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Cross mating studies among five fruit fly parasitoid populations: potential biological control implications for tephritid pests

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Abstract The reproductive compatibility between four different species/populations of the tephritid parasitoid *Psyttalia* (Walker) species from Kenya and individuals of the morphologically identical *Psyttalia concolor* (Szépligeti) (Hymenoptera: Braconidae) from a laboratory culture in Italy used in augmentative biological control of olive fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) was assessed through cross mating tests using single-pair and group mating methods. Reciprocal crosses among the species resulted in the production of viable offsprings up to the second generation. In spite of the successful production of viable offspring in the laboratory, *Psyttalia* species are known to have specific host fruit and/or host fly preferences and populations/species may be isolated in one way or the other. However, it is not known whether these populations/species interbreed in the field. We discuss the ability of these parasitoids to interbreed and the potential effects of that on their use as biological control agents, especially in environments where other closely related species are present or in situations where multiple parasitoid introductions are intended.

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Introduction

Cross mating experiments are 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 and Stouthamer 1994), and for making important biological control decisions (Pinto et al. 1986; Stouthamer et al. 2000). In tephritids, it has also been used to test the compatibility of different populations in a species complex to warrant the use of the right compatible population in sterile insect technique (SIT) programmes (Vera et al. 2006). Decisions on species in groups such as the Eulophidae, Aphelinidae, Trichogrammatidae, and aphidiine Braconidae have often utilized cross mating data (Grissell and Schauff 1997; Stouthamer et al. 2000).

Many factors contribute to variation within species, including use of different host species (Janssen 1989), host size (Salt 1941; Charnov et al. 1981; Charnov 1982; Billah et al. 2005), host age or quality (King 1987; Godfray 1994), host condition and diet (Vinson and Iwantsch 1980; Wajnberg et al. 1990), environmental (Phillips et al. 1993) as well as genetic factors (Diehl and Bush 1984). These factors are further complicated by the limited extent to which the degree of morphological and biological plasticity inherent in single species is understood (Grissell and Schauff 1997). When an insect has a wide geographic distribution, a greater intra-specific variability might be expected (Diehl and Bush 1984), and the importance of intra-specific variability of hymenopterous parasitoids in the biological control of insect pests has long been recognized (Hopper et al. 1993). *Psyttalia* species are widely distributed on the African continent and elsewhere, and comparative studies among these populations would help to reveal intra-specific 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 optimal strains/populations for introductions is often of crucial importance for the successes of such programmes (van den Bosch et al. 1979; Debach and Rosen 1991). For these reasons, comparisons within or among populations of parasitoid species collected from different geographic regions or host-habitats have often been made for sources of variations (Hopper et al. 1993). *Psyttalia* species have been used in several classical and augmentative biological control programmes (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 and 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 20 years (Wharton et al. 2000; Kimani-Njogu et al. 2001;

Wharton and Kimani-Njogu 2002; Copeland et al. 2004, 2006). The paucity of character states of use to taxonomists, coupled with the 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).

To utilize these parasitoids, they need to be characterized and compared with other morphologically similar species. This study is part of a series aimed at establishing the identity and relationship among these potential biological control candidates by assessing their mating compatibility status using the biological species concept (BSC) and the implications for the control of pest fruit flies.

Materials and methods

Sources of biological materials

Psyttalia concolor (Szépligeti) was imported from a laboratory culture maintained in Pisa, Italy (originally described from Tunisia in North Africa) for basic research and augmentative releases against the olive fly, *Bactrocera oleae* (Gmelin) (Raspi and Loni 1994). A colony was initiated at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya and maintained on *Ceratitis capitata* (Wiedemann) larvae as the preferred laboratory host. Field collections of other *Psyttalia* species were obtained from coffee berries—commonly infested with *Ceratitis capitata*, *C. rosa* Karsch, *Trirhithrum coffeae* Bezzi and *T. nigerrimum* (Bezzi). Collections were also made from mango (*Mangifera indica* L.)—mostly infested with *C. cosyra* (Walker) and from cultivated squash (*Cucurbita pepo* L.) which were mostly infested by *Dacus* Fabricius and *Bactrocera* Macquart species. Fruits were held in cages in the insectary until tephritid larvae exited the hosts. Puparia were collected and held until fly or parasitoid emergence. Coffee berries were collected from plantations in Ruiru, Central Province (01°5′72 S, 36°54′22 E; elevation 1,609 m) and Rurima, Eastern province (00°38′39 S, 37°29′69 E; elevation 1,228 m) in the Central and Eastern provinces of Kenya, respectively. The parasitoid populations from these two locations are morphologically similar to *P. concolor* and were reared from *Ceratitis* MacLeay species. Mango samples were collected from Nguruman (01°48′39 S, 36°03′28 E; elevation 817 m) in the Rift-Valley Province of Kenya, and the parasitoids identified as *Psyttalia cosyrae* (Wilkinson). Cultivated squash were obtained from two gardens at ICIPE, Nairobi (Garden 1: 01°13′14 S, 36°53′44 E; elevation 1,626 m and Garden 2: 01°13′29 S, 36°53′51 E; elevation 1,619 m). Parasitoids from squash were identified as *Psyttalia phaeostigma* (Wilkinson). *Psyttalia concolor, P. cosyrae, P. phaeostigma* and the two populations from Ruiru and Rurima are referred to in the crosses as Pcn, Pcs, Pph, Pru and Prm, respectively. *Ceratitis* (*C. capitata* and *C. cosyra*) and *Dacus ciliatus* Loew larvae were obtained from fruit fly colonies maintained at ICIPE by the African fruit Fly Initiative (AFFI). Third instar larvae of these were exposed to mated *P. concolor*, *P.cosyrae* and *P. phaeostigma* females, respectively. Parasitoids from Ruiru and Rurima were also reared on *C. capitata* larvae (which are their preferred hosts) to get the parasitoids as close to their natural performance as possible, and to reduce the number of external factors that may negatively influence their performance (Hall 1993). Each parasitoid population was reared in the laboratory for at least five generations before experiments began. Samples of parasitoid colonies were screened by S. Dupas (Institut de Recherché pour le Développement (IRD), Paris, France) and found to be free of the

intra-cellular parasite, *Wolbachia*—which negatively affects the reproductive fitness of biological control agents.

Identification of biological material

Parasitoids were identified, mostly using available literature and keys to the genus (Wharton and Gilstrap 1983; Wharton 1997a, b), or by RAW, while the flies were identified using literature and keys of White and Elson-Harris (1992) and 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 (Tervuren Museum, Belgium) for confirmation. Routine identifications of the common fruit fly and parasitoid species were performed by MKB. Voucher specimens were deposited at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya

Crossing experiments and mating behaviour

Two methods of crossing were used; single-pair and group mating (Tables 1 and 2). 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 (Liu et al. 2002). 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 × 7.5 cm height), covered at the top with a piece of fine netting material

Table 1 Pairs of parasitoids used in main, reciprocal and back crosses between individuals of *Psyttalia concolor* and *P. cosyrae*

Crosses/backcrosses (♂ × ♀)	No. of parasitoids		Remarks
	Single-pair mating	Group mating	
Cross and reciprocal cross			
Pcn* × Pcs**	11	11 ♂ × 11 ♀	Inter-population crosses
Pcs × Pcn	13	10 ♂ × 10 ♀	
Pcn × Pcn	12	25 ♂ × 25 ♀	Intra-population crosses
Pcs × Pcs	12	10 ♂ × 10 ♀	
Backcrosses			
Pcn × (Pcn × Pcs)	10	17 ♂ × 17 ♀	F_1 females back crossed to parental males
Pcs × (Pcn × Pcs)	10	14 ♂ × 14 ♀	
Pcs × (Pcs × Pcn)	10	16 ♂ × 16 ♀	
Pcn × (Pcs × Pcn)	10	15 ♂ × 15 ♀	
F_1 Crosses			
(Pcn × Pcs) × (Pcn × Pcs)	10	15 ♂ × 15 ♀	F_1 males crossed with F_1 females
(Pcs × Pcn) × (Pcs × Pcn)	10	15 ♂ × 15 ♀	

* Pcn = *Psyttalia concolor*

** Pcs = *Psyttalia cosyrae*

Table 2 Results of group mating crosses among *Psyttalia* parasitoids from three populations in Kenya and individuals of *P. concolor* from Italy

Crosses/back crosses (♂ × ♀)	No. of wasps (group mating)	Remarks
Cross and reciprocal cross		
Pcn × Prm	24 ♂ × 24 ♀	Inter-population crosses
Prm × Pcn	25 ♂ × 25 ♀	
Pcn × Pph	15 ♂ × 15 ♀	
Pph × Pcn	14 ♂ × 14 ♀	
Pru × Pcn	12 ♂ × 12 ♀	
*Pcn × Pru	–	
Prm × Prm	25 ♂ × 25♀	Intra-population crosses
Pru × Pru	19 ♂ × 19 ♀	
Pph × Pph	15 ♂ × 15 ♀	
Pcn × Pcn	25 ♂ × 25 ♀	
Backcrosses		
(Pcn × Prm) × Pcn	15 ♂ × 15 ♀	F_1 females back crossed to parental males
(Pcn × Prm) × Prm	17 ♂ × 17 ♀	
(Prm × Pcn) × Prm	18 ♂ × 18 ♀	
(Prm × Pcn) × Pcn	10 ♂ × 10 ♀	
(Pcn × Pph) × Pcn	14 ♂ × 14 ♀	
*(Pcn × Pph) × Pph	–	
(Pph × Pcn) × Pph	12 ♂ × 12 ♀	
(Pph × Pcn) × Pcn	15 ♂ × 15 ♀	
(Pru × Pcn) × Pru	12 ♂ × 12 ♀	
*(Pru × Pcn) × Pcn	–	
F_1 Crosses		
(Pcn × Prm) × (Pcn × Prm)	15 ♂ × 15 ♀	F_1 males crossed with F_1 females
(Prm × Pcn) × (Prm × Pcn)	14 ♂ × 14 ♀	
(Pcn × Pph) × (Pcn × Pph)	11 ♂ × 11 ♀	
(Pph × Pcn) × (Pph × Pcn)	13 ♂ × 13 ♀	
(Pru × Pcn) × (Pru × Pcn)	14 ♂ × 14 ♀	
*(Pcn × Pru) × (Pcn × Pru)	–	

* Cross died and values not calculated for backcross and/or F_1 cross

Pcn = *Psyttalia concolor* from Pisa, Italy

Prm = *Psyttalia* from Rurima, Eastern Province

Pru = *Psyttalia* from Ruiru, Central Province

Pph = *Psyttalia phaeostigma*, Kasarani, Nairobi Province

and held in place with a rubber band. Group mating involved a group of virgin males and virgin females confined in perspex cages (12 × 12 × 12 cm). Group mating was conducted for all five 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 × 1.8 cm height, Costar®, USA) to prevent mating upon emergence, and the wells covered with perforated

Parafilm® "M" for ventilation. Each experiment consisted of the main inter-population cross and two intra-population crosses as controls. The controls consisted of pairs of virgin males and females from each of the 2 parental populations e.g. for *P. concolor* × *P. cosyrae* experiment, controls were *P. concolor* males × *P. concolor* females and *P. cosyrae* males × *P. cosyrae* females, as done by Kimani and Overholt (1995) and Kimani-Njogu et al. (2001). All trials were conducted between 10:00 and 15:00 h, 25–27°C, 60–65% R. H. and under partial natural light in the ICIPE Quarantine facility. After 4 h of mating observation, single-pair females were individually transferred to small perspex cages (11.5 × 7.5 × 11.5 cm), while group mating females remained in their original cages (12 × 12 × 12 cm). Parasitoids were provided with a 10% honey solution soaked in cotton wool, water soaked in cotton wool and fine droplets of pure honey streaked on the top side of the cage. 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 cages had small openings (5–6.5 cm) at the top fixed with organza material and used for exposure of larvae to the parasitoids. Host larvae (in a ratio of one female parasitoid to ten larvae per day) were aggregated in a thin layer of artificial larval diet (a modification of Hooper's (1987) method, consisting 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) made into a paste) on a modified petri dish serving as oviposition unit. Larvae were left in the oviposition unit for about 20–30 min prior to exposure to avoid the initial reaction of larvae popping out from the unit and to ensure some feeding and moving activity in the diet for easy detection by parasitoids. The oviposition unit was placed (inverted) on the top organza material for 4–6 h with a 15–20 g weight to keep it in place. After oviposition, the larvae were transferred to bigger petri dishes (8.6–9 cm diameter) and provided with fresh diet. The petri dishes were placed in plastic containers (13 cm diameter, 6 cm depth) with a layer of sand at the bottom of each to serve as a pupation medium. The sand was kept moist to prevent pupal desiccation. A 10 cm diameter opening was made in the lid of the container and replaced with fine net. The sand was periodically sieved to recover puparia, which were individually held till flies and/or parasitoids emerged. Emerging parasitoids were counted, sexed and held separately (by sex) till ready for subsequent crosses. Uneclosed puparia were dissected to confirm presence or absence of parasitoids.

Upon successful production of males and females in the crosses, F_1 females were crossed with parental males (as in Kimani-Njogu et al. 2001; Liu et al. 2002). *Psyttalia* species are arrhenotokous and production of both sexes was used to confirm successful egg fertilization. Reproductive compatibility (RC) was assessed by the ability of the inter-population crosses to produce viable female offspring through at least two generations (Tables 1 and 2). Reproductive compatibility was expressed as the relative value of the proportion of females in a progeny of an inter-population cross to that of the corresponding intra-population crosses (Pinto et al. 1991). Mating behaviours of wasps i.e. wing fanning, male approach to females, female response to males, number of attempted mountings and body movements before, during and after copulation were also observed.

Data analysis

Mating data was analyzed with a general linear model (Proc GLM; SAS Institute 2001), and 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. Proportions were arcsine-root

transformed and subjected to ANOVA (Sokal and Rohlf 1995). Relative compatibility (RC) for each inter-population crosses were 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 calculated and a mean value determined (i.e. inter-population cross in relation to each of the two intra-population control crosses; ½{A × B/A × A + A × B/B × B}).

Results

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 a rapid approach toward females. 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 s and started fanning gain. After separation or "grooming", *P. cosyrae* pairs usually stayed motionless for a while and then spent the next 20–45 s cleaning their bodies before walking away.

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 were 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.

Reproductive compatibility

The mean relative reproductive compatibility of the inter-population crosses fluctuated between 0.21 and 1.15 (Tables 3 and 4). For females which produced both sexes, the mean proportion of females in their progeny 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 3). However, the proportion of parasitoids produced differed in various crosses (Table 3). Under single-pair mating, the highest proportions occurred in the two intra-population crosses (Pcn × Pcn {0.61} and Pcs × Pcs {0.51}), which did not differ from each other but differed significantly from the two inter-population crosses (Pcn × Pcs {0.43} and Pcs × 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 3 and Fig. 1). Under group mating, the proportion of parasitoids produced was highest in the intra-population crosses (Pcn × Pcn {0.91} and Pcs × Pcs {0.51}), but differed from each other ($F = 43.62$; df = 3, 49; $P < 0.0001$). Pcn × Pcn also differed from the two inter-population crosses (Pcn × Pcs {0.24} and Pcs × Pcn {0.47}), while Pcs × Pcs differed only from Pcn × Pcs but not from the reciprocal cross Pcs × Pcn. The progenies 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 with the exception of the $F_1 \times F_1$ value for Pph × Pcn {58.8} (Table 5).

Discussion

Mating behaviour

Many courtship behaviours exhibit combinations of features that are unique to groups and can be used for identification purposes, as they are usually species-characteristic (Jervis and Kidd 1996). 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 "song" which is thought to be a way of fanning pheromones toward the females to solicit their response (Sivinski

Table 3 Proportion of females and total number of parasitoids in progenies produced in single-pair and group mating crosses between *Psyttalia concolor* and *P. cosyrae*

Biological parameters	Inter-population cross		Intra-population cross	
	Pcn × Pcs (♂ × ♀)	Pcs × Pcn (♂ × ♀)	Pcn × Pcn (♂ × ♀)	Pcs × Pcs (♂ × ♀)
Single-pair mating				
Number of females	9**	11	11	10
Proportion of females in progeny	0.50 ± 0.06 a*	0.63 ± 0.06 a	0.52 ± 0.05 a	0.57 ± 0.06 a
	$F = 1.03$, df = 3, 35, $P = 0.3918$			
No. of parasitoids produced	0.43 ± 0.06 b	0.34 ± 0.04 b	0.61 ± 0.05 a	0.51 ± 0.04 ab
	$F = 6.46$, df = 3, 39, $P = 0.0012$			
Relative Compatibility	0.93	1.15	–	–
Group mating				
Number of females	8	10	25	10
Proportion of females in progeny	0.50 ± 0.01 a	0.49 ± 0.08 a	0.58 ± 0.02 a	0.54 ± 0.04 a
	$F = 1.49$, df = 3, 44, $P = 0.2306$			
No. of parasitoids produced	0.24 ± 0.02 c	0.47 ± 0.07 b	0.91 ± 0.03 a	0.51 ± 0.04 b
	$F = 43.62$ df = 3, 49, $P < 0.0001$			
Relative Compatibility	0.78	0.79	–	–

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

* 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

** Replicates failing to parasitize host larvae or produce progeny were discounted from analyses, thus accounting for the apparent differences in number of females in Tables 1 and 3

and Webb 1989). Though chemical, tactile as well as visual stimuli are involved in the release and continuation of courtship in parasitic Hymenoptera (van den Assem and 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 females. No matter the species/population used, they started fanning as soon as females were offered to them. The females also exhibited behaviours suggesting eagerness to mate without waiting for males to go through all the features of courtship.

Reproductive compatibility (RC)

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) 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 (Nagarkatti 1975), which have been justified by the role played by reproductive isolation in speciation (Pinto et al. 1991) and the need to formally recognize distinct populations for biological control purposes. Pinto et al. (1991) used relative compatibility to reveal genetic differences between strains of *Trichogramma* and suggested that with their experimental procedure, RC values lower than 0.75 could be taken as evidence of partial incompatibility. In this study, RC levels range from 0.21 to a high value of 1.15, indicating partial to full compatibility between the populations. With the exception of the RC value for Pcn × Pph (0.21), all other crosses have values >0.75 (Table 4). In a similar study between two geographic populations of the pupal parasitoid *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), Liu et al. (2002) suggested that very high RC levels could be assumed to be a result of experimental errors culminating from the fact that cultures are maintained in the laboratory and experiments conducted under forced and no-choice conditions. No significant difference was observed in the mean proportion of females

Table 4 Relative compatibility (RC) values for group mating and reciprocal crosses among individuals of four *Psyttalia* populations from Kenya and those of *P. concolor*

Inter-population cross	Proportion of females (a)	Intra-population cross	Proportion of females(b)	Relative compatibility (a/b)	Mean RC
Pcn × Pcs	42.9	Pcn × Pcn	57.1	0.75	0.78
		Pcs × Pcs	52.6	0.82	
Pcs × Pcn	43.2	Pcn × Pcn	57.1	0.76	0.79
		Pcs × Pcs	52.6	0.82	
Pcn × Prm	43.6	Pcn × Pcn	57.1	0.76	0.78
		Prm × Prm	54.2	0.80	
Prm × Pcn	44.2	Pcn × Pcn	57.1	0.77	0.80
		Prm × Prm	54.2	0.82	
Pcn × Pph	12.5	Pcn × Pcn	57.1	0.22	0.21
		Pph × Pph	61.9	0.20	
Pph × Pcn	61.3	Pcn × Pcn	57.1	1.07	1.03
		Pph × Pph	61.9	0.99	
Pcn × Pru	*	Pcn × Pcn	57.1	*	*
		Pru × Pru	32.4	*	
Pru × Pcn	38.1	Pcn × Pcn	57.1	0.67	0.92
		Pru × Pru	32.4	1.18	

Pcn = *Psyttalia concolor* from Pisa, Italy

Pcs = *P. cosyrae*, Nguruman, Rift-Valley Province

Pph = *P. phaeostigma ;* Kasarani, Nairobi Province

Prm = *Psyttalia* from Rurima, Eastern Province

Pru = *Psyttalia* from Ruiru, Central Province

* Values not calculated due to death of parental cross

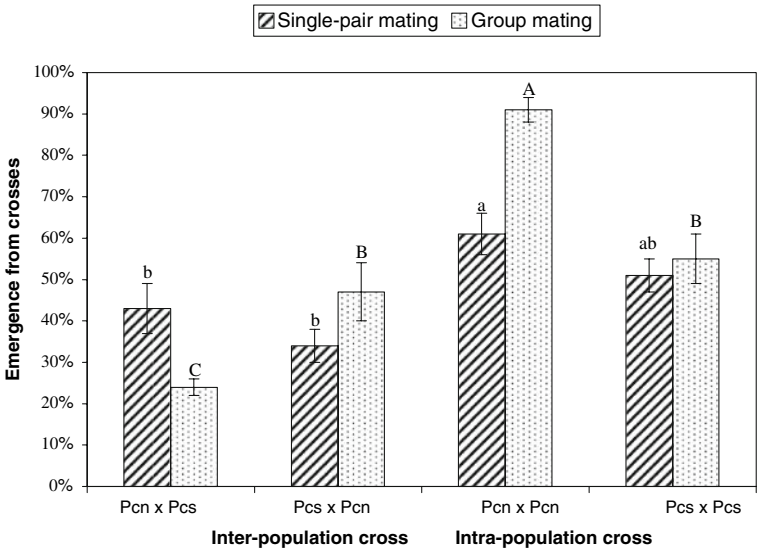

Fig. 1 Parasitoid emergence (± SE) from single-pair and group mating methods*. Bars with same letters under same mating method show no significant difference between means ($P = 0.05$), using Student-Newman-Keuls (SNK) test. *Mean separation was performed separately for each mating method

produced between *P. concolor* and *P. cosyrae* (Table 3), but there were differences in the mean percent emergence from the crosses (inter-population crosses = 24–47%; intra-population crosses = 51–91%).

In nature, mating, host-searching and parasitization take place as long as conditions allow. In this study, the number of larvae exposed to parasitoids was limited to 10 per parasitoid per day, and therefore, the RC values only indicate the existence of the physiological potential of these species/populations to produce hybrids under laboratory conditions. Furthermore, natural hybrids between these populations have never been described and could potentially account for the observed high morphological similarities in

Table 5 Proportion of female parasitoids produced in experimental crosses compared with those produced in F_1 crosses

Cross	Proportion of females		Intra-population cross	Proportion of females
	Inter-population cross	$F_1 \times F_1$		
Pcn × Pcs	42.9	64.0	Pcn × Pcn	57.1
Pcs × Pcn	43.2	60.0	Pcs × Pcs	52.6
Pcn × Prm	43.6	74.5	Pcn × Pcn	57.1
Prm × Pcn	44.2	67.8	Prm × Prm	54.2
Pcn × Pph	12.5	*	Pcn × Pcn	57.1
Pph × Pcn	61.3	58.8	Pph × Pph	61.9
Pcn × Pru	*	*	Pcn × Pcn	57.1
Pru × Pcn	38.1	64.3	Pru × Pru	32.4

* Proportions not calculated due to death of crosses or incomplete data

the genus. Description of some morphological aspects of parental species and their hybrids (F_1, F_2 and F_3) for use in the analysis of natural populations will be of interest in future studies as well as their fitness and/or parasitizing ability, while studies into the stability of these hybrids will also give an indication of their efficacy and survival in the field. Though performance in the inter-population was lower than in the intra-population crosses, the $F_1 \times F_1$ crosses exceeded the average parental performances of both inter- and intra-crosses with higher female proportions (Table 5). In general, higher proportions of females in inter-population crosses relative to proportions in intra-population crosses are preferred—a condition referred to as hybrid vigour or heterosis, which is due to over-dominance of genes in heterozygous individuals. Hybrid vigour is routinely exploited in the development of several crop plants for high yields and other traits, but this has not been exploited much in contemporary agriculture using parasitoids, except through selection (Gujar et al. 2006). In parasitic Hymenoptera, females parasitize hosts and are responsible for host mortality. 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, however, works very well if males normally mate more than once, as a strongly female-biased population in one generation can give rise to a strongly male-biased population in the next generation if a large number of the females are not mated by the few males or if there is insufficient mating.

Identity of parasitoids

Here, we observe the production of viable hybrids between; (1) morphologically similar populations (*P. concolor*, Ruiru and Rurima) and (2) morphologically distinct populations (*P. concolor*, *P. cosyrae* and *P. phaeostigma*). In both cases the high RC values and the ease with which the populations hybridize (coupled with the viability of the hybrids), seem to suggest no evidence of 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 mating was conducted in the laboratory in a no-choice and/or under forced artificial conditions, and further testing would be necessary to determine any pre-copulatory isolating mechanisms. 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, ecological, behavioural or temporal isolation. Nevertheless, the results in this study support the work on morphology and host suitability by Mohamed et al. (2003) as well as morphometric and phylogenetic relations by Billah (2004), to suggest that the genus may comprise a series of very closely related species which cannot be separated on the basis of reproductive compatibility alone.

Biological control considerations

For effective use of natural enemies in biological control, correct identification of species is essential. *Ceratitis capitata* (Medfly) 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) and with 353 plant species reported as hosts or potential hosts (Liquido et al. 1991). In Africa alone, more than 150 host plants are reported attacked by *C. capitata* with certainty (De Meyer et al. 2002). 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 *Bactrocera* genus (a genus far removed from the native range of *Ceratitis*). These parasitoids were introduced to Hawaii and elsewhere 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 two populations from *Ceratitis* species, their study should be of interest for future use on the medfly. Laboratory studies of these populations on medfly and other tephritid pests at ICIPE, Nairobi, in collaboration with Texas A&M University, USA, have shown encouraging results, resulting in the shipment of medfly parasitoids from Kenya to Hawaii, Guatemala and South Africa (for use in St. Helena Islands), 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 (Lopez et al. 2003; Bokonon-Ganta et al. 2005). If these parasitoids successfully attack medfly (and Natal fly) and establish in their new environments, then it will be a re-establishment of relationships between them and their original hosts; and the plausible prediction will be a more effective control of those pests. One 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 has lead to studies in different aspects of colonization, competition, displacement and intra-guild predation (Salt 1963; Lopez et al. 2003; Wang and Messing 2003; Messing et al. 2006) 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 do 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.

The biggest potential problem will be with the morphologically indistinguishable populations, where their introduction in areas with other species already present is likely to give erroneous impressions in post-release sampling results (since the presence of the other populations will add to the frequency and numbers). Secondly, if the introduced species are different, then apart from the possibility of inter-specific hybridization, there will also be competition if they have the same host-larvae (target pest). On the other hand, the morphologically distinct populations (*P. cosyrae* and *P. phaeostigma*) have different host-larvae preferences and competition is likely to be minimal or absent except for the chances of hybridization (if no pre-copulatory isolating mechanisms come into play). 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 are interesting questions beyond the scope of this study.

In the light of these results, cross mating studies alone 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 (Billah et al. 2005). It is therefore, important that information on the background study of natural population types in the targeted environment and their potential interaction with species to be introduced are examined together with host-habitat relationships before introductions are made to achieve better establishment and colonization.

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References

Billah MK (2004) Biosystematic studies of *Psyttalia* species (Hymenoptera: Braconidae): Parasitoids attacking fruit-infesting flies (Diptera: Tephritidae) in Africa. PhD thesis, University of Ghana, Legon-Accra, 236 pp

Billah MK, Kimani-Njogu S, Overholt WA, Wharton RA, Wilson DD, Cobblah MA (2005) The effect of host larvae on three *Psyttalia* species (Hymenoptera: Braconidae): parasitoids of fruit-infesting flies (Diptera: Tephritidae). Intl J Trop Insect Sc 25(3):168–175

Bokonon-Ganta AH, Ramadan MM, Wang X-G, Messing RH (2005) Biological performance and potential of *Fopius ceratitivorus* (Hymenoptera: Braconidae), an egg-larval parasitoid of tephritid fruit flies newly imported to Hawaii. Biol Control 33:238–247

Briggs CJ (1993) Competition among parasitoid species on a stage-structured host and its effects on host suppression. Am Naturalist 141:372–396

Charnov EL (1982) The theory of sex allocation. Princeton University Press, Princeton, USA

Charnov EL, Los-den Hartogh RL, Jones WT, van den Assem J (1981) Sex ratio evolution in a variable environment. Nature 289:27–33

Claridge MF (1991) Genetic and biological diversity of insect pests and their natural enemies. In: Hawksworth DL (ed) Biodiversity of microorganisms and invertebrates; its role in sustainable agriculture. CAB International, Wallingford, pp 183–194

Clausen CP, Clancy DW, Chock QC (1965) Biological control of the oriental fruit fly and other fruit flies in Hawaii. USDA Tech Bull 1322

Copeland RS, White IM, Okumu M, Machera P, Wharton RA (2004) Insects associated with fruits of the Oleaceae (Asteridae, Lamiales) in Kenya, with special reference to the Tephritidae (Diptera). Bis Mus Bull Entomol 12:135–164

Copeland RS, Wharton RA, Luke Q, De Meyer M, Lux S, Zenz N, Machera P, Okumu M (2006) Geographic distribution, host fruit, and parasitoids of African fruit fly pests *Ceratitis anonae*, *Ceratitis cosyra*, *Ceratitis fasciventris* and *Ceratitis rosa* (Diptera: Tephritidae) in Kenya. Ann Entomol Soc Am 99(2):261–278

DeBach P, Rosen D (1991) Biological control by natural enemies, 2nd edn. Cambridge University Press, Cambridge, 440 pp

De Meyer M (1996) Revision of the subgenus *Ceratitis* (*Pardalaspis*) Bezzi, 1918 (Diptera, Tephritidae, Ceratitini). Syst Entomol 21:15–26

De Meyer M (1998) Revision of the subgenus *Ceratitis* (*Ceratalaspis*) Hancock (Diptera: Tephritidae). Bull Entomol Res 88:257–290

De Meyer M (2000) Systematic revision of the subgenus *Ceratitis* MacLeay s. (Diptera: Tephritidae). Zool J Linn Soc 128:439–467

De Meyer M, Copeland RS, Lux SA, Mansell M, Quilici S, Wharton R, White IM, Zenz N (2002) Annotated checklist of host plants for Afrotropical fruit flies (Diptera: Tephritidae) of the genus *Ceratitis*. Musée Roy Afriq Centr Tervuren Belgique 27:1–91

Diehl SR, Bush GL (1984) An evolutionary and applied perspective of insect biotypes. Annu Rev Entomol 29:471–483

Gauld ID (1986) Taxonomy, its limitations and its role in understanding parasitoid biology. In: Waage JK, Greathead D (eds) Insect parasitoids, Academic Press, London, pp 1–22

Godfray HCJ (1994) Parasitoids: behaviour and evolutionary ecology. Princeton Univercity Press, NJ, USA, 473 pp

Greathead DJ (1976) Mediterranean fruit fly, Olive fly. In: Greathead DJ (ed) A review of biological control in western and southern Europe, Commonw Inst Biol Cont Techn Com, vol 7. pp 37–43

Grissell EE, Schauff ME (1997) A handbook of the families of Nearctic Chalcidoidea (Hymenoptera), 2nd edn, Revised. Entomol Soc Wash, 87 pp

Gujar GT, Chandrashekaran S, Kalia V (2006) Hybrid parasitoids for pest management. Opinion Curr Sci 90(7):907

Hall RW (1993) Alteration of sex ratios of parasitoids for use in biological control. In: Wrensch DL, Ebbert MA (eds) Evolution and diversity of sex ratio in insects and mites. Chapman and Hall, New York, pp 542–547

Hooper GHS (1987) Application of quality control procedures for large scale rearing of Mediterranean fruit fly. Ent Exp Appl 44:161–167
Hopper KR, Roush RT, Powell W (1993) Management of genetics of biological control introductions. Annu Rev Entomol 38:27–51
Janssen A (1989) Optimal host selection by *Drosophila* parasitoids in the field. Func Ecol 3:469–479
Jervis M, Kidd N (eds) (1996) Insect natural enemies. Practical approaches to their study and evaluation. Chapman and Hall, London, UK, 491 pp
Kimani-Njogu SW, Wharton RA (2002) Two new species of Opiinae (Hymenoptera: Braconidae) attacking fruit-infesting Tephritidae (Diptera) in western Kenya. Proc Entomol Soc Wash 104:79–90
Kimani SW, Overholt WA (1995) Biosystematics of the *Cotesia flavipes* complex (Hymenoptera: Braconidae): interspecific hybridization, sex pheromone and mating behaviour studies. Bull Entomol Res 85:379–386
Kimani-Njogu SW, Trostle MK, Wharton RA, Woolley JB, Raspi A (2001) Biosystematics of the *Psyttalia concolor* species complex (Hymenoptera: Braconidae: Opiinae): the identity of populations attacking *Ceratitis capitata* (Diptera: Tephritidae) in coffee in Kenya. Biol Control 20:167–174
King BH (1987) Offspring sex ratios in parasitoid wasps. Quarterly Rev Biol 62:367–396
Liquido NJ, Shinoda LA, Cunningham RT (1991) Host plants of the Mediterranean fruit fly: an annotated worldview. ESA Misc Pub 77:1–52
Liu S-S, Gebremeskel FB, Shi Z-H (2002) Reproductive compatibility and variation in survival and sex ratio between two geographic populations of *Diadromus collaris*, a pupal parasitoid of the Diamondback moth, *Plutella xylostella*. BioControl 47:625–643
Lopez M, Sivinski J, Rendon P, Holler T, Bloem K, Copeland R, Trostle M, Aluja M (2003) Colonization of *Fopius ceratitivorus*, a newly discovered African egg-pupal parasitoid (Hymenoptera: Braconidae) of *Ceratitis capitata* (Diptera: Tephritidae). Florida Entomologist 86(1):53–60
Mackauer M (1969) Sexual behaviour and hybridization between three species of *Aphidius* Nees, parasitic on the pea aphid. Proc Entomol Soc Wash 71:339–351
Messing R, Roitberg B, Brodeur J (2006) Effects of competition, displacement and intraguild predation in biocontrol and evaluation methods. In: Bigler F, Babendreier D, Kuhlman U (eds) Environmental impact of invertebrates in biological control of arthropods: methods and risk assessment. Wallingford, UK, CAB Int, pp 64–77
Mitchell WC, Andrew CO, Hagan KS, Hamilton RA, Harris EJ, Maehler KL, Rhode RA (1977) The Mediterranean fruit fly and its economic impact in Central American countries and Panama. Univ. of California/Agency for International Development, Pest Mgt and Rel Envir Prot, 189 pp
Mohamed SA, Overholt WA, Wharton RA, Lux SA, Eltoum EM (2003). Host specificity of *Psyttalia cosyrae* (Hymenoptera: Braconidae) and the effect of different host species on parasitoid fitness. Biol Control 28:155–163
Nagarkatti S (1975). Two new species of *Trichogramma* (Hymenoptera: Trichogrammatidae) from the USA. Entomophaga 20:245–248
Phillips CB, Emberson RM Goldson SL (1993) Host-associated morphological variation in Canterbury (New Zealand) populations of the parasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae, Euphorinae). Proceedings of the 6th Australasian conference grassland invertebrate ecology, pp 405–414
Pinto JD, Stouthamer R (1994) Systematics of the Trichogrammatidae with emphasis on Trichogramma. In: Wajnberg E, Hassan SA (eds) Biological control with egg parasitoids. CAB International, Wallingford, pp 1–36
Pinto JD, Oatman ER, Platner GR (1986) *Trichogramma pretiosum* and a new cryptic species occurring sympatrically in southwestern North America (Hymenoptera: Trichogrammatidae). Ann Entomol Soc Am 79:1019–1028
Pinto JD, Stouthamer R, Platner GR, Oatman ER (1991) Variation in reproductive compatibility in *Trichogramma* and its taxonomic significance (Hymenoptera: Trichogrammatidae). Ann Entomol Soc Am 84:37–46
Pinto JD, Kazmer DJ, Platner GR, Sassaman CA (1992) Taxonomy of the *Trichogramma minutum* complex (Hymenoptera: Trichogrammatidae): allozymic variation and its relationship to reproductive and geographic data. Ann Entomol Soc Am 85:413–422
Pinto JD, Platner GR, Sassaman CA (1993) Electrophoretic study of two closely related species of North American *Trichogramma: T. pretiosum and T. deion* (Hymenoptera: Trichogrammatidae). Ann Entomol Soc Am 86(6):702–709
Raspi A, Loni A (1994) Alcune note sull'allevamenton massale di *Opius concolor* Szépligeti (Hymenoptera: Braconidae) e su recenti tentative d'introduzione della specie in Toscana e Liguria. Frustula Entomologica 30:135–145

Rosen D (1986) The role of taxonomy in effective biological control programs. Agric Ecosys Envir 15: 121–129
Salt G (1941) The effects of hosts upon their insect parasites. Biol Revs 16:239–264
Salt G (1963) The defense reactions of insects to metazoan parasites. Parasitology 53:527–642
SAS Institute Inc. (2001) SAS/STAT® User's Guide, Version 8.2. Cary, NC, USA
Silvestri F (1914) Report of an expedition to Africa in search of the natural enemies of fruit flies. Hawaiian Board of Agriculture and Forestry Division, Entomol Bull No. 3, 176 pp (Translation of 1913 report in Italian)
Sivinski JM, Webb JC (1989) Acoustic courtship signals produced during courtship in *Diachasmimorpha* (= *Biosteres*) *longicaudata* (Hymenoptera: Braconidae) and other Braconidae. Ann Entomol Soc Am 82:116–120
Smith DC, Lyons SA, Berlocher SH (1993) Production and electrophoretic variation of F_1 hybrids between the sibling species *Rhagoletis pomonella* and *R. Cornivora*. Ent Exp Appl 69:209–213
Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research, 3rd edn. W. H. Freeman and Company, USA, 887 pp
Stouthamer R, Jochemsen P, Platner, GR Pinto JD (2000). Crossing incompatibility between *Trichogramma minutum* and *T. platneri* (Hymenoptera: Trichogrammatidae): implications for application in biological control. Environ Entomol 29(4):832–837
van den Assem J, Jachmann F (1982) The coevolution of receptivity signalling and body size in the Chalcidoidea. Behaviour 80:96–105
van den Bosch R, Hom R, Matteson P, Frazer BD, Messenger PS, Davies CS (1979) Biological control of the walnut aphid in California: impact of the parasite, *Trioxys pallidus*. Hilgardia 47:1–15
Vera MT, Caceres C, Wornoayporn V, Islam A, Robinson AS, de La Vega MH, Hendrichs J, Cayol J-P (2006) Mating incompatibility among populations of the South American fruit fly *Anastrepha frateculus* (Diptera: Tephritidae). Ann Entomol Soc Am 99(2):387–397
Vinson SB, Iwantsch GF (1980) Host suitability for insect parasitoids. An Rev Entomol 25:397–419
Wajnberg E, Boulétreau M, Prévost G, Fouillet P (1990) Developmental relationships between *Drosophila* larvae and their endoparasitoid *Leptopilina* (Hymenoptera: Cynipidae) as affected by crowding. Arch Insect Biochem Physiol 13:293–245
Wang X-G, Messing RH (2003) Intra- and interspecific competition by *Fopius arisanus* and *Diachasmimorpha tryoni* (Hymenoptera: Braconidae), parasitoids of tephritid fruit flies. Biol Control 27:251–259
Weems HV (1981) Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Fla Dept Agric And Consumer Service, Div Plant Industry, Entomol Cir No 230, 8 pp
Wharton RA (1989a) Control; Classical biological control of fruit-infesting Tephritidae. In: Robinson AS, Hooper G (eds) Fruit flies; their biology, natural enemies and control. World crop pests, 3(B). Elsevier Science Publishers, Amsterdam, pp 303–313
Wharton RA (1989b) Biological control of fruit-infesting Tephritidae. In: R Cavalloro (ed) Fruit flies of Economic Importance 87. Proceedings of the CEC/IOBC international symposium, Rome, 1987, Balkema, Rotterdam, pp 323–332
Wharton RA (1997a) Generic relationships of opiine Braconidae (Hymenoptera) parasitic on fruit-infesting Tephritidae (Diptera). Contri Am Entomol Inst 30:1–53
Wharton RA 1997b. Key to new world genera of Opiinae. In: RA Wharton, Marsh PM, Sharkey MJ (eds) Manual of the New world Genera of the family Braconidae (Hymenoptera). Special publ Intl Soc Hym No 1
Wharton RA, Gilstrap FE (1983) Key to and status of opiine braconid (Hymenoptera) parasitoids used in biological control of *Ceratitis* and *Dacus s.l.* (Diptera: Tephritidae). Ann Entomol Soc Am 76:721–742
Wharton RA, Trostle MK, Messing RH, Copeland RS, Kimani-Njogu SW, Lux S, Overholt WA, Mohamed S, Sivinski J (2000) Parasitoids of medfly, *Ceratitis capitata*, and related tephritids in Kenyan coffee: a predominantly koinobiont assemblage. Bull Entomol Res 90:517–526
White IM, Elson-Harris MM (1992) Fruit flies of economic significance: their identification and bionomics. CAB International, Wallingford, UK, 602 pp