ x F_1♀)	Remarks
Pcn x Pcs	+	+	+	Production of mixed F_2 progeny
Pcs x Pcn	+	+	+	Production of mixed F_2 progeny
Pcn x Prm	+	+	+	Production of mixed F_2 progeny
Prm x Pcn	+	+	+	Production of mixed F_2 progeny
Pcn x Ppt	+	?	+	Undetermined
Ppt x Pcn	+	+	+	Production of mixed F_2 progeny
Pcn x Pru	?	?	?	Undetermined
Pru x Pcn	+	?	+	Production of mixed F_2 progeny
Pcn x Pmh	?	?	?	Undetermined
Pmh x Pcn	+	+	+	Production of mixed F_2 progeny
Pcn x Psb	?	?	?	Undetermined
Psb x Pcn	+	?	+	Production of mixed F_2 progeny

+ = production of female offspring
? = Cross not done or died out.

Table 6.6 Comparison of the performance (proportion of females) of experimental crosses against F_1 generation.

Cross	Proportion of females		Intra-population cross	Proportion of females
	Inter-population cross	$F_1 \times F_1$		
Pcn x Pcs	42.9	64.0	Pcn x Pcn	57.1
Pcs x Pcn	43.2	60.0	Pcs x Pcs	52.6
Pcn x Prm	43.6	74.5	Pcn x Pcn	57.1
Prm x Pcn	44.2	67.8	Prm x Prm	54.2
Pcn x Ppt	12.5	-	Pcn x Pcn	57.1
Ppt x Pcn	61.3	58.8	Ppt x Ppt	61.9
Pru x Pcn	38.1	64.3	Pcn x Pcn	57.1
			Pru x Pru	32.4
Psb x Pcn	58.1	79.5	Pcn x Pcn	57.1
			Psb x Psb	-
Pmh x Pcn	38.6	74.4	Pcn x Pcn	57.1
			Pmh x Pmh	-

- = Proportions not worked out due to incomplete data.

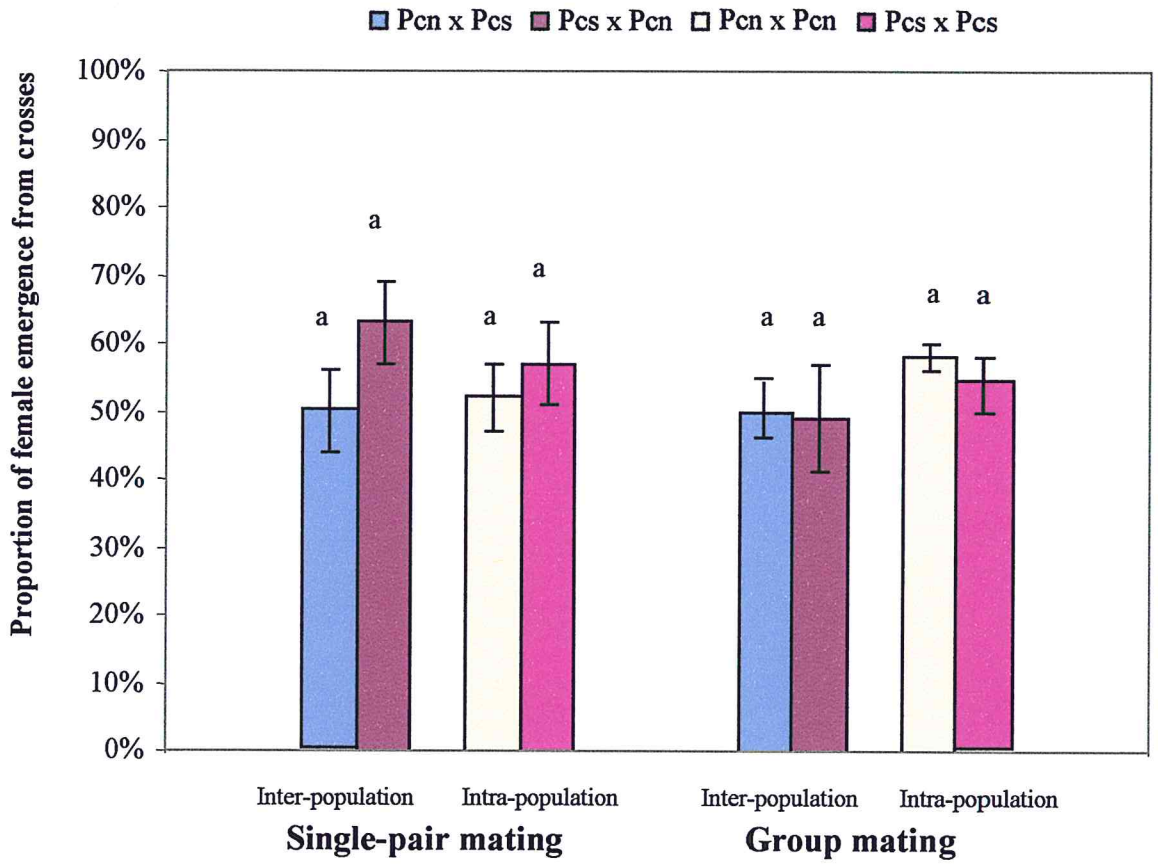


Figure 6.1 Comparison of proportion of female emergence from the two methods of mating* (single-pair and group). Same letters on bars indicate no significant differences between means (P = 0.05), using Student-Newman-Keuls (SNK) test. *Mean separation was performed separately for each mating method.

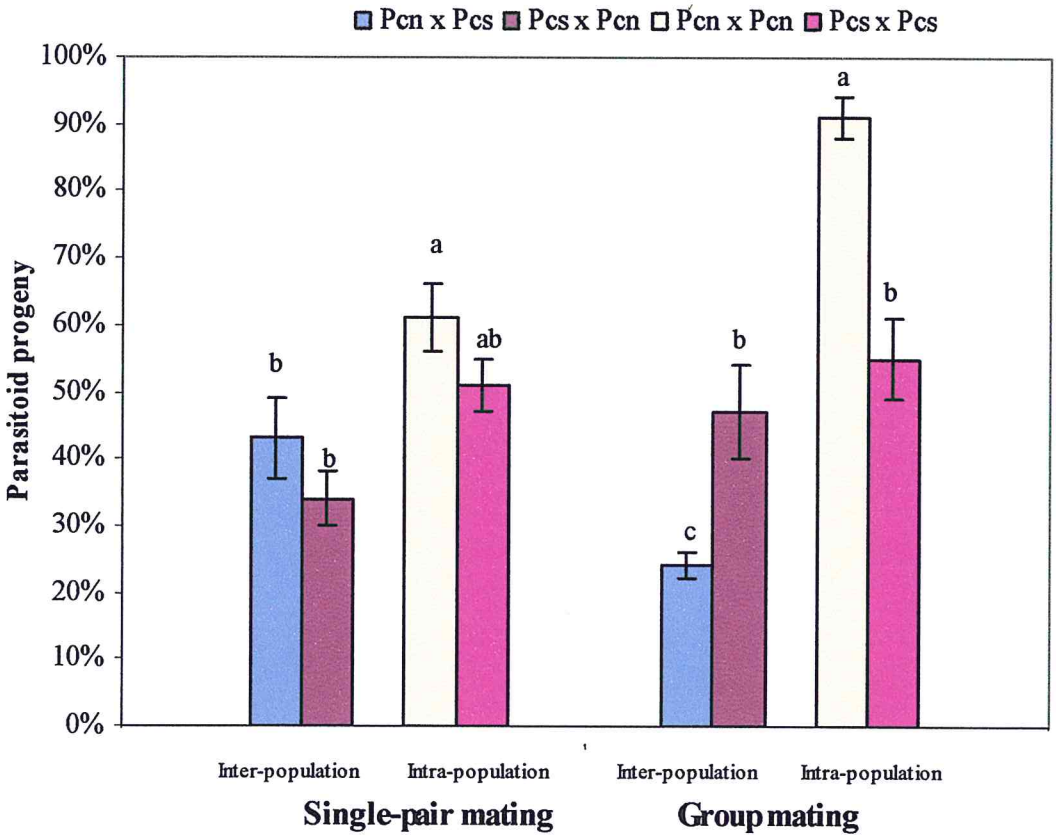


Figure 6.2 Comparison of proportion of parasitoid emergence from single-pair and group mating methods*. Different letters on bars indicate significant differences between means (P = 0.05), using Student-Newman-Keuls (SNK) test. *Mean separation was performed separately for each mating method.

6.5 Discussion

a) Mating behaviour - Many courtship behaviours exhibit combinations of features that are unique to groups and can be used for identification purposes. These are usually species-characteristic and include the length of time in performing features in the sequence of events, and how particular features in the sequence are stressed or carried out for conspecific mates to recognize their kind (Jervis & Kidd, 1996 and references therein). For example, in *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), a solitary koinobiont parasitoid of fruit flies, it has been observed that males beat their wings rapidly during courtship to produce a sound ("song") which is perhaps thought to be a way of fanning pheromones toward the females to solicit their response (Sivinski & Webb, 1989). Analyses of such behaviour are sometimes used as a means of distinguishing between species. This was illustrated by van den Assem and Povel (1973) in the courtship behaviour of *Muscidifurax* species (Hymenoptera: Pteromalidae), parasitoids of the house fly, *Musca domestica* (Diptera: Muscidae). The study showed that what was originally believed to be a single, variable species was in reality a complex of sibling species, thus clearing a long-held erroneous impression and so shedding more light on the taxonomic status of members of the genus.

Though it has been reported that chemical, tactile as well as visual stimuli are involved in the release and continuation of courtship in parasitic Hymenoptera (van den Assem & Jachmann, 1982), the behaviour of *P. concolor* males seems to suggest that more emphasis is placed on visual stimuli, which elicit an aggressive behaviour of physical pursuit of the females. Irrespective of population, they started fanning as soon as females were offered to them. *Psyttalia concolor* females also exhibited behaviours that

suggest eagerness to mate without waiting for males to go through all the features of courtship.

It has been reported (Bartlett, 1984) that the process of colonization and mass-rearing over long periods of time may have detrimental effects on reared species, and can lead to unintentional behavioural changes occurring (Bautista & Harris, 1997). This starts with the initial loss of genetic variability and subsequent selection to induce domestication and adaptation to laboratory environments (Bartlett, 1984; van Lenteren, 1991; Wajnberg, 1991; Leppla, 1993). To minimize the effects of such laboratory rearing, Joslyn (1984) enumerated some precautionary methods which include pooling multiple-founder colonies, maintaining variable laboratory environments, and regular infusion of wild genetic stock. Bautista *et al.* (1999) also suggest that procedures of careful, sustained studies of parasitoid development and behaviour can be identified and incorporated in insectary rearing. Results in this study lend support to the findings of a similar study by Kimani-Njogu *et al.* (2001) in which *P. concolor* was suggested as having mating problems, and its aggressive behaviour was speculated to be associated with either the long period of mass-rearing in cages or a reflection of their behaviour in the wild.

b) Reproductive compatibility - The weight given to cross mating data in taxonomic decision-making depends on whether the results show compatibility or incompatibility (Pinto *et al.,* 1991, 1992, 1993; Stouthamer *et al.,* .2000). According to Smith *et al.* (1993), the compatibility of laboratory cultures must be used with caution before allowing it to contribute to any hypothesis of conspecificity. This is because the laboratory is an artificial habitat in which the organisms are brought together under forced conditions where there may be the elimination of several isolating ecological and

behavioural barriers (Mackauer, 1969; Smith *et al.,* 1993) which prevent the populations in question from ever meeting and mating in the field. On the contrary, reproductive incompatibility between cultures/populations can be more appropriately used to support hypotheses of hetero-specificity as long as simultaneous intra-culture crosses are performed as controls. In some cases, cross mating incompatibility has been used to support cases where minor morphological, life history or allozymic differences (on their own) have not been convincing enough to argue for species recognition (Pinto *et al.,* 1991). In others, it comprises the primary or even the sole source of evidence for the species. For example, two species of *Trichogramma* (Hymenoptera: Trichogrammatidae); *T. minutum* Riley and *T. platneri* Nagarkatti which are commonly used in biological control can be differentiated only on the basis of cross mating incompatibility (Nagarkatti, 1975).

Cross mating data have also been used in other parasitic Hymenoptera such as *Trichogrammatoidea* (Nagaraja, 1978), *Aphytis* (Rao & DeBach, 1969), and *Trioxys* (Hall *et al.,* 1962). The taxonomic weight accorded these crossing data have usually been justified by the important role played by reproductive isolation in the theory of speciation (Pinto *et al.,* 1991), and the need to formally recognize distinct populations for biological control purposes (e.g. DeBach, 1969). However, it is cautioned (Pinto *et al.,* 1992) that defining species on incompatibility criteria alone could be problematic because such species are defined only relative to another taxon (as they lack characters of their own) and their routine identification becomes difficult.

From the results in Tables 6.3 and 6.4, it can be inferred that there is no instance of complete reproductive incompatibility between any of the crosses. Pinto *et al.* (1991) used relative compatibility of reproduction to reveal genetic differences between strains

of *Trichogramma,* and arbitrarily suggested that with their experimental procedure, relative compatibility (RC) values lower than 0.75 for a cross between two strains/populations could be taken as evidence of partial incompatibility. In this study, levels of RC range from as low as 0.21 to a high value of 1.15, which indicate that reproductive compatibility between the populations of *Psyttalia* under study ranges from partial incompatibility to full compatibility. With the exception of the RC value for Pcn x Ppt (0.21), Psb x Pcn (0.51), and Pmh x Pcn (0.34), all other crosses have values > 0.75. In a similar study of the reproductive compatibility between two geographic populations of the pupal parasitoid *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), Liu *et al.* (2002) suggested that the very high RC levels could be assumed to be a result of experimental errors, culminating from the fact that cultures are maintained and experiments conducted under forced and no-choice conditions.

Regardless of the method of mating or the type of cross, no significant difference was observed in the mean proportion of females produced, indicating that the rates of fertilization and host acceptance by mated females were not affected by hybridization (Morales-Ramos *et al.,* 2000). There were, however, differences in the mean percent emergences from the crosses (inter-population crosses = 24-47%, and intra-population crosses = 51-91%). Some females produced only males under single-pair mating. This means under group mating, there could also be chances of some females being missed out on mating (by chance or avoidance) and consequently adding up to the all-male progenies.

In this study, crossing of the wasps was restricted to a maximum of four hours, and the number of larvae exposed to mated females per day was fixed. In nature, mating, host-searching and parasitization take place as long as conditions allow. The RC values are

therefore, based on the laboratory conditions and factors under study; they verify the existence of the physiological potential of these species/populations to produce hybrids. It is worth noting that natural hybrids between these populations have never been described and this could potentially explain or account for the observed high morphological similarities. It will be interesting in future studies to describe some morphological aspects of parental species and their F_1 hybrids for use in the analysis of natural populations.

If hybrids are fertile, introduction of genetic variability through hybridization can result in colonization of new habitats and/or hosts (dos Santos *et al.*, 2001). Additionally, genes introduced from different species can serve as raw material for evolutionary changes with subsequent challenges for management and control.

The results show the physiological potential for hybrid production and the high fertility rates indicate the possibility of introgressive hybridization in these species/populations which should be considered in future introductions of *Psyttalia* species as biological control agents, especially in environments where other species of the genus exist in abundance - "a scenario that reproduces the condition of no choice crosses" (dos Santos *et al.*, 2001).

In general, higher proportions of females in inter-strain/population crosses relative to proportions in intra-strain/population crosses are preferred, a condition referred to as hybrid vigour or heterosis, which is due to over-dominance of genes in heterozygous individuals (Gu & Dorn, 2002). Though the performance of the inter-population crosses was lower than that in the intra-population crosses in this study, the F_1 performance (from F_1 x F_1 crosses) exceeded the average parental performances of both the inter- and intra-population crosses. In parasitic Hymenoptera, females parasitize hosts and they are responsible for host mortality. Therefore, if fecundity remains constant, then a

strong female-biased progeny will result in more "pest killers" (Hall, 1993) and consequently increase their efficacy as biological control agents. This situation works very well if males normally mate more than once, since a strongly female-biased population in one generation can give rise to a strongly male-biased population in the next generation if a good number of the females are not mated by the few males or if there is insufficient mating.

c) Identity of parasitoids - The production of viable hybrids in all the crosses gives rise to two scenarios: 1) production of viable hybrids between morphologically similar populations, and 2) production of viable hybrids between morphologically distinct populations.

The Biological Species Concept (BSC) states that "a species is a group of interbreeding natural populations that is reproductively isolated from other such groups" (Mayr & Ashlock, 1991). Since there was no sign of any reproductive incompatibility in the results presented, it means there is an intercommunicating reproductive link between the populations, and they cannot be separated on this basis. Furthermore, the second scenario gives an indication of a very close relationship between the morphologically distinct populations. The biological species concept may therefore, not apply to these very closely related but morphologically distinct species in this genus. According to Claridge *et al.* (1985), such a case shows that genetic differentiation between the species/populations is slight, and may indicate a state of a recent speciation. It is also reported that organisms in many taxonomic groups which have been separated for long periods of evolutionary time nonetheless may retain the anatomical and physiological capacity for hybrid production (Avise, 1994). The ability to interbreed is a primitive

trait known to transcend well-defined species boundaries and does not, in itself, establish evolutionary units or their components (Cracraft, 1989).

In both scenarios the findings, together with the ease with which the populations hybridize (and the viability of the F_1 and F_2 hybrids), seem to suggest that there is no evidence of either post-copulatory or post-zygotic isolating mechanisms. However, the same conclusion cannot be drawn for the presence or absence of pre-copulatory isolating mechanisms, since cross mating was conducted in the laboratory in a no-choice and/or under forced artificial conditions. Further testing would be necessary to determine whether there are any pre-copulatory isolating mechanisms in nature. The parasitoid populations may or may not encounter each other in nature as they came from different host/host plant systems, and might maintain physical isolation despite reproductive compatibility.

In the view of Godfray (1994), the taxonomic and genetic status of populations from different, often related, hosts has long been an area of controversy, and "where such populations differ clearly in morphology, there has normally been no problem about recognizing them as distinct species" but where host-related populations show no clear-cut diagnostic differences in morphology, difficulties have arisen".

Nevertheless, the reproductive compatibility results in this study support the work on morphology and host suitability by Mohamed *et al.* (2003) to suggest that the genus *Psyttalia* comprises a series of very closely related species, forming a complex that may sort out primarily by host and/or region. It is worth noting that although the existence of intra-specific variation has long been recognized, considerable confusion still exists over terminologies like "subspecies", "variety", "race", and "biotype" which are all being used widely but rarely defined clearly (Berlocher, 1984).

d) Biological control considerations – For effective use of natural enemies in biological control, correct identification of species is essential. Establishing the identity of the parasitoid populations on *Ceratitis* in coffee in Kenya should, therefore, facilitate their use in biological control of the Mediterranean fruit fly and other related *Ceratitis* pests. Medfly is considered to be the most devastating and widespread species of all fruit flies. It attacks hosts in nearly 70 plant families (Mitchell *et al.,* 1977; Weems, 1981), with 353 plant species reported as hosts or potential hosts (Liquido *et al.*, 1991). In Africa alone, more than 150 host plants are reported to be attacked by *C. capitata* with certainty (De Meyer *et al.,* 2002b). The search for natural enemies against these pests for use in classical biological control started in Australia in 1902. According to Wharton (1989a, b), most of the parasitoids obtained in the search develop successfully on medfly and are usually maintained in the laboratory on this host. Interestingly, nearly all the opiine parasitoids against medfly were originally collected and reared from the genus *Bactrocera* (an Australasian genus far removed from the native range of *Ceratitis*). These parasitoids were introduced to Hawaii and other places where they attack medfly and other introduced tephritid pests (Silvestri, 1914; Clausen *et al.,* 1965), thus establishing new host associations with medfly.

With the collection of the Kenyan populations which come from *Ceratitis* (and morphologically similar to *P. concolor*), their study should be in line with proponents of the idea that a logical focus of attention for the search for indigenous parasitoids of medfly is sub-Saharan Africa (Headrick & Goeden, 1996). Laboratory studies of these Kenyan populations on medfly and other tephritid pests at ICIPE, Nairobi, in collaboration with Texas A&M University, USA, have shown encouraging results. This has resulted in the shipment of these medfly parasitoids from Kenya to Hawaii, Guatemala, and St. Helena Islands (South Africa), and both medfly and Natal fruit fly (*C. rosa*) parasitoids to La

Réunion for potential use in the biological control of medfly and other fruit-infesting Tephritidae (PEET, 2003). If these parasitoids successfully attack medfly (and Natal fly) and establish in their new environments, then it will be a re-establishment of the original relationships between the parasitoids and the hosts and the plausible prediction will be a more effective control of those pests.

However, an area of concern in many biological control programmes is the problem of whether or not to introduce one or several species of parasitoids in the same environment. This concern has lead to many studies in different aspects of competition (Salt, 1963; Fisher 1971; Wang & Messing, 2003) resulting in a number of population dynamic models (e.g. Briggs, 1993) aimed at examining the requirements for species competition and coexistence. According to Godfray (1994), no single answer has been found to the problem and all the results suggest that the strategy which gives the greatest or best depression in host equilibrium abundance may depend quite critically on the biological details of the interaction under study.

In view of the identity suggested, different parasitoid populations may exist in the same or different geographic areas and introduction of parasitoids in an area may result in some chances of inter-population mating. The biggest potential problem will be with the morphologically similar populations. The introduction of such species in areas where others already exist is likely to give erroneous impression in the post-release sampling results since there will already be the presence of other morphologically similar populations that will add up to the frequency and numbers of the introduced species. Secondly, if they represent different species, then apart from the possibility of inter-specific hybridization, there will also be the problem of competition since they would have the same host-larvae preferences. On the other hand, since the

morphologically distinct populations (*P. cosyrae* and *P. phaeostigma*) have different host-larvae preferences, competition is likely to be minimal or absent except for the chances of hybridization, if no pre-copulatory isolating mechanisms come into play. As to whether these populations will interbreed in nature when introduced in the presence of others, and if that will result in changes in the ability of one or both to be effective in biological control, these are interesting questions which cannot be answered now without detailed field work to establish the possibility of hybrids in nature; and establishing the status of pre-copulatory or pre-zygotic isolating mechanisms.

In the light of these results, it is important to note that the cross mating studies are not adequate for determining differences among the populations in this genus and a range of other studies (particularly those emphasizing host-habitat relationships) will be important for use of *Psyttalia* species in biological control. It is therefore, important that information on a background study of the natural population types in the targeted environment and their potential interaction with species to be introduced are collected and examined; and host-habitat relationships established before any introductions are made for better establishment and colonization. Concerns in considering candidate biological control agents for release were first expressed by Howard (1917) and Pemberton & Willard (1918), who pointed to the need for more intensive pre-release studies, forms and species in release areas, and stress on critical selection of natural enemies adapted to local conditions (Wharton, 1989a). Although the success of an introduced parasitoid is determined by many factors, the information revealed by this study may add to the bulk of existing knowledge and help in the future planning of conservation and/or introduction of parasitoids for the biological control of fruit-infesting flies.

CHAPTER SEVEN

7.0 MOLECULAR STUDIES USING AMPLIFIED RESTRICTION FRAGMENT LENGTH POLYMORPHISM (AFLP).

7.1 Introduction

For the study of genetic variation in natural populations, deoxyribonucleic acid (DNA) sequences are much more informative than protein sequences or electrophoretic variation of proteins (Nei & Kumar, 2000), because a large part of DNA sequences is not encoded into proteins, and there is degeneracy of the genetic code. DNA sequences also reveal detailed information about the polymorphism due to nucleotide substitution, insertion/deletion, gene conversion, unequal crossing over, horizontal gene transfer, and so forth. The extent of DNA polymorphism may be measured in several different ways (Nei, 1987), but the most commonly used measures are, 1) the number of segregating sites per nucleotide site, and 2) nucleotide diversity (or heterozygosity at the nucleotide level) (Nei & Kumar, 2000). A restriction enzyme recognizes a specific sequence of nucleotide pairs, generally four or six pairs in length, and cleaves to it.

Amplified Restriction Fragment Length Polymorphism (AFLP) is a method of genotyping that combines the specificity of restriction enzyme digestion of DNA with the power of the polymerase chain reaction (PCR).

7.1.1 Origin of AFLP

Originally developed for the typing of crop plants (European patent application 0534858A1) (Zabeau & Vos, 1993), AFLP has been applied to genetic typing in different taxa, ranging from viruses, insect pests through higher plants to variations

among cloned *Trypanosomiasis evansi* (Kinetoplastda: Trypanosomatidae) stocks isolated from camels, as well as for phylogenetic studies within given species (Vos *et al.*, 1995; Heckel *et al.*, 1998; Li *et al.*, 1998; Reineke *et al.*, 1998; Masiga *et al.*, 2000). AFLP methods rapidly generate hundreds of highly replicable markers from DNA of any organism, thus allowing high-resolution genotyping of fingerprinting quality. The time and cost efficiency, replicability and resolution of AFLPs are superior or equal to those of other markers (allozymes, random amplified polymorphic DNA (RAPD), Restriction fragment length polymorphism (RFLP) and microsatellites). The only difference being that, AFLP methods primarily generate dominant rather than co-dominant markers.

AFLP detects DNA polymorphisms at specific restriction enzyme-sites and enables these polymorphisms to be detected at multiple independent restriction sites simultaneously. It is applicable to the detection of variation in DNA of any origin and complexity without prior sequence knowledge (Masiga *et al.*, 2000). The power of AFLP is based upon the molecular genetic variations that exist between closely related species, varieties or cultivars (Liscum & Briggs, 1995). These variations in DNA sequence are exploited by the AFLP technology such that "fingerprints" of particular genotypes can be routinely generated.

7.1.2 AFLP working principle

It is a powerful method of DNA fingerprinting of nuclear genomes (Nei & Kumar, 2000), and a selective PCR amplification of restriction fragments from a total digest of genomic DNA. In the standard or original protocol, the entire DNA is first digested with two restriction enzymes (*Eco*RI and *Mse*I), and double-stranded oligonucleotides adaptors ligated to both ends of the restriction fragment. PCR primers (complementary

to the adaptors and parts of the restriction site) are then used for amplification of the fragment that is flanked by the adaptors (i.e. the adaptor oligonucleotides are ligated to the restriction enzyme fragments and used as target sites for primers in the subsequent PCR amplification process). Only those restriction fragments that perfectly match the primer sequences are amplified, and they are electrophoretically separated to give strain-specific band profiles. See Figure 7.1 for schematic presentation of the process. These band patterns can be used for monitoring or determining the identity of specific DNA samples/isolates or to assess the degree of similarity among samples/isolates. According to Weising *et al.* (1995) and Hoelzel (1998), they can also be used as the source for genetic markers to generate linkage maps, as polymorphism in band patterns map out to specific loci, thereby allowing individuals to be genotyped or differentiated based on the alleles they carry.

Usually, 50-100 restriction fragments are amplified and detected on denaturing polyacrylamide electrophoretic gels. Gel autoradiographs are scored visually using a binary data matrix by scoring the bands as present or absent among different individuals, which indicate polymorphism at that locus.

In summary, AFLP involves four main steps; a) Digestion of extracted DNA with restriction enzymes, b) Ligation of an adaptor to each "sticky" end of the digestion fragments (designed to disrupt the enzyme restriction-site), c) PCR amplification of the adaptor-tagged fragments with complementary primers to the adaptor sequence, and d) Electrophoretic separation (usually through denaturing gels) and the detection of the amplified fragments.

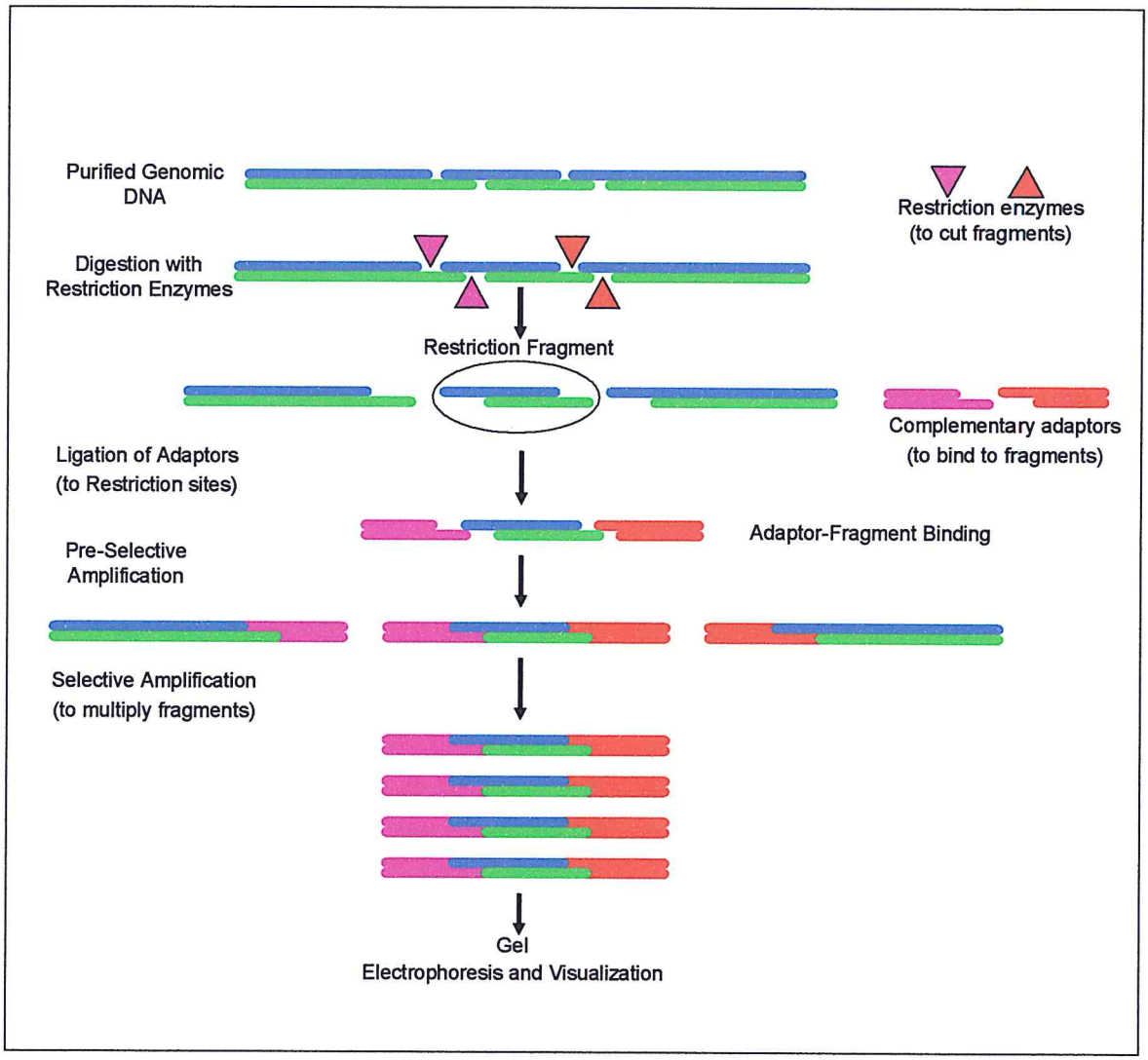


Figure 7.1 Schematic diagram showing steps involved in Amplified Restriction Fragment Length Polymorphism (AFLP). (Scheme: M. Billah).

7.1.3 Applicability of AFLP

A number of different AFLP-based techniques have been described (Janssen *et al.*, 1996; Mazurak *et al.*, 1996; Valsangiacomo *et al.*, 1995; Vos *et al.*, 1995). Originally used in plant analyses, it has now found application in entomology (Reineke *et al.*, 1998), and has been used in linkage-mapping studies of pest moths (Heckel *et al.*, 1998). Use was mostly restricted to the study of genetic diversity within and among a few species such as the gypsy moth *Lymantria dispar* (Linnaeus) populations (Lepidoptera: Lymatriidae) (Reineke *et al.*, 1999). But because of their high replicability and ease of use, AFLP markers have emerged as a major new type of genetic marker with broad application in systematics, pathotyping, population genetics, DNA profiling and quantitative trait loci (QTL) mapping (Mueller & Wolfenbarger, 1999).

7.1.4 Advantages of AFLP

AFLP provides enhanced performance in terms of reproducibility, resolution, and time efficiency. It is sensitive to polymorphism detection at the total-genome level without prior sequence knowledge. This is of particular benefit when studying organisms where very little DNA marker information is available or those from the field without any information at all. AFLP markers usually exhibit Mendelian inheritance, indicating that they are unique DNA fragments. They detect DNA polymorphisms at specific restriction enzyme sites and enable further detection of these polymorphisms at multiple independent restriction sites simultaneously. The technique, however, involves the presence or absence of restriction fragments, and it is therefore not possible to deduce immediate homozygosity or heterozygosity at a locus from those bands (Carlson *et al.*, 1991). With no prior sequence knowledge of the species, the first line of action was to

look for a method that could compare species across the whole genome and show similarities and differences (as opposed to comparison at particular loci and/or nucleotides on the genome). Thus, Amplified Restriction Fragment Length Polymorphism (AFLP) which provides a means of examining DNA segments distributed over the entire genome of an organism, and offers this advantage over methods such as PCR-RFLP techniques (Gibson *et al.*, 1998) were used. The plausible expectation being that when similarities and differences are known, further separation methods can be developed based on specific loci.

7.2 Materials and methods

7.2.1. Source of biological materials

Parasitoid populations were obtained from various sources for this study (see Chapter three). These included *Psyttalia concolor* (Szépligeti) (from Pisa, Italy), *Psyttalia* populations from Rurima (Kenya), Ruiru (Kenya), Shimba Hills (Kenya), *Psyttalia cosyrae* (Wilkinson) (from Nguruman, Kenya), *Psyttalia lounsburyi* (Silvestri) (from Burguret Forest, Kenya), *Psyttalia perproximus* (Silvestri) (from Nkolbisson, Cameroon), and a population from Tafo (Ghana).

7.2.2 DNA extraction and Polymerase Chain Reaction (PCR)

Total DNA was extracted separately from both males and females of the eight populations, using the protocols of Sheppard *et al.* (1992) and Han & McPheron (1997) for fresh or frozen specimens and alcohol-preserved specimens, respectively (Appendix 5).

7.2.3 Digestion and Ligation of total DNA

7.2.3.1 Restriction Digest

Total DNA was double-digested using the restriction enzymes *Hind*III (Roche Diagnostics, GmbH) and *Taq*I (Fermentas) in a total reaction volume of 20 µl. Digestion was started with *Hind*III and incubated at 37 °C for 3 hours, after which *Taq*I was added to each sample tube, and further incubated at 65 °C for 3 hours.

The digestion mixture consisted of 4.0 µl 10X Y+/Tango™ Buffer (with BSA, MBI Fermentas), 1U *Hind*III Restriction enzyme and 10 µl of the DNA sample.

7.2.3.2 Adaptor-Ligation

After the digestion period, end-specific complementary adaptors (double-stranded oligonucleotides) were ligated to the digested samples by the addition of 0.1 µl T_4 Ligase (10 U/ µl), 6.0 µl T_4 Ligase Buffer (5X), and 1.0 µl *Hind*III adaptor (H-adaptor 1 + H-adaptor 2) at 37 °C for 4 hours. The adaptors serve as recognition sequences or sites for subsequent amplification reactions.

7.2.3.3 Verification of digestion

After digestion, the product was run on 1.0 % standard agarose gel in 1X TAE buffer, using 1µl Ethidium bromide (as visualizing agent) per mixture of the product and 2µl loading dye. A 50 base-pair (bp) DNA ladder (GeneRuler™, Fermentas) was used as a size marker. The electrophoresis was run using a Bio-Rad 200/2.0 power supply device at 80V for 60 minutes in a 1X TAE buffer solution. The gel was then visualized under ultra violet (UV) light and photographed (Plate 7.1).

7.2.4. Amplification of digested samples

In AFLP, amplification of restricted fragments is accomplished in two steps; a first amplification - known as Pre-selective amplification, which involves the use of primers with a single selective nucleotide at the 3'-end e.g. H-Core (GAC TGC GTA CCA GCT T) (Invitrogen™, Life Technologies), and a second reaction known as Selective amplification, which employs primers with two or three selective nucleotides e.g. H-CTA (GAC TGC GTA CCA GCT T-CTA). These primers are usually 17-21 nucleotides in length. PCR reactions were carried out in a Programmable Thermal Controller machine (PTC-100™, M. J. Research Inc., U.S.A.). Table 7.1 shows the detailed sequences of adaptors and primers used.

Table 7.1 Detailed sequences of adaptors, primers and their selective extension bases used in this study.

Sequence Name	Recognition Sequence	Selective Extension
H-adaptor 1	5'-CTC GTA GAC TGC GTA CC-3'	
H-adaptor 2	5'-AGC TGG TAC GCA GTC TAC-3'	
H-Core	5'-GAC TGC GTA CCA GCT T-3'	+ **O**
[FAM] H-CTA*	5'-GAC TGC GTA CCA GCT TCT A-3'	+ **CTA**
*Taq*I-CA	5'-GAT GAG TCC TGA GCG AAC A-3'	+ **CA**
*Taq*I-CC	5'-GAT GAG TCC TGA GCG AAC C-3'	+ **CC**
*Taq*I-TT	5'-GAT GAG TCC TGA GCG AAT T-3'	+ **TT**

*H-CTA was fluorescent-labelled with a blue dye [FAM] at the 5'-end to facilitate visualization.

7.2.4.1 Pre-selective amplification

The PCR amplifications were carried out in a 20 µl total reaction volume. This consisted of 1.0µl digested and ligated mix (diluted 1:3), 2.0 µl 10 X PCR buffer (without $MgCl_2$) (MBI Fermentas), 100 ng of H-core primer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.5U of *Taq* DNA polymerase (MBI Fermentas). This was then set to a thermal profile of 4 °C for 2 min, followed by 35 cycles of 94 °C for 30 sec, 50 °C for 30 sec, 72 °C for 1 min and a final holding temperature of 4 °C. The pre-amplified samples were diluted 1:20 in sterile double-distilled water (ddH_2O) for use in selective amplification.

7.2.4.2 Selective amplification

This was also carried out in a 20 µl total reaction volume as in the Pre-selective process. Three different primer combinations were used in this reaction; 10 ng of a fluorescent-labelled primer, ([FAM] H-CTA) (a blue dye obtained from MWG-Biotech, Germany), and 100 ng each of the *Taq*I primers (*Taq*-CA, *Taq*-CC and *Taq*-TT). The three primer combinations were as follows; ([FAM] H-CTA + *Taq*-CA), ([FAM] H-CTA + *Taq*-CC) and ([FAM] H-CTA + *Taq*-TT).

Selective amplification primers usually have two or three additional bases at the 3'-end (compared with the core or pre-selective primers). When the primers are fluorescent-labelled, the dyes are usually attached to the 5'-end. The PCR profile was 94 °C for 2 min, followed by 94 °C for 30 sec, 66 °C for 30 sec, and 72 °C for 1 min. The annealing temperature (66 °C) was then decreased by 1.0 °C in the next 9 cycles, followed by another 20 cycles of 94 °C for 30 sec, 56 °C for 30 sec, 72 °C for 2 min, 60 °C for 30 min and a final holding temperature of 4 °C.

Both the pre-selective and selective amplification reactions were performed with negative controls (NC). These consisted of all the reaction components except the digested-ligated and pre-amplified products.

7.2.5 Loading and electrophoresis of PCR products

Each PCR product was diluted 3-fold (i.e. 1:2). A volume of 0.3µl of the diluted product was then added to 1.5 µl loading buffer (one part GeneScan 500 ROX size standard, three parts deionized formamide, one part loading dye i.e. 1:3:1). The samples were heated to 95°C for 3 min in microAmp® PCR plates and quickly chilled on ice until and during loading. The entire sample was loaded onto a 5% denaturing LongRanger polyacrylamide gel with 1X TBE running buffer, and electrophoresed using an ABI PRISM 377 Sequencing machine (Applied Biosystems) at 55W for 4 hours.

7.3 Data analysis

This was done using a Genotyper 2.0 machine (Applied BioSystems) for scoring binary data (present or absent). The program goes through each lane and extracts the data, thus making an individual sample file for that lane. Once all sample data are extracted, the files are added together and peaks automatically generated for each lane, and the size standard applied to all samples. GeneScan goes through each sample and does the size calling for all peaks, based on the size standard selected (GeneScan-500 ROX). When this is done, the accuracy of size calling is checked by examining all the red data (which indicate peaks). If samples do not line up correctly, a new size standard for those samples is selected and re-analyzed. Only peaks with good signals (i.e. 300 fluorescent-units) were scored (in at least one individual).

All peaks were then scaled and labelled with the size standard in base pairs. All individuals in the categories were then scored by labelling all other peaks (and not just those over 300 fluorescent-units).

The scored data was then analyzed using the Population Genetic Analysis software (PopGen32, Release version 1.31, 1997). Nei's (1978) genetic distances (D) were

calculated with individual genotypes as input (Table 7.2) and a phenogram based on genetic distances generated using UPGMA clustering (Figure 7.2).

7. 4 Results

It was observed that different levels of amplification were achieved with the different primer combinations used (Plate 7.2). The first and third primer combinations ([FAM] H-CTA + *Taq*-CA and [FAM] H-CTA + *Taq*-TT) were more sensitive in the detection of bands than the second combination ([FAM] H-CTA + *Taq*-CC). Between the two sensitive primers however, the first combination seemed slightly over-sensitive, detecting bands at almost all loci in all populations – a situation that makes it very difficult for analysis. The third combination, though sensitive too, was more selective in its detection (Plate 7.2).

The majority of the similarities were observed to occur in *P. concolor* and the populations from Rurima and Ruiru, followed by the populations from Shimba Hills, Cameroon (*P. perproximus*) and Tafo, Ghana, while the major differences were in the *P. lounsburyi* and *P. cosyrae* populations at loci slightly above or below those from the other groups (Pate 7.3). Different levels of sensitivity were indicated by the different primer combinations used in the AFLP, and a closer look at the first two combinations reveals a change only in the position of one base nucleotide (from *Taq*-CA to *Taq*-CC) – a basis for detection of polymorphisms by AFLP (i.e. detecting same base sequences at different loci across the whole genome). These polymorphisms can then be used to measure the intra- as well as inter-specific variations (Nei & Kumar, 2000). However, if a new mutation occurs at a restriction-recognition site (due to changes in the base sequence), then the restriction enzyme will not recognize the site; and a restriction

fragment corresponding to the restriction site will not show up on the gel (Nei & Kumar, 2000).

From Table 7.2, it is observed that the genetic distances (D) between *P. concolor* and the Ruiru and Rurima populations are 0.1023 and 0.1335, respectively; and the genetic identity values are also 0.9028 and 0.8750, respectively. The Shimba Hills population (of unknown identity) has the smallest genetic distance (0.0572) between it and the population from Ghana and the highest genetic identity value (0.9444).

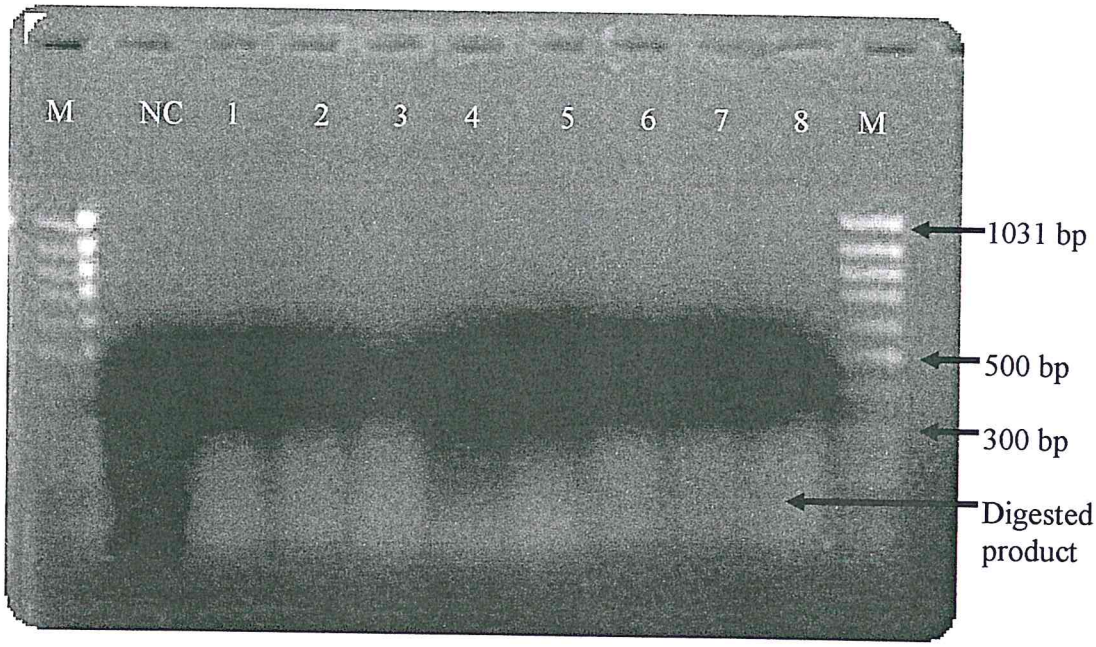


Plate 7.1 Standard agarose electrophoresis gel (1.0%) of the eight *Psyttalia* populations run to verify digestion of DNA before selective amplification. Presence of smears between 150-600 bp indicates digestion of products. Lane M = 50 bp Ladder (MBI Fermentas), NC = Negative Control, 1 = *P. cosyrae* from Nguruman, Kenya, 2 = *P. lounsburyi* from Burguret Forest, Kenya, 3 = *Psyttalia* from Tafo, Ghana, 4 = *P. perproximus* from Nkolbisson, Cameroon, 5 = *Psyttalia* from Shimba Hills, Kenya, 6 = *Psyttalia* from Ruiru, Kenya, 7 = *Psyttalia* from Rurima, Kenya, 8 = *P. concolor* from Pisa, Italy.

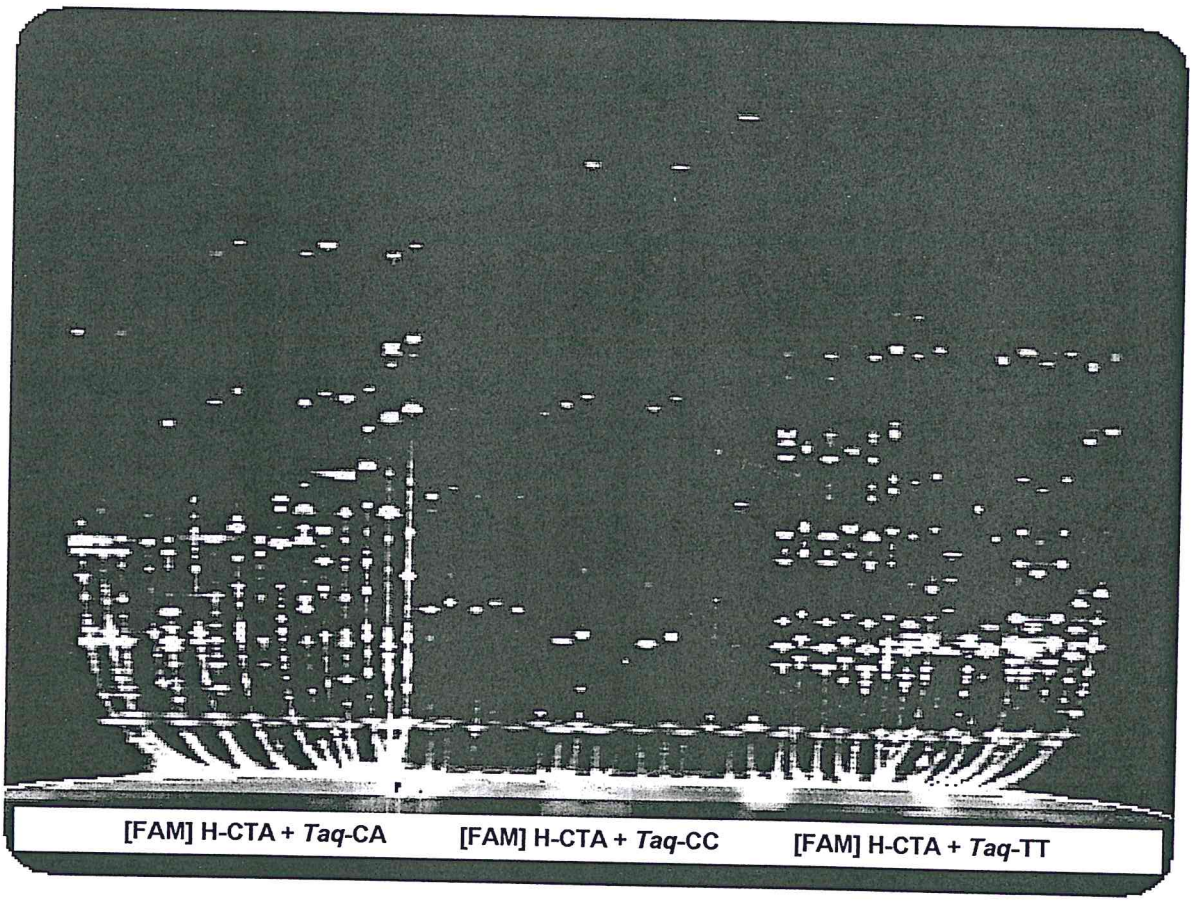
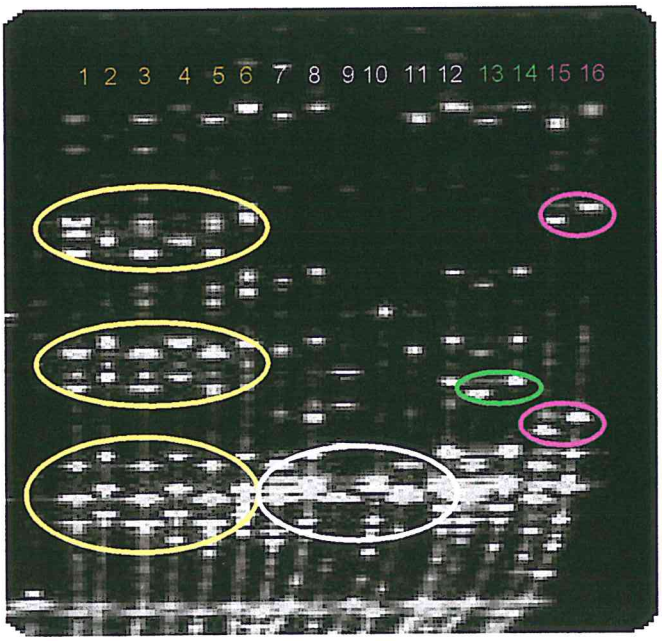


Plate 7.2 Florescent-labelled AFLP products on an ABI PRISM 377 DNA Sequencing machine (using different primer combinations). [All sample fragments were sized using GeneScan-500 ROX size standard (Applied BioSystems)].



1, 2 *P. concolor* – Male & female
3, 4 Rurima *Psyttalia* – Male & female
5, 6 Ruiru *Psyttalia* – Male & female
7, 8 Shimba *Psyttalia* – Male & female
9, 10 *P. perproximus* – Male & female
11, 12 Ghana *Psyttalia* – Male & female
13, 14 *P. lounsburyi* – Male & female
15, 16 *P. cosyrae* – Male & female

Plate 7.3 Florescent-labelled AFLP products on an ABI PRISM 377 DNA Sequencing machine (primer combination [FAM] H-CTA + *Taq*-TT). Fragments were sized using GeneScan-500 [ROX] size standard (Applied BioSystems).

Table 7.2 Nei's (1978) unbiased measures of genetic identity (upper diagonal) and genetic distance (lower diagonal) between the eight *Psyttalia* populations used in this study.

Population	*P. concolor*	Rurima *Psyttalia*	Ruiru *Psyttalia*	Shimba Hills *Psyttalia*	*P. perproximus*	Ghana *Psyttalia*	*P. lounsburyi*	*P. cosyrae*
P. concolor	-	0.8750	0.9028	0.7639	0.8194	0.7639	0.8333	0.7778
Rurima *Psyttalia*	0.1335	-	0.8611	0.6944	0.7778	0.7222	0.7639	0.6806
Ruiru *Psyttalia*	0.1023	0.1495	-	0.8056	0.8611	0.8056	0.8194	0.7639
Shimba Hills *Psyttalia*	0.2693	0.3646	0.2162	-	0.8611	0.9444	0.8472	0.7917
P. perproximus	0.1991	0.2513	0.1495	0.1495	-	0.8889	0.8194	0.7639
Ghana *Psyttalia*	0.2693	0.3254	0.2162	0.0572	0.1178	-	0.8750	0.8194
P. lounsburyi	0.1823	0.2693	0.1991	0.1658	0.1991	0.1335	-	0.8333
P. cosyrae	0.2513	0.3848	0.2693	0.2336	0.2693	0.1991	0.1823	-

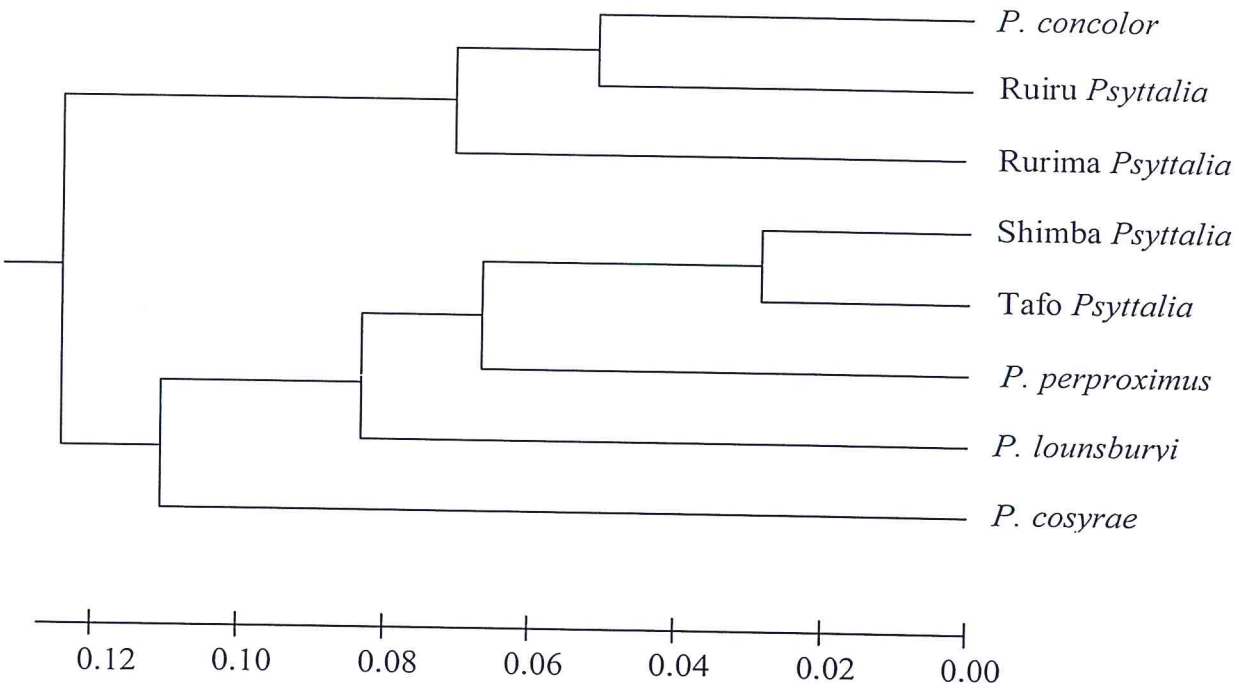


Figure 7.2 Phenogram (UPGMA clustering from Table 7.2) of Nei's (1978) genetic distances among the eight *Psyttalia* populations used in this study.

7.5 Discussion

Though complete separation of the species/population was not achieved, the common loci similarities revealed in the scan are more peculiar to certain populations than others, which coincidentally, match the groupings defined by the morphometric analyses.

The genetic distance values obtained in this study put the Ruiru, Rurima and *P. concolor* populations very close to each other. These values support the hypothesis that the three populations are the same species unless it can clearly be demonstrated otherwise. The information obtained from the distance table also corroborates findings from the morphological studies, and allows placement of the Shimba Hills population with that from Tafo (Ghana) and *P. perproximus*. The results show the usefulness of the AFLP assay in the study of genetic variation of *Psyttalia* parasitoids and contribute to the general picture which is developing on the genetic relationships within species of the *Psyttalia* genus. The AFLP, therefore, lends genomic support to the morphometric data, and strengthens the fact that the use of different identification tools, when used together, can play complementary roles to the elucidation of long standing problems (E. Harley, University of Cape Town, S. Africa, personal communication).

Furthermore, a spin-off of this preliminary genomic comparison is the fact that the common loci similarities revealed can be used as the source for genetic markers to generate linkage maps, as polymorphism in band patterns map out to specific loci, thereby allowing individuals to be genotyped or differentiated, based on the alleles they carry (Weising *et al.,* 1995; Hoelzel, 1998). Microsatellite studies, which are mainly based on the use of loci (or regions within DNA sequences) where short sequences of DNA are repeated one right after the other (i.e. in tandem arrays) (Anon, 2003c) are particularly favoured. What makes microsatellite studies useful is the fact that at the

same location within the genomic DNA, the number of times the sequence is repeated often varies between individuals, within populations, and/or between species.

Despite the minute or virtually hidden differences revealed by the AFLP, the present findings can together with morphological and/or ecological data, play very important complementary roles to make any differences useable, and give them practical use in the field. According to Manhart and McCourt (1992), until there is `the development of a "molecular species concept" that employs the disembodied sequences as the *sine qua non* of species definition`, molecular data will be used to test established species concepts (principally from morphological data).

CHAPTER EIGHT

8.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General discussion

Sub-Saharan Africa is the native home to several well-known fruit pests (Silvestri, 1914). Due to the conducive climatic conditions and the potential for the cultivation of a wide range of horticultural crops (Purcell, 1998), Africa is also now home to one invasive genus *Bactrocera* Macquart (Silvestri, 1914) from the Indo-Australian region. Heavy fruit fly infestation has consequently been named (among others) as the main biotic factor affecting the horticultural industry (Zalom, 1997). The fruit fly problem is aggravated by the prevailing warm weather in the tropics, which is conducive to asynchronous fruiting patterns, resulting in overlapping generations and the potential of fruit fly infestation all year round (Purcell, 1998). These constraints do not make Africa realise its full horticultural potential. Several strategies are used to combat fruit-infesting pests, but chemical control has been the most widely used method because of the higher intensity of pests in these climatic conditions (Hutson & Roberts, 1985; Pimentel, 1997). The use of chemical insecticides has, however, become a subject of controversy (Gary & Mussen, 1984) and a need, therefore, exists to find more environmentally acceptable tactics to the management and control of these flies.

A resurgence of interest in biological control as a management tool for medfly and other tephritid pests has been advocated by Knipling (1992), Wong *et al.* (1992), Waterhouse (1993b) Sivinski (1996), and Purcell (1998); and the main control strategy proposed has been the classical biological control option (Clausen, 1978; Wharton, 1989a). The opiine Braconidae, which has the largest diversity of parasitoids attacking tephritid fruit flies (Clausen, 1978), have a long record of use as biological control

agents against tephritid pests (Silvestri, 1914; Fullaway, 1915; Bianchi & Krauss, 1937; Clausen *et al.*, 1965; Wharton 1989a), and are among the few parasitoids that are presently receiving increased attention for biological control in the neotropics (Messing, 1996; Sivinski, 1996; Kimani-Njogu *et al.*, 2001).

Since the number of fruit fly parasitoids on the continent is high and diverse (Silvestri, 1914), studies on distinguishing between the parasitoids and their association with the different fly hosts will provide valuable information for their use as distinct entities in biological control programmes.

In order to utilize these native parasitoid species more effectively, a clear knowledge of their systematics and behaviour need to be solved to separate them from other species, and be able to determine their true identities for use in future biological control programmes. The study was therefore carried out with the aim of identifying species-specific features of *Psyttalia* species to facilitate their reliable use as biological control agents, by comparing the populations from Kenya with those from other parts of Africa. This focused on identifying species-specific diagnostic characters through the study of morphology and morphometrics of adult populations, studying host-larvae effect on the species/populations, testing the validity of the selected species/populations using the biological species concept (BSC), and examining the relationship between them using molecular studies (Amplified restriction fragment length polymorphism, AFLP).

Morphological comparison of ovipositor to tibia ratio (OTR) and ovipositor sheath to tibia ratio (STR) for all populations, as well as for progenies reared from different host species (larvae) were, to a large extent, useful discriminatory features. The STR was slightly better than the ovipositor/tibia ratio (OTR) and could clearly distinguish between the species. Both ratios can, therefore, be used as reliable tools for

differentiating the species/populations. Species separation into natural groups was also achieved using flagellomere counts. Rearing on different host species showed significant changes in linear measurements as well as body colour (in the case of *P. lounsburyi*). The results provide experimental evidence to the observation by Kimani-Njogu *et al.* (2001) that body size and colour of *Psyttalia* species are largely influenced by the hosts on which they develop.

In the morphometric studies, univariate and bivariate analyses could not distinguish between the populations, but the bivariate plots showed a good number of wing variables with very strong positive correlations between them, and this could be used as supplemental information. The results indicate that the veins enclosing the submarginal cell of the fore wing and the adjoining ones showed the highest weights, and thus served as the main contributing variables in the diagnostic differentiation or separation of the species/populations. The populations from central Kenya were found to be the closest to each other, and also to *P. concolor*, with their clusters usually lying very close to each other in all principal component and canonical variate projections. The lack of complete separation between these three populations, even with the use of canonical variate analysis, is indicative of the very close relationship among the populations with respect to their morphological similarities.

In the cross mating studies, all crosses produced viable female offspring once mating was successful, indicating the absence of complete reproductive incompatibility, as well as the absence of any post-copulatory isolating mechanisms or barriers. This status was also noted in the work of Kimani-Njogu *et al.* (2001) between individuals of *P. concolor* and those of populations from the central highlands of Kenya (Ruiru and Rurima). Interestingly, the clusters defined by morphometric analysis somehow also reflected the host-larvae from which the parasitoids were reared. Members of the *P.*

concolor cluster (*P. concolor*, Rurima and Ruiru) were all reared from *Ceratitis* species, while those of the *P. perproximus* cluster (*P. perproximus*, Tafo and Shimba Hills) were reared from *Trirhithrum* species. Members of the *P. cosyrae* cluster (*P. cosyrae* and *P. phaeostigma*) on the other hand, were either from the *Ceratitis* group of a different size and species (i.e. *C. cosyra* or from the *Dacus* species). This again, brings to the fore the vital role which host-size and species play in the morphological development of parasitoids and, indirectly, lends support to the host-habitat specificity status of the parasitoids. For example, *P. cosyrae* and *P. phaeostigma*, which always differed from the other species, utilize *Ceratitis cosyra* and *Dacus ciliatus*, respectively as their natural hosts. These host flies are known to infest mangoes and cucurbits, and it is thought that due to the bigger size and more fleshy nature of those fruits, the host larvae feed deep into the pulp and parasitoids that access them for parasitization must have ovipositors long enough for insertion into the pulp to reach them. The converse also seems to be the case for individuals of *P. concolor*, Rurima, Ruiru, *P. perproximus*, Tafo, Shimba Hills, and *P. lounsburyi*. These utilize host larvae found in smaller fruits or berries such as coffee and olives, which contain very little pulp and have thin skins. The larvae in these fruits are found at relatively shallow depths and within easy reach of the parasitoids. It is therefore believed that ovipositor length (relative to fruit size) is one area where some form of microevolution might have taken place in the parasitoids. Again, the larvae of *Bactrocera oleae* and *Trirhithrum nigerrimum* like the adult flies; are darker in colour and tend to produce parasitoids with dark body markings (e.g. *P. lounsburyi, P. perproximus*, Tafo and Shimba Hills) as compared to the creamy-white larvae of *Ceratitis* species, which produce parasitoids with the usual yellowish-brown body colour. This observation in nature was experimentally proven to be so with the change in body colour when *P. lounsburyi* was reared on *C. capitata* larvae. These two

natural occurrences might probably explain why there is a strong association between parasitoid species and the hosts from which they develop.

With the revelation of common loci similarities in the genomic comparison, a spin-off of this genomic comparison is the fact that the common loci similarities can be used as the source for genetic markers to generate linkage maps and make it possible for individual populations to be genotyped or differentiated based on the alleles they carry (Weising *et al.,* 1995; Hoelzel, 1998). Microsatellite studies, which are mainly based on the use of loci (or regions within DNA sequences) where short sequences of DNA are repeated one right after the other (i.e. in tandem arrays) (Anonymous, 2003c) will be particularly enhanced - an area to be considered in the future. A summary of the species-specific characters of the representative parasitoid species used in this study is shown in Table 8.1.

Despite the difficulties associated with separation of the individual populations, laboratory studies on these populations have shown that they can cause parasitism rates ranging from 30-70 % (Billah, unpublished data), and that they can be considered among the list of potential or candidate biological control agents in the fight against fruit-infesting flies (at least until new candidates are recruited). Bearing in mind that laboratory studies are usually conducted under selected pre-defined conditions, observations based on such results should be interpreted with caution (McDonald *et al.*, 1998). The performance of some species/populations (Rurima and *P. phaeostigma*) has, however, been considered as high enough and has warranted shipments from ICIPE, Kenya (in collaboration with Texas A&M University, USA) to Hawaii, Guatemala, South Africa (for use in St. Helena's Island) and Le Réunion for potential use in the biological control of medfly and other fruit-infesting Tephritidae (PEET, 2003).

The study has shown that body colour and size are influenced by host characteristics, and should be used with caution when describing species in this group. Ovipositor/tibia, sheath/tibia ratios and the number of flagellomeres are discriminatory features; and together with morphometric analysis, can be used to separate the populations into their natural groups.

The populations from Shimba Hills and Tafo always clustered with that from Cameroon (in the morphometric and molecular studies) and since the populations from Cameroon and Tafo have on different occasions been identified as *P. perproximus* by Steck *et al.* (1986) and by R. A. Wharton (from samples sent by M. K. Billah for identification), there is a strong inclination that the population from Shimba Hills belongs to *P. perproximus* or is very closely related to it.

8.2 CONCLUSIONS

In the light of the findings in these studies, the following conclusions can be drawn;

- Host-larvae colour and size can affect parasitoid morphology (size and colour), and thus should be used with caution when describing species in this group.
- Morphology (ovipositor/tibia, sheath/tibia ratios and flagellomere count), morphometrics and molecular studies indicate that members of *Psyttalia cosyrae* and *P. phaeostigma* are distinct species which are different from the *P. concolor*-group despite their similarity in body colour and shape.
- Increase in size in *Psyttalia concolor* showed significant difference only in the linear measurements, while *P. cosyrae* showed differences in both linear and ratio measurements.
- Ovipositor/tibia and ovipositor sheath/tibia ratios are discriminatory and can be used in separating species.

- Flagellomere count and morphometric analysis were able to separate the species/populations into their natural groups.
- Morphometric separation also indicated the relationship between parasitoids and the host-larvae from which they developed.
- Populations from Rurima and Ruiru may be considered as the same species as *Psyttalia concolor* (i.e. *Psyttalia* cf. *concolor* or Kenyan population of *P. concolor*).
- The population from Shimba Hills shares many morphological and molecular features with *P. perproximus*. They also utilize the same host-larvae resource (*Trirhithrum* species) from the same host fruit/plant (*Coffea canephora* Pierre ex. Froehner), and it is suggested as belonging to *P. perproximus*; thus, representing a new occurrence record from Kenya and East Africa.
- Reproductive compatibility is common among the species studied.
- Production of viable females in all crosses indicates the inadequacy of using the Biological Species concept alone to separate members of this genus.
- Laboratory experiments (based on levels of parasitism in the cross mating studies) indicate that the populations from Kenya are promising biological control candidates for control of *Ceratitis* species.
- The groupings defined by morphometric analysis (based on morphological characters) also share common molecular properties, thus lending genomic support to the observed morphological characters.

8.3 RECOMMENDATIONS

Further research in the areas of ecology, taxonomy as well as the systematics of these African fruit fly parasitoids is still important if sound future biological control programmes are to be implemented.

i. Taxonomic research should continue and include all the presently recognized African species (and most of the undescribed species) reared under similar conditions. This will permit an all-inclusive revision of the African species.

ii. Rearing of the same species on both smaller and larger host larvae may be required to confirm the effects of host size on the parasitoids.

iii. Since it is not known whether or not individuals of the populations interbreed in nature, studies to establish the possibility of pre-copulatory (physical) or pre-zygotic isolating mechanisms will be required.

iv. Future studies on revision of the genus should consider describing the population from Shimba Hills and including it as *P. perproximus* from Kenya.

v. Studies to establish the possibility of hybrids in nature and their characterization will also enhance decision-making from cross mating studies.

vi. Since these African species hold a good biological control potential, research on habitat management will enhance their activity and increase their efficiency.

vii. Molecular studies to develop markers that can help elucidate differences in the species/populations will be required. Since the present study has revealed common loci similarities, microsatellite studies will be particularly favoured as it is mainly based on the use of such repeated loci sequences of DNA.

Table 8.1 Characterization features of *Psyttalia* species used in study.

	Ovipositor/tibia ratio (OTR)	Sheath/tibia ratio (STR)	No. of flagellomeres	Tibia length (mm)	Morphometric grouping	Cross mating	Molecular grouping
Ruiru *Psyttalia*	2.2	1.5	29-38	0.80-1.15	Formed the *P. concolor*-cluster	Produced viable females in all crosses. Therefore not possible to separate species in this genus.	Differentiated between *P. concolor*, *P. perproximus* and *P. cosyrae* clusters matching with the morphometric grouping.
Rurima *Psyttalia*	2.4	1.6	28-39	0.93-1.21			
P. concolor	2.5	1.7	26-36	0.87-1.17			
Shimba *Psyttalia*	2.5	1.8	30-38	0.76-1.01	Formed the *P. perproximus*-cluster		
P. perproximus	2.6	1.8	29-34	0.79-0.92			
Ghana *Psyttalia*	2.7	1.9	32-38	0.85-1.09			
P. phaeostigma	2.8	2.0	40-50	1.26-1.71	Formed the *P. cosyrae*-cluster		
P. cosyrae	3.5	2.5	42-47	1.27-1.49			

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APPENDIX 1

List of all species names known to have been used in association with *Psyttalia* Walker.

A. Names from Africa

Species name	Year	Original Description
advenator (Fischer)	1963	Mujenje, Uganda.
africanus (Szépligeti)	1910	Wellington, Stellenbosch, S. Africa
agreutretae (Wilkinson)	1927	East London, S. Africa.
alleni (Fischer)	1964	Mweru, Brit. E. Africa (Kenya)
bisulcata (Szépligeti)	1914	Bismarck-burg, Togo
colombina (Fischer)	1972	Pietermaritzburg, S. Africa
concolor (Szépligeti)	1910	Souse, Tunisia
cosyrae (Wilkinson)	1927	Morogoro, Tanzania
dacicida (Silvestri)	1911	Eritrea, Ethiopia.
dexter (Silvestri)	1913	Dakar, Senegal
distinguenda (Granger)	1949	Rogez, Madagascar
efoveolatus (Szépligeti)	1913	Arusha-Ju, Tanzania
fuscitarsis Szépligeti	1913	Voi, Tanzania
gigantura (Fischer)	1972	Central uplands, Kenya
hemicauda (Fischer)	1972	Rutshuru Territory, Congo
humilis (Silvestri)	1913	Constantia, (Cape Colony) S. Africa
hypopygialis (Szépligeti)	1913	Ste. Marie, Madagascar
inconsueta (Silvestri)	1913	Olokemeji, Nigeria
infuscata (Granger)	1949	Ankaratra, Madagascar
insignipennis (Granger)	1949	Diego-Suares, Madagascar
inquirendus (Silvestri)	1914	Victoria, Cameroon
kirstenboschensis (Fischer)	1972	Kirstenbosch near Cape Town, S. Africa
kolomani Fischer	1996	Santiago, Cabo Verde
lindbergiana (Fischer)	1971	Tarrafal; S. Antao; Cape Verde Islands
lounsburyi (Silvestri)	1913	Transvaal, S. Africa
ngomeensis (Fischer)	1972	South Ngome forest, S. Africa
niloticus (Fischer)	1972	Egypt
palpalis (Szépligeti)	1902	Aschanti, Nigeria
papagena (Fischer)	1972	Mahembe, Rwanda
paralleni (Fischer)	1972	Rutshuru Territory, Congo
perproximus (Silvestri)	1913	Segboroué, Benin.
perproximus var. *modestior* (Silvestri)	1913	Aburi, Ghana
phaeostigma (Wilkinson)	1927	Natal, Durban, S. Africa
phorelliae (Wilkinson)	1929	Petronella, Transvaal, S. Africa
prothoracalis (Fischer)	1972	Gillits near Durban, S. Africa
puncticranium (Fischer)	1972	Port St. Johns, S. Africa
pusilla (Szépligeti)	1913	Arusha-Ju, Tanzania
sanctamariana (Fischer)	1978	Ste.-Marie, Réunion.
scleroticus (Fischer)	1972	N. Lake Kivu, Rwankwi, Congo
somereni (Fischer)	1972	C. Karura, Kenya
subsulcata (Granger)	1949	Bekily, Madagascar
tshuapana (Fischer)	1972	Tshuapa, Bokuma, Congo
urogramma (Fischer)	1972	Rutshuru Territory, Congo
vittator (Brues)	1926	Umbilo, Durban, Natal, S. Africa.
yangambiana (Fischer)	1972	Yangambi, Congo.

APPENDIX 1 – Continued.

B. Names from outside Africa

Species name	Year	Original Description
acidoxanthicida (Fullaway)	1949	Zamboanga, Philippine Island
amboinensis (Fullaway)	1919	Amboina [Indonesia]
carinata (Thomson)	1895	Gottland, Sweden
danumicus Fischer	2000	Sabah near Danum Valley, Malaysia
fijiensis (Fullaway)	1936	Noainee, Fiji Islands
fletcheri (Silvestri)	1916	India
flexicarina (Fischer)	1975	Oak DForest, Philippines
haemaelaeineni Fischer	2001	Khaeo Khieo Wildlife Sanctuary, Thailand
incisi (Silvestri)	1916	South Coorg, Pollibette, India
javana (Szépligeti)	1908	Semerang, Java
kuchingicola Fischer	1996	Kuching, Semengo, Sarawak, Malaysia
lemiensis (Szépligeti)	1900	Papua New Guinea
leveri (Fullaway)	1953	Kuala Lumpur, W. Malaysia
makii (Sonan)	1932	Tainan (Taiwan)
manilensis Fischer	1963	Manila, Philippines
muesebecki (Fischer)	1963	Noumea, New Caledonia
novaguineensis(Szépligeti)	1900	Lemen (Berlinhofen), New Guinea
novoirlandica (Fischer)	1971	Lemkamin, Bismarch Archipelago, New Ireland
ophthalmica (Tobias)	1977	Eastern Russia
ovaliops (Fischer)	1980	Repress Rio Grande, Guanabara, Brazil
papuensis (Fischer)	1966	Mafula, Papua New Guinea
philippinensis (Ashmead)	1904	Manila, Philippines
ponerophaga (Silvestri)	1916	Cherat, Northern Pakistan
psyttaloides (Fischer)	1971	Lemkamin, Bismarch Archipelago, New Ireland
rhagoleticola (Sachtleban)	1934	Naumburg a. d. Saale, Germany
rufoflava Fischer	2001	El Batey, Puerto Plata, Sosua, Dominican Republic
sabhayanus(Fischer)	1966	Arun Valley, Nepal
sapamoroanus (Fischer)	1971	Sapamoro, Mindanao, Philippines
siculus Monastero	1931	Altavilla, Milan, Sicily, Italy
tamurensis (Fischer)	1966	Taplejung District, East Nepal
testacea Walker	1860	Ceylon
testaceipes (Cameron)	1905	Pasuruan, Java [Indonesia]
walkeri (Muesebeck)	1931	Ceylon [Sri Lanka]

APPENDIX 2

Generic names used in association with tephritid parasitoids and their current status. Citations to original descriptions obtained from Fischer (1972, 1977 and 1987).

Generic name	Current status
Austroopius Szépligeti 1900	Synonym of *Psyttalia* (Wharton 1987). Tephritid parasitoids formerly included here have usually been referred to as *Opius* in the biological control literature.
Biosteres Förster, 1862	Valid genus. Tephritid parasitoids formerly included here have now been transferred to either *Diachasmimorpha* or *Fopius*. See Wharton (1988).
Bracanastrepha Brèthes, 1924	Synonym of *Utetes* (Wharton 1987, 1988). Tephritid parasitoids formerly included here were referred to as *Opius* in most of the biological control literature prior to 1979.
Desmiostoma Förster, 1862	Synonym of *Opius* (Wharton 1983, 1988). Tephritid parasitoids formerly included here have usually been referred to as *Opius*.
Diachasma Förster, 1862	Valid genus (but see Tobias, 1977). Most tephritid parasitoids formerly included here have been transferred to either *Fopius* or *Diachasmimorpha*. Members of the *ferrugineum* species group are provisionally retained in *Diachasma*.
Diachasmimorpha Viereck, 1913	Valid genus for some of the most widely used parasitoids in tephritid biocontrol. Prior to 1987, most of these species were placed in either *Biosteres* or *Opius*, and more rarely *Parasteres* (Wharton 1987).
Doryctobracon Enderlein, 1920	Valid genus. Prior to 1987, these species were placed either in *Opius* or *Parachasma* (Fischer, 1977; Wharton & Marsh, 1978).
Fopius Wharton, 1987	Valid genus, originally described as a subgenus of *Rhynchosteres* (Wharton, 1987; van Achterberg & Maetô, 1990). Includes some of the most significant parasitoids used in tephritid biocontrol. Most of these species have been placed in *Biosteres* in recent literature and in *Opius* prior to about 1970.
Hedylus Marshall, 1891	A junior homonym of *Hedylus* Förster, 1862 – an ichneumonid, and considered a junior subjective synonym of either *Biosteres* or *Gnamptodon* Haliday by Fischer (1972). One species (*giffardii*) was originally described in *Hedylus*.
Opius Wesmeal, 1835	Valid genus. Most of the tephritid parasitoids formerly included in *Opius* have been transferred elsewhere.
Parachasma Fischer, 1967	Junior subjective synonym of *Doryctobracon* (Fischer, 1972; Wharton & Marsh, 1978).
Parasteres Fischer 1967	Junior subjective synonym of *Diachasmimorpha* (Wharton & Marsh, 1978; Wharton, 1987).
Psyttalia Walker, 1860	Valid genus for some of the most widely used parasitoids in tephritid biocontrol (Wharton, 1987, 1988). Most of these species have been referred to as *Opius* in the biocontrol literature.
Utetes Förster, 1862	Valid genus. Tephritid parasitoids have been referred to as *Opius* (Wharton, 1988). Treated by Fischer as a subgenus of *Opius*.

Source: Reproduced from Wharton (1997a, 34).

APPENDIX 3

Objective lens calibration of Leica WILD M3Z microscope used for measuring the length and width of fruit fly puparia.

Objective lens magnification	Matching values	One unit	Conversion factor / mm	Factor /µm
6.5X	26 units = 2.00 mm	1/26 x 2.00	0.07692	76.92
10X	39 units = 2.00 mm	1/39 x 2.00	0.05128	51.28
16X	62 units = 2.00 mm	1/62 x 2.00	0.03226	32.26
25X	96 units = 2.00 mm	1/96 x 2.00	0.02083	20.83
40X	100 units = 1.29 mm	1/100 x 1.29	0.0129	12.90

APPENDIX 4

Counts of flagellomeres (antennal segments without scape and pedicel) in *Psyttalia* species/populations used in study.

	Species/Population											
	P. concolor		*P. concolor* ex *C. cosyra*		*P. cosyrae*		*P. cosyrae* ex *C. capitata*		Rurima *Psyttalia*		Ruiru *Psyttalia*	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
1	33	31	37	34	46	44	43	44	38	30	32	32
2	34	31	33+	32	44	46	43	42	36	34	36	30
3	32	30	37	32	44	42	44	44	35	29	37	36
4	32	29	38	33	44	44	45	43	34	39	33	35
5	33	30	37	34	46	43	43	45	34	28	38	36
6	33	29	38	33	45	43	42	42	33	35	35	34
7	34	30	36	34	46	44	43	42	36	34	37	31
8	36	30	-	33	42	45	42	43	37	29	37	32
9	34	33	-	33	44	43	45	43	35	31	36	34
10	34	28	-	32	41	43	46	43	36	34	36	34
11	35	33	-	31	43	43	-	44	35	34	37	34
12	35	29	-	32	47	44	-	43	31	31	35	34
13	36	26	-	33	46	44	-	42	37	32	35	33
14	32	32	-	33	43	44	-	40	38	37	39	32
15	35	28	-	32	44	46	-	44	34	35	34	36
16	34	32	-	32	43	43	-	41	35	31	33	33
17	35	29	-	32	46	43	-	44	36	34	36	36
18	31	31	-	34	45	42	-	43	34	34	34	32
19	33	30	-	34	46	46	-	44	36	32	33	35
20	33	29	-	30	45	43	-	44	37	35	37	34
21	33	31	-	-	44	42	-	-	36	34	33	36
22	35	28	-	-	43	43	-	-	37	38	31	32
23	34	29	-	-	43	46	-	-	37	33	35	32
24	34	32	-	-	44	45	-	-	37	34	31	32
25	32	30	-	-	46	43	-	-	38	34	32	37
26	33	30	-	-	45	44	-	-	37	35	33	34
27	35	32	-	-	47	45	-	-	36	37	33	33
28	34	31	-	-	46	46	-	-	34	36	36	35
29	33	32	-	-	47	46	-	-	34	29	36	33
30	34	31	-	-	44	43	-	-	36	36	34	29
31	34	29	-	-	-	-	-	-	-	-	-	-
32	35	32	-	-	-	-	-	-	-	-	-	-
33	33	31	-	-	-	-	-	-	-	-	-	-
34	34	33	-	-	-	-	-	-	-	-	-	-
35	32	32	-	-	-	-	-	-	-	-	-	-
36	33	32	-	-	-	-	-	-	-	-	-	-
37	32	29	-	-	-	-	-	-	-	-	-	-
38	36	31	-	-	-	-	-	-	-	-	-	-
39	34	30	-	-	-	-	-	-	-	-	-	-
40	31	28	-	-	-	-	-	-	-	-	-	-

+ = Number of segments counted in broken antennae.

APPENDIX 4 - Continued.

	Species/Population*											
	Shimba Hills *Psyttalia*		*P. perproximus*		*P. phaeostigma*		Mrima Hill *Psyttalia*		Ghana *Psyttalia*		*P. lounsburyi*	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
1	37	36	30	28+	43	48	46	48	32	38	36	34
2	31	36	33	29	44	47	46	lost	33	34	36	34
3	33	36	33	27+	45	43	45	48	-	35	35	32
4	33	35	34	23+	46	43	46	48	-	30+	35	32
5	35	35	32	26+	44	47	44+	48	-	34	33	33
6	33	37	30	-	45	47	45	46	-	-	35	34
7	33	36	31	-	41	50	46	39+	-	-	33	34
8	34	35	33	-	44	47	47	24+	-	-	33	33
9	32	33	31	-	44	49	45	47	-	-	33	32
10	31	37	30	-	46	45	45	50	-	-	36	35
11	36	38	-	-	43	48	47	-	-	-	35	35
12	34	33	-	-	46	44	45	-	-	-	35	35
13	30	35	-	-	44	43	45	-	-	-	36	35
14	34	35	-	-	42	45	46	-	-	-	35	34
15	32	35+	-	-	44	47	47	-	-	-	36	35
16	35	35	-	-	43	47	46	-	-	-	34	32
17	33	34	-	-	41	45	41	-	-	-	36	34
18	33	37	-	-	43	48	43	-	-	-	35	36
19	33	33	-	-	43	44	47	-	-	-	36	34
20	35	35	-	-	41	44	44	-	-	-	35	35
21	35	35	-	-	49	47	-	-	-	-	33	32
22	35	35	-	-	43	44	-	-	-	-	36	33
23	33	34	-	-	44	47	-	-	-	-	35	34
24	33	31	-	-	42	44	-	-	-	-	35	34
25	-	31	-	-	40	44	-	-	-	-	34	33
26	-	33	-	-	41	48	-	-	-	-	37	36
27	-	35	-	-	42	43	-	-	-	-	35	35
28	-	35	-	-	36+	43	-	-	-	-	37	34
29	-	35	-	-	32+	38+	-	-	-	-	36	34
30	-	34	-	-	40	33+	-	-	-	-	34	35

+ = Number of segments counted in broken antennae.
* = Population from South Africa (not indicated) - had all antennal segments lost.

APPENDIX 5

Chemical components of DNA extraction and gel running Buffers

a) For Fresh or Frozen insect specimens

Grinding Buffer

- 10mM Tris-HCl (pH 8.0)
- 60mM NaCl
- 1% sucrose
- 100mM EDTA (pH 8.0)

Lysis Buffer

- 300mM Tris-HCl (pH 8.0)
- 5% sucrose
- 100mM EDTA (pH 8.0)
- 1.24% (w/v) SDS
- 1.2-2% freshly prepared DEPC (diethyl pyrocarbonate)

b) For pinned or alcohol preserved specimens

Grinding buffer

- 10mM Tris-HCl (pH 8.0)
- 60mM NaCl
- 150mM or 5% sucrose
- 10mM EDTA
- 0.5% SDS

Lysis Buffer

- 300mM Tris-HCl (pH 8.0)
- 100mM EDTA (pH 8.0)
- 150mM sucrose
- 60mM NaCl,
- 0.75% (w/v) SDS
- 1.2-2% freshly mixed DEPC (diethyl pyrocarbonate)

Gel Buffers

a) 1 X TAE Buffer

Prepare 50X:

- 242 g Tris base
- 57.1 ml glacial acetic acid
- 100 ml 0.5 M EDTA (pH 8.0)
- Dilute to 1 X TAE working solution with dH_2O

b) 1 X TBE Buffer

Prepare 5X:

- 54g Tris base
- 27.5 g Boric acid
- 20 ml 0.5 M EDTA (pH 8.0)
- Dilute to 1 X TBE working solution with dH_2O