Propositions

1. Biocontrol workers often seem ignorant that the success of a natural enemy following its release into a new area is partially determined by the genetic composition of the founder population.
   This thesis.
   Van Driesche, R.G. & Bellows, T.S., 1996. *Biological control.* New York: Chapman & Hall.

2. *Cotesia flavipes*, like other effective natural enemies, is paradoxically numerically scarce where most effective.
   This thesis.
   Huffaker, C.B. & Messenger, P.S., 1976. *Theory and Practice of Biological Control.* Academic Press: New York.

3. Incest promotes survival of some insects.
   This thesis.

4. Stemborer body odours can be lethal.
   This thesis.

5. 'Copy and paste' saves time but should be applied to biological control with caution.

6. Development in Africa is often addressed by symptoms and not causes.

Propositions with the thesis '**Genetic variability in *Cotesia flavipes* and its importance in biological control of lepidopteran stemborers**'.

Emmanuel Iyamulemye Niyibigira Wageningen, 26 May 2003.

Genetic variability in *Cotesia flavipes* and its importance in biological control of lepidopteran stemborers

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Genetic variability in *Cotesia flavipes* and its importance in biological control of lepidopteran stemborers

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Abstract

Lepidoperan stemborers are a major constraint to increasing the production of maize and sorghum under subsistence farming conditions in sub-Saharan Africa. Classical biological control is considered as the most cost-effective form of pest management but it has not attained the desired success rate. It has been postulated that one major reason for the failures in classical biological control is related to the genetic diversity of released individuals of natural enemies. The aim of this study was to examine the importance of genetic variability in the establishment and performance of the parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae) as a classical biological control agent of lepidopteran stemborers. Field surveys carried out on Unguja and Pemba islands of Zanzibar showed that the introduced stemborer *Chilo partellus* (Crambidae) was the most abundant and widespread species in the stemborer complex. Although a number of indigenous natural enemies were recorded, they had a low impact on stemborer populations and hence, classical biological control was considered as an option. The endoparasitoid *C. flavipes*, an old association natural enemy of *C. partellus*, was collected from central India and imported in Kenya for laboratory and field studies. Olfactometer studies to examine the effect of duration of laboratory rearing on the parasitoid's responses to host- and host plant-associated odours showed that the overall behavioural response was stable over many generations regardless of the genetic diversity of the population. A reduced genetic variability at the sex locus of some Hymenoptera can result in biocontrol failures due to the production of diploid males from fertilized eggs as a consequence of single locus complementary sex determination mechanism (sl-CSD). In species with sl-CSD, inbreeding that may occur during rearing and release in the field will result in the production of diploid males and the associated reduction in population growth rate. Models were developed to predict the frequency of matched matings in populations with different frequencies of sib mating and egg fertilization. The models showed that sl-CSD could be detected from brood sex ratios if the diploid male offspring survives. Analysis of sex ratios from field-collected data showed that sex ratio was highly female-biased. Sex ratio frequency followed a unimodal distribution instead bimodal distribution that is expected if sl-CSD with diploid male survival exists in this parasitoid species. However, the results suggested that the presence of sl-CSD with diploid male mortality could not be excluded. Inbreeding crosses to deduce further evidence for the existence of sl-CSD in *C. flavipes* revealed that brood sizes resulting from matched matings were not smaller than those from crosses among unmatched matings, suggesting that sl-CSD with diploid male mortality did not occur in crosses involving matched matings. Moreover, inbreeding of populations for several generations did not result in male-biased sex ratios. The importance of genetic variability to the colonization and establishment of *C. flavipes* was investigated through the release of three genetically impoverished populations (isofemale lines) and one genetically diverse (mixed) population on two islands of Zanzibar. The mixed population showed higher colonization of stemborers than one of the isofemale lines but it was not different from the other two isofemale lines. This suggests that genetic variability may not have been an important factor in the colonization of *C. flavipes*, possibly due the similarity in climate of the area of origin in central India and the release areas in Zanzibar. Suggestions for future research to improve the biological control of lepidopteran stemborers in maize and sorghum are presented.

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Chapter 1

General Introduction

One of the greatest challenges facing the people of sub-Saharan Africa is the production of sufficient food to feed a rapidly increasing population. Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) are the two most important food crops grown by small-scale farmers in the region (Krebs, 1988; Carr, 1989). The crops are grown primarily for human consumption but they are also important sources of income in most households. Crop yields tend to be very low, and in 2001 average yields in sub-Saharan Africa were 1,660 kg/ha for maize and 870 kg/ha for sorghum, compared to the world averages of 4,420 and 1,360 kg/ha for maize and sorghum respectively (FAO, 2002). A major constraint to increasing the production of maize and sorghum is attack by insect pests, among which lepidopteran stemborers are generally considered to be the most injurious (Nye, 1960; Seshu Reddy, 1988; 1991; Youdeowei, 1989).

1.0 Lepidopteran stemborers

1.1 Distribution

In East Africa, 12 stemborer species have been recorded on maize and sorghum (Seshu Reddy, 1998). However the most economically important and widely distributed species are the spotted borer, *Chilo partellus* (Swinhoe) (Crambidae), the coastal borer *Chilo orichalcociliellus* (Strand) (Crambidae), the maize stalkborer *Busseola fusca* Fuller (Noctuidae), the pink stalk borer *Sesamia calamistis* Hampson (Noctuidae) and the sugarcane borer *Eldana saccharina* Walker (Pyralidae) (Seshu occasionally at elevations between 1500 and 2300 m (Zhou *et al.*, 2001a). *Busseola fusca* is dominant in highland areas at elevations above 600 m (Nye, 1960; Zhou *et al.*, 2001a) while *C. orichalcociliellus* occurs in the coastal areas and a few inland areas at elevations below 600 m (Nye, 1960; Sithole, 1989; Zhou *et al.*, 2001a). *Sesamia calamistis* is reportedly found in low, mid and high elevation maize and sorghum growing regions (Ingram, 1958; Nye, 1960). *Eldana saccharina* is found throughout tropical Africa and is considered as a pest of sugarcane although it also attacks maize (Atkinson, 1980; Sithole, 1988; Maes, 1998).

All the above stemborer species are indigenous to Africa, except *C. partellus* which is of Asian origin (Bleszynski, 1970). *Chilo partellus* was introduced into Africa in the previous century and was first recorded in Malawi around 1930 (Tams, 1932). Since its introduction, *C. partellus* has spread to most countries in eastern and southern Africa (Harris, 1990). In many of the areas it has invaded, *C. partellus* is considered to be the most economically important pest of maize and sorghum (Seshu Reddy & Walker, 1990). Recent reports indicate that *C. partellus* may be displacing the indigenous species (Ofomata *et al.*, 1999a; b; 2000; Kfir *et al.*, 2002). The factors which are responsible for the competitive superiority of *C. partellus* over some native borers were recently reviewed by Kfir *et al.*, (2002) and they include: 1) shorter generation times than *C. orichalcociliellus*, which may result in a higher population growth rate, 2) rapid termination of diapause thus allowing *C. partellus* to colonize host plants before *C. orichalcociliellus*, or *B. fusca* at the beginning of the growing seasons, 3) more *C. partellus* successfully complete development than *C. orichalcociliellus*, when they infest the same plant suggesting superiority during direct competition, 4) higher dispersal of first instar *C. partellus* larvae and greater distances of dispersal than *C. orichalcociliellus*, which may allow *C. partellus* to colonize more plants than the native borer.

1.2 Biology and life cycle

Although each stemborer species has a slighdy different life history, a typical generalized picture of the life cycle can be described. Adult moths emerge in late afternoon and early evening and are active at night. Females mate soon after emergence and start laying eggs during 2-3 subsequent nights. Female moths live for 4-10 days during which they lay eggs in batches of varying numbers. Crambid eggs are oval and flattened and are laid in overlapping rows anywhere on the plant, but most often on the underside of mature leaves near the midribs (Harris, 1990; Pats & Ekbom, 1994). Noctuid eggs are semi-globular and are laid singly or in batches under the inner surfaces of the leaf shealths (van Rensburg, 1980). The eggs are creamy white when first laid, but after 3-6 days, they turn black just before hatching. Eggs hatch in the early morning, 4-8 days after being oviposited. The first instar larvae disperse from the emergence sites and migrate to the leaf whorls where they feed on young leaf tissues. In some species, e.g. *C. partellus*, larvae disperse as first instar larvae by spinning silken threads and ballooning to neighbouring plants. Older larvae that have reached the third instar tunnel into the stem tissue and feed internally until larval development is completed. When feeding inside the stem, the larvae deposit their frass outside the entrance of the tunnel. Larva stage passes through 6-8 instars in 25-50 days. Fully-grown larvae chew exit holes for the adult moth just before they pupate in a small chamber within the stem. Some species e.g. *E. saccharina* and *S. calamistis,* sometimes pupate outside or partially outside of the stem. *Eldana*

saccharina pupates in a silken cocoon attached to the plant. Pupation typically takes 5-10 days and adults are short lived. Under adverse conditions, the larvae may enter diapause in stems or crop residues only pupating when favourable conditions return. During diapause, larvae are protected from natural enemies and adverse climatic conditions (Kfir, 1988; 1991).

1.3 Damage

Most stemborer species produce similar symptoms to attacked maize and sorghum plants. Stemborers are destructive in their larval stage. Newly hatched larvae feed initially in leaf whorls, producing characteristic 'windows'. Older larvae tunnel into the stem tissue and feed internally, which disrupts the translocation of water and nutrients and may result in death of the growing point, producing 'deadhearts'. The larvae also bore into maize cobs and feed on the developing grains. The severity and nature of stemborer damage depend upon the borer species, plant growth stage, number of larvae feeding on the plant and the plant's reaction to larval feeding. Feeding by borer larvae on maize and sorghum plants usually results in stunted growth and crop losses as a consequence of death of the growing point, early leaf senescence, reduced translocation, lodging and direct damage to the ears (Brenire, 1971; Leuschner, 1989; Bosque-Perez & Mareck, 1991). Stem tunneling also provides entry points for plant pathogens especially the stalk rots (Sithole, 1988). Estimated yield losses due to stemborers in sub-Saharan Africa vary greatly among ecological zones, regions and seasons, but are in the range of 20-40% of the potential yield (Youdeowei, 1989; Seshu Reddy & Walker, 1990).

1.4 Control

Cultural control, which is considered the first line of defence against pests, is the most economic method of stemborer control available for resource-poor farmers in Africa. It includes techniques such as crop rotation, intercropping, destruction of crop residues, stalk shredding and deep ploughing to increase mortality of diapausing larvae. Early planting to avoid heaviest losses from second-generation stem borers and early harvest to reduce yield losses from dropped ears and lodged plants remain as attractive measures. Prevention through crop rotation with non-host crops involves a three-year rotation scheme. Although intercropping maize or sorghum with other crops may suppress stemborer populations (Skovgard & Pats, 1996; Oloo & Ogedah, 1990), no studies have recommended the best crop combination for maize or sorghum. Recent studies in Kenya have reported the effectiveness of controlling stemborers by intercropping maize with the non-host molasses grass, *Melinis minutiflora* (Khan *et al.*, 1997a,b, 2001). Although cultural control options for stemborer management may be effective, most farmers have not adopted them (Nwanze & Mueller, 1989). Cultural control is severely constrained by high labour

demands and lack of management capabilities of farmers, especially in areas where farming communities lack the support of an adequate extension service (Harris, 1989). Pheromone-baited traps are useful for monitoring moth population levels of stemborers but using pheromone traps for mass trapping moths may not give satisfactory control (reviewed in Campion & Nesbitt, 1983). Botanical formulations such as neem are effective against early larval instars of stemborers (Ganguli *et al.*, 1997; 1998; Akbar *et al.*, 1999) but their use may be limited by the proper timing of application. Chemical control using pesticides can be effective against first generation borers (van den Berg & van Rensburg, 1991). However, pesticides are too expensive or not available to the small-scale farmers and are associated with user hazards. In addition, the use of pesticides is not economically justified considering the low prices of cereal crops particularly when grown for subsistence. Stemborer larvae are also protected from pesticides due to the feeding behaviour in stem tunnels (Ampofo, 1986; Seshu Reddy & Sum, 1992). Resistant hybrids provide an inherent control that involves no environmental problems, and they are generally compatible with other insect-control methods. Unfortunately resistant hybrids that exhibit tolerance to stemborer damage are not widely available to farmers. Genetically modified plants with insecticidal properties are widely grown in other areas of the world and may eventually be adopted in Africa (Ahl Goy & Duesing, 1995; Bagnara, 2000; Zuppiroli & Mancini, 2000). Various indigenous natural enemies including parasitoids, predators, nematodes and pathogens attack stemborers. A recent review by Bonhof *et al.* (1997) listed the natural enemies of cereal stemborers in East Africa. The impact of predators on maize stemborers has been extensively studied in coastal Kenya (Bonholf, 2000). However, the mortality levels inflicted by indigenous natural enemies on stemborers are often too low to effectively regulate their densities at acceptable levels (Oloo & Ogeda, 1990; Kfir, 1992).

2.0 Classical biological control of *Chilo partellus*

Because of the low impact of indigenous natural enemies on stemborer populations, classical biological control may be a feasible option particularly in a stemborer complex in which the exotic stemborer *C. partellus* is most abundant and widespread species. Classical biological control is a pest management approach that involves the importation of natural enemies from one geographic area of the world, and the release of the natural enemies in another area of the world, where they formerly did not exist, for the sustainable suppression of specific pest populations (Ehler, 1982). Once a natural enemy is permanently established in a new area, it will have a self-perpetuating impact on the target pest population, with no additional external input (Huffaker & Messenger, 1976). The greatest efforts in classical biological control have

been against introduced pest species, whereby a pest's natural enemies in its aboriginal home are introduced into the geographic area the pest has invaded.

2.1 The parasitoid *Cotesia flavipes* Cameron

Cotesia flavipes Cameron (Hymenoptera: Braconidae), a 2-mm wasp, is an old association natural enemy of *C. partellus*. It is a gregarious endoparasitoid that is thought to originate from the Indo-Australian region where it is known to parasitize various species of noctuid and crambid stemborers, including economically important stemborers in maize, sorghum, sugarcane and rice (Singh *et al.*, 1975; Mohyuddin *et al.*, 1981; Neupane *et al.*, 1985; Kishore, 1986). Its host range also includes defoliators in the families of Noctuidae, Actiidae and Lymantriidae as well as reafrollers (Crambidae) (Overholt *et al.*, 1997). Parasitism of *C. partellus* by *C. flavipes* in its native environment is high. This is shown by reports that it parasitizes up to 80% of *C. partellus* populations in some locations in Asia (Singh *et al.*, 1975) (Table 1). *Cotesia flavipes* has been introduced into more than 40 countries in the tropics for biological control of *C. partellus* with varying success (Alam *et al.*, 1972; Overholt *et al.*, 1994; 1997, Omwega *et al.*, 1995; 1997).

Cotesia flavipes has a fairly narrow host range in East Africa, as it is restricted to parasitizing a few species of gramineous stemborers. Laboratory studies by Ngi-Song *et al.* (1995) showed that *C. flavipes*, in addition to *C. partellus*, was able to parasitise the indigenous stemborers *C. orichalcociliellus* and *S. calamistis*. In East Africa, *C. flavipes* has been reared from the above three stemborers in the field (e.g. Overholt *et al.*, 1994; Omwega *et al.*, 1997; Ofomata *et al.*, 1999a; b; 2000). *Cotesia flavipes* was not able to successfully develop in *B. fusca* (Ngi-Song *et al.*, 1995) or *E. saccharina* (Overholt *et al.*, 1997) in laboratory studies, although there are reports that some *B. fusca* populations may be susceptible (Matama-Kauma *et al.*, 2001; Getu *et al.*, 2001).

3.0 Genetic variability in parasitoid populations

Individuals within a population usually vary genetically and this variation is often expressed both in the insects' morphology and a range of biological attributes (phenotypic variation). These include, among others, behaviour (Roush, 1990a; Prevost & Lewis, 1990; Fleury *et al.*, 1995), life history traits (e.g. Pintureau *et al.*, 1995; Cronin & Strong, 1996; Chenot & Raffa, 1998), sex ratio (Pintureau *et al.*, 1999), host finding ability (Urquijo, 1951; Wajnberg & Colazza, 1998), progeny allocation (Wajnberg *et al.*, 1989), egg laying (Chassain & Bouletreau, 1987) and insecticide resistance (Rosenheim & Hoy, 1988; Chinnici, 1980). Genetic variability may thus have considerable influence on the killing efficiency of predators and parasitoids (Powell *et al.*, 1996). It has been suggested that most measurable traits are variable

Table 1: Field parasitism by *Cotesia flavipes* on stemborers and a reafroller* reported in different host plants and locations in Asia

Country	Location	Host plant	Family	Species	Parasitism	Reference
India	Andra Pradesh	sorghum	Crambidae	*Chilo partellus*	5.2-57.0%	Rao & Ali, 1976
	Assam	sugarcane	Crambidae	*Chilo tumidicostalis*	0-21.0%	Borah & Arya, 1995
	Assam	sugarcane	Crambidae	*Chilo tumidicostalis*	27.3-31.7%	Borah & Sarma, 1995
	Bangalore	sorghum	Crambidae	*Chilo partellus*	3.3-24.5%	Rao *et al.*, 2001
	Dehli	maize & sorghum	Crambidae	*Chilo partellus*	5.3-42.8%	Subba Rao *et al.*, 1969
	Haryana	sorghum	Crambidae	*Chilo partellus*	16.2-29.1%	Singh, 1999
	Haryana	forage sorghum	Crambidae	*Chilo partellus*	2.0-33.2%	Mohan *et al.*, 1991
	Madhya Pradesh	sorghum	Crambidae	*Chilo partellus*	0-9%	Barpete & Shinde, 1991
	Madhya Pradesh	sorghum	Crambidae	*Chilo partellus*	21.4-44.8%	Sharma & Pathak, 1999
	New Dehli	sorghum	Crambidae	*Chilo partellus*	0-20.76%	Kishore, 1986
	North India	sorghum	Crambidae	*Chilo partellus*	25-44%	Nagarkatti & Nair, 1973
	Punjab	maize	Crambidae	*Chilo partellus*	0.2-80%	Singh *et al.*, 1975
	Punjab	sugarcane	Crambidae	*Bissetia steniella*	0.5-4.2%	Bindra & Chand, 1973
	southern India	sorghum	Crambidae	*Chilo partellus*	35%	Chandy, 1955
	Tamil Nadu	sugarcane	Crambidae	*Chilo sacchariphagus*	0-8.3%	Srikanth *et al.*, 1999
	Tamil Nadu	sorghum	Crambidae	*Chilo partellus*	0-17.9%	Srikanth *et al.*, 1999
	Tamil Nadu	sugarcane	Crambidae	*Chilo infuscatellus*	0-1.1%	Srikanth *et al.*, 1999
	Uttar Pradesh	*Coix larchryma-jobi* L.	Crambidae	*Chilo partellus*	90.56-100%	Nair, 1988
	Uttar Pradesh	sorghum	Crambidae	*Chilo partellus*	0-36.7%	Chaudhary & Sharma, 1987
Indonesia	?	sugarcane	Crambidae	*Chilo auricilius*	7.35%	Mohyuddin, 1987
	?	sugarcane	Crambidae	*Chilo sacchariphagus*	35.2%	Mohyuddin, 1987
Japan	Okinawa Island	sugarcane	Noctuidae	*Sesamia inferens*	61.2-80.1%	Abdul & Iwahashi, 1999
Nepal	Chitwan Valley	maize & sorghum	Crambidae	*Chilo partellus*	up to 30%	Neupane *et al.*, 1985
Pakistan	Yousafwala, Rawalpindi	maize	Crambidae	*Chilo partellus*	78.6-80.4%	Attique *et al.*, 1980
Papua New Guinea	Gusap	sugarcane	Noctuidae	*Sesamia grisescens*	up to70%	Kuniata & Sweet, 1994
Taiwan	Taiwan SRI station	sugarcane	Crambidae	*Chilo infuscatellus*	0.5%	Cheng *et al.*, 1999
	Taiwan SRI station	sugarcane	Crambidae	*Chilo sacchariphagus*	8.5%	Cheng *et al.*, 1999
	Taiwan SRI station	sugarcane	Crambidae	*Chilo infuscatellus*	4%	Cheng *et al.*, 1987
	Taiwan SRI station	sugarcane	Noctuidae	*Sesamia inferens*	1.9%	Cheng *et al.*, 1987
	Taiwan SRI station	sugarcane	Tortricidae	*Argyroploce schistaceana*	1.4%	Cheng *et al.*, 1987
Sri Lanka	southern part	rice	Crambidae	*Cnaphalocrocis medinalis**	53.0%	Rajapakse & Kulasekare, 1982.

(Bartlett, 1984) and abundant variation in traits occurs in native populations (e.g. Prakash, 1973) and even in inbred populations, high amounts can remain (Yamazaki, 1972).

Genetic variation also occurs at the molecular level. Although the existence of intraspecific variation has long been recognized, considerable confusion still exists over terminology, with subspecies, variety, race, strain and biotype all being widely used but rarely defined clearly (Berlocher, 1984). Gonzalez *et al.*, (1979) proposed that the term biotype alone should be used to designate genetic variants in parasitoids (but see Claridge & Hollander, 1983). Because of this confusion, electrophoresis has been proposed as a reliable tool to describe and quantify intraspecific variability. Relative assessments of genetic variability within insect populations can be obtained using allozymes and electrophoresis, randomly amplified polymorphic DNA (RAPDs) and restriction flagment length polymorphism (RFLP) markers (Fong et al., 1995, Gong *et al.*, 2001; Nagaraju *et. al.*, 2001; Kumar *et. al.*, 2001a). Levels of heterozygosity obtained using these techniques can be used to compare different populations (Unruh *et al.*, 1989). Simple sequence repeats (SSR), also known as microsatellites are a relatively new class of molecular markers based on tandem repeats of short (usually 2-5 bp) DNA sequences (Litt & Luty, 1989). These repeats are highly polymorphic, even among closely related individuals, due to mutations causing variation in the number of repeating units and thus provide co-dominant markers with a high degree of allelic polymorphism (Schlotterer *et al.*, 2000; Gyllenstrand *et al.*, 2002; Endsley *et al.*, 2002). However, these markers are obtained through a difficult and labour-intensive procedure, principally because they require prior DNA sequence information for primer design. Recently, a relatively novel molecular technique that permits the detection of polymorphisms in microsatellite or inter-microsatellite loci without previous knowledge of the DNA sequence has been described as intersimple sequence repeats (ISSR) (Zietkiewicz *et. al.* 1994). The technique involves the use of a single primer composed of a microsatellite sequence plus a short (2-4 arbitrary nucleotides) sequence anchored at the 3'or 5'end, which targets a subset of SSRs and amplifies the region between two closely spaced and oppositely oriented simple sequence repeats (Wolfe *et al.*, 1998). The production of large numbers of fragments, reproducibility, and low cost are advantages of these ISSR markers (Moreno *et. al.*, 1998; Kumar *et. al.*, 2001b).

3.1 Why is genetic variability relevant?

Genetic variability is necessary for adaptive evolutionary change. New variation enters a population through mutation or through interbreeding between native and genetically distinct individuals (Carvalho, 1993; Joslyn, 1984). The possession of heritable variation in fitness traits provides phenotypic flexibility for the introduced

population to adapt to a new habitat (Allen, 1958) and changing environments (Joslyn, 1984). Genetic variability in an introduced population of a natural enemy may allow natural selection of sub populations that have greater local survival value and effectiveness (Messenger *et al.*, 1976). Thus, genetic variation is necessary if the newly colonized population must adapt genetically to achieve success, and there is evidence that such adaptation occurs in biological control programmes. For example, Peschken (1972) compared adults of the chrysomelid *Chrysolina quadrigemina* (Suffr.) from a location in British Columbia, where this species was introduced for the control of the weed *Hypericum perforatum* L., with others from California, and found differences in behaviour, physiology and colour in association with adaptation to the long cold winters at the former locality. It was found that the chrysomelids from British Columbia tended to seek shelter from freezing temperatures more readily than those from California. They also laid more eggs, as measured during 4.5 months of their oviposition period. Myers & Sabath (1981) also detected post colonization changes in polygenic traits of the cinnabar moth *Tyria jacobaeceae* (Linnaeus), a herbivore of Tansy ragwort (*Senecio jacobaea* Linnaeus).

3.2 Genetic variability and importation of biological control agents

The general sequence of procedures followed for natural enemy introductions has been described by many authors (e.g. Bartlett & van den Bosch, 1964; Zwölfer *et al.*, 1976; Bellows & Legner, 1993; van Driesche & Bellows, 1996). This sequence includes the collection of the natural enemy in the native home of the pest, shipment, quarantine processing, rearing, field release and evaluation of the natural enemy against the target pest in the new environment.

Maintaining genetic fitness[1] and adaptability of the natural enemy are crucial for the success of biological control introductions (Unruh & Wooley, 1999). Unfortunately, the practices used in the collection, importation, quarantine, rearing (Unruh, *et al.*, 1983), and release (Hopper & Roush, 1993) phases of an introduction of a natural enemy may lead to reduced fitness or, at least, reduced genetic variability (Roush, 1990a; Hopper *et al.*, 1993; Unruh & Messing, 1993).

3.3 Changes in genetic variability

During establishment of colonies of natural enemies, changes in the population's level of genetic variability can take place (Huettel, 1976; Bartlett, 1984; 1985). Electrophoretic evidence has been used to evaluate the genetic changes in laboratory cultures of parasitoids, e.g. in *Aphidus ervi* (Unruh *et al.*, 1983) and in *Cotesia flavipes* (Omwega & Overholt, 1996) but the degree to which such predominantly neutral variation indicates the fate of variation for more adaptive traits is unclear (Roush,

1990a; Hopper *et al.*, 1993). In contrast, changes in allele frequency in mass cultures of screwworm were related to a loss in flight ability and competitiveness compared with field populations (Bush & Neck, 1976). Spurway (1955), in describing the forces that can change gene frequency during domestication[2] of species, called this process 'winnowing', an analogy to sorting and sifting of suitable genotypes in the new environment. The processes that may lead to reduced fitness or losses in genetic variability of a founder population are both random events (founder effect and genetic drift) and directed processes (inbreeding and selection for adaptation) (Bartlett & van den Bosch, 1964; Mackauer, 1976; Messenger & van den Bosch, 1971; Roush, 1990a; Hopper *et al.*, 1993; Unruh & Messing, 1993). These processes, which are presented in Figure 1, are briefly described below.

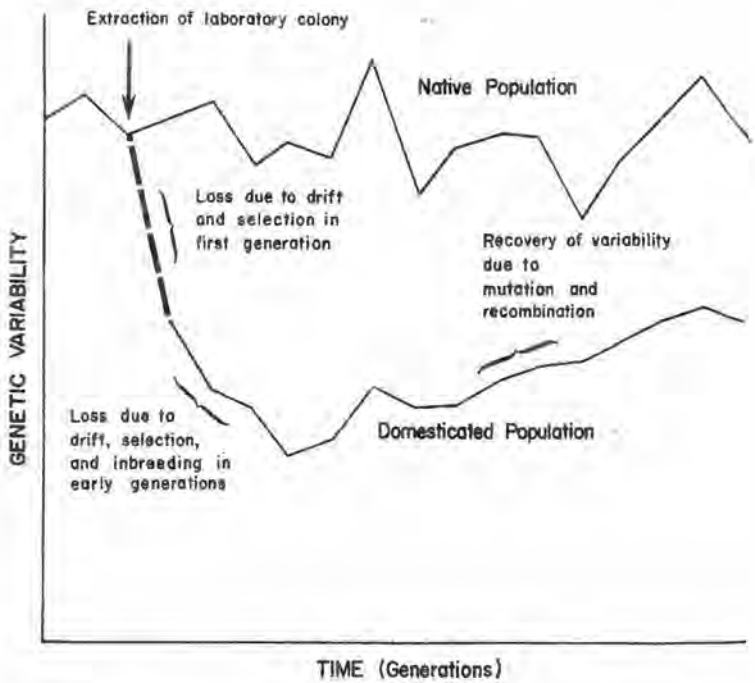

Figure 1. A conceptualization of the changes in genetic variability that may take place during domestication of an insect species (Adapted from: Bartlett, 1984).

3.3.1 Founder effect

Natural enemies vary in nature and capturing this variation may be crucial to successful biological control, as different genotypes may express different biological attributes (Hopper *et al.*, 1993; van Driesche & Bellows, 1996). During collection of

[1] Fitness is the relative ability of an organism to survive and pass its genes to the next generation
[2] Domestication in this case refers to the process of establishing and maintaining an insect colony

exotic natural enemies from their native areas, only a limited number of individuals can be collected and processed. Thus, by the chance effects of sampling, a fraction of the total genetic variation of the original population will be obtained (Joslyn, 1984) which may therefore lack the ability to adapt to their new environment (Messenger & van den Bosch, 1971; Messenger *et al.*, 1976; Roush, 1990a). However, there is insufficient evidence to suggest a significant relationship between the size of the founder population and successful establishment (Mackauer, 1976). In one study, founder effects due to small initial population sizes were found to cause genetic differentiation between isolated populations of *Drosophila* (Dobzhansky & Pavlovsky, 1957 cited in Roush, 1990a) but it did not show whether the founder effect reduced the ability of populations to adapt successfully. It has been found that founder effect is not severe when the traits are polygenic i.e. controlled under many loci (e.g. Lande, 1980). Roush (1990a), for example, suggested that to collect a founder population of a biological control agent that comprises a reasonable expression of a species' diversity, 20 individuals may form an absolute minimum while populations of over 100 individuals probably offer little additional advantage. This issue is still debated hotly in the biocontrol community. Collection of samples from as many sites as possible has also been emphasized.

3.3.2 Genetic drift

Genetic drift is the random change of allelic frequencies which can occur from such sampling effects in small populations and can result in the loss of alleles (Josyln, 1984; Roush, 1990 a, b; Hopper *et al.*, 1993; Roush & Hopper, 1995). Drift, because it is a chance effect, will have its greatest impact in the first few generations when the initial population size is small. Thus genetic drift is small when populations are maintained in big numbers (Hanrahan *et al.*, 1973; Franklin, 1980; Bartlett, 1984; Joslyn, 1984; Briscoe *et al.*, 1992; Roush & Hopper, 1995). It has been speculated that genetic drift in laboratory cultures is more extreme than the number of individuals actually sampled would indicate because the effective population sizes are smaller (Unruh *et al.*, 1983). Effective population size (N_e), which is a weighted average of the number and degree to which individuals participate in the reproductive effort, has been estimated to be one half the number of individuals used to renew the cultures each generation (Unruh *et al.*, 1983). Changes in laboratory cultures due to genetic drift can be detected by measuring allele frequencies. Genetic drift can be avoided by maintaining large numbers of breeding adults (Franklin, 1980; Joslyn, 1984; Roush, 1990a). Unruh *et al.* (1983) suggested that the best way to retain heterozygosity and prevent genetic drift in laboratory cultures is to maintain relatively large population sizes (>100) in the laboratory.

3.3.3 Inbreeding

Inbreeding results from matings that occur between close relatives, which can be expected to happen frequently in populations where the number of breeding individuals has been small for many generations. Because close relatives share alleles, mating between them produces relatively more homozygous offspring than would be expected in a random mating population. Thus, inbreeding changes genotype frequencies: it increases the frequency of homozygotes and decreases the frequency of heterozygotes (Josyln, 1984; Hopper *et al.*, 1993; Hartl & Clark, 1997). However, inbreeding does not directly change allelic frequencies. The detrimental effect of inbreeding, known as inbreeding depression, is caused by the expression of deleterious recessive alleles that have become homozygous (Hartl & Clark, 1997). One kind of problem that occasionally results from inbreeding in the Hymenoptera is the distortion of sex ratio due to the production of diploid males. Some Hymenoptera exhibit a multiple allele single-locus complementary sex determination, whereby individuals which are heterozygous at a single sex locus develop into females, whereas hemizygotes (haploids) and homozygotes (diploids) develop into males (Whiting, 1943). Inbreeding in such circumstances can cause an increased proportion of diploid males, which in most cases, are inviable, sterile or produce sterile (triploid) daughters (Stouthamer *et al.*, 1992; Cook, 1993; Cook & Crozier, 1995). So far single-locus complementary sex determination (sl-CSD) has been identified in more than 40 species within four superfamilies of Hymenoptera. However inbreeding is not always a problem during culturing of insects. For example, in some hymenopteran species such as *Muscidifurax raptor* and *M. zaraptor*, various degrees of inbreeding did not cause any genetic decay after 18-110 generations (Legner, 1979). It was thus concluded that sl-CSD was not operative in these species. In addition, Sorati *et al.* (1996) found that the egg parasitoid *Trichogramma* nr *brassicae* did not undergo inbreeding depression after four generations of sib mating. Inbreeding, like genetic drift, can be avoided by maintaining large numbers of breeding adults (Franklin, 1980; Josyln, 1984; Roush, 1990a).

3.3.4 Selection for adaptation

Selection is the preferential reproduction or survival of one genotype over other genotypes, and is thus, unlike drift, nonrandom. Selection, like genetic drift, can change the allelic frequencies but unlike drift which is random, selection changes allelic frequencies in a directed way by increasing the relative frequencies of favoured genotypes, i.e. it changes allelic and genotypic frequencies. Selection occurs in small and large populations, but is most effective in large populations, especially those maintained over many generations (Hartl & Clark, 1997). Inadvertent selection can easily make common and presumably field adapted alleles become rare (e.g. Bush, 1979). Thus, depending on the strength of selection, availability of genetic variability

and the number of generations involved (Hartl & Clark, 1997), selection has the potential to become the most important influence on a large population. Loss of common alleles (which are important for adaptation in the field) by selection in the laboratory can be minimized by maintaining natural enemy cultures as a number of isofemale lines (Delpuech *et al.*, 1993; Roush & Hopper, 1995). The number of lines needed to prevent loss of common alleles in a population was calculated to be 22 for arrhenotokous species (Roush & Hopper, 1995).

However genetic changes are not the only cause of colony deterioration; many kinds of environmental factors such as nutritional deficiencies, host size, disease, effects of treatment and handling and absence of developmental stimuli can cause similar effects (Chambers, 1977, Roush, 1990a).

4.0 Problem definition and research objectives

Classical biological control is considered as the most cost-effective form of pest management (e.g. Huffaker *et al.*, 1976; Greathead, 1986; Tisdell, 1988; Habeck *et al.*, 1993; Fowler *et al.*, 1994; van den Berg *et al.*, 2000). About a third of all attempts to introduce natural enemies have been successful and about half of those have provided some degree of control of the target pest population (Waage 1990). This is a very good success rate when compared to the development of chemical insecticides where 1 out of 20,000 compounds make it to the market. Still, biocontrol workers would like to attain a higher success rate. Although many factors have been proposed to account for failures, low genetic variability in the founder population was considered as one of the main causes (Mackauer 1976, Stouthamer *et al.*, 1992, Hopper *et al.*, 1993). The value of genetic diversity in released natural enemies for establishment and the effectiveness of biological control has been discussed but there is lack of experimental fieldwork to test its importance. Loss of genetic variability can take place during importation and laboratory rearing of natural enemies (Unruh *et al.*, 1983). However little is known on how to maintain the genetic variability in mass cultures of natural enemies.

The International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya executes a Dutch-funded project of classical biological control of cereal stemborers in Africa. This project has focused on the introduction of *C. flavipes*, a parasitoid of *C. partellus*. In 1993, *C. flavipes* was introduced in the coastal area of Kenya (Overholt *et al.*, 1994) and recent impact analysis has shown that the stemborer population has declined and larval mortality due to the parasitoid has increased (Zhou *et al.*, 2001b). The success of the releases of *C. flavipes* in Kenya resulted in the ICIPE project extending collaborative activities with National Agricultural Research Systems

(NARS) in other East and southern African countries through which releases of *C. flavipes* in these countries have been facilitated.

The field research described in this thesis was conducted on Zanzibar Islands through a collaborative effort between the ICIPE project and Zanzibar Plant Protection Division. It involved field surveys and the release of the exotic stemborer parasitoid. *flavipes* on the islands. Zanzibar is an archipelago made up of Unguja and Pemba Islands, and several islets. It is located in the Indian Ocean about 35 km off the coast of mainland Tanzania and lies between latitudes 4°50'and 6°30' South and between longitudes 39°10'and 39°50'East. Unguja has a total area of 1500 km^2 while Pemba covers 850 km^2. Zanzibar has a tropical climate with two main seasons. Rice, maize and sorghum are the important cereal crops grown on these islands.

Field research also included collaboration with scientists at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, who were involved in the collection and importation of original material of the parasitoid *Cotesia flavipes* which formed a nucleus for establishment of colonies for laboratory experiments and for field releases on Zanzibar islands.

4.1 The objectives of the research presented in this thesis were the following:

1. To determine the main stemborer species of maize and sorghum, their abundance and distribution and associated indigenous parasitoids in Zanzibar
2. To determine whether foraging behaviour of *C. flavipes* changes over time in laboratory colonies and whether genetic variability is related to changes
3. To investigate effect of the allelic diversity at the sex locus of *C. flavipes* on its population growth.
4. To investigate the relationship between the level of genetic variability of a colonizing population of *C. flavipes* and its probability of establishment in the field.

5.0 Outline of the thesis

Background information on the most important stemborer pests of maize and sorghum in East Africa, their biology and control is given in **Chapter 1.** Genetic variability as an important factor for establishment of natural enemies introduced in classical biological control programmes is also discussed along with the genetic changes that may occur during the introduction and rearing of the natural enemies.

In **Chapter 2,** results are presented of field studies conducted in several sites on the two main islands of Zanzibar that were aimed at determining the species composition, abundance and distribution of lepidopteran stemborers and their indigenous parasitoids in maize and sorghum and to assess the levels of parasitism by

indigenous parasitoids. It was revealed that the introduced stemborer *C. partellus* was the most abundant species and that the levels of parasitism by larval and pupal parasitoids were very low. It is suggested that the introduction of exotic natural enemies of *C. partellus* would supplement parasitism by the indigenous parasitoids.

Laboratory rearing can lead to changes in the life history characters and behaviour of natural enemies and this can reduce their effectiveness when they are released in the field. In **Chapter 3,** results are reported concerning the responses of a genetically impoverished (isofemale lines) and of genetically diverse populations of the introduced cereal stemborer parasitoid, *Cotesia flavipes,* to host- and host plant-associated odours measured over several generations. These studies were aimed at examining the effect of duration of laboratory rearing on parasitoid foraging behaviour and whether genetic variability is related to changes. Changes in behaviour were noticed in early generations of laboratory rearing but the overall behavioural responses in most populations remained stable in the 45 generations in which the tests were conducted. The apparent changes in behaviour during early generations were attributed to behavioural plasticity using a 'hand-stinging' method in rearing of the parasitoid.

A reduced number of alleles at sex loci in some hymenopteran species can hamper population growth during laboratory rearing or after field release due to the production of diploid males. This can occur as a result of a sex determination system called single locus complementary sex determination (sl-CSD). In species that exhibit sl-CSD, matched matings, i.e. matings between males and females that share a sex allele, result in the production of diploid males leading to a male biased sex ratio. Investigations were conducted to find evidence for the production of diploid males in the field and hence, the existence of sl-CSD, in the braconids *Cotesia flavipes* and *Cotesia sesamiae* (**Chapter 4**). Models to predict the frequency of matched matings in populations with different frequencies of sib mating and egg fertilization rates were developed. The predictions of these models were used to determine if brood sex ratio distributions could be used to determine whether a species exhibits sl-CSD. The models showed that sl-CSD could be detected from brood sex ratios if the diploid male offspring survives. Sex ratios of field populations were also analysed to find evidence for the existence of sl-CSD and the production of diploid males in nature.

Laboratory experiments to further deduce evidence for sl-CSD in *C. flavipes* are described in **Chapter 5.** The existence of sl-CSD in *C. flavipes* was investigated using a series of inbreeding crosses among five isofemale lines. In addition, sex ratios were monitored for several generations of inbreeding of these populations. The findings provide new evidence that CSD does not exist in *C. flavipes.*

The importance of genetic variability in colonization of natural enemies was tested through field releases of three genetically impoverished populations (isofemale lines) and one genetically diverse population of *Cotesia flavipes* on two islands of Zanzibar (**Chapter 6**). Populations of *C. flavipes* were collected from India, which is the presumed origin of *C. partellus*, and they were imported, quarantine processed and reared in the laboratory in Kenya. In a randomized complete block design, the four populations were released in a total of 28 maize and sorghum plots on Unguja and Pemba islands. Releases of an equal number of cocoon masses of each population were made once in each plot during the long rainy season in 2000. Monitoring for colonization was done every two weeks in long rainy seasons of 2000 and 2001. Results from surveys in 2000 showed that colonization of stemborers by *C. flavipes* was higher for the genetically diverse population than one of the isofemale lines, but was not different from the other two isofemale lines, suggesting that genetic diversity may not have been all that important for colonization of *C. flavipes* in Zanzibar. All the populations were recovered one year after release, confirming that the parasitoid was firmly established on the islands.

In the final chapter (**Chapter 7**), a synthesis is presented of all previous chapters on the genetic variability in *C. flavipes* and its importance in biological control of stemborers. The most important results are briefly summarized, the implications for the research are discussed. My main conclusion from this thesis is that genetic diversity exists in populations of natural enemies but may not be an important factor for their colonization if the natural enemies are introduced in an area with a climate similar to the area of origin. During the rearing of *C. flavipes*, behavioural responses to host finding cues in most populations remained stable over many generations regardless of the genetic variability of the wasp population. Furthermore, the allelic diversity at the sex loci of *C. flavipes* may not affect its population growth both during laboratory rearing and after release in the field as no evidence for complementary sex determination was found in this braconid. Suggestions for future studies to improve the biological control of stemborers in Zanzibar are given.

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Chapter 2

Distribution and abundance of lepidopteran stemborers and associated indigenous parasitoids in maize and sorghum in Zanzibar

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Abstract

Studies to determine the relative abundance and distribution of stemborer species and their associated parasitoids in maize and sorghum were carried out during the 1995-1996 and 1999-2000 cropping seasons on Unguja and Pemba, the two main islands of Zanzibar. Three stemborer species were found on both islands. The exotic species *Chilo partellus* (Swinhoe) (Crambidae) was the most abundant and accounted for 75.3% of stemborer attack. It was followed by *Sesamia calamistis* Hampson (Noctuidae) and *Chilo orichalcoceliellus* Strand (Crambidae). Stemborer density was higher on Pemba (1.47±0.20 borers per plant) than on Unguja (0.85±0.05 borers per plant). Overall stemborer density in Zanzibar, during the period of study was 1.03±0.08 borers per plant. Stemborer infestation was significantly higher during the short rainy season than in the long rainy season but did not vary between maize and sorghum or coral rag and plantation zones. Indigenous parasitoids recorded included an egg/larval parasitoid *Chelonus* sp. and ten larval parasitoids: *Bassus* sp., *Cotesia* sp., *Cotesia sesamiae* Cameron, *Cotesia ruficrus* (Haliday), *Dolichogenidea* sp., *Dolichogenidea aethiopica* Wilkinson, *Dolichogenidea polaszeki* Walker, *Megaselia* sp. and an unidentified Tachinidae. Among these, the braconid *C. sesamiae* Cameron was the most common parasitoid, attacking all the stemborer species and was recorded from 85.2% of parasitized larvae. However, the efficiency of *C. sesamiae* was reduced by two hyperparasitoids *Aphagnomus fijiensis* Ferrière and *Elasmus* sp. Seven pupal parasitoids were recorded: *Dentichasmias busseolae* Heinrich, *Brachymeria* sp. Westwood, *Brachymeria olethria* Waterston, *Pediobius furvus* Gahan, *Psilochalsis soudanensis* Steffan, *Syzeuctus ruberrimus* Benoit and an unidentified Chalcididae. The overall parasitism by larval and pupal parasitoids was too low to effectively regulate the stemborer densities at acceptable levels.

Introduction

Maize, *Zea mays* L. and sorghum, *Sorghum bicolor* (L.) Moench are important cereal crops in Zanzibar especially in the coral rag zone (Borsa, 1987; Koenders, 1992) where smallholder farmers have no access to rice growing areas (SMZ, 1987). Maize and sorghum are also grown in the plantation zone (Keulen, 1990). Because of two rainy seasons, these crops can be cultivated twice a year as well as between the main seasons. Some farmers plant maize and sorghum in the short rainy season (*vuli*) to ensure that they have seed to plant in the long rainy season (*masika*). Arendse (1990) reported that 31% of farmers on Unguja grow maize.

Maize was introduced into East Africa in the 17th century with varieties originating from the Caribbean which were only suited to the coastal strip of E. Africa (Seshu Reddy, 1998). Although it is not known when maize first arrived in Zanzibar, the earliest available record indicates that by the 1870s it was being grown (Burton, 1872). Sorghum, on the other hand, is a native African cereal reported to have originated in the Sudan/Ethiopian boarder region (De Wet, 1978) and was already being grown in Zanzibar by 1860s (Rigby, 1861).

In Zanzibar, maize yields are generally low, varying between 300-500 kg/ha (Keulen, 1990). One of the major constraints limiting yields is insect pests, with stemborers being the most important (Briant, 1961; Allertz *et al.*, 1988; Keulen, 1990; Bezemer, 1994). Grain yield losses due to stemborer attack range between 30-40% (Allertz *et al.*, 1988; Keulen, 1990) and infestation levels up to 70% have been reported (Briant, 1959). Arendse (1990) observed that 91% of the farmers on Unguja considered stemborers as the most serious constraint to maize production, with 89% of them attributing moderate to high losses in maize to stemborers. In Pemba, Bezemer (1994) reported 25-51.9% stemborer infestation in maize and 40-73.7% in sorghum the during long rains of 1994.

Stemborer attack begins from the leaf whorls, where young larvae mine leaf sheaths (windowing). Older larvae migrate down to stems where they excavate tunnels. Tunneling of young plants on meristematic tissue may result in destruction of the growing point, typically referred to as 'dead heart'. Stems attacked in a later growth phase become stunted in growth and cobs will not develop well. Stemborer attack reduces the number of plants and decreases numbers and weight of cobs, resulting in a lower final yield. Occurrence of dead hearts is mainly responsible for a decrease in plant density. Mohyuddin and Attique (1978) observed that yield reduction caused by *C. partellus* was due to dead hearts and stunting. Stem tunneling also provides points for

entry of plant pathogens and renders the plant more susceptible to lodging. The second-generation larvae are often associated with cobrot disease *Fusarium moniliforme* (Sithole, 1988). Stemborer infestation may also lead to quality loss in cobs (Eveleens, 1990). Keulen (1990) reported that during the long rains of 1989 on Unguja, the highest increase in attacked plants occurred between 3 and 6 weeks after sowing. In some fields, a second peak occurred at about 9 weeks after sowing.

Previous research on stemborers in Zanzibar has concentrated on yield loss assessments (Allertz *et al.* 1988), chemical control (Briant, 1961; Selby, 1963), effect of fertilizer (Briant, 1952a; 1952b; 1953) and a combination of fertilizer and insecticide application (Keulen, 1990). Information on the stemborer complex, their abundance and associated parasitoids in Zanzibar, is lacking. Such information is important for the development of an integrated pest management strategy for stemborers in small-holder cropping systems based on tactics such as habitat management, use of resistant varieties and biological control. The objectives of the study were to (i) determine the relative abundance of stemborers, and (ii) to determine the distribution of stemborer species and their associated native parasitoids in maize and sorghum in Zanzibar.

Materials and methods

Study sites

Studies were conducted in major maize and sorghum growing areas of Unguja and Pemba between 1995 and 2000. In 1995 and 1996, sampling was conducted only on Unguja at 3 sites (Tazari, Bambi and Kizimbani), while in 1999 and 2000 data were collected from 12 sites on Pemba and 20 sites on Unguja located in three and four districts, respectively (Table 1). The districts represented the northern, central and southern parts of each island.

Two rainy seasons can be distinguished in Zanzibar: March-June (long rainy season, *masika*) and October-December (short rainy season, *vuli*). The long rains (900-1000 mm) tend to be more reliable than the more variable short rains (400-500 mm). In general, the pattern of rainfall of Unguja is similar to that of Pemba. However, Pemba receives more rain, with annual average of 1900 mm than Unguja with 1600 mm (FAO/IFAD, 1987). The mean annual temperature is 30.3°C on Unguja and 23.5°C on Pemba and relative humidity ranges from 87% in April (long rains) to 76% in November (short rains) (Wirth *et al.*, 1988).

Study sites were farmers' fields of approximately 0.5-1.0 ha in size and at least 5 km apart, randomly selected from the coral rag and plantation zones, which are the major maize and sorghum growing areas. The plantation zone, characterized by relatively deep and rich soils, is located on the western side of the islands while the coral rag zone

Table 1: Location of study sites where stemborers and their parasitoids were monitored on Unguja and Pemba islands in 1995-96 and 1999-2000

Site	Location	Altitude	District	AEZ[1]	Crop
Unguja Island					
Zone 1					
Tazari	05°46.766'S, 039°18.666'E	18 m	North	coral rag	maize
Mkwajuni	05°53.238'S, 039°17.536'E	29 m	North	plantation	maize
Kinyasini	05°58.078'S, 039°18.769'E	29 m	North	plantation	maize
Mahonda	05°59.243'S, 039°15.622'E	28 m	North	plantation	maize
Kiwengwa	05°59.549'S, 039°21.031'E	30 m	North	coral rag	maize
Zone 2					
Selem	06°02.717'S, 039°14.569'E	23 m	West	plantation	maize
Kizimbani	06°05.498'S, 039°14.524'E	28 m	West	plantation	maize
Shakani	06°15.008'S, 039°14.210'E	20 m	West	coral rag	maize
Kibonde Mzungu	06°11.384'S, 039°16.558'E	16 m	West	plantation	maize
Jumbi	06°12.039'S, 039°16.953'E	14 m	West	coral rag	maize
Zone 3					
Bambi	06°04.596'S, 039°21.615'E	24 m	Central	plantation	maize
Mpapa	06°06.303'S, 039°20.177'E	22 m	Central	coral rag	maize
Kitumba	06°06.725'S, 039°18.415'E	26 m	Central	coral rag	maize
Bungi	06°14.957'S, 039°20.169'E	08 m	Central	coral rag	maize
Unguja Ukuu	06°17.960'S, 039°22.454'E	09 m	Central	coral rag	maize
Zone 4					
Ubago	06°09.197'S, 039°18.191'E	25 m	South	coral rag	maize
Jendele	06°10.475'S, 039°22.437'E	21 m	South	coral rag	maize
Ndijani	06°11.272'S, 039°20.981'E	29 m	South	coral rag	maize
Kitogani	06°17.220'S, 039°26.755'E	12 m	South	coral rag	maize
Makunduchi	06°24.763'S, 039°32.005'E	23 m	South	coral rag	maize
Pemba Island					
Zone 1					
Makangale	04°53.616'S, 039°41.234'E	08 m	Micheweni	coral rag	maize
Kiuyu Mbuyuni	04°57.840'S, 039°51.331'E	10 m	Micheweni	coral rag	sorghum
Mziwanda	05°00.313'S, 039°49.183'E	19 m	North	plantation	maize
Matangatuani	04°58.531'S, 039°44.047'E	56 m	North	plantation	maize
Zone 2					
Kiwani	05°07.071'S, 039°49.322 'E	16 m	Wete	plantation	sorghum
Kangagani	05°10.119'S, 039°50.486'E	12 m	Wete	plantation	maize
Dodeani[2]	05°11.714'S, 039°49.001'E	29 m	Wete	plantation	maize
Vitongoji	05°13.116'S, 039°50.347'E	08 m	Chake Chake	coral rag	maize
Zone 3					
Pujini	05°18.222'S, 039°49.225'E	32 m	Chake Chake	coral rag	maize
Kibaridi	05°19.082'S, 039°48.963'E	38 m	Chake Chake	coral rag	maize
Ndooni	05°16.958'S, 039°48.646'E	51 m	Chake Chake	plantation	maize
Muwambe	05°25.446'S, 039°45.010'E	12 m	Mkoani	plantation	maize

[1]AEZ: Agro-ecological zone, [2]Sorghum was grown at Dodeani for one season (long rainy season of 1999).

occupies the eastern side and has poorer soils consisting of weathered coral rocks with pockets of fertile soils (NCDP, 1980). Additional sites were located on agricultural stations at Kizimbani, Kibonde Mzungu, Bambi, Makunduchi on Unguja and Matangatuani and Mziwanda on Pemba. At two sites, Kiuyu Mbuyuni and Kiwani, sorghum was grown for two seasons and at Dodeani for one season. In all other sites, maize was grown. Surveys were conducted in fields that were prepared and managed by farmers according to local agricultural practices. Local varieties of seeds were sown and no organic fertilizer or pesticides were applied in all study sites.

Sampling and rearing of stemborers

Sampling was conducted during the two main rainy seasons. For each site, studies were conducted for at least two seasons, commencing during the 1994/1995 short rainy season. Data collected during the 1994/1995 short rainy season only served to facilitate planning of subsequent surveys after gaining information on the prevalence of stemborers and their natural enemies. A stratified random sampling procedure was followed. Each field was divided into four quadrants and an equal number of plants with putative stemborer symptoms were randomly removed from each quadrant along x/y coordinates. Sampling was conducted on three occasions in each season and began 5-6 weeks after sowing to coincide with the infestation of medium-sized and larger larvae. A sampling interval of two weeks between sampling occasions was maintained. Small larvae (first/second instars) were not reared for parasitism because generally only parasitoid eggs from the subfamily Cheloninae can be found, but they emerge from late instars (Smith *et al.*, 1993). A total of 52-60 plants were removed from the field for destructive sampling on each sampling occasion. Plants with purative stemborer symptoms were uprooted from the field and dissected to remove medium to large (3rd – 6thinstar) larvae, pupae and parasitoid pupal stages from stems, tassels or cobs. All larvae and pupae were identified, counted and placed in glass vials (8.5 x 2.7 cm). Larvae were reared individually on small pieces of maize stems in glass vials at room temperature (27.0±1°C) and inspected every two days for mortality or parasitoid emergence. Maize stems provided suitable conditions for parasitoid larvae to form cocoons after exit from the hosts, while the larvae could still feed on the stem. Stems also maintained a high relative humidity in the vials. Stems were replenished at each inspection to avoid fungal attack. Any larvae that died before forming cocoons, escaped, or were injured, were excluded from the calculation to determine the rate of parasitism. Pupae were placed in vials plugged with cotton wool. All emerging moths were identified and then discarded. Specimens of each parasitoid species were labeled and preserved in 100% alcohol and sent to International Centre of Insect Physiology and Ecology (ICIPE) and Wageningen University for identifications.

Statistical analysis

For each site, average stemborer and parasitoid numbers were calculated over the sampling occasions per season, which represented the reproductive and mature growth stages (Oloo, 1989). The percentage of each stemborer species and that of larval/pupal stages was calculated for each site per season. The number of all stemborer species was pooled for each site and percentage parasitism was calculated from the pooled susceptible stemborer stage for each parasitoid species. Descriptive statistics were performed on stemborer numbers and species. Distribution and abundance of stemborers were compared between seasons, districts, agro-ecological zones and crop types using ANOVA (PROC GLM, SAS Institute 1988). Comparison of variables was performed on data obtained in the same seasons. Significant differences in mean stemborer per infested plant were separated using the Least Significant Difference Test (LSD) at a probability level of 5%.

Results

Stemborer species composition

Three stemborer species were found, with the introduced species, the spotted stalk borer, *Chilo partellus* (Swinhoe) (Crambidae), being the most abundant and accounting for 75.3% of the stemborer larvae and pupae collected during this period. The two other stemborer species were native species, the pink stalk borer *Sesamia calamistis* Hampson (Noctuidae) and the coastal borer *Chilo orichalcociliellus* Strand (Crambidae) which accounted for 16.4% and 8.0% of borers respectively (Table 2). Often two or more stemborer species were found together in the same part of a plant. All the stemborer species were collected from stems, tassels and cobs and attacked both maize and sorghum. In contrast, cobs harvested in the field were often attacked by *S. calamistis*. *Chilo partellus* comprised 71.3 and 85.3% of stemborer species on Unguja and Pemba, respectively. All the three stemborer species occurred on both islands. A stalk-eyed borer, *Diopsis longicornis* Macquart (Diptera: Diopsidae) was collected once in the long rainy season of 1999 from a maize stem at Mpapa on Unguja.

Stemborer abundance and distribution

A total of 11,355 stemborer larvae and pupae were collected from 10,189 putatively infested plants of maize and sorghum. The number of stemborers per infested plant was significantly higher on Pemba than on Unguja (df=1; F=6; p=0.0001), with 0.85±0.05 and 1.47±0.20 stemborers/plant on Unguja and Pemba, respectively. Stemborer numbers did not vary significantly between the districts on the two islands, except on Pemba where the infestation was significantly higher in Wete than Micheweni district. The overall man stemborer density on Zanzibar during the period of study was 1.05±0.08 stemborers/plant. Stemborer numbers also varied with season (Table 2 and 3). Levels of stemborer attack on Unguja were significantly higher during

Table 2. Distribution and percentage composition of stemborer species recorded from two agro-ecological zones on Unguja islands between 1995 and 2000 seasons

Island/AEZ[a]	Mean (±SE)	% composition by species			Season*	Mean (±SE)	% composition by species		
		Cp	Co	Sc			Cp	Co	Sc
Unguja									
Coral rag	0.77±0.08a	68.6	10.9	20.5	LR1999	0.58±0.07a	64.2	11.4	24.4
Plantation	0.83±0.08a	76.8	7.9	15.8	SR1999	1.08±0.11b	63.3	10.7	26.0
					LR2000	0.72±0.07a	78.2	11.7	10.1
Total	0.85±0.05A	71.5	9.9	18.6					
Pemba									
Coral rag	1.45±0.30a	70.4	6.6	13.0	LR1999	1.05±0.32	73.1	7.3	19.6
Plantation	1.55±0.36a	89.8	0.1	10.1	LR2000	1.84±0.30	94.5	0.1	5.4
Total	1.47±0.20B	85.3	3.2	11.5					
Zanzibar	1.03±0.08	75.3	8.0	16.7					

AEZ[a], Agro-ecological zone; *LR, long rainy season; SR, short rainy season; Cp, *Chilo partellus;* Co, *Chilo orichalcociliellus;* Sc, *Sesamia calamistis.*

Means in a column followed by the same lowercase letter are not significantly different (LSD, $p<0.05$). Means in a column followed by the same uppercase letter are not significantly different (LSD, $p<0.05$).

the short rainy season than long rainy season (df=1; F=9.92; p=0.0029), with 1.08±0.11 and 0.76±0.05 stemborers/plant in the short and long rainy season, respectively. Such comparison could not be made on Pemba as sampling was done only during the long rainy season. However, no significant differences in stemborer infestation in maize and sorghum or between coral rag and plantation zones were detected. The average number of stemborers per infested plant did not vary significantly among the years of study.

Table 3. Percentage composition of stemborers collected from two agroecological zones on Unguja in 1995-1996 cropping seasons

Site+ and season	AEZ++	Crop	No. of borers	% Composition		
				Cp	Co	Sc
Tazari						
LR1995	Coral rag	Maize	557	92.3	2.9	4.8
SR1995		Maize	163	86.5	4.3	9.2
LR1996		Maize	304	82.6	2.6	14.8
Bambi						
LR1995	Plantation	Maize	522	90.8	4.8	4.4
SR1995		Maize	378	84.4	2.1	13.5
LR1996		Maize	242	82.2	4.1	13.6
Kizimbani						
LR1995	Plantation	Maize	242	75.6	8.3	16.1
SR1995		Maize	388	80.2	3.1	16.8
LR1996		Maize	349	83.1	4.3	12.6
Total			3,145	84.2	4.1	11.8

+Three sites were surveyed in 1995-1996 seasons. ++Agroecological zone
Cp, *Chilo partellus;* Co, *Chilo orichalcociliellus;*. Sc, *Sesamia calamistis.*

Larval and pupal parasitoids

Tables 4, 5 and 6 provide a list of parasitoids recorded from stemborer larvae and pupae. The parasitoids were represented mainly by the family Braconidae and included ten larval parasitoids and seven pupal parasitoids. Parasitoids recorded included an egg/larval parasitoid *Chelonus* sp. and ten larval parasitoids: *Bassus* sp., *Cotesia* sp., *Cotesia sesamiae* Cameron, *Cotesia ruficrus* (Haliday), *Dolichogenidea* sp., *Dolichogenidea aethiopica* Wilkinson, *Dolichogenidea polaszeki* Walker, *Megaselia* sp. and an unidentified Tachinidae. Of these the braconid *C. sesamiae* was the most abundant and widespread parasitoid. It attacked all the stemborer species.

Table 4. Larval parasitoids and associated hyperparsitoids recorded on Unguja islands between 1995 and 2000

Parasitoid species	No. of parasitoids recovered	Borer species	Borer stage	District and season in which recovered							
				North		West		Central		South	
				LR	SR	LR	SR	LR	SR	LR	SR
Larval parasitoids											
Hymenoptera: Braconidae											
Bassus sp. Cameron	1	Cp	Larva	-	-	-	+	-	-	-	-
Cotesia sp.	18	?, Cp, Co, Sc	Larva	+	-	+	+	+	-	+	-
Cotesia sesamiae (Cameron)	225	Cp, Co, Sc	Larva	++	+	++	++	++	++	+	+
Dolichogenidea aethiopica Wilkinson	1	?	Larva	-	-	-	-	-	-	-	+
Dolichogenidea polaszeki Walker	1	Co	Larva	-	-	-	+	-	-	-	-
Hyperparasitoids											
Hymenopetra: Ceraphronidae											
Aphanogmus fijiensis (Ferrière)	36	ex. *C. sesamiae*	cocoons	-	-	++	+	+	-	+	-
Hymenoptera:Elasmidae											
Elasmus sp.	1	ex. *C. sesamiae*	cocoons	-	-	+	-	-	-	-	-

Cp, *Chilo partellus;* Co, *Chilo orichalcociliellus;* Sc, *Sesamia calamistis.*
?, Unknown or unidentified species
Larva, 3rd - 6th instars; ++, abundant; +, present; -, absent; LR, long rainy season; SR, short rainy season.

Table 5. Larval parasitoids and associated hyperparsitoids recorded on Unguja islands between 1995 and 2000

Parasitoid species	No. of parasitoids recovered	Borer species	Borer stage	District and season in which recovered							
				North		West		Central		South	
				LR	SR	LR	SR	LR	SR	LR	SR
Hymenoptera: Chalcididae											
Brachymeria sp. Westwood	1	Cp	Pupa	-	-	-	+	-	-	-	-
Brachymeria olethria Waterston	2	Cp	Pupa	-	-	-	-	+	-	-	-
Psilochalchis soudanensis (Steffan)	1	Cp	Pupa	+	-	-	-	-	-	-	-
Hymenoptera: Eulophidae											
Pediobius furvus (Gahan)	22	Co, Cp, Sc	Pupa	+	-	+	+	+	+	-	-
Hymenoptera: Ichneumonidae											
Dentichasmias busseolae Heinrich	4	? Cp	Pupa	+	-	+	+	+	-	-	-
Syzeuctus ruberrimus Benoit	8	?, Cp	Pupa	-	-	-	+	+	+	-	-

Cp, *Chilo partellus;* Co, *Chilo orichalcociliellus;*.Sc, *Sesamia calamistis.*
?, Unknown or unidentified species
++, abundant; +, present; -, absent. LR, long rainy season; SR, short rainy season.

Table 6. Larval and pupal parasitoids recorded from stemborers in maize and sorghum on Pemba islands in 1999-2000

Parasitoid species	No. of parasitized borers	Borer species	Borer stage	District and Season in which recovered					
				Micheweni		Wete		Chake Chake	
				LR1999	LR2000	LR1999	LR2000	LR1999	LR2000
Hymenoptera: Braconidae									
Chelonus sp.	2	Cp	Larva	-	-	-	2	-	-
Cotesia sp.	29	?, Cp, Co, Sc	Larva	-	-	-	-	-	29
Cotesia ruficrus (Haliday)	1	?	Larva	-	-	1	-	-	-
Cotesia sesamiae(Cameron)	22	?,Cp, Co, Sc,	Larva	1	2	10	9	2	7
Dolichogenidea sp.	1	?	Larva	-	1	-	-	-	-
Diptera: Phoridae									
Megaselia sp.	1	?	Larva	-	-	-	-	1	-
Diptera: Tachinidae									
Unidentified	1	Cp	Larva	-	-	-	-	-	1
Hymenoptera: Ichneumonidae									
Syzeuctus ruberrimus Benoit	4	?, Cp	Pupa	-	1	-	-	3	-
Hymenoptera: Chalcididae									
Unidentified	1	Sc	Pupa	-	-	-	-	-	1

Cp, *Chilo partellus;* Co, *Chilo orichalcociliellus;*. Sc, *Sesamia calamistis.*
?, Unknown or unidentified species
Larva, 3rd - 6th instars; ++, abundant; +, present; - , absent; LR, long rainy season; SR, short rainy season

The pupal parasitoids recorded were the hymenopterans *Dentichasmias busseolae* Heinrich (Ichneumonidae), *Brachymeria sp.* Westwood (Chalcididae), *Brachymeria olethria* Waterston (Chalcididae), *Pediobius furvus* (Gahan) (Eulophidae), *Psilochalsis soudanensis* Steffan (Chalcididae), *Syzeuctus ruberrimus* Benoit (Ichneumonidae), and an unidentified Chalcididae. *Pediobius furvus* was the dominant pupal parasitoid on Unguja. Some parasitoid species were only recorded on one island. *Cotesia sesamiae, Dolichogenidea* sp. and *S. ruberrimus* were recorded on both islands. A higher diversity of parasitoid species was recorded on Unguja than Pemba.

Field parasitism

Cotesia sesamiae was the most common parasitoid recorded during this study. It was recovered in all sites and was recorded from 85.2% of the parasitized larvae. Stemborer parasitism by *C. sesamiae* was low and in the long rainy season of 2000, it averaged 7.1% on Unguja and 1.1% on Pemba. Its efficiency was, however, reduced by two hyperparasitoids *Aphagnomus fijiensis* (Ferrière) (Hymenoptera: Ceraphronidae) and *Elasmus* sp. (Hymenoptera: Elasmidae). *Aphagnomus fijiensis* was frequently reared from field-collected cocoons of *C. sesamiae*. Parasitism by *A. fijiensis* was high and during the long rainy season in 1999 it reached up to 50% at Kibonde Mzungu. *Elasmus* sp. was collected once from cocoons of *C. sesamiae* at Jumbi (Table 4). Larval parasitoids were more abundant from 6 weeks after crop emergence whereas pupal parasitoids were first recorded at tassel and boot stages of maize and sorghum, respectively. Larval parasitism was higher than pupal parasitism on each island. Pupal parasitism was recorded on Pemba only during the long rainy season in 2000 and even then it was lower (mean 2.3%) than on Unguja (mean 8.3%). The overall stemborer parasitism levels were low and averaged 3.9% on Unguja and 1.9% on Pemba. The parasitism levels of various stemborers were related to the species abundance.

Discussion

Chilo partellus was the most widespread and abundant stemborer on both islands. The species is a new introduction to Zanzibar, as there is no evidence that it was collected prior to 1930s. Although Aders (1913) reported an infestation of *Chilo* sp. in Zanzibar as early as 1912, it is likely to have been the native species *C. orichalcociliellus*. *Chilo partellus* was accidentally introduced into Africa from Asia and was first recorded in Malawi around 1930 (Tams, 1932). As a result of its superior colonising potential in new habitats (Overholt *et al.*, 1994b; Kfir, 1997; Ofomata *et al.*, 2000), *C. partellus* colonises suitable feeding niches much earlier than the indigenous stemborers, hence reducing the number of other stemborer species that successfully colonise these habitats.

In Zanzibar, *C. partellus* also attacks rice where it has been reported to cause infestations of up to 12.7% (Bezemer, 1984). On the Kenyan coast, studies show that *C. partellus* has been gradually displacing the native species *C. orichalcociliellus* (Ofomata *et al.*, 1999a; b; 2000). The maize stalk borer *Busseola fusca* Fuller (Noctuidae) was not recorded in these surveys although it has been reported in Zanzibar (Aders, 1913; Niyibigira, pers. obs.) and seems to be of minor importance in maize (Arendse, 1990). This is not unexpected because in East Africa, *B. fusca* usually occurs at high altitudes above 900-1500 m, whereas most areas on Unguja are below 90 m and the hills on Pemba reach only a height of over 60 m above sea level.

Stemborer infestation was significantly higher on Unguja in the short rainy season than in the long rainy season. The high number of stemborers in the short rainy season may be due to a building up of densities from the previous season. Moreover, in the short rainy season, when the rains are poor and cereals are dwarfed and thin-stemmed, damage can be severe and sometimes cause total loss of the crop.

It has been shown that soil moisture influences crop damage by insect pests through its effect on plant vigour and growth. In Somalia, it was shown that sorghum grown under drought stress suffered greater damage from *C. partellus* than in the rain-fed areas (A.F. Nur, cited in Polaszek, 1998). Moyal (1995) observed that more vigorously growing maize plants within water-stressed plantings were heavily attacked by *B. fusca* and *Eldana saccharina*. In Ghana, Scheibelreiter (1980) reported that *Sesamia* spp. and *E. saccharina* reached their highest densities in sugar cane during the dry season. Similarly, a higher infestation of stemborers on maize was recorded in the short rainy season compared to the long rainy season in Bukoba district of Northern Tanzania (Ndile, 1997). Probable explanations for the deleterious effect of rainfall on stemborers are hindrance of adults to fly for mating (Kaufmann, 1983), stopping or depressing egg laying, and causing mortality to moths and larvae due to attack by fungi (Atkinson, 1980). The incidence of stemborers was significantly higher on Pemba than Unguja and this may be attributed to the differences in climate.

In the present study, field parasitism of stemborer larvae was mainly due to braconids including *C. sesamiae,* which was the dominant and most widely distributed parasitoid. All parasitoids listed above are indigenous to Africa (Mohyuddin & Greathead, 1970; Oloo, 1989; Kfir, 1990; 1992; Overholt *et al*, 1994b), and their association with the exotic *C. partellus* is relatively new. These parasitoids expanded their host ranges to utilize *C. partellus* as a new resource (Kfir, 1992, Overholt *et al*, 1994b). Although indigenous parasitoids play an important role in reducing the population levels of *C. partellus*, they are not able to reduce density to acceptable damage levels (Oloo, 1989; Kfir, 1990; 1992; Overholt *et al.*, 1994b). The parasitoids could not prevent the dispersal and subsequent wide distribution of *C. partellus* after its introduction into Africa. Most

of the parasitoids attacked *C. partellus* and are therefore probably more habitat-specific than host specific.

Egg parasitoids were not collected in these studies. Feijen *et al.* (1988) recorded the egg parasitoid *Telenomus* sp. in Zanzibar, but from *Maliarpha separatella* in rice. *Telenomus* sp. has been reported on stemborer eggs in maize and sorghum (Oloo, 1989; Polaszek & Kimani, 1990; Polaszek *et al.*, 1993; Polaszek, 1995; Bonhof, 2000). Further research is needed to explore the role of egg parasitoids in stemborer mortality. A ceraphronid hyperparasitoid, *A. fijiensis* caused high mortality of *C. sesamiae* cocoons. The high levels of hyperparasitism may further reduce the efficiency of *C. sesamiae* (Polaszek & LaSalle, 1995). In S. Africa, Kfir (1992) reported parasitism rates of 50-100% on *C. sesamiae* by *A. fijiensis*. Polaszek (1998) reported parasitization of *C. sesamiae* cocoons by *A. fijiensis* in many other parts of Africa. These findings therefore suggest that biological control by conservation of *C. sesamiae* may not to be highly productive in Zanzibar.

Unguja island supports slightly more parasitoid species than Pemba, perhaps due to its larger size. The total of 17 parasitoid species recovered from Unguja and Pemba constitutes less than 50% of the species found on stemborers on Tanzania mainland (Le Pelly, 1959; Nye, 1960; Robertson, 1965; Milner, 1967; Mohyuddin & Greathead, 1970; Mathez, 1972; Walker, 1994; Polaszek, 1995; van Achterberg & Polaszek, 1996; Ndile, 1997; Zwart, 1998) and over 40 parasitoid species reared from stemborers in East Africa (Bonhof *et al.*, 1997; Zhou *et al.*, 2003). Overall, islands support fewer species per unit of habitat than are found on the mainland. One of the most obvious observations one can make about factors affecting species diversity is that the larger the area, the more species it is likely to contain as a result of the greater habitat diversity associated with larger geographical areas. The equilibrium theory of biogeography (Preston, 1962; MacArthur & Wilson, 1963; 1967) proposes another explanation, that the species diversity of an area is the result of equilibrium between colonization and extinction. If we compare islands with mainland, we expect extinctions less frequently on mainland because of larger local population size.

Control of stemborers in Zanzibar is attempted mainly through cultural practices such as residue management, manipulation of planting date and mechanical measures. However, many types of cultural control methods require education and labour (Arendse, 1990). The control of stemborers through the use of synthetic organic pesticide, endosulfan (Thionex 3G), was shown to be cost effective in 1990 with a benefit-cost ratio of 17.25:1 (Keulen, 1990). However government subsidies on pesticides have recently been removed which has greatly increased their cost to farmers. It is unlikely that pesticides are still economically justified considering the low value of cereals particularly when grown for subsistence. Moreover, farmers do not follow the recommendations of applications (Allertz *et al.*, 1988). Stemborers are also protected

from pesticides due to their cryptic feeding behaviour in stem tunnels and this makes chemical control an unrealistic option.

Considering that *C. partellus* is the most widely distributed and dominant stemborer in both islands, releases of the co-evolved parasitoid, *Cotesia flavipes* Cameron could help to regulate the stemborer density. Moreover, *C. flavipes* was shown to attack the indigenous stemborer species *C. orichalcoceliellus* and *S. calamistis* (Ngi-Song *et al.*, 1995). Nye (1960) suggested that *C. partellus* probably originally came from India and proposed that the introduction of some of the Asian parasitoids, even though unlikely to produce spectacular results, would possibly increase the rate of parasitism. In India this parasitoid is considered to be effective against stemborers (Rao & Ali, 1977; Shenhmar & Brar, 1996). *Cotesia flavipes* has been introduced in more than 40 countries for the biological control of stemborers in the genera *Chilo* and *Diatraea* (Polaszek & Walker, 1991). *Cotesia flavipes* has also recently been introduced into several countries in the East and Central Africa for biological control of *C. partellus* in maize and sorghum, and establishment has been reported in many countries (Omwega *et al.*, 1995; 1997; Overholt, *et al.*, 1997; Overholt, 1998). On coastal Kenya where *C. flavipes* was released in 1993, impact analysis has shown that the stemborer population has declined consecutively for four seasons since its introduction, and that the stemborer mortality due to the parasitoid has increased exponentially during the same period (Zhou *et al.*, 2001).

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Chapter

3

Temporal changes in behavioural response to host- and host plant-associated odours with laboratory rearing of a stemborer parasitoid *Cotesia flavipes* (Cameron) (Hym.: Braconidae)

E.I. Niyibigira, Overholt W.A. & Stouthamer R.

Abstract

The responses of genetically impoverished and genetically diverse populations of the stemborer parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), to host- and host plant-associated odours were measured over several generations in a Y-tube olfactometer. The study aimed to examine the effect of duration of laboratory rearing on parasitoid behaviour and whether genetic variability of the population was related to changes in behaviour. Responses of wasps to uninfested maize plants and to maize plants infested with larvae of *Chilo partellus* (Lepidoptera: Crambidae) were significantly different among populations. In general, the response of *C. flavipes* females was higher in early generations than in later generations but the overall behavioural response in four of the five genetically impoverished and in the genetically diverse population remained stable throughout the study. There was also little change in response time with duration in culture. The mean response of wasps from all populations was higher than 80% indicating that the ability of the wasps to discriminate host- and host plant-associated odours was high and it could persist over many generations of laboratory rearing. It is suggested that further studies to monitor gene frequency changes over time be carried out and related to changes in other biological characters that may be observed.

Introduction

In classical biological control, natural enemies are imported from one geographical area of the world and released into another area where they formerly did not exist, for sustainable suppression of specific pest populations. Because initial collections are

often very small, natural enemies are typically reared to increase their numbers before field releases. Mass-rearing conditions can cause selective changes which result in behavioural features that are different from those of wild individuals (Boller, 1972; Mackauer, 1972; 1976; Lecomte *et al.*, 1998). Two genetic processes can occur during laboratory rearing that may have a negative influence on the establishment and biological control potential of the natural enemy: loss of genetic variation by random genetic drift and unintentional selection for adaptation to the mass-rearing environment (Roush, 1990; Hopper *et al.*, 1993; Roush & Hopper, 1995). Genetic drift can be avoided by maintenance of large population sizes (Hanrahan *et al.*, 1973; Franklin, 1980; Bartlett, 1984; Joslyn, 1984; Unruh *et al.*, 1983; Briscoe *et al.*, 1992). Selection for adaptation can be minimized by maintaining natural enemy cultures as a number of isofemale lines (Delpuech *et al.*, 1993; Roush & Hopper, 1995). Each isofemale line lacks genetic variability and therefore the potential of such lines to genetically adapt to the laboratory environment is reduced. The number of lines needed to prevent loss of common alleles in a population was calculated to be 22 for arrhenotokous species (Roush & Hopper, 1995). Common alleles are the ones that are most likely to be important to field performance.

Cotesia flavipes Cameron (Hymenoptera: Braconidae) is a gregarious larval endoparasitoid of lepidopteran stemborers. It attacks medium and large instar larvae by ingressing into the stem through the entrance hole to the stemborer tunnel (Smith *et al.*, 1993). *Cotesia flavipes* is indigenous to the Indo-Australian region where it attacks several stemborer species in maize, sorghum, rice, sugarcane and various wild grasses. This parasitoid has been widely used in classical biological control programmes against *Chilo partellus* (Lepidoptera: Crambidae) (Alam *et al.*, 1972; Overholt *et al.*, 1994); *Diatraea saccharalis* (Gifford & Mann, 1967; Alam *et al.*, 1971; Fuchs *et al.*, 1979) and *Chilo sacchariphagus* (Betbeder-Metibet, 1971). In Africa, *C. flavipes* has been introduced against *C. partellus* (Omwega *et al.*, 1995; 1997; Overholt *et al.*, 1997), a widespread and destructive borer of maize and sorghum, which was introduced into Africa from the Indo-Australian region (Bleszynski, 1970) and first reported in Malawi in the 1930s (Tams, 1932). Since arriving on the continent, *C. partellus* has spread to nearly all countries in East and southern Africa, often becoming the predominant and most damaging stemborer of maize and sorghum (Kfir *et al.*, 2002).

In the field, females of *C. flavipes* use volatile cues emitted by stemborer-infested plants to locate hosts (Leerdam *et al.*, 1985; Potting, *et al.*, 1995; Ngi-Song *et al.*, 1996). The current standard rearing procedure for *C. flavipes* largely circumvents host finding and the need to recognize host associated odours. Hosts are removed from pieces of maize stem and offered to the parasitoid by holding the stemborer larva with soft forceps and positioning it next to a female *C. flavipes* in a cage. The female typically moves quickly onto the larva and immediately begins to oviposit. This method has been referred to as 'hand stinging' (Overholt *et al.*, 1994). There is concern that the

response of *C. flavipes* to stemborer-infested plants may decline after several generations of laboratory rearing.

The objective of this study was to investigate whether response to host- and host plant- associated odours by *C. flavipes* females changed over time during laboratory rearing and whether genetic variability was related to changes in behaviour. The relationship between response time and number of generations in culture was also examined. Response time was defined as the total time taken by wasps to respond to an odour source (i.e. make a choice) after release in a Y-tube olfactometer.

Materials and Methods

Plants: Maize plants (*Zea mays* L., hybrid 5-11) were grown in plastic buckets (ø20 cm) in a nursery at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. Potted plants were kept under large field cages (2 x 2 x 2 m), which were covered with fine mesh (400 µm) netting to protect them from insect attack. Additional maize plants were grown in the field. Plants used in the tests were 6-8 weeks old and 40-50 cm tall.

Insects: Populations of *C. flavipes* were established from cocoon masses reared from parasitized *C. partellus* larvae which were collected from sorghum at several locations in Andra Pradesh, Maharastra and Karnataka states in India between 11 December 1998 and 2 January 1999. These locations were 20 –160 km apart. Cocoon masses were imported to a quarantine unit at ICIPE in Nairobi, Kenya. Each cocoon mass was kept separately in a glass vial for adult emergence. It was assumed that the field-collected larvae were each parasitized by a single female wasp suggesting that one female was the founder of each isofemale line. The isofemale lines were named according to the order of collection and the state in India where it was collected. For the behavioural assays, we used isofemale lines Mah2, Mah20, Karn6, Karn8 and Karn16 based on the population size of their cultures. The number of F1 siblings in the above mentioned isofemale lines were 2 males and 25 females in Mah2, 1 male and 19 females in Mah20, 2 males and 19 females in Karn6 and 1 male and 13 females in Karn8 and 1 male and 24 females in Karn16. Freshly emerged male and female wasps from each isofemale line were released in a cage and after 24 h, mated female wasps were each offered two fourth-instar larvae per day for parasitization until the females died. In addition to these isofemale cultures, a genetically diverse culture (hereafter referred to as the "mixed population") was established in the 3rd generation from 26 isofemale lines. At least ten females from each isofemale line were crossed with males from other lines. Cultures of isofemale lines and the mixed population were maintained under similar conditions. *Cotesia flavipes* was reared on fourth-instar *C. partellus* larvae using the "hand-stinging" method described earlier. In each generation, an average of 600 host larvae were exposed per population culture.

Chilo partellus larvae were reared on artificial diet (Ochieng *et al.*, 1985) at 25°C, 70-80% RH and a photoperiod of 12L:12D. Parasitoid cocoons were harvested and placed in vials containing a 20% honey/water solution and held at 25°C for adult emergence. For the tests, we used 1-2 day old mated *C. flavipes* females that were naïve with respect to oviposition or contact with host-related cues. Wasps were assayed for their responsiveness to host- and host plant-associated odours at intervals of approximately 10 generations of laboratory rearing. One generation of *C. flavipes* is completed in 17-21 days (Potting *et al.*, 1997). *Chilo partellus* larvae for tests were removed from artificial diet 48 h in advance and were allowed to feed on fresh maize stems.

Bioassay procedure: Behavioural assays were carried out in a closed-system glass Y-tube olfactometer. The olfactometer consisted of a glass Y-shaped tube. The two arms of the Y-tube were connected to flow meters and two Perspex chambers (30 x 30 x 120 cm) which were sufficiently large to accommodate one whole maize plant (40-50 cm tall). Odour sources were placed in the chambers. A vacuum pump drew and pushed air through the closed system. Air was pushed through an activated charcoal filter into the two chambers and drawn into the arms of Y-tube. The airflow was set at 2.5 l/min for each Y-tube arm. For a more detailed description of the olfactometer, see Ngi-Song *et al.* (1996). Females of *C. flavipes* were released individually in the stem of the Y-tube and allowed 5 min to choose one of the odour sources at the end of the olfactometer arms. When a test female moved towards the odour source, crossed the 'choice line' (4 cm after division of the stem) and stayed beyond the choice line for more than 15 seconds, it was recorded as a 'choice'. The time taken by insects to make a choice was recorded. Individuals that did not make a choice within five minutes were recorded as 'no choice'. To avoid any asymmetrical bias in the olfactometer or its surroundings, the connections of the odour source chambers to the arms of the olfactometer were reversed after testing every five insects. Tests were conducted at 25-28°C, 65-75% RH and a light intensity of 1200-1300 lux. A cream-white curtain was used to separate the testing area from the surroundings. The responses of female wasps from five isofemale lines and the mixed population were tested during the 10th, 19th, 25th, 35th, and 45th generations. Line Karn16 was not tested in the 10th generation due to a paucity of insects. For each odour source combination, 3 replicates (2 replicates in the 10th generation) of 20 females each were tested.

Odour sources: In dual-choice tests, female wasps were offered the following three odour combinations:-

Test 1: Uninfested maize plants vs air. Individual *C. flavipes* females were given a choice between potted uninfested maize plants and air drawn over a pot containing soil only.

Test 2: Infested maize plants vs air. An infested plant was obtained by introducing one fourth-instar *C. partellus* larva into each of two holes artificially bored in a maize stem

(1 cm deep) at a distance of 10 cm from each other. Each larva was placed in a hole and allowed to feed overnight. Tests were conducted 18-20 h after infestation.
Test 3: Infested vs uninfested maize plants. Maize plants were artificially infested with two fourth-instar *C. partellus* larvae as described for test 2 and compared to uninfested maize plants. Holes were also made in control plants, but no larvae were introduced.

In each of the above tests, potted plants were placed into the olfactometer chambers 30 minutes before tests were conducted in order to allow time for volatiles to be released.

Statistical analysis

The behavioural data was expressed as the proportion of females that responded to the odour source. Positive response was calculated in each test for each population and each generation. A positive response was when wasps responded to the Y-tube arm containing the odour source with putatively stronger attractiveness, otherwise it was recorded as negative if the choice was made for the odour source thought to have less attractiveness. In the test between uninfested plants against air, a choice for uninfested plant was considered as positive; in test 2 and 3, infested maize plants were the positive choice. The proportion of positive responses was subjected to arcsin-square root transformation (Sokal & Rohlf, 1995). Analysis of variance (ANOVA, PROC GLM, SAS Institute, 1999-2000) was used to test for differences among generations and populations in the mean proportion of positive responses. When ANOVAs were significant, means were separated using Student-Newman-Keuls tests (SNK). A regression analysis was conducted to examine relationships between response time and the number of generations spent in laboratory culture.

Results

Test 1: Response to uninfested maize plants vs air. The percentage of females that responded to volatiles from uninfested maize plants was dependent on population ($F=7.21$, $df=5$; $p<0.0001$) and generation ($F=31.73$, $df=4$; $p<0.0001$). The interaction between population and generation was also significant ($F=5.87$; $df=19$; $p<0.0001$) indicating that the generation effect was inconsistent among populations. Overall, the wasps showed a stronger response in the 10th generation than in other generations, but there were no significant differences in the responses in the 25th, 35th, and 45th generations (Figure 1 A). Wasps from lines Karn6 and Karn8 responded more strongly than those from Mah2, Mah20 or the mixed population while individuals from Karn16 exhibited an intermediate response (Figure 2 A).

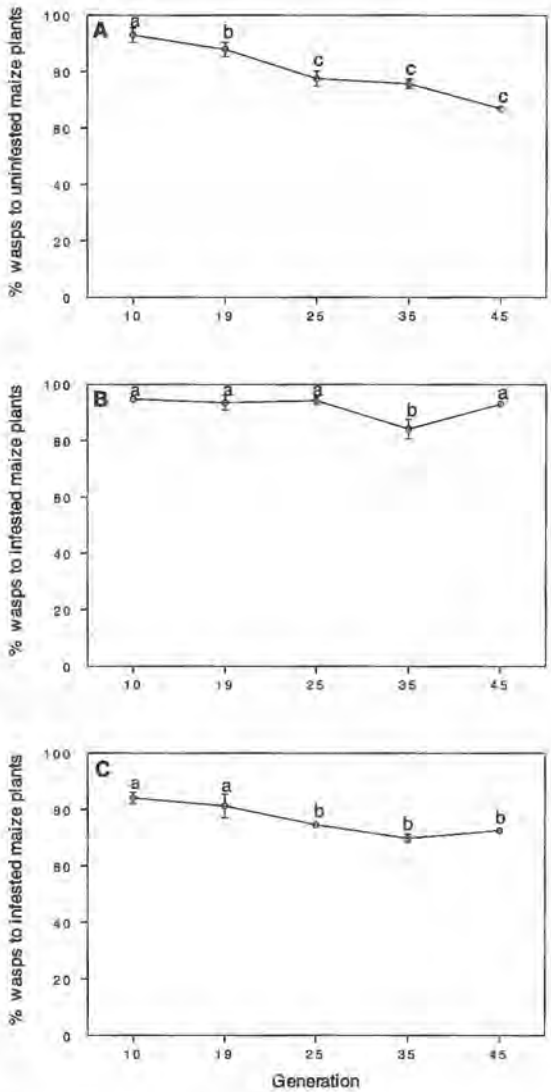

Figure 1. Change in response to host associated cues by females of *C. flavipes* during the first 45 generations in the laboratory. In dual-choice tests, females were offered three odour combinations in a Y-tube olfactometer: **A.** uninfested maize plants vs air; **B.** *C. partellus*-infested maize plants vs air; **C.** *C. partellus*-infested maize plants vs uninfested maize plants. Mean (± SE) percentages of females that chose the volatiles from uninfested maize plants and *C. partellus*-infested maize plants in the olfactometer in each generation are shown. Each data point represents a mean response for six populations. Different letters at data points in the same test indicate significant differences among generations (SNK). The number of olfactometer tests per combination was 10 in the 10th generation and 18 in other generations, with 20 wasps each.

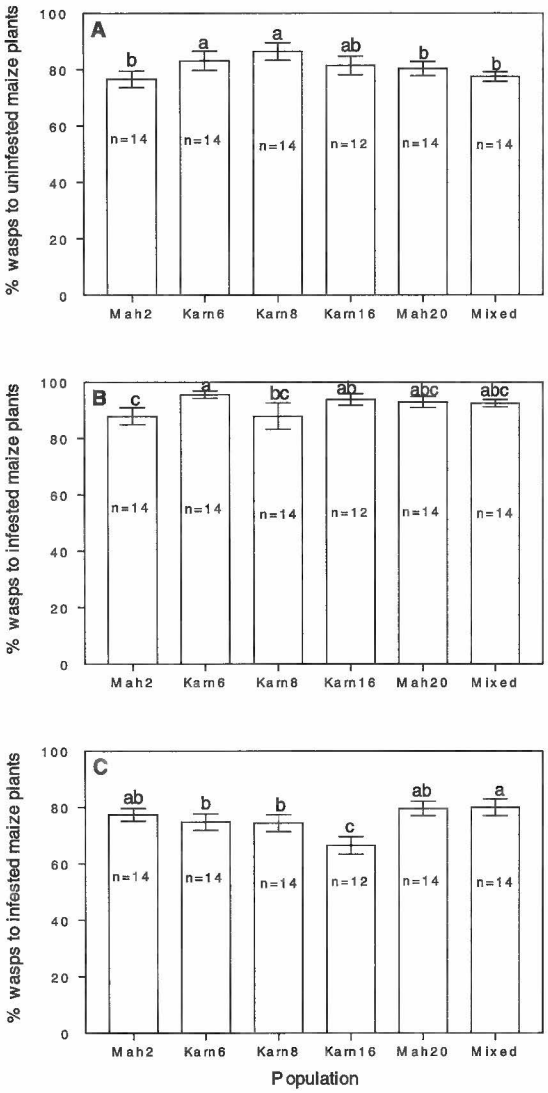

Figure 2. Response to host-associated cues by females of *C. flavipes* from six different populations. In dual-choice tests, females were offered three odour combinations in a Y-tube olfactometer: **A.** uninfested maize plants vs air; **B.** *C. partellus*-infested maize plants vs air; **C.** *C. partellus*-infested maize plants vs uninfested maize plants. Each bar represents the mean (± SE) percentage of females from each population that chose the volatiles from uninfested maize plants and *C. partellus*-infested maize plants in the olfactometer. The means were calculated from data obtained in tests conducted in the 10th, 19th, 25th, 35th and 45th generations of each population. Different letters above bars in the same test indicate significant differences among populations (SNK). n= number of olfactometer tests with 20 wasps each.

The generation at which tests were conducted influenced the response of wasps from Mah2 ($F=11.95$; $df= 4$; $p=0.0012$); Karn8 ($F=6.03$; $df=4$; $p=0.0121$; Karn6 ($F=43.87$; $df=4$; $p<0.0001$) and Karn16 ($F=14.69$; $df=3$; $p=0.0013$) but not from Mah20 ($F=3.77$; $df=4$; $p=0.05$) or the mixed population ($F=0.83$; $df=4$; $p=0.540$). The highest mean response (97.1± 2.9)% was recorded in the 10th generation for wasps from Mah2 (Figure 3 A), the 19th generation for those from Karn16 (Figure 3 B) and both the 10th and the 19th generations for the individuals from Karn6 (Figure 3 C). The percentage of wasps from Karn8 that responded to volatiles from uninfested maize was lower in the 45th generation (Figure 3 D). Although the responsiveness of females from Mah20 in the 10th generation was higher than in 35th generation, it did not differ from the 45th generation, indicating that the responsiveness in this line remained largely unchanged throughout the duration of rearing (Figure 3 E). Similarly, the generation at which females from the mixed population were tested did not affect their response to volatiles from uninfested maize plants (Figure 3 F).

Test 2: Response to C. partellus-infested maize plants vs air. As in test 1, the response of females was highly dependent on the generation at which they were tested ($F=12.31$; $df=5$; $p<0.0001$), population ($F=4.28$; $df=5$; $p=0.0024$) and the interaction between generation and population ($F=9.59$; $df=19$; $p<0.0001$). The strong interaction between the two factors shows that differences among populations with respect to generation were inconsistent. The percentage of wasps that responded to *C. partellus*-infested plants was less in the 35th generation than in other generations but it did not differ among other generations. This indicates that the responsiveness to *C. partellus*-infested plants between the 10th and the 45th generations remained largely unchanged (Figure 1 B). Wasps from Karn6 were more responsive than those from Mah2 (Figure 2 B).

The percentage of wasps that responded to *C. partellus*-infested maize plants remained unchanged throughout the 45 generations of lab rearing in four of the six populations. The response of females from Mah2 was significantly affected by generation ($F=17.82$; $df=4$; $p=0.0003$). Their highest response was recorded in the 25th generation and surprisingly, they responded least in the 19th generation. However, they showed similar responses in the 10th and 45th generations (Figure 4 A). For Karn6, generation significantly affected the female response ($F=7.15$; $df=4$; $p=0.0071$). All the females from Karn6 (100%) were attracted in the 10th and 19th generations while a lower attraction was observed in the 35th and 45th generations (Figure 4 B). The response of females from Karn8 was lower in the 35th generation than in other generations ($F=17.77$; $df=4$; $p=0.0002$). However the responses in the 10th and 45th generations were similar indicating that responsiveness remained largely unchanged during the generations in which tests were carried out (Figure 4 C). Behavioural assay of wasps from Karn16 was not carried out in the 10th generation. Regardless, generation had a significant influence on the response of females from

this line (F=14.62; df=3; p=0.0013). Wasps from Karn16 were more attracted to volatiles from *C. partellus*-infested maize plants in the 19th and 35th generations than in other generations (Figure 4 D). The response of females from Mah20 was weakly affected by generation (F=4.24; df=4; p=0.0336).

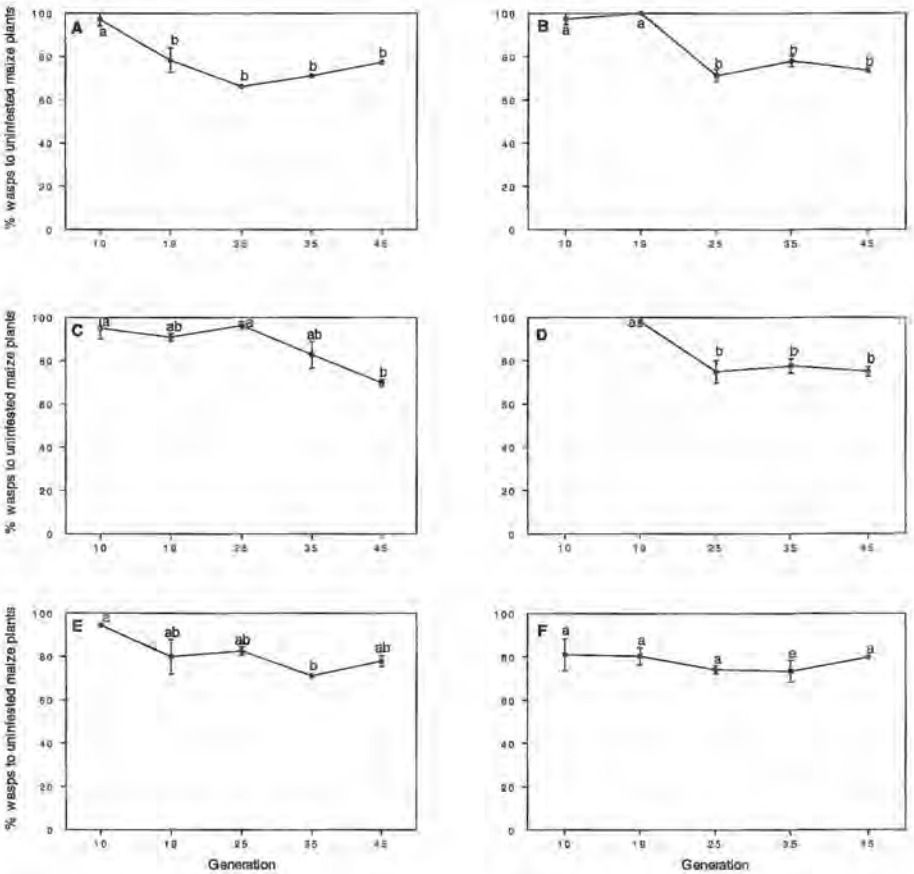

Figure 3. Change in response of *C. flavipes* to uninfested maize plants in the first 45 generations in the laboratory rearing. In dual-choice set up, females were tested to uninfested maize plants and air at intervals of approximately 10 generations (Test 1). Tests consisted of six populations: **A.** Mah2; **B.** Karn6; **C.** Karn8; **D.** Karn16; **E.** Mah20; and **F.** mixed population. The percentage of wasps that chose uninfested maize plants were calculated as the total number of wasps that chose uninfested maize plants divided by the sum of those that chose uninfested maize plants and air, multiplied by one hundred. Mean (± SE) percentages of females from each population that chose uninfested maize plants in each generation are shown. A total of 40 wasps were tested in the 10th generation and 60 wasps in other generations, with 20 wasps being tested per day. Different letters at data points of the same population indicate significant differences in responses among generations (SNK).

Although wasps from Mah20 were more attracted in the 19th generation than in the 35th generation responses in the 10th and 45th generations were similar suggesting that responsiveness remained largely unchanged through throughout the duration of rearing (Figure 4 E). Furthermore, it was observed that the response of females from the mixed population to volatiles from maize plants infested with *C. partellus* did not change over time (F=3.35; df=4; p=0.0611) (Figures 4 F).

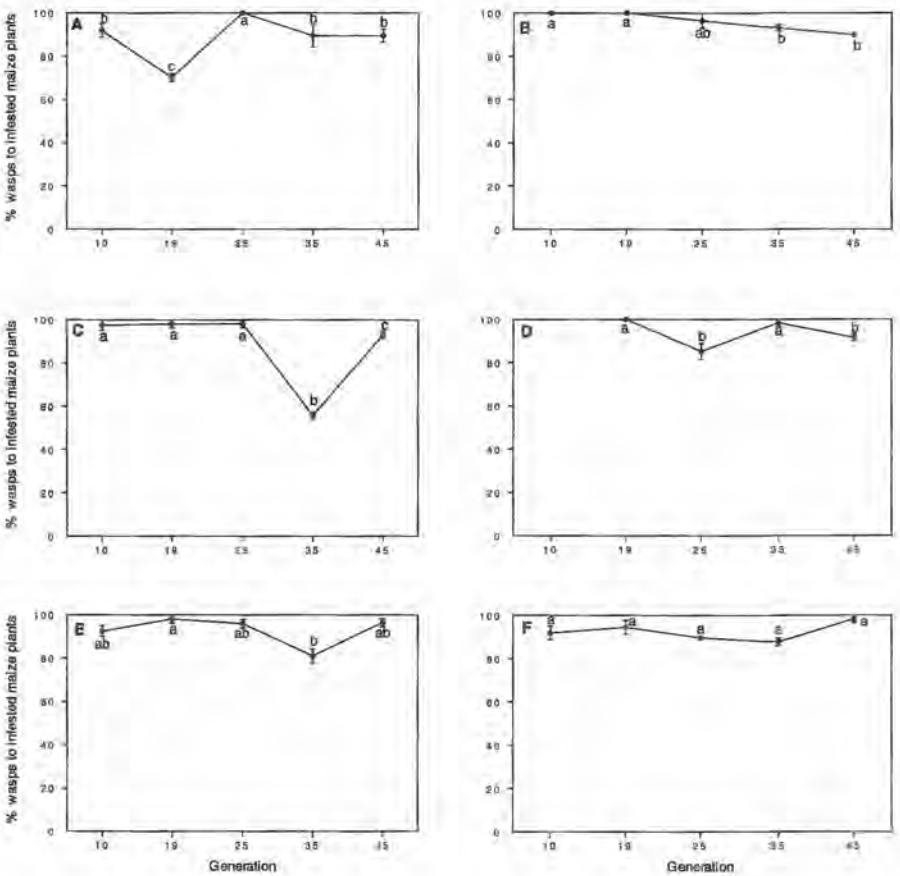

Figure 4. Change in response of *C. flavipes* to volatiles from *C. partellus*-infested maize plants in the first 45 generations in the laboratory rearing. In dual-choice set up, females were tested against *C. partellus*-infested maize plants and air in a Y-tube olfactometer at intervals of approximately 10 generations (Test 2). Tests consisted of six populations: **A**. Mah2; **B**. Karn6; **C**. Karn8; **D**. Karn16; **E**. Mah20 and **F**. mixed population. Different letters at data points of the same population indicate significant differences in responses among generations (SNK).

Test 3: Response to infested vs uninfested maize plants. In this test, generation ($F=17.63$, $df=4$; $p<0.0001$), population ($F=7.48$, $df=5$; $p<0.0001$) and the interaction between population and generation ($F=7.37$; $df=19$; $p<0.0001$) had significant influence on the degree of response of wasps. Wasps showed the highest response in the 10th and 19th generations while response in other generations did not differ (Figure 1 C). Wasps from Karn16 showed the lowest response (Figure 2 C).

The percentage of wasps that responded to *C. partellus*-infested maize plants was similar in the 10th and the 45th generations for all populations except Karn6. Although the generation at which tests were carried out influenced the response of females from Mah2 ($F=16.46$; $df=4$; $p=0.0003$) (Figure 5 A); Karn8 ($F=16.48$; $df=4$; $p=0.0004$) (Figure 5 C); Mah20 ($F=5.55$; $df=4$; $p=0.0156$) (Figure 5 E) and the mixed population ($F=16.46$; $df=4$; $p=0.0003$) (Figure 5 F), the overall response did not change between the 10th and 45th generations. In contrast, a higher percentage of wasps from Karn6 were attracted in the 10th generation than other generations ($F=11.69$; $df=4$; $p=0.0013$) (Figure 5 B). The response of wasps from Karn16 was not significantly affected by generation ($F=1.91$; $df=3$; $p=0.2057$) (Figures 5 D).

Relationship between response time and number of generations in culture

Response time (i.e. time taken by wasps to make a choice) to volatiles from uninfested maize plants vs air decreased for wasps from Karn16 ($R^2=0.0202$, $p=0.0279$) (Figure 6 A). It also decreased for wasps from the mixed population ($R^2=0.0310$, $p=0.0031$) in response to volatiles from *C. partellus*-infested maize plants vs air (Figure 6 B). In contrast, increases in response time with generation were found for wasps from Karn6 ($R^2=0.0261$, $p=0.0068$) (Figure 6 A) in their response to uninfested maize plants vs air; and from Karn8 in response to *C. partellus*-infested maize plants vs air ($R^2=0.0271$, $p=0.0058$) (Figure 6 B). Similarly, response time by wasps from Karn6, Karn16 and the mixed population increased with generations during responses to *C. partellus*-infested vs uninfested maize plants ($R^2=0.0238$, $p=0.0097$; $R^2=0.0533$, $p=0.0003$; $R^2=0.0300$, $p=0.0037$, respectively) (Figure 6 C). There was no significant relationship between response time and time in culture by wasps from other populations.

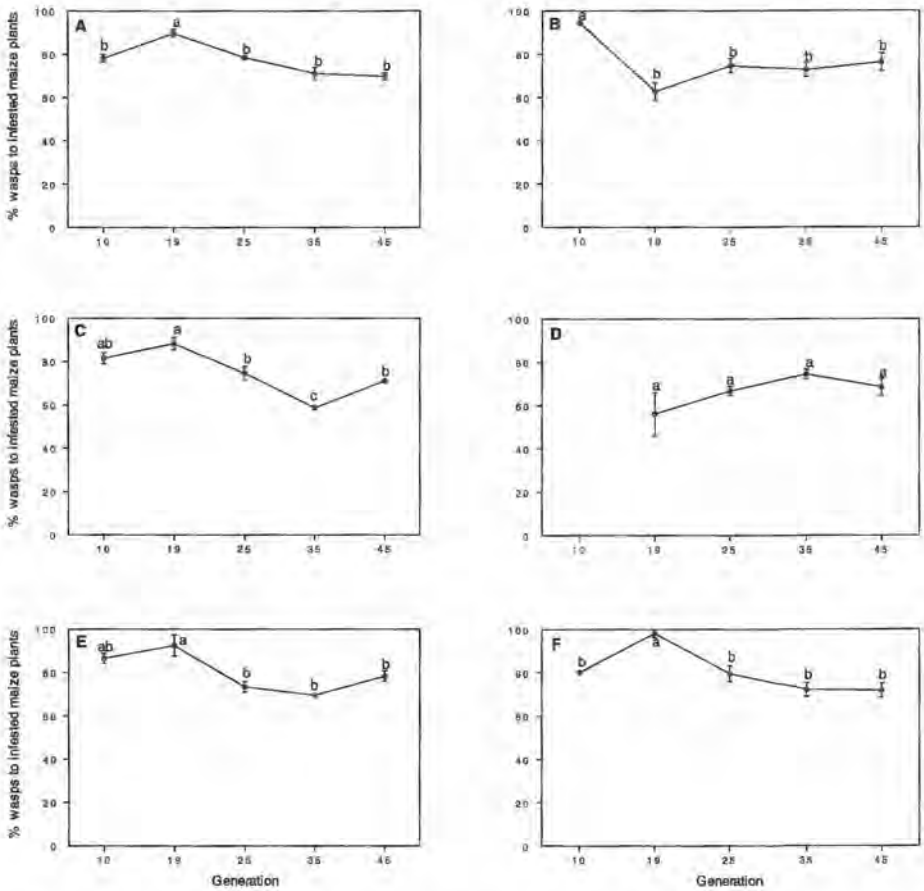

Figure 5. Change in response of *C. flavipes* to volatiles from *C. partellus*-infested maize plants in the first 45 generations in the laboratory rearing. In dual-choice set up, females were tested against *C. partellus*-infested and uninfested maize plants in a Y-tube olfactometer at intervals of about 10 generations (Test 3). Tests consisted of six populations: **A**. Mah2; **B**. Karn6; **C**. Karn8; **D**. Karn16; **E**. Mah20 and **F**. mixed population. Different letters at data points of the same population indicate significant differences in responses among generations (SNK).

Discussion

In general, the response of *C. flavipes* females to uninfested maize plants and maize plants infested with *C. partellus* larvae was higher in early generations than in later generations (Figure 1), but the overall behavioural response in most populations remained stable throughout the study (Figure 3, 4, 5). After the early shifts in response, the mean percentage of responses varied little with time.

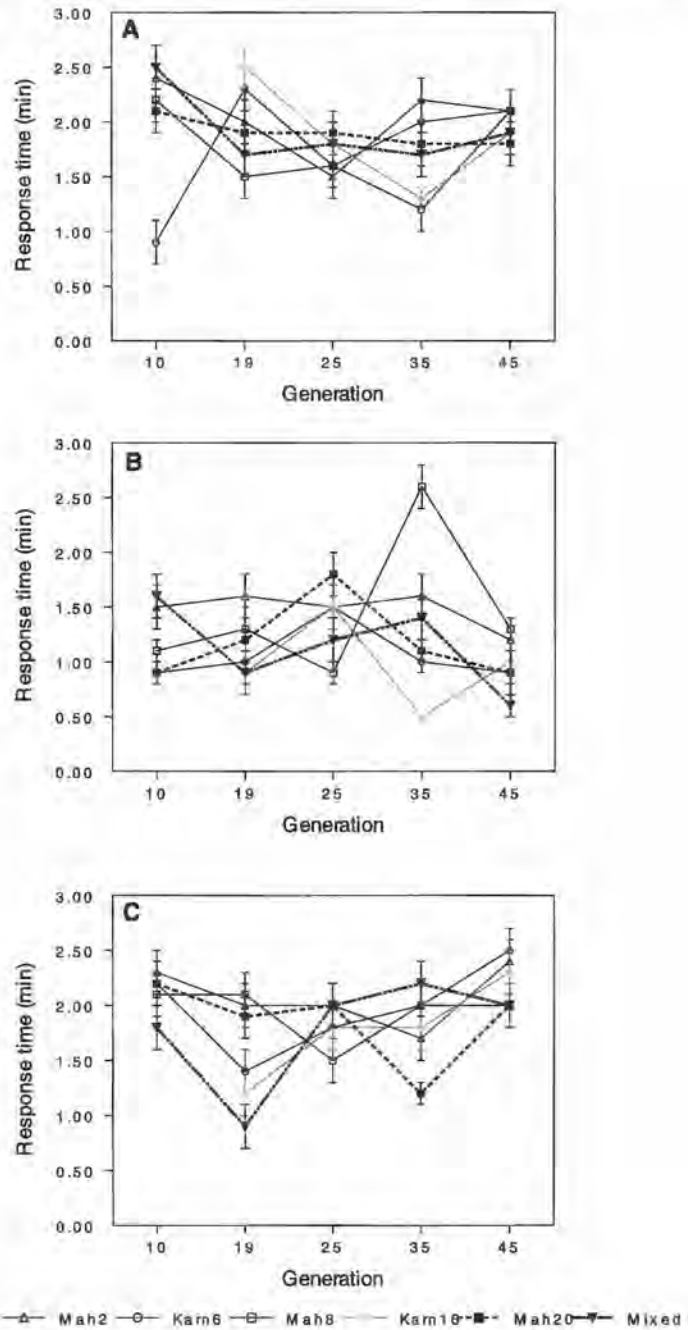

Figure 6. Change in response time with duration in culture by females of *C. flvaipes* to: **A.** uninfested maize plants vs air, **B.** *C. partellus*-infested maize plants vs air, and **C.** *C. partellus*-infested maize plants vs uninfested maize plants.

There was a strong interaction between population and generation in each of the three tests suggesting that variation in response of populations was inconsistent with generation. Variability in response among populations was found throughout the duration of laboratory rearing and may have a genetic basis (Via, 1987; Lewis *et al.*, 1990; Prevost & Lewis, 1990; Gu & Dorn, 2000).

There was little and inconsistent change in response time over time in culture (Figure 6), suggesting that for those wasps that responded positively, the response time did not change over generations, i.e. once the response behaviour was initiated, the time required to move to odour source did not vary.

The observed changes in response in early generations could have been caused by at least three factors: First, the laboratory could be a novel environment to which a population may adapt (Hoffman and Merila, 1999). Secondly, small population sizes and sampling (i.e. founder effects) may result in random genetic drift (Bijsma *et al.*, 1999). Finally, the laboratory rearing methods could directly affect phenotypically plastic traits (Krebs *et al.* 2001). The most likely explanation may be that behavioural plasticity (i.e. variation in behaviour that is not genetically based) using the 'hand stinging' method during rearing caused the apparent changes in behavioural response during early generations because the wasps did not need to respond to host-related cues in order to successfully leave progeny for the next generation. Random genetic drift due to small population sizes cannot explain the changes in behaviour because there was limited genetic variation in the isofemale lines on which there may have been change (Roush & Hopper, 1995). Delpuech *et al.* (1993) proposed that maintenance of laboratory populations as isofemale lines could conserve genetic variability.

Our results indicate that wasps from all the isofemale lines (except Kam6) as well as from the mixed population showed stable behavioural responses during laboratory rearing. However, wasps from most populations showed more stable and higher responses to volatiles emitted from *C. partellus*-infested maize plants compared to those from uninfested maize plants. This can be explained by the variable response model, which postulates that the variability of the response to a stimulus is inversely related to its response potential (Vet *et al.*, 1990). Thus, variability is low towards stimuli with a high response potential and high towards stimuli with a low response potential. The most reliable stimuli with the highest response potential in the case of *C. flavipes* are volatiles emitted by *C. partellus*-infested maize plants because they are directly associated with the presence of a host. Volatiles from uninfested maize plants on the other hand, are believed to have low reliability and therefore have only low innate response potential which was shown in the higher variable response of *C. flavipes* towards them.

The mean response of wasps from all populations was higher than 80% indicating that the ability of the wasps to discriminate host- and host plant-associated odours was high and it could persist over many generations of laboratory rearing. It could also indicate that all isofemale lines which were used to initiate colonies were highly responsive. Similar results were obtained in the gypsy moth parasitoid *Cotesia melanoscela* (Ratzeburg) (Hymenoptera: Braconidae). Weseloh (1987) observed that females of *C. melanoscela* from cultures maintained in the laboratory for 6 or 25 generations responded similarly to gypsy moth silk kairomone and he concluded that the orientation behaviour was not modified by laboratory rearing.

In conclusion, the rearing of *C. flavipes* for a number of generations as a number of isofemale lines did not result in deterioration of the parasitoid's response to host- and host plant-associated odours. Additionally, the response of the wasps from the mixed population was stable over time, suggesting that laboratory adaptation to the rearing procedure did not occur. There may not have been sufficient genetic variability at loci involved in host finding to allow for selection in the laboratory. Further investigations to monitor gene frequency changes over time should be carried out and related to changes in other biological traits, such as fitness, that may be observed.

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Chapter

4

Cotesia flavipes Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) do not exhibit Complementary Sex Determination (i) Evidence from field populations

E.I. Niyibigira, Overholt W.A. & Stouthamer R.

Abstract

Diploid males are expected to occur in natural populations of some Hymenoptera as a consequence of single locus complementary sex determination (sl-CSD) mechanism. Heterozygotes at a single sex determination locus develop into females whereas hemizygotes (haploids) and homozygotes (diploids) develop into males. If sl-CSD is found in a species that produces gregarious broods and sib mates at a high frequency, we expect a fraction of the matings to be between males and females that share an sex allele. In the offspring of this mating the diploid males should be formed which results in a more male biased sex ratio. We developed models to predict the frequency of such matched matings in populations with different frequencies of sib mating and egg fertilization. The predictions of these models are used to determine if we can use brood sex ratio distributions to determine if a species determines its sex by sl-CSD. The models show that sl-CSD can be detected from these brood sex ratios if the diploid male offspring survives. We report an analysis of sex ratios of *Cotesia sesamiae* and its exotic congener *Cotesia flavipes* (Hymenoptera: Braconidae), to find evidence for the existence of sl-CSD and the production of diploid males in field populations of these species. Parasitoids were reared from stemborer larvae sampled from maize fields in the Coast province of Kenya between 1992 and 1999. Sex ratio (% females) was highly female-biased in *C. sesamiae* (79.0%) and in *C. flavipes* (75.0%). The frequency distribution of sex ratio was unimodal with a peak at 70-95% instead of an expected bimodal distribution if sl-CSD existed. We found no evidence for the presence of sl-CSD with survival of diploid males in both braconid species. However,

we cannot exclude the possibility that sl-CSD with diploid male mortality takes place in these species.

Introduction

Sex determination in the order Hymenoptera is mostly based upon haplo-diploid arrhenotoky (Bull, 1981; Poirie *et. al.*, 1992), where unfertilised eggs develop as haploid males and fertilised eggs as diploid females. However, diploid males can also be produced as a result of inbreeding in some Hymenoptera, which display a genetic mechanism of sex determination called single-locus complementary sex determination (Whiting, 1943). Single-locus complementary sex determination (sl-CSD) involves a single co-dominant multiallelic locus (Whiting, 1943). In species with sl-CSD, diploid individuals that are heterozygous for any two alleles at the sex locus develop as females, while individuals that are hemizygous (haploid) or homozygous (diploid) develop as males. Under the sl-CSD model, well-defined proportions of diploid males are expected following sib matings (Whiting, 1943; Crozier, 1971; Adams *et. al.*, 1977; Beukeboom *et. al.*, 2000; Butcher *et. al.*, 2000; Noda, 2000). Adams *et. al.* (1977) called the crosses "matched" matings if matings took place between individuals that share a sex allele, and "unmatched" matings in those involving individuals that did not have a sex allele in common. In matched matings, a more male-biased offspring sex ratio is expected because 50% of the diploid eggs become diploid males. In unmatched matings, the offspring sex ratio (% females) should be substantially higher because 100% of diploid eggs will be heterozygous and develop into females.

Diploid males either die as embryos or if they survive they are generally effectively sterile (Glancey *et. al.*, 1976; El Agoze *et. al.*, 1994; Holloway *et. al.*, 1999) and their frequency increases with small populations or inbreeding (Stouthamer *et. al.* 1992). Production of diploid males leads to more male-biased sex ratios and thus represents a reduction in the reproductive rate of the parents and population growth. How severely the population growth is affected depends on the number of alleles present in the population and the mating system. The fewer the number of sex alleles, the lower the relative rate of increase of the wasp population (Stouthamer *et. al.* 1992). A reduced potential rate of increase can lead to a lower rate both of establishment and of successful biological control.

Diploid males have been found in over 40 hymenopteran species in Tenthredinoidea, Ichneumonoidea, Vespoidea and Apoidea (reviewed in Stouthamer *et. al.*, 1992; Cook, 1993; Cook and Crozier, 1995; Periquet *et. al.*, 1993; Butcher *et. al.*, 2000). The widespread occurrence of sl-CSD in four out of five superfamilies so far tested has

led to a suggestion that sl-CSD must have been the ancestral sex determination mechanism in the Hymenoptera (Crozier, 1977; Cook, 1993b).

Inbreeding in Hymenoptera does not always result in production of diploid males. For example in Chalcidoids which exhibit strong natural inbreeding, diploid males apparently never occur (Hardy, 1994) and apparently the Chalcidoidea do not have sl-CSD (Cook, 1993a;b; Luck *et. al.*, 1992, Stouthamer and Kazmer, 1994). Male-biased sex ratios were not obtained after prolonged inbreeding in some chalcidoid species (reviewed in Stouthamer *et. al.*, 1992; Periquet *et. al.*, 1993). This suggests that these species have some other mechanisms of sex determination. In the family of Braconidae, so far four of six members tested were shown to have sl-CSD: *Bracon brevicornis* (Speicher and Speicher, 1940), *B. hebetor* (Whiting, 1943), *B. serinopae* (Clark and Rubin, 1961) and *Microplitis croceipes* (Steiner and Teig, 1989). However, sl-CSD was found to be absent in the braconids *Asobara tabida* and *Alysia mandicator* (Beukeboom *et al.*, 2000).

Distribution of sex ratios in sib matings can reveal evidence for sl-CSD (Whiting, 1943; Crozier, 1971; Periquet *et. al.*, 1992; Cook, 1993a;b, Beukeboom *et al.*, 2000). The braconid wasp species *Cotesia flavipes* and *Cotesia sesamiae* (Braconidae) exhibit high sib mating frequency in nature (Arakaki and Gahana, 1986). If sl-CSD is the mode of sex determination in these species, inbreeding through sib mating is expected to result in production of diploid males (Periquet *et. al.*, 1992; Butcher *et. al.*, 2000) and hence more male-biased sex ratios and a bimodal distribution of offspring sex ratios should be obtained from field populations (Stouthamer *et. al.*, 1992). *Cotesia flavipes* has been released in a biocontrol program and has therefore experienced population bottle-necks that may have resulted in a reduction in the number of sex-alleles present in the introduced population compared to the source population. In contrast, *C. sesamiae* is a native species and natural numbers of sex alleles are expected to be present in its population.

In this chapter, we first develop models to determine the relationship between relative frequencies of sib mating in a population and the equilibrium frequency of matched matings. Next, we study the sex ratios produced in the populations of the parasitoid wasps *C. flavipes* and *C. sesamiae* parasitizing stemborer larvae in Kenya, to determine if evidence can be found for the existence of sl-CSD and the occurrence of diploid males in these species. We show that sex ratio distributions in *C. flavipes* and *C. sesamiae* are female-biased. We find no evidence for the presence of sl-CSD with diploid males survival, but we cannot exclude the possibility of sl-CSD with diploid male mortality in both braconid species.

Materials and methods

Derivation of the relationship between number of sex alleles, the level of sib mating and the frequency of matched matings in populations.

Assume that a population contains **n** different alleles at the sex determination locus. The frequency of each of these alleles is assumed to be 1/n. The frequency of matings between sibs (**s**) is defined as the chance that a female mates with her brother. We also assume that females only mate once and that no superparasitization occurs. If a mating happens between a male and a female that share a sex allele ("matched mating"), half of the fertilized eggs could develop into diploid males. In the following we assume that these males do not participate in the matings because they either die as diploid male embryos or are sterile. In such a population, two types of matings can take place: matched matings when the mates share a sex allele and unmatched mating i.e. when three different sex alleles are involved in the mating.

The outcome of a matched mating:
AB female crossed with A male results in: AB daughters, AA diploid sons, A haploid sons and B haploid sons. First of all, in such a matched mating only half of the diploid individuals are females and such a mating will contribute only half as many females to the next generation as an unmatched mating (see below). When sib mating takes place among the progeny of a matched mating, all of the resulting matings will also be matched matings: AB daughter mated with A son and AB daughter mated with B son. If a female offspring of a matched mating does not sib mate, but mates with a male as they occur in the population, then the chance that she will mate with a male sharing one of her alleles is 2/n. Similarly, the chance for her to mate with a male that carries a different sex allele equals (n-2)/n.

The outcome of an unmatched mating:
AB female crossed with a C male results in: AC and BC daughters and A and B sons. When sib mating takes place among the offspring of an unmatched mating, this results in half of the matings being matched (AC * A and BC * B) and half of the matings being unmatched (AC * B and BC * A). When a female offspring of a unmatched mating does not sib mate, the chance for her to mate with a male sharing one of her alleles is again 2/n and her chances for an unmatched mating are (n-2)/n.

If we assume that the frequency of matched matings in a population is **x**, then we can derive this frequency at equilibrium as follows. The frequency of matched mating in generation t+1 (x_{t+1}) equals:

(1) $x_{t+1} = [\frac{1}{2} * s * x_t + \frac{1}{2} * (1-s) * x_t * 2/n + \frac{1}{2} * s * (1-x_t) + (1-s)*(1-x_t) * 2/n]$/ total number of females in generation t

Similarly the frequency of unmatched mating at generation t +1 equals:
(2) $(1-x_{t+1}) = [½ * (1-s) * x_t * (n-2)/n + (1-s)*(1-x_t) * (n-2)/n + ½ *s * (1-x_t)]$/ total number of females in generation t

The equilibrium frequency of matched matings can be derived by dividing equation (1) by equation (2) and setting x_t equal to x_{t+1}.

The resulting equation:
(3) $-n * x^2 + (2n-2s+2) * x - s*n - 4 + 4s = 0$

has the following solution:
(4) $x_{1,2} = [s-n-1 \pm \sqrt{(2*n*s - 2* n + s^2 - 2*s + 1-s*n^2)}]/-n$

If there is random mating in the population (s=0) this equation collapses to: $x= 2/n$. This is identical to the frequency of matched matings derived by Owen and Packer (1994).

Similarly if we set the sib mating frequency to 1, the equation (4) results in a frequency of matched matings of 1.

Effects of diploid male survival on sex ratio and brood size produced by matched matings.

The fate of the diploid male embryos has a substantial effect on the offspring sex ratio of the matched mating. Assume that the fertilization frequency equals f, then the frequency of females in the offspring equals f in unmatched matings. However, in matched matings the frequency of female offspring will be ½ *f if diploid male survive, and (½ *f)/[(1-f) + ½ * f] if they die. If diploid males die the brood size of the unmatched matings (n) should be larger than those of matched matings [n(1-½ *f)].

Simulation studies

We did simulations to determine the brood size and sex ratio distribution in populations with and without sl-CSD. For these simulations we used the random number generator function of the program Excel to generate a large population of brood sizes and sex ratios each with their own mean and standard deviation, drawn from normal distributions. Thirty percent of the broods were assigned to the "matched" population and the remainder formed the "unmatched" population. Broods were removed from this simulated population if the brood size was below 1 or if their sex ratio was larger than 1 or smaller than 0. The sex ratio distribution was plotted to compare the distributions gained from the situation with sl-CSD with diploid male mortality, sl-CSD without diploid male mortality and populations

without sl-CSD. Similarly the brood size distributions were plotted for the cases with diploid male mortality and without diploid male mortality.

Biology of *Cotesia flavipes* and *Cotesia sesamiae*

Cotesia flavipes and *Cotesia sesamiae* are gregarious endoparasitoids of the larvae of lepidopteran stemborers that attack maize and sorghum. Both parasitoids are ecologically similar, attacking medium and large-sized stemborer larvae (Smith *et. al.*, 1993; Ngi-Song *et. al.*, 1996). The two parasitoids can complete development in the exotic stemborer *Chilo partellus* (Swinhoe) (Crambidae) and two indigenous stemborers *Chilo orichalcociliellus* (Strand) (Crambidae) and *Sesamiae calamistis* Hampson (Noctuidae) (Ngi-Song *et. al.*, 1995), which are the main stemborer species in the coastal area of Kenya. *Cotesia sesamiae*, a major larval parasitoid of these pests, is indigenous to Africa, while *C. flavipes* and its co-evolved host *C. partellus* are thought to be indigenous to Asia (Blezynski, 1970). As part of a stemborer biological control programme, *C. flavipes* was introduced into the Coast Province of Kenya from Pakistan and released in three locations in 1993 and at one additional site in 1994 (Overholt *et. al.*, 1994b). Recent surveys clearly indicate that *C. flavipes* is established and has spread in many areas of southern Kenya (Zhou and Overholt, 2001). Both parasitoids have similar biology and lifecycle. *Cotesia flavipes* is pro-ovigenic and has about 150 eggs available for oviposition with each female laying a brood of 20-25% of the available egg load in the host larvae (Potting *et. al*, 1997a). *Cotesia flavipes* has been found to experience a high level of sib mating directly after emergence from the stemborer tunnel (Arakaki and Gahana, 1986). The emergence of *C. flavipes* is usually concentrated in the morning and light stimulus plays an important role in promoting the adult emergence. Males generally emerge first and mate with their sisters soon after emergence from cocoons (Niyibigira, unpublished data). Males mate with many females and one male *C. flavipes* is capable of inseminating at least 12 sisters (Arakaki and Gahana, 1986). *Cotesia flavipes* females are mostly monogamic (Kajita and Drake, 1969) and a second mating does not influence parasitoid fitness (Campos and Chaud, 1998). Due to haplodiploidy, unmated females will readily parasitize hosts but produce male offspring only (Godfray, 1990). Moderately dispersed hosts and the tendency of males to mate with their sisters soon after emergence from cocoons reduces the likelihood of outbreeding. Thus, their mating behaviour suggests that matings occur most often between siblings from the same brood. Under these circumstances, theory predicts a female biased sex ratio with low sex ratio variances (Hardy and Cook, 1995).

Field sampling

Cocoons of *C. flavipes* and *C. sesamiae* were reared from stemborers collected from 162 maize fields located in four districts of Kilifi, Kwale, Malindi and Taita Taveta in the Coast Province of Kenya between 1992 and 1999. This region has a bimodal rainfall

pattern with rains typically occurring from April to June (long rainy season) and from October to December (short rainy season). Surveys were initiated in the long rainy season of 1992 and continued through the long rains of 1999. In both the long and short rainy seasons of 1992 and the short rainy seasons of 1994, 100 randomly selected plants were sampled weekly at each site. In the long and short rainy seasons of 1993 and since the long rainy season of 1995, 20 plants were examined bi-weekly at each sampling site. Data for the short rains of 1996 was unavailable. The sampling methodology was described in Overholt *et. al.* (1994a). Plants were sampled starting at plant emergence up to senescence by excising and dissecting randomly selected plants from the field. Larvae removed from stems were identified to species and reared individually in glass vials (7.5 cm x 2.5 cm) on pieces of maize stems until they died, pupated or parasitoids emerged. After emergence, adult *Cotesia* spp. were identified to species level by the shape of the male genitalia (Polaszek and Walker, 1991) or the propodia in all-female broods. Individuals from each brood were sexed, counted and the sex ratio (proportion of females) determined.

Statistical analysis

Data for long and short rainy seasons 1992-1999 were analysed, with exception of data for short rainy season 1996 that were not available. Statistical analyses were performed using logistic regression model (PROC GENMOD, SAS Institute, 1999-2000). In the model we specified the distribution function as binomial and link function as logit to facilitate the analysis of "proportional" (e.g. sex ratio) and "count" (brood size, number of males, number of females) data, since these need not be transformed. The assumption of a binomial error distribution was checked by dividing the residual scaled deviance by the residual degrees of freedom to calculate a heterogeneity factor (HF). Under the binomial model, the expected value of HF is 1, and significant departures from this value indicate overdispersion (greater than binomial variance) or underdispersion (less than binomial variance) of the data (Hardy and Cook, 1995).

In this chapter, we use brood size to refer to the number of adult offspring emerging from a cocoon. For each brood, the number of male and female progeny of *C. flavipes* and *C. sesamiae* was determined. A total of 2591 and 2065 broods of *C. sesamiae* and *C. flavipes*, respectively, were analysed. Sex ratio was expressed as proportion of female progeny at emergence. Sex ratio of all broods at adult emergence was examined. However, data from 115 and 89 all-male broods in *C. sesamiae* and *C. flavipes* respectively, were excluded from the analysis to prevent confusion of all-male progenies of virgin females from diploid males produced under sl-CSD. Also the data for parasitoids whose host stages and host species were unknown were excluded from the analysis. Host stages were defined as small, medium and large-

sized larvae which roughly correspond to first and second instars, third and fourth instars, and fifth and sixth instars, respectively. To determine the relationships between brood size and sex ratio, and numbers of females and males, regression lines were fitted to describe the data. Total progeny and sex ratios produced by the two parasitoids on three host stages for each host species were subjected to ANOVA (PROC GLM, SAS Institute, 1999-2000) and means were separated by Student Newman-Keuls (SNK) multiple range test when ANOVA was significant ($p=0.05$).

Results

Relationship between sib mating, number of sex alleles and frequency of matched matings. Figure 2 shows the relationship between the sib mating frequency and the frequency of matched matings assuming the 4 different levels of sex alleles present in the population. The number of sex alleles present in populations with sl-CSD was estimated to be in the range of 10-100 (for review see Cook and Crozier, 1995). The results indicate that at sib mating frequencies of 60%, between 30-40% of all mating in the field should be matched matings.

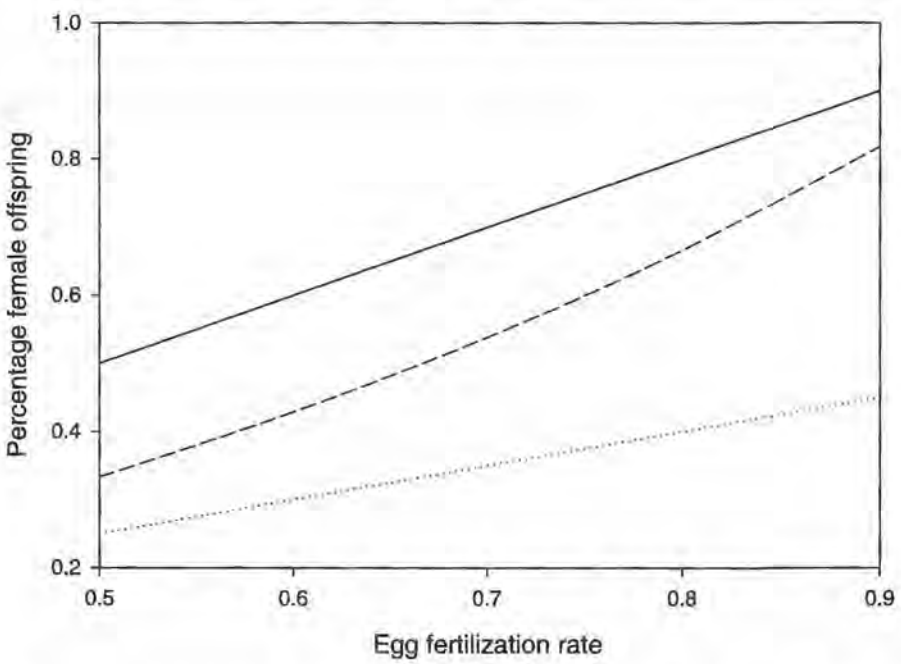

Figure 1. The relationship between egg fertilization rate and brood sex ratios assuming different types of sex detemination. Dotted line for matched matings with sl-CSD and diploid male survival, dashed line for matched matings with sl-CSD and diploid male mortality, and solid line for unmatched matings and sl-CSD.

If the diploid males survive, the sex ratio distributions of populations should be bimodal. The simulations show that this bimodal distribution remains visible even when there is a substantial variation both in the fertilization proportion of eggs and the brood size (see Figure 3). However, when diploid males die the sex ratio of matched broods differed less from the sex ratio of an unmatched brood (Figure 1) and consequently the bimodality of the sex ratio distribution will only be noticeable when there is very little variation in the egg fertilization proportion. Similarly the expected bimodal distribution of the brood sizes will also only be noticeable when the variation in brood size is small (see Figure 3 A2).

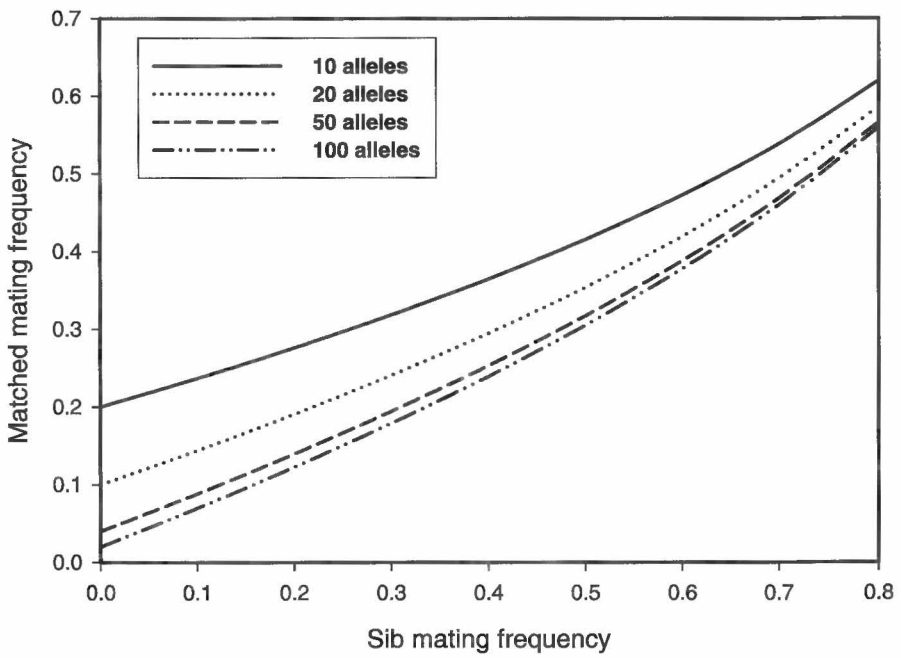

Figure 2. Equilibrium matched mating frequencies for different levels of sib mating, assuming the presence of different number of sex alleles in the population for sl-CSD. The frequency of the individual sex alleles in the population is assumed to be 1/n with n being equal to the number of alleles.

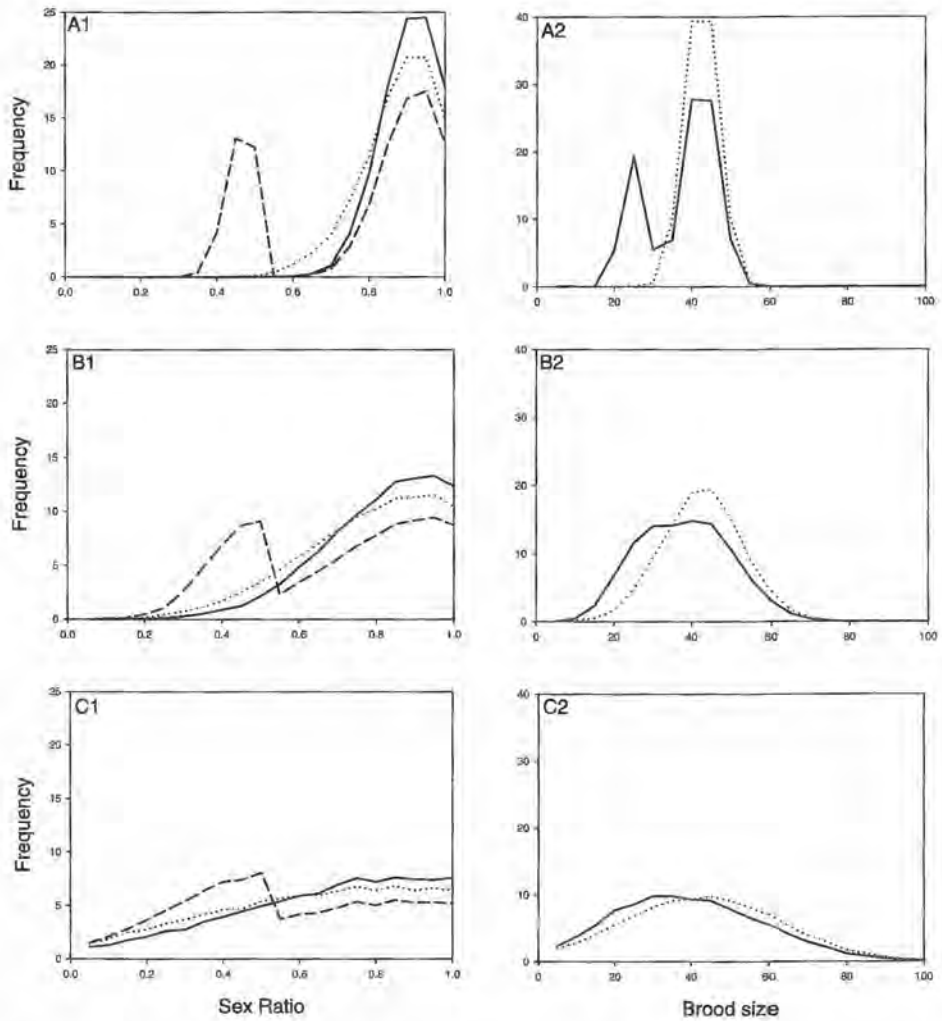

Figure 3. Frequency of broods falling in a class of sex ratios (% female offspring) or brood sizes, each class consists of increments of 0.05 and 5 for sex ratio and brood size respectively. Data are based on simulations using different means and standard deviations for normal distributions (**A** sex ratio mean 0.9 s.d. 0.09, brood size mean 40, s.d 4; **B** sex ratio mean 0.9, s.d 0.225; brood size 40, s.d 10; and **C** sex ratio mean 0.9 s.d. 0.45, brood size mean 40, s.d 20. In figure A1, B1 and C1 the solid line represents the result of simulations where all the diploid eggs become females, the dotted line the situation where the diploid males die and the dashed line the situation where the diploid males survive. In figure A2, B2 and C2 the dotted lines are the result of simulations without egg mortality (i.e. all diploid eggs either develop in diploid males or diploid females), while the solid line represents the result of simulations where diploid males die.

Sex ratio distribution: Sex ratio was highly female biased in both parasitoid species (mean percentage of females = 79.10, SE=0.48, HF=7.53 in *C. sesamiae* and 74.76, SE= 0.41, HF=6.64 in *C. flavipes*). The high values of the heterogeneity factor in both parasitoids indicates that their sex ratios were distributed with a greater than binomial variance. Most broods were female-biased and less than 13% were male-biased in both species. Because of the many female-biased broods, female-skewed progenies (skewness of -1.92 in *C. sesamiae* and -1.84 in *C. flavipes*) and a unimodal distribution with a peak frequency at a sex ratio of 75-95% were obtained (Figure 4). About 15.4% (399/2591) of the broods were all-female in *C. sesamiae* and 5.8% (120/2065) in *C. flavipes* in the field. In both parasitoids, very few all-male broods were present in the field population: 4.4% (115/2591) in *C. sesamiae* and 4.3% (89/2065) in *C. flavipes*. Therefore approximately 95% of the females of both parasitoid species were inseminated.

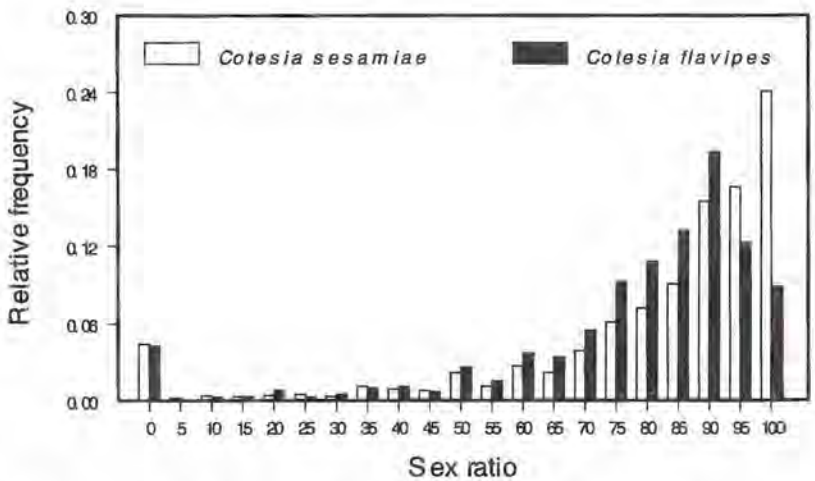

Figure 4. Frequency distribution of sex ratio (% female offspring) of *Cotesia sesamiae* and *Cotesia flavipes* collected from coastal Kenya between 1992 and 1999.

Brood size: The mean brood size in *C. sesamiae* and *C. flavipes* was 27.3±0.4 (range 159) and 31.1±0.4 (range 154) offspring, respectively. Sex ratio (proportion of females) was significantly positively correlated with brood size; r=0.80, y=0.16x+73.0, p=0.0005 in *C. flavipes* (Figure 5A) and r=0.72, y=0.10x+80.0, p=0.004 in *C. sesamiae* (Figure 5A). Sex ratio increased with brood size, and 64.5% of the variation in sex ratio was explained by brood size in *C. flavipes* and 51.3% in *C. sesamiae*.

In both parasitoid species, there were several broods containing large broods (maximum 160 offspring in *C. sesamiae* and 155 in *C. flavipes*) suggesting that

superparasitism may have occurred in the field. In order to discriminate between singly parasitized and superparasitized hosts, the cut-off point was taken to be the halfway point between the number of offspring in one oviposition (38.8) and two ovipositions (57.2) (Sallam *et al.*, 2002), which is around 48 offspring. Assuming that the large broods containing 48 offspring and above resulted from superparasitism, the rate of superparasitism can be estimated to be 15.2% (393/2591) in *C. sesamiae* and 18.5% (383/2065) in *C. flavipes*. However, because some small broods may have resulted from superparasitism, the true rate might be higher. Alternatively, the true rate may be lower because some broods containing more than 48 offspring may have been a result of one oviposition. The sex ratios of broods with 48 or more offspring were female biased (82.9 ±1.1 percent and 78.8±1.0 percent in *C. sesamiae* and

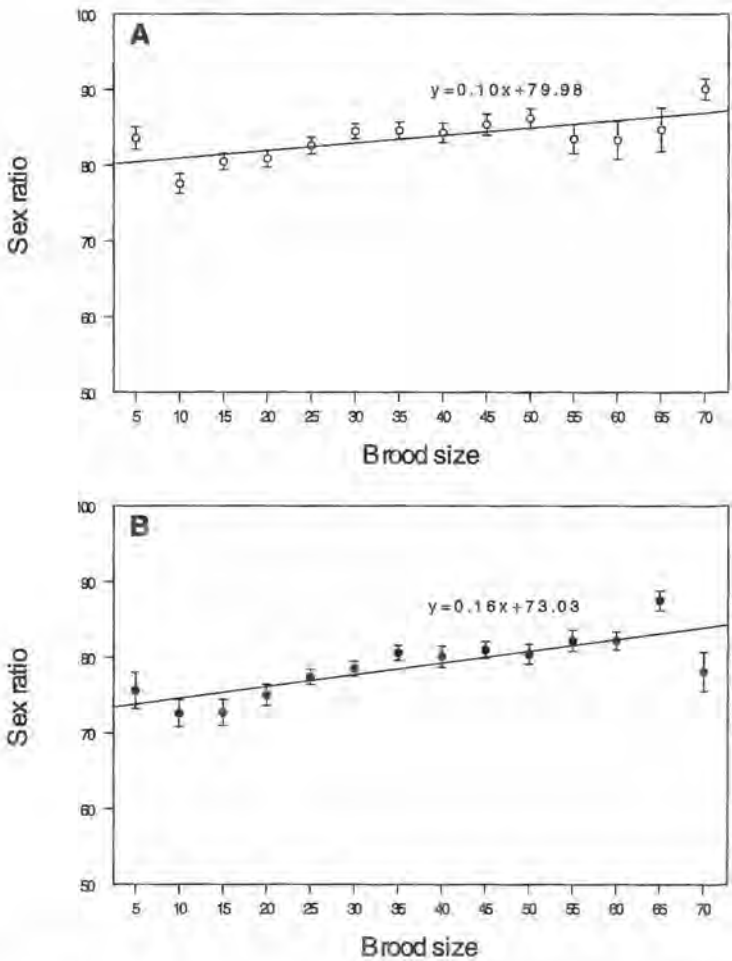

Figure 5. Relationship between brood size and sex ratio (all-male broods omitted) in **A:** *Cotesia sesamiae* (open circles) and **B:** *Cotesia flavipes* (filled circles).

C. flavipes, respectively). Host stage influenced brood sizes ($F=23.50$, $df=2$, $p<0.0001$) and number of females ($F=25.18$, $df=2$, $p<0.0001$) in *C. sesamiae* as well as in *C. flavipes*: brood size ($F=28.17$, $df=2$, $p<0.0001$; number of females ($F=25.58$, $df=2$, $p<0.0001$). However, host species did not affect brood size in *C. sesamiae* ($F=0.59$, $df=2$, $p=0.51$) or in *C. flavipes* ($F=0.21$, $df=2$, $p=0.81$), nor the number of females per brood ($F=0.70$, $df=2$, $p=0.50$ and $F=0.87$ $df=2$, $p=0.42$ in *C. sesamiae* and *C. flavipes*, respectively). Both parasitoid species produced larger brood sizes and more females on large-sized larvae than on medium- or small-sized larvae. However, the brood sizes and numbers of females produced in medium- or small-sized larvae by *C. sesamiae* and by *C. flavipes* did not differ. In *C. flavipes*, broods of 21-25 offspring were more frequent (Figure 6A), while in *C. sesamiae* broods containing 11-15 offspring were more common (Figure 6B).

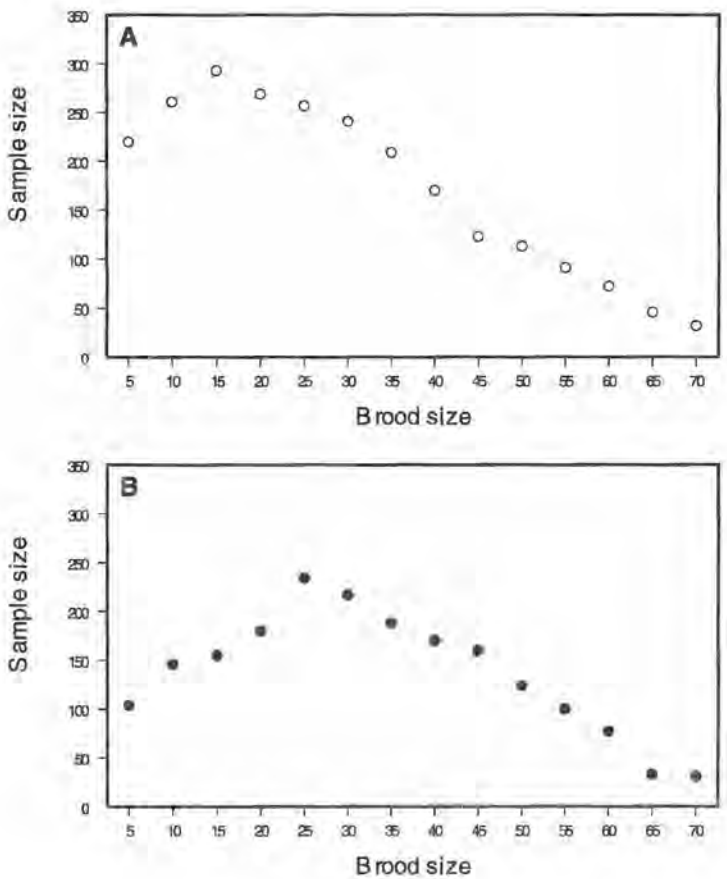

Figure 6. Relationship between brood size and sample size (all-male broods omitted) in **A**: *Cotesia sesamiae* (open circles) and **B**: *Cotesia flavipes* (filled circles).

Discussion

Stouthamer *et al.* (1992) suggested that bimodal sex ratio distributions would result from sib matings, but also indicated that these two populations of sex ratios would be distinguishable only if there was a low variation in sex ratio. Here we show that the difference in sex ratios between matched and unmatched broods are a function of the fertilization rate and the survival of the diploid males. If diploid males die, the difference between the sex ratio of matched and unmatched broods will become smaller. If the diploid males survive, the sex ratio of a matched brood is always half of that of an unmatched brood. If we assume that in populations there is a substantial amount of sib mating as is indicated for a number of gregarious braconids (e.g. Tagawa & Kitano, 1981), then the expected frequencies of matched matings are a function of the number of sex alleles present in the population. The fewer sex alleles present in the population the higher the matched mating frequency. However the influence of the number of sex alleles present in the population on the matched mating frequency declines with increasing sib mating (Figure 2). If we assume a sib mating frequency of 60% for *C. flavipes* and *C. sesamiae* as was indicated by Arakaki and Gahana (1986), figure 2 indicates that the frequency of matched matings in the field should be higher than 30%. Using the figure of 30% matched matings, we did a series of simulations to determine if the distributions of sex ratio and brood size would be able to show a difference between populations with and without sl-CSD. Our simulations show that if we distinguish sl CSD with male mortality from that without male mortality, the sex ratio distribution of sl-CSD with diploid male survival will result in a bimodal sex ratio distribution that is recognizable even when the variation in sex ratio and brood size is substantial (Fig 3, A1, B1, C1). However if sl-CSD results in diploid male mortality then the bimodality of the sex ratio is only noticeable when the variation in fertilization proportion is very small (not shown), while the bimodality in the brood size distribution expected for sl-CSD with diploid male mortality is noticeable only when the variation in brood size is low (Figure 3 A2). From our simulations, we expected to see a clear bimodal pattern in the offspring sex ratio if the *Cotesia* species studied here exhibit sl-CSD and the diploid males survive. However the brood sex ratios of *C. flavipes* and *C. sesamiae* did not show a bimodal distribution making it very unlikely that these species have sl-CSD with diploid male survival. However based on the results of the simulations, we were not able to distinguish the case where the populations do experience sl-CSD but where there is a high level of male mortality.

However are these data sufficient to exclude even the possibility of sl-CSD with diploid male survival in these species? Several assumptions were made that may obscure the expected patterns. First of all we are assuming a high sib mating frequency. What is the evidence for high levels of sib mating in these species? The

evidence is based on laboratory and field observations of matings between individuals emerging from the same cocoon mass, but it is possible that females mate several times after they have left their natal patch and that therefore the frequency of diploid males in individual broods is much lower than we predict. If sperm mixing takes place in the spermatheca of females, only a fraction of the eggs of an individual brood may be fertilized with sperm from a sib. However it was suggested that *C. flavipes* females are mostly monogamic (Kajita & Drake, 1969; Arakaki & Gahana, 1986), although a second mating can take place in the laboratory. Another factor that may influence the level of sib mating is superparasitization. Because of many large broods obtained in the field, superparasitism seems to occur in these species. Brood size in *C. flavipes* is about 40 offspring but highly variable when laboratory reared wasps are allowed to parasitize hosts in the laboratory (Overholt *et. al.*, 1994b; Potting *et. al.*, 1997b). Sex ratio in broods that were considered to result from superparasitism was female-biased like the smaller broods. This agrees with earlier findings that superparasitism has no effect on the sex ratio of *C. flavipes* (Varma & Bindra, 1973; Potting *et. al.*, 1997b; Sallam *et al.*, 2002). The large number of wasps emerging from these "superparasitized" hosts are in contrast to the findings of Potting *et al.* (1997b) who found that, under laboratory conditions, if two females parasitize the same *C. partellus* host within an interval of 4 hours, the superparastized host results in a number of adult wasps that are not significantly higher than those from a host parasitized by a single female. However, Sallam *et al.* (2002) found that progeny production of *C. flavipes* increased with superparasitism by one to three ovipositions per host larva when *C. partellus* was the host but was not affected by superparasitism when *S. calamistis* was the host species. In addition, the work by Potting *et al.* (1997b) also showed that although females were reluctant to superparasitize hosts, they did so in approximately 20% in their experimental set up, indicating that some of the hosts we classified as superparasitized may in fact have been cases of self superparasitism that have taken place shortly after the first oviposition. Self superparasitism does not influence the level of sib mating in the population.

Cotesia flavipes and *C. sesamiae* both belong to the family Braconidae. Complementary sex determination has been suggested as the ancestral sex determination mechanism in the Hymenoptera (Crozier, 1977; Cook, 1993b). In the family of Braconidae both sl-CSD and another sex determination system not vulnerable to the production of diploid males is present (Beukeboom *et. al*, 2000). Given the high level of sib mating in *C. flavipes* and *C. sesamiae*, we expect that sl-CSD would be selected against. Obviously we need additional laboratory studies to exclude sl-CSD with male mortality in these species.

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Chapter

5

Cotesia flavipes Cameron (Hymenoptera: Braconidae) does not exhibit Complementary Sex Determination (ii) Evidence from laboratory experiments

E.I. Niyibigira, Overholt W.A. & Stouthamer R.

Abstract

Sex determination in the order Hymenoptera is based on haplodiploid arrhenotoky; in which males develop from unfertilized eggs and are haploid, whereas females develop from fertilized eggs and are diploid. However, some hymenopteran species produce diploid males through a mechanism known as single-locus complementary sex determination (sl-CSD). In these species, heterozygous individuals at a single sex locus develop into females, whereas hemizygotes (haploids) and homozygotes (diploids) develop into males. Inbreeding leads to homozygosity and consequent production of diploid males. We investigated the presence of single-locus complementary sex determination in the braconid *Cotesia flavipes* using a series of inbreeding crosses among five isofemale lines. Sex ratio (proportion of females) did not differ among the within-line crosses, between-line crosses and crosses carried out between isofemale lines and a mixed (outbred) colony. Brood size of within-line and between-line crosses did not differ. This indicates that sl-SD with diploid male mortality does not take place in within-line crosses. Furthermore, the proportion of males in within-line crosses differed significantly from the expected proportion of males if sl-CSD was present both under the assumptions that diploid males survive or that they died as embryos. Inbreeding of these populations for 25 generations did not result in male-biased sex ratios suggesting that multilocus-CSD was unlikely to occur in *C. flavipes*. We conclude that CSD does not exist in *C. flavipes*. The implications of absence of CSD in *C. flavipes* are discussed in context of mass rearing for classical biological control programmes.

Introduction

In Hymenoptera males are generally produced from unfertilized haploid eggs, whereas females arise from fertilized (diploid) eggs. However two variants of sex determination are known within the Hymenoptera. The apparent ancestral type of sex determination called complementary sex determination (CSD) in which unfertilized eggs always become males, whereas fertilized eggs can become either male or female depending on whether the diploid individual is homozygous or heterozygous at the sex locus. Homozygous diploid individuals become diploid males and heterozygous diploid individuals become females. In the apparent derived sex determination system, fertilized diploid eggs always become females and males only arise from unfertilized haploid eggs. How these two systems are related and the exact molecular pathways involved in the sex determination within these two systems are as yet unknown.

So far sl-CSD has been identified in >40 species within four superfamilies of Hymenoptera, including the sawflies (Tenthredinoidea), parasitoid wasps (Ichneumonoidea), ants and wasps (Vespoidea), bees (Apoidea) (Stouthamer *et al.*, 1992; Cook 1993a, Periquet *et al.*, 1993; Cook & Crozier, 1995; Butcher *et al.*, 2000a, Beukeboom, 2001; Zayed & Parker, 2001). This widespread occurrence of sl-CSD in species belonging to major superfamilies of Hymenoptera suggests that CSD is likely to be the ancestral means of sex determination in Hymenoptera (Schmieder & Whiting, 1947; Crozier, 1977; Cook, 1993a). However single-locus-CSD has been refuted in two braconid wasps *Asobara tabida* and *Alysia manducator* (Ichneumonoidea) (Beukeboom *et al.*, 2000). Therefore within this superfamily two different sex determination systems are found. In most cases, diploid males are inviable, sterile or produce sterile (triploid) daughters, and as a consequence the deleterious effects of inbreeding under sl-CSD are severe (Stouthamer *et al.*, 1992; Cook, 1993; Cook & Crozier, 1995). In some cases, diploid males were reported to produce viable progeny in *Athalia rosae* (Naito & Suzuki, 1991) and in *Diadromus pulchellus* (Agoze *et al.*, 1994). The number of sex alleles estimated in populations that do exhibit CSD is typically 10-100 (Cook & Crozier, 1995).

CSD has been ruled out in other hymenopteran species in which prolonged inbreeding does lead to diploid male production such as in *Muscidifurax raptor* (Pteromalidae) (Fabritius, 1984), *M. zaraptor* (Pteromalidae) (Legner, 1979), *Melittobia* sp.'c' (Eulophidae) (Schmieder & Whiting, 1947) and *Nasonia vitripennis* (Pteromalidae) (Skinner & Werren, 1980). All these species belong to the superfamily Chalcidoidea in which sib mating is prevalent. Also inbreeding did not lead to diploid male production in the bethylid *Goniozus nephantidis* in superfamily Chrysidoidea and single- and multilocus-CSD have been ruled out (Cook, 1993). In addition, Stouthamer &

Kazmer (1994) provided evidence for the absence of any form of complementary sex determination in the genus *Trichogramma*. In these thelytokous wasps, gamete duplication leads to complete homozygosity which would lead to all-male production under sl-CSD instead of all-female offspring.

Therefore, the current phylogenetic distribution of CSD is not exactly defined and there is need for more species to be tested for a better understanding of sex determination mechanisms in Hymenoptera. The Braconidae in particular are interesting because both sex determination systems appear to be present in this family.

It has been suggested that sl-CSD is unlikely to operate in inbreeding species as it would lead to production of a large number of diploid males (Whiting, 1945; Crozier, 1971; Bull, 1981). In cases where diploid males mate, a strong male-biased sex ratio would be generated, resulting in decreased population growth, with consequent potential for extinction of the population (Stouthamer *et al.*, 1992). To allow for some form of CSD in inbreeding species Crozier (1971) proposed a multilocus-complementary sex determination (multilocus-CSD) model in which individuals that are heterozygous for one or more of these loci become females while males are hemizygous or homozygous at all loci. Under this model, sl-CSD would be a special form of multilocus-CSD in which all but one of the loci are monomorphic. Because a diploid individual must be homozygous at several loci to develop as a male, several generations of inbreeding may be required to cause appreciable diploid male production. Crozier (1977) hypothesized that under multilocus-CSD diploid males might remain rare in inbreeding family lines as occasional outcrosses restored heterozygosity. Multilocus-CSD has yet to be demonstrated in any species (Crozier, 1977; Cook, 1993a; Luck *et al.*, 1992). In those species that were specifically tested to determine if multilocus-CSD applied no evidence to support this hypothesis was found. Cook tested the multilocus-CSD model in the bethylid *Goniozus nephantidis* by conducting inbreeding experiments for 22 generations. This did not lead to diploid male production and with this result he concluded that sl-CSD and multilocus-CSD were absent in *G. nephantidis* (Cook, 1993). Multilocus-CSD is also absent in species that undergo gamete duplication such as *Muscidifurax uniraptor* (Legner, 1985); *Diploleps is rosae* (Stille & Davring, 1980) and in several *Trichogramma* species (Stouthamer & Kazmer, 1994).

The presence of CSD in a species may have important consequences for its use in biological control. Precautions need to be taken to avoid losing sex alleles in mass rearing. A reduction in the number of sex alleles present in the population may lead to a reduction in population growth rate and the failure of biological control (Stouthamer et al 1992). Controlled inbred crosses are typically used to detect sl-CSD

(Whiting, 1943; Crozier, 1971; Cook 1993b; Beukeboom, 1999; 2000; Noda, 1999; Butcher *et al.*, 2000). Under sl-CSD models, well-defined proportions of diploid males are expected following crosses between relatives. In mother-son crosses, the mother mates with a son that carries one of her sex alleles, so 50% of fertilized eggs will be diploid males while in brother-sister crosses, 50% of the crosses will be matched (two-allelic) and yield diploid males. The other half will be unmatched (three allelic) and yield no diploid males.

Cotesia flavipes is a gregarious endoparasitoid of the larvae of noctuid and crambid stemborers. It attacks medium and large-sized stemborer larvae (Smith *et. al.*, 1993; Ngi-Song *et. al.*, 1996). *C. flavipes* is a native to the Indo-Australian region but has been widely introduced in more than 40 countries in the tropics and subtropics for biological control of lepidopteran stemborers in maize, sorghum and sugarcane (Polaszek & Walker, 1991). It has recently been introduced into Africa for biological control of *Chilo partellus* (Overholt, 1993). *C. flavipes* is pro-ovigenic and has about 150 eggs available for oviposition with each female laying a brood of 20-25% of the available egg load (Potting *et. al*, 1996). Due to haplodiploidy, unmated females produce male offspring only. *C. flavipes* has been found to exhibit a high level of sib mating directly after emergence from the stemborer tunnel (Arakaki & Gahana, 1986). Such mating behaviour suggests that inbreeding occurs regularly in this species.

In an earlier study (Niyibigira *et al.*, unpublished), we used field brood sex ratios to find evidence for the occurrence of CSD in this species. Our results showed that sl-CSD with survival of diploid males was unlikely in this species, however we could not exclude the possibility of sl-CSD with diploid male mortality. Here we investigate the presence of sl-CSD in *Cotesia flavipes* by conducting brother-sister crosses. The observed number of males in these inbred crosses was compared to the number expected under the assumption of sl-CSD. Furthermore, changes in sex ratio were monitored in laboratory cultures of isofemale lines and a mixed colony (under mass rearing) for at least 25 generations to determine if a more male biased sex ratio would arise possibly as a consequence of the loss of sex alleles in the rearing. None of these tests revealed any evidence for diploid male production in *C. flavipes*.

Materials and Methods

Insects

Cotesia flavipes strains originated from field material collected from Karnataka, Andra Pradesh and Mahrastra states in India between 11 December 1998 and 2 January 1999. It was assumed that the field-collected larvae were each parasitized by a single female wasp suggesting that one female was the founder of each isofemale

line. The isofemale lines were named according to the order of collection and collection state. For our experiments we used lines Mah2, Mah20, Karn6, Karn8 and Karn16. These lines were founded from cocoons collected from different locations between 20 –160 km apart, suggesting that they may have carried different alleles at sex locus. A mixed colony formed by hybridization of 26 isofemale lines was used in control crosses. Cultures of isofemale lines and the mixed colony were maintained under similar conditions in the laboratory. *Cotesia flavipes* was reared on fourth instar larvae of *Chilo partellus* according to the "hand-stinging" method described in Overholt *et al.* (1994). *C. partellus* larvae were maintained on artificial diet (Ochieng *et al.*, 1985) at 25±2°C, 70-80% RH and 12:12 (L:D) h photoperiod.

Detection of diploid males

Diploid males can be detected by crossing sons and daughters of one mother i.e. brothers and sisters. In brother-sister matings, such crosses can be matched (shared allele) or unmatched (no shared allele), depending on whether the brothers inherited the same or different allele as their sisters (Figure 1). If both share an allele, half of the diploid offspring are homozygous and male, but if they carry different alleles 100% of diploid eggs are heterozygous and female. Because half of the brother-sister matings will by chance be matched and half are unmatched, on average 25% of the fertilized eggs are expected to become diploid males in such crosses. This should be manifested through an increase in the proportion of male offspring in within-line (inbred) crosses. However, because diploid males may be unviable, we also compared the brood sizes of within-line (inbred) crosses with those from between-line and control crosses in which females were taken from the same isofemale line as the inbred line, i.e. offspring from three cross types that shared a common mother line.

Crosses

Three types of crosses were made: (i) Within-line crosses whereby males were mated with females from the same line i.e. brother-sister mating; (ii) Between-line crosses. In this case, males of one isofemale line were mated with females from a different line. Reciprocal crosses were performed as well. Thus, if two isofemale lines do not share a sex allele (they are unmatched), the progeny of their crosses are not expected to contain diploid males. (iii) Control crosses: Here, males from isofemale lines were mated with females from the mixed (outbred) colony and vice versa. Crosses were conducted in 20 replicates.

Crosses were initiated by isolating individual cocoons that were about to emerge (in black stage) one day before the expected date of emergence. Adult male and females were paired immediately after adult emergence in glass vials containing a strip of cotton wool saturated with a 20% sugar-water solution as a food source. The wasps

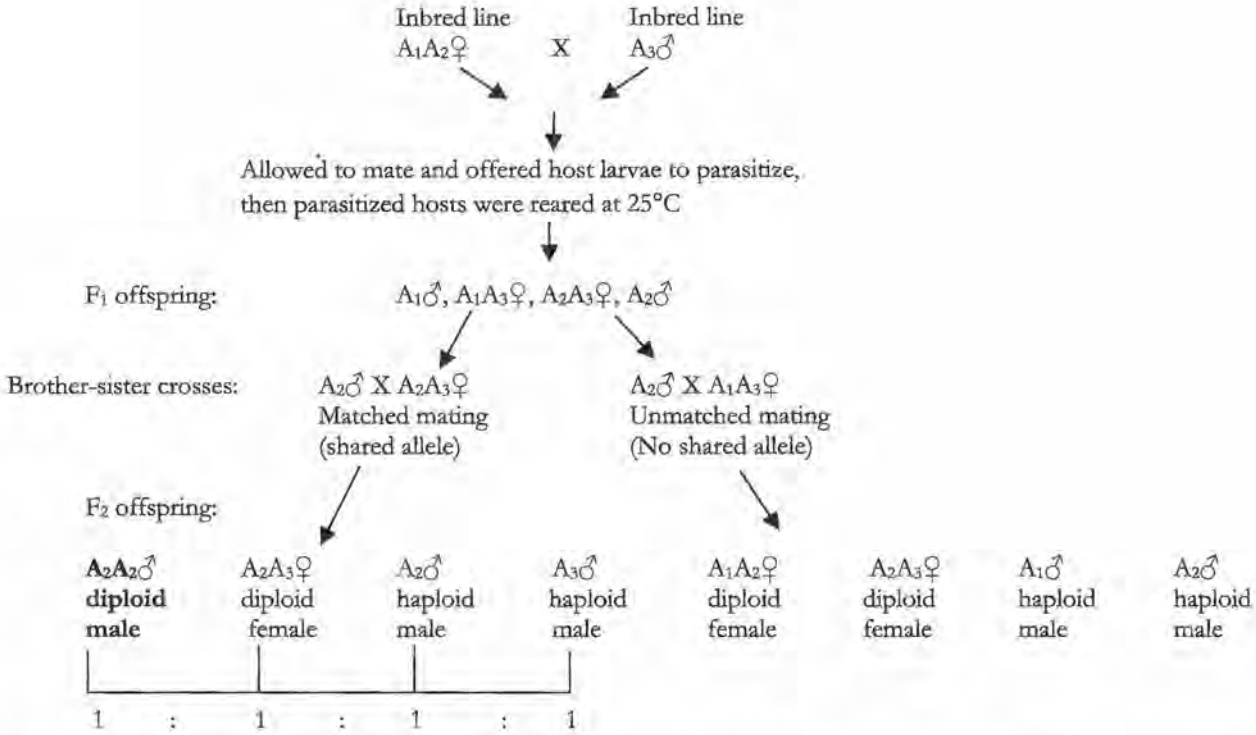

Figure 1: The procedure of cross experiments to detect diploid males in *C. flavipes*. Diploid heterozygous females are mated with their hemizygous brothers. Two of these crosses are matched (two-allelic) and result in 50% diploid homozygous males among fertilized eggs, whereas the other two combinations are unmatched (three-allelic) and do not result in diploid males. For clarity, only one of the two possible outcomes in matched and unmatched crosses are shown above. On average, 25% of fertilized eggs are expected to become diploid males in brother-sister crosses.

were allowed to mate at 25°C for 24 hours. Mated females were then offered two fourth-instar *C. partellus* larvae (one in the morning and another in the afternoon) for oviposition until they died. We used a standard host size in these experiments because *C. flavipes* lays more eggs in medium- and large- larvae (Ngi-Song *et al.*, 1996; Niyibigira, unpublished). Parasitized larvae were reared on artificial diet (Ochieng *et al.*, 1985) at 25±2°C, 70-80% RH and 12:12 (L:D) h photoperiod until cocoon formation, a process which took 12-15 days. Cocoon masses were collected in glass vials (2.5 cm x 7.0 cm) and placed in an incubator at 25°C for adult emergence. Adult male and female offspring were counted and the brood size (total number of offspring) and sex ratio (proportion of females) in each cross determined. We tested for the existence of sl-CSD in *C. flavipes* by comparing brood size and sex ratio of inbred crosses with those of crosses in which females used were taken from the same line as inbred cross. Brood sizes and sex ratios were compared among crosses using a General Linear Model of ANOVA (PROC GLM, SAS Institute, 1999-2000). Means were separated using Student-Newman-Keuls Test (SNK) when ANOVAs were significant ($p<0.05$). Sex ratios were arcsine transformed before analysis. Crosses that resulted in all-male broods were excluded from the analysis because their mothers were considered to be unmated (virgin).

Secondly, we tested for the presence of sl-CSD by comparing the observed number of males in each within-line cross to the expected number under the assumption of sl-CSD, both with viable diploid males and unviable diploid males. The expected number of males in the presence of sl-CSD was calculated from the expected fertilization proportion (proportion of females) which is the fertilization proportion in those cases where all fertilized eggs are expected to develop into females and all unfertilized eggs into males. The fertilization proportion was calculated for each isofemale line by using the sex ratio obtained in those crosses involving females from that same line with males from other isofemale lines (unmatched crosses). Next we used the mean of estimated fertilization proportion in Chi-square tests to determine if the observed number of males deviated significantly from the estimated frequencies. The expected number of males in both cases were calculated as:

Case 1: (No. of haploid males + No. of diploid males)/No. of total offspring, if diploid males are viable;

or

Case 2: No. of haploid males/(No. of total offspring - No. of diploid males); if diploid males are unviable.

For instance if the fertilization proportion for line 1 was 80%, then the with-in line cross in which the brood size was 40 would consist of 8 males and 32 females. In the case of sl-CSD, from a matched mating we would expect 8 haploid males, 16 diploid

males and 16 diploid females. If the diploid males die, then the resulting brood would be 8 haploid males and 16 diploid females, resulting in a sex ratio of 67%. The expected number of haploid males, diploid males and diploid females was calculated for all the brood sizes in within-line crosses, with assumption of with and without diploid male mortality. Deviations of the observed from the expected number of males were tested using Chi-square test, which evaluated the null hypothesis that the number of males observed is consistent with a matched mating.

Monitoring sex ratio changes in laboratory cultures

Samples of dead wasps were randomly taken from laboratory cultures of isofemale lines and mixed colony in each generation. Individuals from each sample were sexed, counted and the sex ratio (proportion of females) was determined. This procedure was conducted for 25 generations for all colonies except Karn16 which was monitored from the 20th to the 25th generation. We compared sex ratio of mixed colony with isofemale lines using a G-test of independence with a William's correction (Sokal & Rohlf, 1995). The relationship between sex ratio and time in culture was determined using using PROC REG (SAS Institute, 1999-2000).

Results

Brood size and sex ratio in crosses

Of the total crosses, 20% (18 females from within-line, 79 between-line and 23 control) of the crosses produced all-male broods. These females were considered to be unmated and consequently data from these crosses was discarded from the analysis. In addition, 23.8% females produced no offspring because their hosts died or pupated before cocoons formed.

Table 1 shows the brood size and sex ratio of all the different crosses. In crosses in which females were taken from Mah2, brood size was significantly different among the crosses ($F=5.13$, $df=5$, $p=0.0004$) but sex ratio did not differ ($F=1.74$, $df=5$, $p=0.136$). This was the reverse among crosses in which females originated from Karn6: brood size did not differ among the crosses ($F=1.45$, $df=5$, $p=0.220$) while sex ratio was significantly different ($F=6.76$, $df=5$, $p<0.0001$). However, for crosses in which females were taken from Karn8, there were no differences in both brood size ($F=0.76$, $df=5$, $p=0.585$) and sex ratio ($F=0.82$, $df=5$, $p=0.541$). Brood size in crosses that involved females from Karn6 were not different ($F=0.64$, $df=5$, $p=0.638$) while the differences in sex ratio were weakly significant ($F=2.71$, $df=5$, $p=0.037$). In contrast, crosses that involved females from Mah20 were significantly different in brood size ($F=6.22$, $df=5$, $p=0.0001$) and sex ratio ($F=3.43$, $df=5$, $p=0.0084$). Among the cross types, brood sizes in within-line crosses, between-line

Table 1: Brood size and sex ratio of *C. flavipes* offspring from different crosses

Cross type	Cross Males x Female	N	Brood size[1] (Total offspring) Mean±SE)	Sex ratio[2] (% of females) Mean±SE)
Within-line	Mah2 x Mah2	15	34.1±2.9 bc	68.1±6.0
Between-line	Karn6 x Mah2	11	44.5±4.7 ab	87.9±1.5 a
	Karn8 x Mah2	19	48.3±3.1 a	70.4±6.1 a
	Karn16 x Mah2	13	40.2±4.6 abc	67.7±6.4 a
	Mah20 x Mah2	12	30.2±4.5 c	77.0±2.2 a
Control	Mixed x Mah2	13	27.4±3.0 c	73.5±5.3 a
Within-line	Karn6 x Karn6	14	33.0±3.4 a	71.7±7.7 a
Between-line	Mah2 x Karn6	7	41.6±5.6 a	65.3 ±10.8 a
	Karn8 x Karn6	9	37.1±5.6 a	80.4±4.1 a
	Karn16 x Karn6	13	37.6±3.3 a	65.2±5.4 a
	Mah20 x Karn6	13	32.2±3.9 a	71.2±3.7 a
Control	Mixed x Karn6	7	24.9±4.0 a	37.4±9.8 b
Within-line	Karn8 x Karn8	14	24.0±2.0 a	63.4±7.4 a
Between-line	Mah2 x Karn8	7	35.2±10.2 a	82.2±3.2 a
	Karn6 x Karn8	4	28.3±4.6 a	60.6±12.5 a
	Karn16 x Karn8	9	27.1±4.3 a	63.5±7.5 a
	Mah20 x Karn8	8	29.1±5.4 a	68.1±4.7 a
Control	Mixed x Karn8	11	24.2±2.6 a	65.9±8.7 a
Within-line	Karn16 x Karn16	18	35.2±2.8 a	58.8±6.2 ab
Between-line	Mah2 x Karn16	14	40.1±6.3 a	56.4±9.1 ab
	Karn6 x Karn16	15	43.3±3.5 a	37.6±8.1 b
	Karn8 x Karn16	7	36.6±5.2 a	78.4±5.3 a
	Mah20 x Karn16	17	37.4±3.1 a	58.1±6.1 ab
Control	Mixed x Karn16	9	28.2±4.0 a	78.4±4.7 a
Within-line	Mah20 x Mah20	19	36.2±1.3 a	69.6±3.5 ab
Between-line	Mah2 x Mah20	8	31.9±7.4 ab	78.0±8.1 a
	Karn6 x Mah20	11	29.3±5.3 ab	66.3±6.8 ab
	Karn8 x Mah20	7	25.4±7.0 ab	54.0±10.5 b
	Karn16 x Mah20	13	28.5±3.5 ab	59.2±6.9 ab
Control	Mixed x Mah20	10	14.9±2.2 b	43.9±6.7 b

[1]Means of brood size followed by the same letter in same column for crosses involving females from the same isofemale line are not significantly different (SNK, $p<0.05$). [2]Means of sex ratio followed by the same letter in same column of crosses involving females from the same isofemale line are not significantly different (SNK, $p<0.05$). Brood size of crosses with females taken from line Mah2: $F=5.13$, $df=5$, $p=0.0004$; Karn6: $F=1.40$, $df=5$, $p=0.2386$; Karn8: $F=0.76$, $df=5$, $p=0.5847$; Karn16: $F=1.26$, $df=5$, $p=0.2891$; Mah20: $F=3.49$, $df=5$, $p=0.0076$. For sex ratio of crosses with females taken from line Mah2: $F=1.74$, $df=5$, $p=0.1355$; Karn6: $F=3.50$, $df=5$, $p=0.0079$; Karn8: $F=0.84$, $df=5$, $p=0.5414$; Karn16: $F=3.49$, $df=5$, $p=0.0070$; Mah20: $F=3.45$, $df=5$, $p=0.0081$.

Table 2: Brood size and sex ratio of *C. flavipes* from different cross types. The between-line cross types are crosses in which females and males used were taken from different isofemale lines while in the within-line crosses, males and females were taken from the same isofemale lines. Control crosses are those in which females were obtained from isofemale lines and males from the mixed colony.

Cross type	N	Brood size (Mean±SE)	Sex ratio (% of females) Mean±SE)
Between-line	217	36.35 ± 1.13 a	66.30 ± 1.67 a
Within-line	80	32.86 ± 1.20 a	66.26 ± 2.72 a
Control	50	23.98 ± 1.52 b	61.72 ± 3.74 a

Means followed by the same letter in same column are not significantly different (SNK, $p<0.05$). Means followed by the same letter in same column are not significantly different (SNK, $p<0.05$). Brood size of cross types: $F=12.30$, $df=2$, $p<0.0001$; Sex ratio between cross types: $F=1.88$, $df=2$, $p=0.1543$

Table 3: Comparison of the observed and calculated expected proportions of males in within-line crosses of *C. flavipes* if sl-CSD is present. Expected number of diploid males in each cross were calculated based on the assumption that (i) diploid males survive, and (ii) diploid males die during development

With-in line cross		Fertilization proportion (%)	Diploid males survive		Diploid males die	
Male x Female	N	Mean ± SE	χ^2_1	p-value	χ^2_2	p-value
Mah2 x Mah2	15	74.5±2.5	63.03	<0.0001	39.23	0.0003
Karn6 x Karn6	14	65.6±3.2	86.19	<0.0001	56.10	<0.0001
Karn8 x Karn8	14	68.2±3.5	45.80	<0.0001	29.87	0.0049
Karn16 x Karn16	18	58.0±3.8	73.26	<0.0001	55.64	<0.0001
Mah20 x Mah20	19	60.0±3.6	36.69	0.0057	28.67	0.0500

crosses and control crosses were significantly different ($F=12.30$, $df=2$, $p<0.0001$) but sex ratio did not differ ($F=1.88$, $df=2$, $p=0.154$) and was female-biased (Table 2).

Observed and expected sex ratios in sib-sib matings

The expected proportion of males if sl-CSD is present in *C. flavipes* differed significantly from the observed proportion of males in within-line crosses, under both the assumptions of viable diploid males and unviable diploid males (Table 3). From these results, we conclude that matched mating in *C. flavipes* did not result in the production of diploid males and further confirm that sl-CSD is not the mode of sex determination mechanism in this species. Under sl-CSD, the expected number of diploid males is 1/3 of the number of females, because 25% of diploid eggs are expected to develop into diploid males.

Sex ratio of laboratory cultures

Sex ratio of *C. flavipes* from isofemale lines (pooled data) was higher than that from the mixed colony in the 25th generation of laboratory rearing (G-test; $G_{adj}=36.84$; $p<0.001$). Sex ratio increased i.e. became female-biased, with number of generations in culture in Mah2, Karn16 and Mixed colony but it decreased in Karn6 ($p=0.16$) (Table 4).

Table 4: Relationship between number of generations in culture and sex ratio (proportion of females) of *C. flavipes* in laboratory cultures of isofemale lines and a mixed colony[a]

Laboratory[b] culture	Regression line	R-square	F-value[c]
Mah2	Y= 2.00*X + 24.211	0.627	34.88 ***
Karn6	Y= -0.23*X + 44.75	0.007	0.16 NS
Karn8	Y= 0.398*X + 31.02	0.037	0.77 NS
Karn16	Y= 0.76*X + 51.51	0.037	0.16 NS
Mah20	Y= 0.28*X + 34.40	0.016	0.33 *
Mixed	Y= 1.63*X + 31.78	0.359	12.32 **

[a]Mixed colony: a culture formed by hybridization of several isofemale lines which was being maintained under mass rearing conditions. [b]Cultures were monitored from 2nd to 25th generation (from 20th to 25th generation for Karn16). In each generation a random sample of dead wasps was taken from each culture and number of males and females in each sample were counted. All cultures were female-biased. [c]NS, not significant at $p=0.05$ level; *$p < 0.01$; **$p< 0.005$; *** $p< 0.0001$.

Discussion

Our results showed no evidence suggesting that diploid males are produced in *C. flavipes*. We demonstrated that inbreeding of *C. flavipes* did not result in diploid male production, implying that sl-CSD is not the mode of sex determination in this species. In those species that possess sl-CSD, matched mating should result into diploid male production which is reflected in an increased proportion of males (male-biased broods).

Brood sizes and sex ratios did not differ between within-line crosses and crosses involving unrelated males and females and sex ratios in all crosses were female-biased, which indicates that diploid males were not produced in matched matings. The number of diploid males produced increases with relatedness of individuals participating in the crosses (Stouthamer *et. al.*, 1992). Even in populations containing several alleles, a substantial number of diploid males should be produced (Crozier, 1977; Cook, 1993). By using formulas derived for a large, randomly mating population containing >20 sex-alleles, Stouthamer and colleagues were able to demonstrate the effects of a reduced number of alleles on sex ratio both in the case of viable and unviable diploid males. In all cases, they showed that the sex ratio became more male-biased and population growth rate was depressed with a reduced number of alleles (Stouthamer *et. al.*, 1992).

Furthermore, we found that sex ratio of *C. flavipes* in five isofemale line cultures that were maintained in the laboratory for several generations under inbreeding did not result in male-biased sex ratios. In the rearing unit at ICIPE, several cultures of *C. flavipes* have been maintained under mass rearing conditions since 1992 for a biological control programme against cereal stemborers (Overholt *et al.*, 1994). In these cultures (some which are over 180 generations in rearing), there has not been any detectable shift in progeny sex ratios towards male-bias (Ochieng J.O., pers. comm.), despite the fact they undergo occasional population fluctuations particularly during periods of field releases for biological control purposes.

In multilocus-CSD, the number of loci at which alleles are fixed increases with the number of generations of inbreeding. Cook (1993) derived formulas for the calculation of expected levels of diploid male production in species that possess multilocus-CSD. Using these formulas, he concluded that multilocus-CSD was absent in *G. nephantidis* and proposed that 10 generations of inbreeding would be adequate to test even a 15 locus model (Cook, 1993). Based on this model, 25 generations of inbreeding in each isofemale line of *C. flavipes* should have lead to a higher number of males per generation than the mixed colony that was maintained under mass-rearing (outbreeding) conditions. Cook (1993b) derived a model based on brood survival and

secondary sex ratio data that can be used to test for the presence of sl-CSD in any parasitoid species with female biased sex ratios and measurable mortality. From the results of inbred crosses in isofemale lines Mah2, Karn6 and Mah20, the proportion of males was 0.239 and brood survival was 0.800, which falls outside the critical region stated in Cook (1993b). This indicates that there is insufficient mortality to generate the degree of female bias observed. Therefore, the highly female-biased sex ratios and low brood mortality in *C. flavipes* is inconsistent with the sl-CSD requirement that 50% of fertilized eggs are male.

We have demonstrated from experimental crosses that no evidence exists for diploid male production in *C. flavipes*. From field data, it has already been shown sl-CSD was unlikely if the diploid males survived, our experiments here show that there is no evidence for sl-CSD with diploid male mortality either. These findings have implications for classical biological control because *C. flavipes* has been widely introduced against lepidopteran stemborers attacking maize, sorghum and sugarcane. The absence of CSD in *C. flavipes* implies that it can be reared for several generations of inbreeding without negative consequences of male-biased sex ratios due to diploid male production. Secondly, once this parasitoid has been released in the field, sib mating cannot lead to reduction in population growth through CSD, thus allowing the successful colonization of the parasitoid population and increased parasitism of the target pest.

Sex determination in species of Hymenoptera without CSD is not clearly understood. Two other models of hymenopteran sex determination have been proposed (Cook, 1993; Cook & Crozier, 1995): (1) Imprinting which occurs in inbreeding species that have lost CSD (Poirié *et al.*, 1992), and (2) the genetic balance model (da Cunha & Kerr, 1957). The imprinting hypothesis (Poirié *et al.*, 1992) proposes that the sex locus (X), binds an active product that is present in the egg or zygote. Females imprint the X locus so that the active product is not bound and is inactive (X_i) in unfertilized eggs, which become males. The imprint is erased during development. Males do not imprint so the locus is active (X_a) in fertilized eggs, which develop as females. In contrast to CSD, only the paternal allele would be transcribed and synthesize a product (Poirié *et al.*, 1992). In the genetic balance model, the sex of an offspring is determined by the effects of male-determining genes (m) and additive female-determining genes (f). In haploids, the total effect of maleness genes (1m=M) outweighs that of femaleness genes (1f=F), while in diploids the additivity of femaleness genes (2f=2F) outweighs the male effect (2m=M). These individuals become females. The accumulation of evidence for diploid males and sl-CSD in several species contradicts the genetic balance hypothesis (Cook, 1993b) and several authors have argued that the supposedly supportive evidence from bees is open to multiple interpretations (Cook, 1993; Cook & Crozier, 1995; Woyke, 1986).

In conclusion, sl-CSD does not operate in the inbreeding species *C. flavipes*. Single-locus-CSD has so far been found only in outbreeding species. In the family of Braconidae, so far four out of six tested members namely *Bracon brevicornis* (Speicher & Speicher, 1940), *B. hebetor* (Whiting, 1943), *B. serinope* (Clark & Rubin, 1961) and *Microplitis croceipes* (Steiner & Teig, 1989) were shown to have sl-CSD. However, it is clearly absent in other Braconidae as was shown here and earlier by Beukeboom *et. al.*(1999). Further tests should be done to determine the phylogenetic distribution of sl-CSD and to see if some outbreeding braconids have lost CSD.

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Chapter

6

Importance of genetic variability in the colonization of the stemborer parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae)

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Abstract

The importance of genetic variability to colonization and establishment of natural enemies was examined through the release of three genetically impoverished populations (isofemale lines) and one population of high genetic diversity (mixed population) of the parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae), a biological control agent of the introduced stemborer *Chilo partellus* (Lepidoptera: Crambidae) on two islands of Zanzibar. *Cotesia flavipes* was collected from India, imported, quarantine processed and reared in Kenya. Genetic variability of four *C. flavipes* populations was investigated using intersimple sequence repeat (ISSR) markers. A dendrogram constructed using the UPGMA method revealed that individuals belonging to the same isofemale line were grouped together suggesting that there was little variation within populations, except for the mixed population. The similarity of different individuals from the isofemale lines was high: about 90% from Mah2, 94% from Karn8, and 98-100% from Mah20. Individuals from the mixed population had the lowest similarity of 66%. All four populations were 82% similar to each other. Gene diversity was 0.54±0.08, 0.41±0.10, 0.46±0.07 for Mah2, Karn8 and Mah20 respectively which was low compared to the gene diversity of 0.88±0.03 for the mixed population. In a randomized complete block design, the four populations were released in maize and sorghum plots located in 16 sites on Unguja and 12 sites on Pemba. Releases of 250 cocoon masses of each population in each plot were made once in long rainy season of 2000. Monitoring of colonization was done fortnightly by random removal and dissection of plants with putative symptoms of stemborer infestation from the field. *Cotesia flavipes* was first recovered two weeks after release and was the most common parasitoid throughout the season. The genetically diverse population showed higher colonization of stemborers than Karn8, but was not different from the other two isofemale lines. This suggests that genetic variability may not have been an important factor in the

colonization of *C. flavipes*. Climatic similarities of origin of the parasitoids in central India and release areas in Zanzibar may offer a possible explanation, suggesting that all the parasitoids collected were pre-adapted to the conditions in Zanzibar. Colonization of *C. flavipes* was higher on Pemba than on Unguja. *Cotesia flavipes* was recovered one year after releases were made confirming, that the parasitoid was firmly established on the islands. To our knowledge, this is the first field study to investigate the importance of genetic diversity within a population of natural enemy for the colonization process.

Introduction

Classical biological control is a pest management approach that involves the importation of natural enemies from one geographic area of the world, and the release of the natural enemies in another area of the world, where they formerly did not exist, for the sustainable suppression of specific pest populations (Ehler, 1982). Once a natural enemy is permanently established in a new area, it will have a self-perpetuating impact on the target pest population, with no additional external input (Huffaker & Messenger, 1976). The greatest efforts in classical biological control have been directed against introduced pest species, whereby a pest's natural enemies in its aboriginal home are introduced into a geographic area that the pest has invaded.

Although the rate of failure of classical biological control is low compared to identification of chemical control agents, there is need to improve it. For example, of 2189 attempts to introduce natural enemies, only 860 have resulted in establishments, and only about half of those have provided some degree of control of the target pest population (Waage 1990). The often cited reasons for the low success rate include, the inability of the natural enemy to adapt to the new environment, inappropriate release procedures (low numbers released, releases not repeated, all releases made in one area, bad timing of releases, etc.) and poor synchronization of the natural enemy with the pest population (Beirne 1975; Hopper & Roush, 1993, Hopper, 1996). Low genetic variability in the founder population has also been suggested as a cause of many failures (Mackauer 1976, Stouthamer *et al.* 1992, Hopper *et al.* 1993).

Candidate natural enemies often occur in several geographical areas and ecosystems in the aboriginal home of the pest. These areas are likely to have different climates, and the natural enemy may attack different host/prey species in the different areas. It is likely that natural enemies from different geographical and ecological areas have diverged in response to local selection pressures and stochastic processes, and thus, occur as genetically distinct populations. However, little is known about the genetic diversity within species of natural enemies, or of its importance to successful biological control.

Many theoretical studies have described in general terms the relationship between genetic variability, population size and extinction probability. In a number of cases these theories have been tested using as a model system populations of *Drosophila* species that are kept in laboratory

cages (Briscoe *et al.* 1992, Loebel *et al.* 1992, Frankham & Loebel 1992, Spielman & Frankham 1992). Most of the work done on field populations has been exclusively descriptive. The lack of experimental fieldwork is caused by the difficulties of manipulating genetic variability and population size in the field. In only a few studies related to biological control has the relationship between genetic variability and population size been determined. Again most of these studies are either based on theory (Stouthamer *et al.* 1992, Roush, 1990; Hopper *et al.* 1993) or on the loss of genetic variability during rearing in the laboratory (Unruh *et al.*, 1983). Recently, the lack of knowledge of the relationship between genetic variability and chances for successful biological control was reviewed by Hopper *et al.* (1993) who suggested that experimental field work on this topic is of utmost importance.

One of the most important pests of maize and sorghum in East Africa is the stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), a species accidentally introduced from South-East Asia sometime before 1930 (Tams, 1932). Since arriving on the continent, *C. partellus* has spread to nearly all countries in East and southern Africa, often becoming the predominant and most damaging stemborer of maize and sorghum (Kfir *et al.*, 2002). In Zanzibar, *C. partellus* is the most serious pest of maize and sorghum, contributing between 72-85% of stemborer attack (Niyibigira *et al.*, 2001). Yield losses due to stemborer attack vary with location and season but are typically in range of 30 – 40% (Allertz *et al.*, 1988; van Keulen, 1990). In the aboriginal home of *C. partellus*, an important natural enemy is *Cotesia flavipes* (Hymenoptera: Braconidae), a gregarious larval endoparasitoid that attacks medium and large instar larvae. This parasitoid has been widely used in classical biological control programmes against *C. partellus* (Alam *et al.*, 1972; Overholt *et al.*, 1994; 1997, Omwega *et al.*, 1995; 1997). In coastal Kenya where *C. flavipes* was introduced in 1993 (Overholt *et al.* 1994), recent impact analysis has shown that the stemborer population has declined and larval mortality due to the parasitoid has increased (Zhou *et al.*, 2001).

Intersimple sequence repeats (ISSRs) markers are among the molecular techniques that have been developed in recent years for assessing the genetic variability in populations. ISSR technique involves the use of a single primer composed of a microsatellite sequence or simple sequence repeats (SSR) plus a short (2-4 arbitrary nucleotides) sequence anchored at the 3'or 5'end, which targets a subset of SSRs and amplifies the region between two closely spaced and oppositely oriented SSRs (Wolfe *et. al.*, 1998). Simple sequence repeats are highly polymorphic, even among closely related individuals, due to mutations causing variation in the number of repeating units. Thus ISSRs are co-dominant markers which provide a high degree of allelic polymorphism (Zietkiewicz *et. al.* 1994). The advantages of these ISSR markers are in terms of speed, the production of large numbers of fragments, reproducibility, and low cost (Wolfe *et. al.*, 1998). Although ISSRs rely on the presence of SSRs, no previous knowledge of the DNA sequence in the flanking regions of the SSRs is required for primer design, as is the case for microsatellite markers (Zietkiewicz *et. al.* 1994). ISSR markers have proved to be useful in assessing genetic variability in both plants (Joshi *et al.*, 2000; Fernandez *et al.*, 2002) and animals (e.g. Bai & Li, 2001) including insects (Reddy *et al.*, 1999; Kumar *et al.*, 2001; Nagaraju *et al.*, 2001).

In the present study, ISSR markers were used to estimate the genetic variability in four Indian populations of the stemborer parasitoid *C. flavipes*. Three of these populations were genetically impoverished (isofemale lines) while the fourth population was a hybrid of several isofemale lines (i.e. a genetically diverse population). Secondly, we determined the relationship between genetic variability and chances of colonization through field releases of these populations on two islands of Zanzibar. Colonization was defined as the presence of the parasitoid in the release fields at the end of one growing season. The parasitoids that were recovered at the end of the season were tested using the ISSR markers to determine that they indeed were the offspring of the lines that had been released.

Materials and methods

Importation, quarantine and rearing of *Cotesia flavipes*

Cotesia flavipes cocoon masses were reared from parasitized *C. partellus* larvae that had been collected from sorghum plants at 28 locations in Andra Pradesh, Maharastra and Karnataka states in India between 11 December 1998 and 2 January 1999 (Figure 1). The distance between any two locations was at least 20 km. A total of 44 cocoon masses that egressed from stemborer larvae were shipped to the quarantine unit at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya (Table 1). Each cocoon mass was kept separately in a glass vial for adult emergence. It was assumed that the field-collected larvae were each parasitized by a single female wasp suggesting that one female was the founder of each isofemale line. The isofemale lines were named according to the order of collection and the states where they were collected. Three isofemale lines were selected for field releases: Mah2, Mah20 and Katn8, based on the population size of their cultures while ensuring that parasitoids from a wide geographical range were released.

Table 1: Location of collection sites and number of cocoon masses of *C. flavipes* collected from in India, 11 December 1998 - 2 January 1999

District	State	# cocoon masses
Warangal	Andra Pradesh	4
Medak	Andra Pradesh	11
Mahbubnagar	Andra Pradesh	3
Raichur	Karnataka	4
Bijapur	Karnataka	3
Raichur	Karnataka	4
Solapur	Maharastra	6
Osmanabad	Maharastra	6
Ahmadnagar	Maharastra	2
Bid	Maharastra	1
Nanded	Maharastra	4
Total		**44**

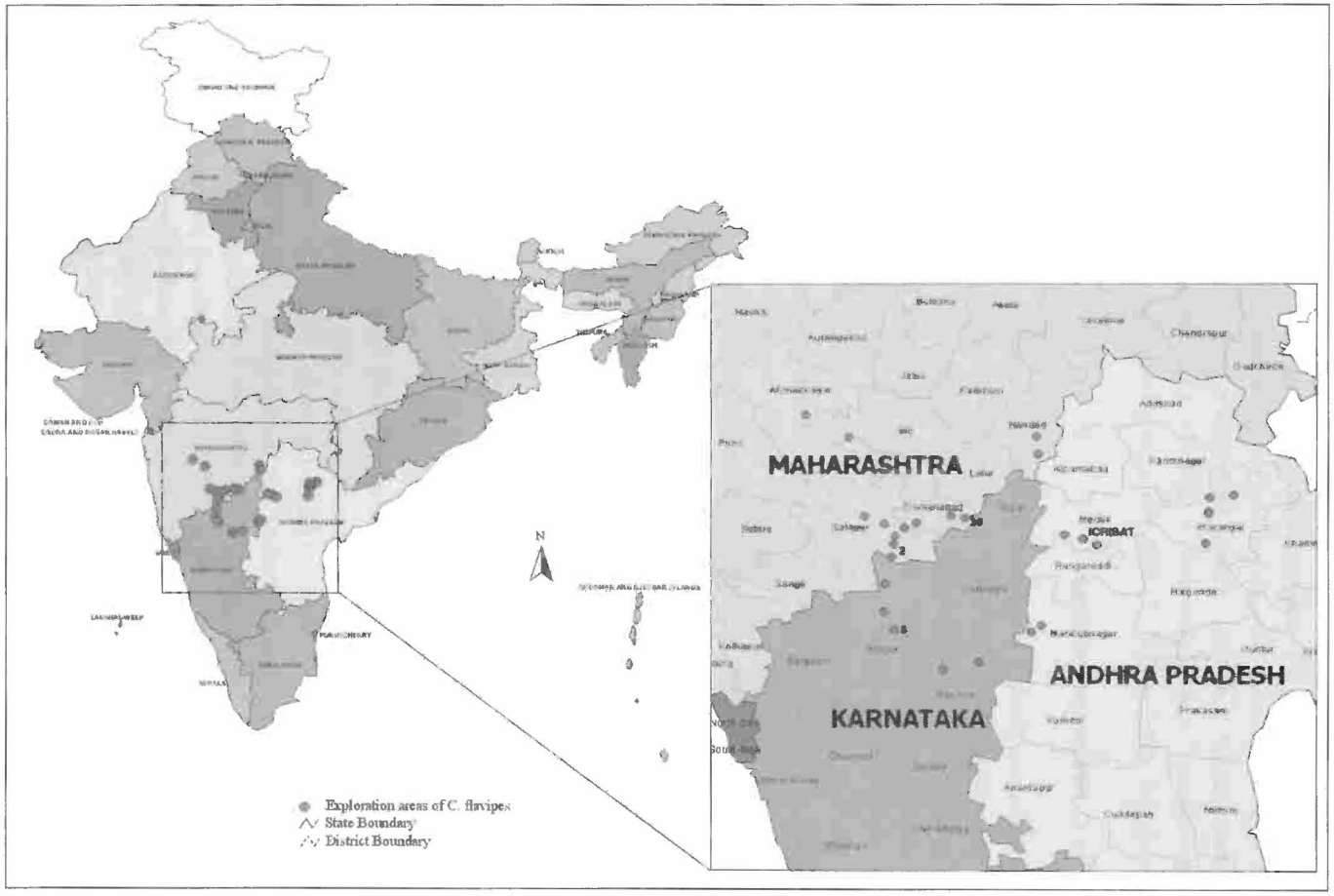

Figure 1: Map of India showing the collection sites of *C. flavipes* in Andra Pradesh, Maharastra and Karnataka states. The collection sites for populations that were imported for field releases are shown: **2** for Mah2; **8** for Karn8 and **20** for Mah20. Populations collected from 26 sites were used to constitute the mixed population.

Newly emerged male and female wasps were aspirated in a cage for mating and after 24 h, each mated female was offered two fourth-instar *C. partellus* larvae per day for parasitization until death. In the 3rd generation, a genetically diverse culture (hereafter referred to as the "mixed population") was established from the remaining 26 isofemale lines by crossing at least ten females from each isofemale line with males from other lines. Cultures of isofemale lines and the mixed population were maintained under similar conditions. *Cotesia flavipes* was reared on fourth-instar *C. partellus* larvae using a "hand-stinging" method (Overholt *et. al*, 1994). *Chilo partellus* larvae were reared on artificial diet (Ochieng *et al.*, 1985) at 25°C, 70-80% RH and a photoperiod of 12L:12D.

Genetic diversity in *C. flavipes* populations

DNA extraction: Genomic DNA was isolated from single female wasps (24 individuals per population) following a slightly modified form of the phenol-chloroform method described by Reineke *et al.* (1998). Individual wasps were placed in vials and ground in liquid nitrogen to a fine powder using a glass rod closed at the tip. Then 100 µl of ice cold Bender buffer [0.1M NaCl, 0.2M Sucrose, 0.1M Tris-HCl (pH=7.5), 0.05M EDTA (pH=8.0), 0.5% SDS], Proteinase K (20 mg/ml) and Rnase H (10 mg/ml) were added and incubated overnight at 37°C. The next morning 100 µl of phenol was added and the mixture was gently shaken for 30 min on a rocking table, followed by centrifugation for 5 min at 4,000 rpm. The supernatant was emulsified with 100 µl of chloroform:isoamyl alcohol (24:1), gently mixed for 15 minutes and centrifuged for 5 minutes at 4,000 rpm. The resulting supernatant was precipitated with 200 µl of 100% Ethanol followed by centrifugation for 10 minutes at 14,000 rpm. The pellet was washed once with 100 µl of 70% ethanol and centrifuged for 5 minutes at 4,000 rpm. Subsequently, the DNA pellet was dried at room temperature for 15-20 minutes after which it was re-dissolved in TE (Tris-EDTA) buffer. A working solution of template DNA (25ng/µl) was used for PCR amplification.

PCR amplification and electrophoresis: One hundred ISSR primers obtained from the Biotechnology Laboratory, University of British Columbia (UBC Primer set no. 9) were tested for polymerase chain reaction (PCR) amplification of the wasp DNA. Seven ISSR primers that showed a clear and reproducible banding patterns were used in this study (Table 2). PCR reactions were carried out in a total volume of 25 µl containing 50 ng of template DNA, 200 µM of dNTP, 0.6 µM of primer, 0.5 U of *Taq* polymerase (SphaeroQ), 2.5µl of 10x buffer (10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$ and 50 mM KCl). PCR amplifications was performed in a Master Cycler gradient (Epperdorf), programmed for an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 35.1-66.6°C for 1 min and primer extension at 72°C for 2 min with a final extension at 72°C for 10 min. Amplified products were separated by electrophoresis on 1.5% agarose gels in 1xTAE (Tris-Acetate-EDTA) buffer (pH 8.0) containing ethidium bromide (0.5 µg/ml). The gels were visualized and photographed under UV light using Kodak Digital Science 1D software. The molecular weight of fragments was estimated by reference to a 100-bp DNA ladder (Biozym).

To detect genetic patterns representative from each of the four populations, a DNA template-mixing strategy was adopted (Furman *et al.*, 1997; Gilbert *et al.*, 1999; Martin & Sanchez-Yelamo, 2000). By this approach, five individuals from each population were pooled and ground together to obtain a working solution DNA as the 'mixed template DNA' prior to electrophoresis. The 'mixed template DNA' was used in PCR reactions to optimize all ISSR primers.

Table 2: ISSR primers used in the study of genetic diversity of *C. flavipes* populations

UBC Primer No.	Sequence	Annealing temperature, Ta (°C)
UBC-817	$(CA)_8$+A	44.2
UBC-835	$(AG)_8$+YC	37.8
UBC-855	$(AC)_8$+YT	35.1
UBC-862	$(AGC)_6$	62.1
UBC-865	$(CCG)_6$	66.6
UBC-888	BDB$(CA)_7$	46.6
UBC-889	DBD$(AC)_7$	52.0

where Y= (C, T), B= (C, G, T) (i.e. not A), D= (A, G, T) (i.e. not C).

Release sites

The study was conducted on Unguja and Pemba, the two main islands of Zanzibar (Figure 2). Release sites (16 on Unguja and 12 on Pemba) were selected as follows: four zones each consisting of four sites were established on Unguja while three zones of four sites each were selected on Pemba. The sites were maize and sorghum plots of approximately 0.5 - 1.0 ha in size and at least 5 km apart, located in the major maize and sorghum growing areas of Zanzibar. All plots were farmers' fields (except in five sites where plots were located at Ministry of Agriculture stations). Sorghum was planted in two plots (both on Pemba) while the other 26 plots were planted with maize. Local seed varieties were sown and no artificial fertilizer or pesticide was applied. All other agricultural methods such as weeding were practiced.

Field release procedure

Cotesia flavipes was reared at ICIPE and cocoon masses were air-freighted to Zanzibar for field releases. Cocoon masses were harvested from cultures in the 15th and 16th generations. Immediately after arrival in Zanzibar, cocoon masses were transported to the field for releases. In a randomized complete block design, Mah2, Mah20, Kam8 and the mixed population were released on Unguja and Pemba islands. Two rainy seasons can be distinguished in Zanzibar: March-June (long rainy season) and October-December (short rainy season). Releases were made in the long rainy season on the 15th and 16th May 2000 on Unguja and Pemba respectively, about 4-5 weeks after plant emergence, to synchronise with the occurrence of medium and large instar larvae (3rd –6th) (Kuelen, 1990) which are preferred host stages of *C. flavipes* (Smith *et al.*, 1993). Releases of 250 cocoon masses of each population were made once in each plot. Assuming approximately 30 females per

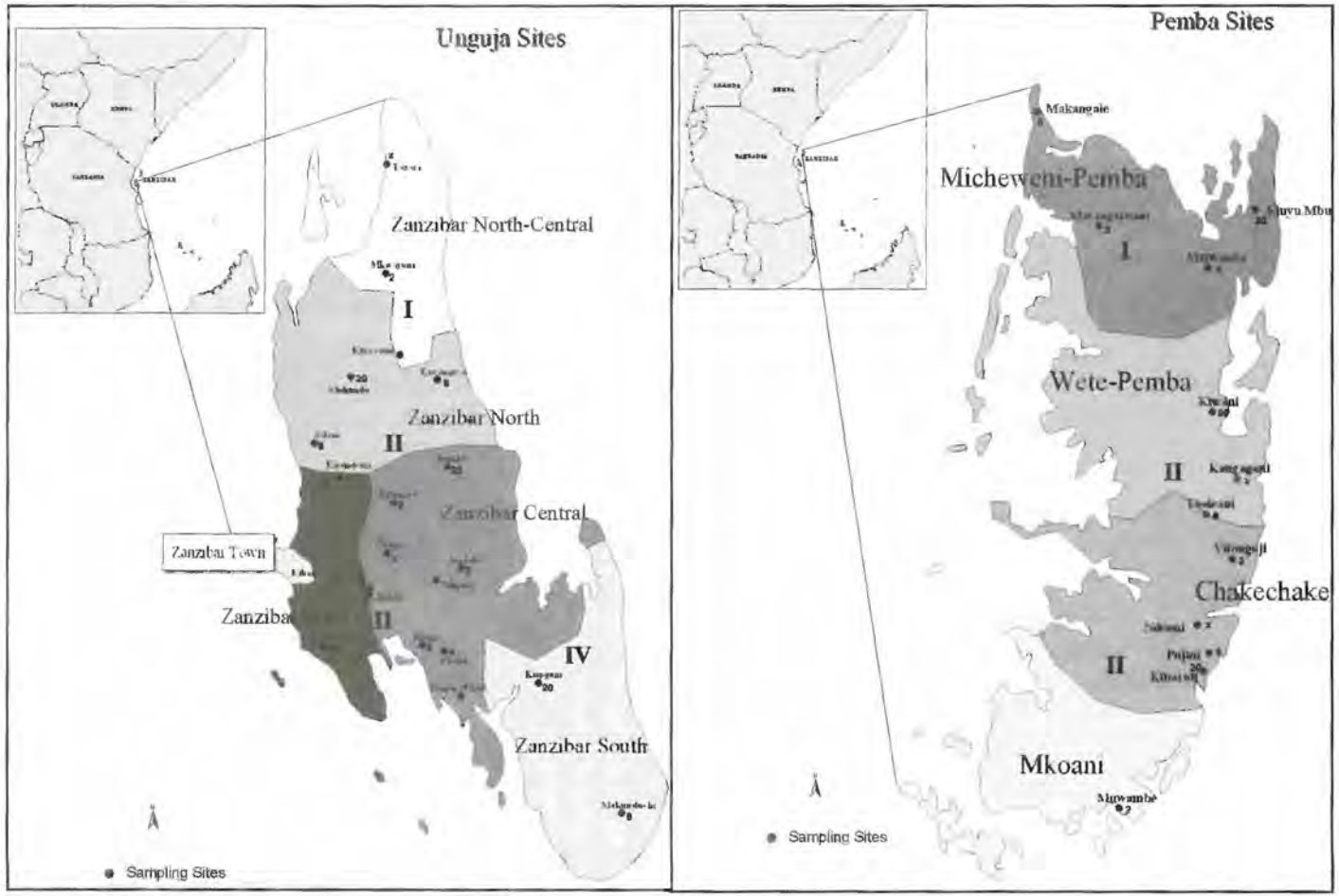

Figure 2: Map of Zanzibar showing the sites on Unguja and Pemba islands where *C. flavipes* was released and sampling was conducted to recover the parasitoid during the long rainy seasons of 2000 and 2001. Dots indicate the sites. A population that was released at each site is shown: **2** stands for Mah2; **8** for Karn8; **20** for Mah20 and **x** for the mixed population. Zones are shown as **I, II, III, IV** and are arbitrary drawn and they do not follow administrative boundaries.

cocoon mass (Overholt, *et al.*, 1994), about 7,500 females were released per plot. To protect the cocoons from predators and rainfall, they were placed in 'release stations' (Figure 3). The 'release stations' were a modification of the structure described in Overholt *et al.* (1994) for field releases of *C. flavipes*. It consisted of a 20 cm x 15 cm x 15cm cuboid chamber fabricated from a plastic container which was obtained locally.

Seventy 6-mm diameter holes were drilled in a 2 cm x 2 cm grid pattern on each side of the chamber to allow parasitoids to exit the station. A plastic lid was provided to cover and allow access to the chamber. The outside of the chamber was painted green to darken the interior and stimulate the parasitoids to exit as there is evidence that *C. flavipes* exhibits positive phototropism (Gifford & Mann, 1967). The station was covered with a metallic sheet (40-cm x 60-cm) painted white to provide shade and shelter, and was hung on a wooden stand 1.5 m above the ground from a 2-m wire. The wire and stand were coated with automotive grease to prevent the entry of ants and other predators. Cocoon masses were placed in a Petri dish (9-cm dia. x 1.5-cm depth) lined with moist filter paper and then placed at the bottom of the chamber. One release station was placed in the middle of each plot.

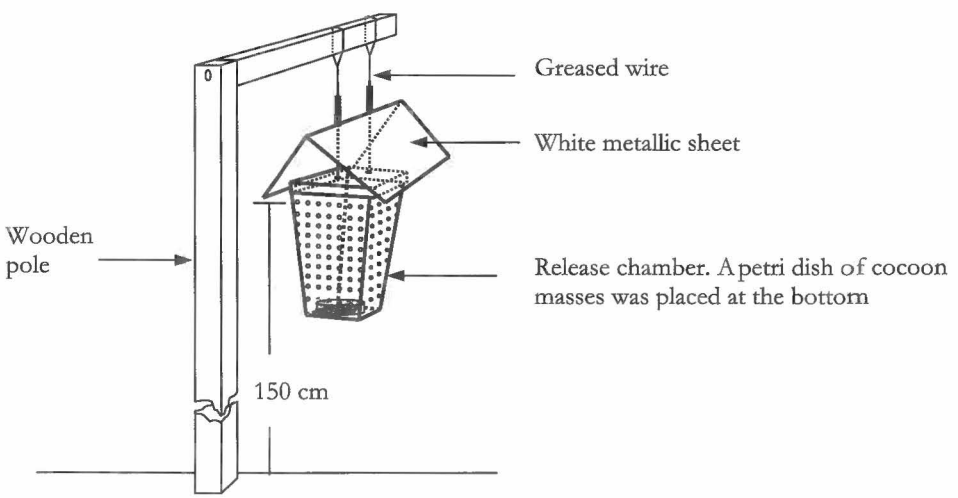

Figure 3: Release station for *Cotesia flavipes*

Monitoring of colonization

The colonization of *C. flavipes* at the release sites was monitored during season of parasitoid releases (LR2000) and one year later during the long rains of 2001 (LR2001). The same sampling protocol was used during both seasons. In LR2000, sampling began two weeks after parasitoid release and was repeated every two weeks thereafter. A stratified random sampling procedure was followed. Each plot was divided into quadrants and on each sampling occasion, 13 plants exhibiting symptoms of stemborer infestation were randomly selected, excised and removed from each

quadrant (a total of 52 plants per plot). Sampling was conducted three times per plot during the release season and three to four times in LR2001. All plants excised from the field were dissected to remove medium to large (3rd –6th instar) larvae from stems, leaf sheaths, ears, ear husks and tassel stems. Larvae were identified, counted and reared individually for parasitoid emergence on pieces of maize stems in glass vials (2.5-cm x 7.5 cm) at room temperature (26.9±2°C). Larvae were inspected for parasitoid cocoon formation, pupation or parasitoid emergence every two days. The stem pieces were replenished at each inspection to avoid fungal attack. Only larvae larger than the 2nd instar were considered for data analysis as *C. flavipes* generally parasitizes 3rd to 6th instar larvae (Ngi-Song, *et al.*, 1995). Parasitoids that were reared from stemborers were identified at the Biosystematics unit of ICIPE in Nairobi, Kenya.

Samples of *C. flavipes* that were recovered at the end of the season were tested using the ISSR markers to determine that they indeed were the offspring of the populations that had been released. DNA was extracted from a single female wasp per cocoon mass that was recovered from each plot using the phenol-chloroform method as described above for genetic diversity analysis. Positive controls, which consisted of genomic DNA of wasps from released populations, were used throughout the PCR analysis.

Statistical analysis

The amplified DNA fragments of each population were scored as present (1) or absent (0). The data was entered into a binary matrix and subsequently analyzed using NTSYS-pc (Rohlf, 1997). Smeared and weak bands were excluded. Pairwise similarities were calculated among individuals within each population using NTSYS-pc with Dice similarity coefficient and the resulting similarity matrices were then used to construct dendrograms using the unweighted pair group method average (UPGMA) clustering procedure. In all populations, individuals were placed into groups of similar genotypes by assigning an arbitrary cut-off point on each dendrogram. The cut-off points, and hence the number of subsequent clusters, varied among populations and depended on the level of diversity within each population. The goodness of clustering of the data matrix was calculated with the COPH and MXCOMP procedures in NTSYS-pc (Rohlf, 1997). To estimate the partitioning of genotypic variation among and within populations, analysis of molecular variance (AMOVA) was performed using the ARLEQUIN program, version 2000 (Schneider *et al.*, 2000). ARLEQUIN program was also used to determine gene diversities.

Colonization was expressed as total parasitism of stemborers in each plot at the end of one season. Parasitism was calculated as the number of cocoon masses that were recovered from the release plots where populations of similar genetic identity were released divided by the number of susceptible stemborer larvae that were collected from the release plots multiplied by one hundred. Data for both islands was pooled. The percentage of parasitized larvae was subjected to arcsin-square root transformation (Sokal & Rohlf, 2001). Analysis of variance (ANOVA, PROC GLM, SAS Institute, 1999-2000) was used to test for differences among mean parasitism by populations during LR2000. When ANOVAs were significant, means were separated using Student-Newman-

Keuls tests (SNK at 1% level). Data for LR2001 was not considered for statistical analysis because the number of cocoon masses recovered during the season were low and sampling was conducted in few sites.

Results

Genetic diversity

The seven primers used generated 50 bands and the most polymorphic pattern was obtained using UBC-865. UPGMA dendrogram showed that there was little variation within populations, except for mixed population, and individuals belonging to the same isofemale population were grouped together. However, the Mah2 came out on two branches with the Karn8 nested within the Mah2. For the mixed population, the individuals did not cluster together, but they were all outliers to the three isofemale populations. Individuals in Mah2 (M) were at least 90% similar, Karn8 (K) about 94% or more, Mah20 (H) about 98-100% similar but the mixed population (X) was only 66% similar. All four populations were 82% similar to each other (Figure 3). The cophenetic correlation value for the dendrogram was 0.958, indicating a very good fit of the clusters to the data. Although AMOVA (Table 4) revealed that the greater part (57.3%) of the variation was found within the populations than among them (42.7%) ($p<0.0001$), in fact, there was an astounding amount of among-population variance in the data as shown by a high Fst value of 0.427. Most of the within population variation can be attributed to the mixed population, in which most of the individuals were more similar to members of isofemale population than to each other (Figure 3). The gene diversity was 0.54 ± 0.08, 0.41 ± 0.10, 0.46 ± 0.07 for Mah2, Karn8 and Mah20 respectively which was low compared to the gene diversity of 0.88 ± 0.03 for the mixed population. The number of polymorphic bands in each population was 1 for Mah20, 4 for both Mah2 and Karn8 and 34 for mixed population.

Table 4: Analysis of molecular variance (AMOVA) of individuals from four populations of *C. flavipes*

Source of variation	d.f.	SSD	Variance components	% of variation	P-value
Among populations	3	16.219	0.21332 Va	42.66	<0.0001
Within populations	92	26.375	0.28668 Vb	57.34	
Total	95	42.594	0.50000		

%, percentage molecular variation explained by the hierarchical level, P-value, level of significance for the distribution of variation for the hierarchical level being different from random.

Stemborer species

The stemborer species that attacked maize and sorghum on Unguja and Pemba during each of two seasons, in descending order of abundance were *Chilo partellus, Sesamiae calamistis* (Hampson

(Lepidoptera: Noctuidae) and *Chilo orichalcociliellus* (Strand) (Lepidoptera: Crambidae). The introduced stemborer, *C. partellus* was the most widely distributed species and represented 78.2% and 94.5% of the stemborer species on Unguja and Pemba, respectively (Table 5).

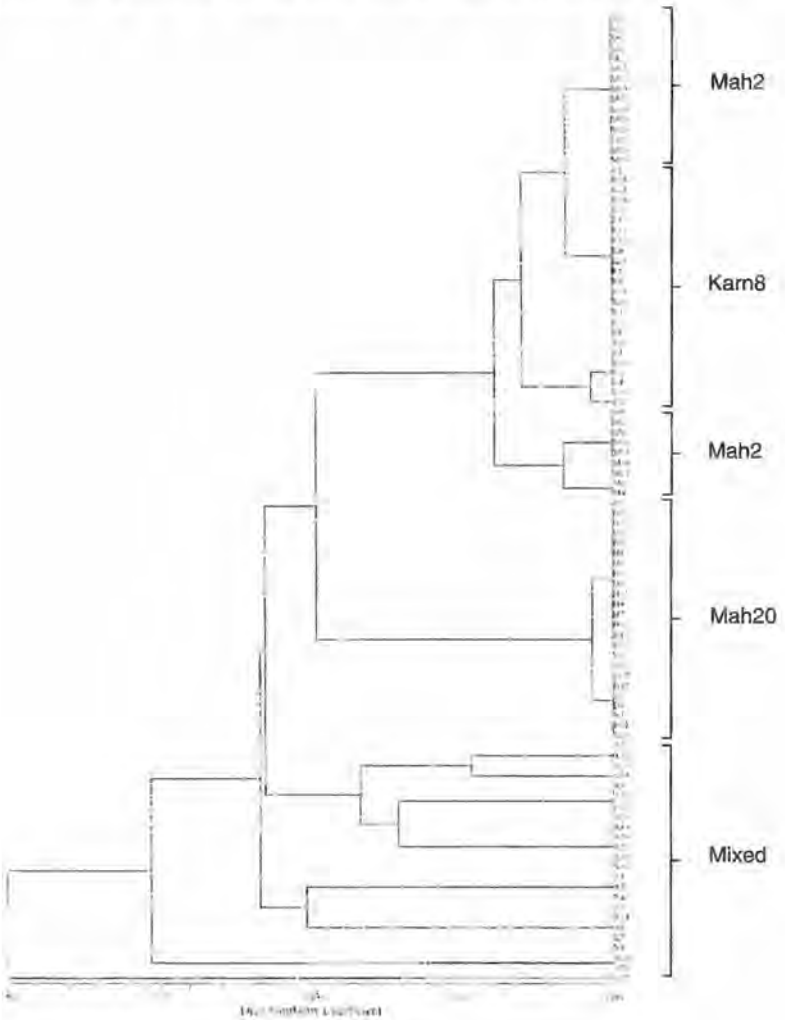

Figure 4. A UPGMA dendrogram of the four *C. flavipes* laboratory populations based on Dice similarity coefficient using ISSR markers. Individuals indicated as M were from Mah2, K from Karn8, H from Mah20 and X from the mixed population.

Colonization of *C. flavipes*

Cotesia flavipes was recovered from all the plots where sampling was conducted (15 plots on Unguja and 11 plots on Pemba) during the release season. Sampling did not take place in two plots (one on each island) because maize plants were harvested from the field before sampling was conducted. *Cotesia flavipes* parasitised all the three stemborer species and it was recovered on each of the three

sampling occasions. All populations were recovered in each sampling occasion, except Karn8 which was not found during the third sampling (Figure 5). A total of 99 and 513 cocoon masses of *C. flavipes* were recovered in LR2000 on Unguja and Pemba, respectively. Parasitism by wasps from the mixed population was higher than that of Karn8 ($F= 4.66$; $df=4$; $p= 0.007$), but it did not differ from parasitism of Mah2 or Mah20 (Figure 6). Higher parasitism was recorded on Pemba (16.7±3.7%) than on Unguja (6.1±1.1%) ($F= 10.4$; $df=1$; $p= 0.004$).

Table 5: Number of stemborers in each zone and percentage of stemborer species recovered from maize and sorghum on Unguja and Pemba islands during LR2000

Island/ Zone	Number of borers	Percent abundance		
		Chilo partellus	*Chilo orichalicociliellus*	*Sesamia calamistis*
Unguja				
Zone I	472	76.5	14.9	8.6
Zone II	569	73.7	17.6	8.6
Zone III	326	79.4	10.4	10.2
Zone IV	521	83.4	3.9	12.4
Total	1888	78.2	11.7	10.1
Pemba				
Zone I	701	99.0	0.0	1.0
Zone II	1428	98.2	0.0	1.8
Zone III	1003	86.4	0.2	13.4
Total	3132	95.4	0.1	5.4

Establishment of *C. flavipes*

During LR2001 season, *C. flavipes* was recovered in 9 of the 15 plots where maize was planted on Unguja and in one plot out of 8 planted plots on Pemba. All the four population were recovered: 10 cocoon masses of Karn8, 4 of Mah2, 3 of Mah20 and 6 of the mixed population were recovered.

Five cocoon masses of the mixed population were recovered in a non-release plot at Tazari in the northern part of Unguja. On Pemba, *C. flavipes* was recovered once from a *C. partellus* larva collected from sorghum in a non-release plot at Dodeani. The point of recovery of *C. flavipes* was about 38 m from the release point of Karn8. No releases were made in this plot in LR2000. The UPGMA dendrogram obtained from cluster analysis of similarity matrices gave similar results as the genetic diversity analysis, with the identification of four main clusters corresponding to the four populations that were recovered during LR2000 and LR2001 (Figure 7).

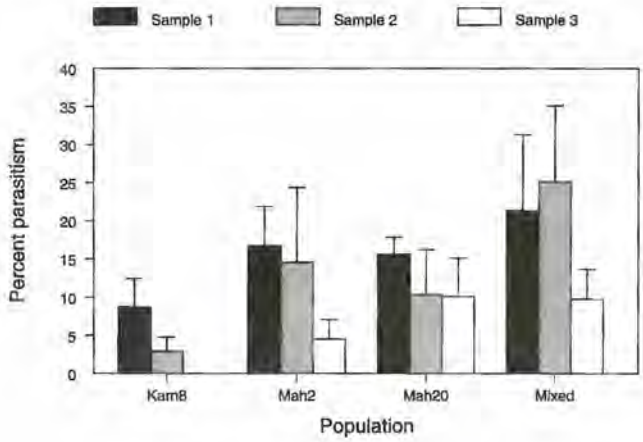

Figure 5. Mean percent parasitism of stemborers by *C. flavipes* from different populations per sampling occasion in the long rainy season of 2000.

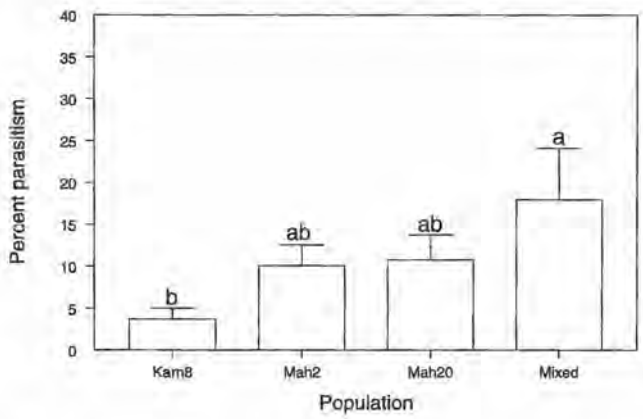

Figure 6: Mean percent parasitism of stemborers by *C. flavipes* from different populations during the long rainy season of 2000.

Discussion

Our results were not conclusive in determining the importance of genetic diversity to colonization and establishment of *C. flavipes*. During the season of release, parasitism by the mixed population was higher than one of the isofemale lines but not significantly different from parasitism caused by the other two isofemale lines. The ISSR technique was found to be useful in assessing the genetic diversity in *C. flavipes*. Higher genetic variability (expressed as gene diversity) was found in the mixed population than in individual isofemale lines. Although the greater part of the variation was detected within the populations than among them, this was mainly attributed to the mixed

population, in which most of the individuals were more similar to members of the isofemale populations than to each other. The similarity values for the isofemale lines were higher than 90% suggesting that little variation within these lines was detected.

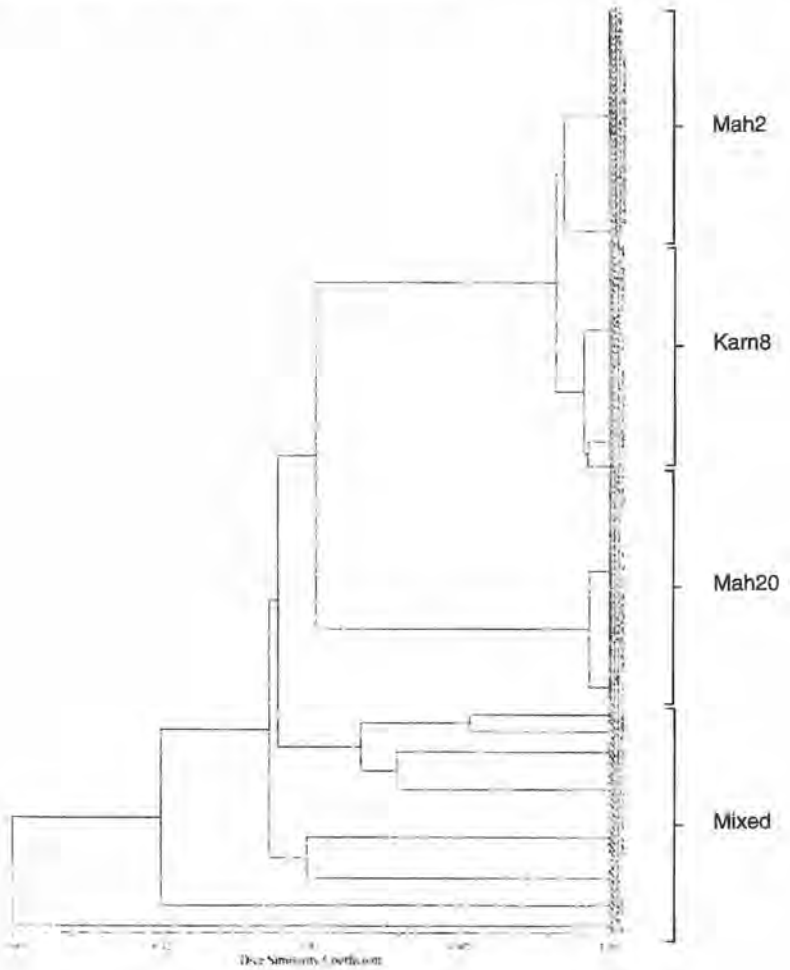

Figure 7. A UPGMA dendrogram of the field recovered samples of *C. flavipes* based on Dice similarity coefficient. Individuals indicated as M were from Mah2, K from Karn8, H from Mah20 and X from the mixed population.

Cotesia flavipes was recovered from the release plots in each of three sampling occasions during the release season. This clearly indicates that the parasitoid populations colonized stemborers in field plots on both islands. All four populations were recovered during the season of release, further indicating that wasps in each population had the ability to search and locate the stemborer hosts in the field. Furthermore, the recovery of *C. flavipes* one year after release confirmed that the

parasitoid was firmly established on the islands. Colonization success, which includes dispersal, search and successful parasitization, was higher for the mixed population than Kam8, but was not different from the other two isofemale lines. This suggests that genetic diversity may not have been an important factor in the colonization process of this species.

It has been proposed that high genetic diversity increases the likelihood of adaptation to the new environment and efficacy of natural enemies introduced for biological control (reviewed in Roush, 1990). However, as far as establishment was concerned, it appears that genetic diversity was not an important factor: Kam8, which caused the lowest parasitism in LR2000, was the most recovered population in LR2001. One possible explanation is that the origin of the parasitioids (central India) has a climate (hot/humid) similar to Zanzibar [21.9 - 30.3°C and 76 - 87% RH (Wirth *et al.*, 1988)], suggesting that all the parasitoids collected in India were pre-adapted to the conditions in Zanzibar, and thus, little difference was seen in their performance. But that is not to say that genetic diversity is not important for colonization. Genetic variability may be more important when a natural enemy is introduced into an area of dissimilar climate from the area of origin.

Genetic variability in individuals within a population exists for different characteristics. Hopper *et al.* (1993) listed examples of variation within populations of biological control agents in traits likely to affect their colonization and efficacy. The list includes traits such as fecundity, field parasitism, longevity, development time, temperature tolerance, temperature preference, insecticide resistance, search rate, locomotion and host acceptance. However, it is not known whether genetic variability for one or more of the above traits exists within these *C. flavipes* populations. The lack of difference in colonization and establishment between the mixed population and some of the isofemale lines suggests that genetic diversity in traits important for performance of *C. flavipes* in Zanzibar was not sufficiently large to be detected in our experimental set up.

Higher parasitism by *C. flavipes* was recorded on Pemba than on Unguja in LR2000. This may be due to the higher proportion of *C. partellus* in the stemborer complex on Pemba (95.4%) than on Unguja (78.2%). Although it was demonstrated under laboratory conditions that *C. partellus*, *C. orichalcociliellus* and *S. calamistis* are suitable for development of *C. flavipes* (Ngi-Song *et. al*, 1995; Ngi-Song & Overholt, 1997), field studies showed that parasitism of *C. partellus* by *C. flavipes* was generally higher than of the native stemborer species suggesting that *C. flavipes* exhibits preference for its co-evolved host under field conditions (Sallam *et al.*, 2001b; Zhou *et al.*, 2003).

There were fewer recoveries in the non-release season of LR2001 than in LR2000. It has been reported that initial parasitoid numbers are often too low to detect a few seasons after release (van Driesche & Bellows, 1996). For instance, in Barbados, where *C. flavipes* was established in sugar cane on *D. saccharalis*, the first recovery was not made until more than 1 year after releases (Alam *et al.*, 1971). Similarly in Madagascar, *C. flavipes* remained rare during the first year after releases (Greathead, 1971). At the Kenya coast, where between 18,100 and 24,200 females of *C. flavipes* were released, recoveries were made at all three sites during the season of release (Overholt *et al.*, 1994).

However, its density was extremely low and difficult to detect one year after the release (Overholt, 1998). In Honduras, 670 cocoon masses of *C. flavipes* were released once in seven sugarcane and four sorghum fields and it was established only in three locations after one year (Marenco *et al.*, 1988).

The spread and survival of introduced natural enemies can be affected by an Allee effect (Hopper & Roush, 1993), due to small release numbers resulting in dispersal and failure to find mates. However, this constraint may not be an important factor in establishment of a gregarious species such as *C. flavipes* which exhibit sibmating. It has been shown that individuals of *C. flavipes* mate with their siblings soon after emergence (Arakaki & Gahana, 1986) and field studies demonstrated that there were few all-male broods recovered in the first generation after field releases of this parasitoid (Overholt *et al.*, 1994; Sallam *et al.*, 2001a), suggesting that they mate before dispersal.

To our knowledge, this is the first field trial to investigate the importance of genetic diversity for colonization and establishment of a natural enemy. The recovery of the parasitoid from almost all the release sites confirms that colonization of stemborers by *C. flavipes* occurred on both islands and its recovery from two non-release plots, one year after field releases were made, indicates that the parasitoid is established and spreading to other areas. Continued monitoring of *C. flavipes* in release and non-release areas will provide information on the spread and impact on stemborer populations. Genetic identification of recovered individuals will be useful in determining the longer term influence of diversity on establishment and performance, as well as provide insights on the mating structure of *C. flavipes*.

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Chapter 7

Summarizing Discussion

The study described in this thesis concerned the importance of genetic variability of the endoparasitoid *Cotesia flavipes* to its performance as a classical biological control agent of lepidopteran stemborers of maize and sorghum. It was part of a larger project on classical control of cereal stemborers in eastern and southern Africa. The project had previously introduced *C. flavipes* into several countries, and establishment has been confirmed in many areas, with variable levels of impact on stemborer populations. Although the success rate is much higher in biological control than in chemical control (1 out of 20 in biological control compared to 1 out of 30,000 in chemical control) (van Lenteren, 1986), it would still be important to increase the success rate further. It has been postulated that one reason for the failures in classical biological control is related to the genetic diversity of released individuals. The current studies were designed to examine the importance of genetic diversity in the establishment and performance of biological control agents. The field studies were conducted on two islands of Zanzibar because *C. flavipes* did not occur there, and thus results would not be confounded with established populations on the mainland. Success may be improved if more is known about the influence of genetic variability on establishment and performance of released biological control agents.

The specific objectives addressed in this research were to: (1) determine the main stemborer species of maize and sorghum in Zanzibar and to identify their parasitoids and levels of parasitism, (2) determine whether foraging behaviour of *C. flavipes* changes over time in laboratory colonies and whether genetic variability is related to changes, (3) investigate the effect of the allelic diversity at the sex locus of *C. flavipes* on its population growth and hence its biological control potential, (4) determine the relationship between the level of genetic variability of a colonizing population of *C. flavipes* and probability of establishment in the field. Each of these subjects are discussed below:

Distribution of stemborers, their parasitoids and levels of parasitism

Three stemborer species were collected from maize and sorghum in Zanzibar of which the exotic stemborer *Chilo partellus* (Swinhoe) (Crambidae) was the most abundant species. *Chilo partellus* is a recent introduction in Africa, having invaded the continent sometime before 1930 when it was first recorded in Malawi (Tams, 1932). Since its invasion, *C. partellus* has spread to nearly all countries in East and southern Africa, often becoming the most damaging species of maize and sorghum particularly in warmer low land areas (Nye, 1960; Van Hamburg, 1979). The earliest available records found in Zanzibar archives indicate that before 1913, *Chilo* sp. was the commonest borer of maize causing crop losses of 50% in some fields (Aders, 1913). However the identity of stemborer species was not stated. Thus the exact origin and period when *C. partellus* invaded Zanzibar remains unclear. Regardless, *C. partellus* may have displaced the indigenous species due to its competitive superiority (Chapter 1).

A total of 17 larval and pupal parasitoid species were reared from stemborers between 1995 and 2000 (Chapter 2). The most common parasitoids included the larval parasitoid *Cotesia sesamiae* and pupal parasitoids *Pediobius furvus*, *Dentichasmias busseola* and *Syzeuctus ruberrimus*. *Cotesia sesamiae* was the most abundant and widely distributed parasitoid and it was reared from all stemborer species. However, parasitism by *C. sesamiae* was low and in the long rainy season of 2000 averaged 7.1% on Unguja and 1.1% on Pemba. Overall parasitism was 3.9% on Unguja and 1.9% on Pemba. Similarly, Overholt (1998) found that although *C. sesamiae* was the most common larval parasitoid in maize in the coastal area of Kenya, generational mortality of *C. partellus* was never greater than 3% and was typically <0.5%. However, in South Africa, Kfir and Bell (1993) found parasitism of *Busseola fusca* in sorghum was as high as 75% at certain times of the year. It has been reported that despite the high number of native parasitoids that attack stemborers, they do not maintain stemborer populations at levels acceptable to farmers (Oloo, 1989; Kfir; 1992; Kfir & Bell, 1993; Overholt *et al.*, 1994).

The underlying reasons to explain the low parasitism rates by indigenous parasitoids in cultivated crops have been discussed by some authors. Conlong (1994) speculated that indigenous parasitoids may have a greater impact on stemborer populations residing in wild grasses than in populations that periodically invade annual crops. In perennial grass habitats, as opposed to cultivated grasses, there is no need for natural enemies to migrate periodically to re-colonize the habitat, and thus stemborers and their natural enemies may be able to approach stability. Secondly, because stemborer survival is much lower in wild grasses than in maize, natural enemies may be better able to maintain stemborer populations below the epidemic growth phase (Wiedenmann & Smith, 1993). Thirdly, there is some evidence that stemborer larval growth is slower in wild plants (Bowden, 1976; Ofomata *et al.*, 2000), which would result in a wider

temporal window of susceptibility to stage-specific parasitoids. Lastly, it has also been reported that natural enemies may experience physical and chemical constraints to host finding in cultivated grasses. Ectoparasitoids that attack concealed hosts by drilling through the stems of small-diameter wild grasses may be unable to parasitize stemborers in the relatively thicker-stemmed maize (Smith *et al.*, 1993; Zhou *et al.*, 2003). Further, olfactory responses of parasitoids to volatiles emitted by wild plants and cultivated grasses may differ (Overholt, 1998).

In this thesis, I did not investigate the parasitism of stemborers in wild grasses but from what is generally known about the biology of stemborers and their parasitoids, the above factors may explain the low stemborer parasitism in most cultivated areas, including Zanzibar. For example, detailed investigations to determine the natural enemies of lepidopterous borers on maize and elephant grass in Cameroon showed that the parasitoid diversity was higher on elephant grass than maize (Ndemah *et al.*, 2001). Similar findings have been reported in India where comparisons of the degree of parasitism of the borers on sugar-cane with that of borers on the wild grasses showed that the larval parasitoids of *Chilo* spp. and *Tryporyza nivella* (F.) were unable to hold these borers in check on sugar-cane. However, eggs and larvae of *T. nivella* on the wild host plants were attacked by a complex of parasites, but only a few species attacked this borer in sugar-cane (Nagarkatti *et al.*, 1973). In order to fill this gap of knowledge for the situation in Zanzibar, I propose that detailed studies be carried out to quantify and compare the degree of parasitism of the borers by indigenous parasitoids on cultivated cereal crops with that of borers on the wild grasses in different locations. The results from such studies would be useful in designing strategies to enhance natural biological control e.g. through habitat management.

Changes in response to host and host plant cues by *C. flavipes* during rearing

Natural enemies used for classical biological control are reared to increase their numbers before field releases. The ability of natural enemies to maintain a high response to host associated cues during laboratory rearing is important for their effectiveness in controlling target pests after release in the field. In Chapter 3, responses of genetically impoverished (isofemale lines) and a genetically diverse population of the introduced stemborer parasitoid, *Cotesia flavipes,* to host finding cues were measured over several generations to examine the effect of duration of laboratory rearing on parasitoid behaviour. Our hypothesis was that the changes in behaviour over time would be expected in the genetically diverse population (if there was adaptation to laboratory conditions) and not fromisofemale lines.

We found that response to host and host plant associated cues by females from isofemale lines and a genetically diverse population decreased in early generations but the overall response varied little with the duration in culture. The mean response of wasps from all populations was higher than 80% indicating that the ability of the wasps to discriminate host- and host plant-associated odours was high and it could persist over many generations of laboratory rearing. Variability in response among populations was found throughout the duration of laboratory rearing and may have a genetic basis (Prevost & Lewis, 1990; Gu & Dorn, 2000). The time taken to make a choice between odours i.e. response time changed little with duration in culture suggesting that once the response behaviour was initiated, the time required to move to odour source did not vary. Behavioural plasticity could be the cause of the apparent shifts in response in the early generations as the rearing procedures did not require wasps to respond to host related cues in order to successfully leave progeny for the next generation.

Our results indicate that wasps from all the isofemale lines (except Karn6) as well as from the mixed population showed stable behavioural responses during laboratory rearing. However, wasps from most populations showed more stable and higher responses to volatiles emitted from *C. partellus*-infested maize plants compared to those from uninfested maize plants. This can be explained by the variable response model, which postulates that the variability of the response to a stimulus is inversely related to its response potential (Vet *et al.*, 1990). Thus, variability is low towards stimuli with a high response potential and high towards stimuli with a low response potential. The most reliable stimuli with the highest response potential in the case of *C. flavipes* are volatiles emitted by *C. partellus*-infested maize plants because they are directly associated with the presence of a host. Volatiles from uninfested maize plants on the other hand, are believed to have low reliability and therefore have only low innate response potential which was shown in the higher variable response of *C. flavipes* towards them. In a related species, Weseloh (1987) observed that females of *Cotesia melanoscela* from cultures maintained in the laboratory for 6 or 25 generations responded similarly to the to gypsy moth silk kairomone.

It can be concluded that the rearing of *C. flavipes* for a number of generations as a isofemale lines and as a mixed population did not result in deterioration of the parasitoid's response to host- and host plant-associated odours. This suggests that laboratory adaptation to the rearing procedure did not occur in the mixed population, possibly due to insufficient genetic variability at loci involved in host finding to allow for selection in the laboratory. I suggest that a study to compare the hand-stinging method with a rearing method by which wasps would have to respond to host volatiles in order to be successful be carried out. Furthermore, there is need to carry out studies to determine the effect of long term maintenance of *C. flavipes* in culture

on other traits e.g. life history characters, that are important for the effectiveness of a biological control agent in the field.

Genetic variability at sex locus of *C. flavipes* and biological control

Populations of biological control agents often go through genetic bottlenecks during collection, rearing and subsequent establishment in the field which results in reduced genetic variability. A reduced genetic variability at the sex locus of some Ichneumonidae and Braconidae can result in biocontrol failures due to: (1) a severe reduction in the population growth, and (2) a male biased sex ratio due to the production of diploid males from fertilized eggs as a consequence of single locus complementary sex determination mechanism (sl-CSD) (Stouthamer *et al.*, 1992). In species with sl-CSD, inbreeding (brother-sister mating) which often occurs during culturing and release in the field, results in production of diploid males.

Cotesia flavipes and *Cotesia sesamiae* (Braconidae) are gregarious and naturally inbreeding species (Arakaki & Gahana, 1986) used in biological control of lepidopteran stemborers (Chapter 2 and Chapter 6). Inbreeding is expected to result in production of diploid males (Periquet *et. al.*, 1992; Butcher *et. al.*, 2000) and hence more male-biased sex ratios and a bimodal distribution of offspring sex ratios should be obtained from field populations of these species if they exhibit sl-CSD (Stouthamer *et. al.*, 1992). Models can be used to predict the frequency of matched matings in populations with different frequencies of sib mating and egg fertilization. The predictions of these models are used to determine if we can use brood sex ratio distributions to determine whether a species determines its sex by sl-CSD. The models showed that sl-CSD can be detected from these brood sex ratios if the diploid male offspring survives. However models need to be supplemented by field data. We supplemented model prediction by analysing the sex ratios of *Cotesia sesamiae* and its exotic congener *Cotesia flavipes* to find out if evidence for the existence of sl-CSD and the production of diploid males in field populations of these species could be found. Our results showed that sex ratio was highly female-biased in *C. sesamiae* (79.0%) and in *C. flavipes* (75.0%). Furthermore, the frequency distribution of sex ratio was unimodal with a peak at 70-95% instead of an expected bimodal distribution if sl-CSD existed. Thus, we found no evidence for the presence of sl-CSD in both braconid species if diploid males survive, but we could not exclude the possibility of presence of sl-CSD if diploid males die before adulthood. Laboratory experiments to determine whether diploid males die may be warranted to further deduce evidence of sl-CSD. Controlled inbred crosses can be used to detect sl-CSD (e.g. Cook 1993; Beukeboom, 1999; 2000; Noda, 1999; Butcher *et al.*, 2000; Chapter 5).

Through crossing experiments, we found that the number of males produced in inbred crosses was different from the number expected in the presence sl-CSD. Inbreeding of *C. flavipes* for >25 generations did not result in male-biased sex ratios. Because none of these tests revealed any evidence for diploid male production in *C. flavipes,* it was concluded that sl-CSD is absent in this species. These results enforce the concept that characteristically inbreeding species such as *C. flavipes, C. sesamiae* and some chalcidoid species do not exhibit sl-CSD (Luck *et al.*, 1992; Stouthamer *et al.*, 1992; Cook, 1993, Cook & Crozier, 1995). It has been proposed that the sl-CSD mechanism poses a genetic load on the population because diploid males are typically sterile and sometimes inviable (Whiting, 1943; Cook & Crozier, 1995). Because inbreeding increases the chance of matched matings with consequent production of diploid males, selection will most strongly favour loss of sl-CSD among naturally inbreeding species (Crozier, 1971).

It can be concluded that a reduced genetic variability at the sex loci of *C. flavipes* may not affect its population growth, and hence its biological control potential, as this species does not exhibit sl-CSD. The sex determination mechanism in *C. flavipes* and other species with similar biologies that do not exhibit CSD remains to be investigated. Regardless, our results show that the parasitoid can be maintained in laboratory cultures for several generations without leading to male-biased sex ratios, thus allowing the production of sufficient numbers for field releases. The methods mentioned in Chapters 4 and 5 can be used to test sex determination in other species.

Genetic variability and colonization of *C. flavipes*

Controversy exists as to the influences of low (homozygosity) and high genetic diversity (heterozygosity) on the fitness and capacity of biological control agents to be effective. Some biocontrol workers (e.g. Remington 1968; Legner 1979; Legner & Warkentin, 1985) do not entirely support the requirement of heterozygosity. Others (e.g. Messenger *et al.*, 1976; Bartlett, 1984; Hopper *et al.*, 1993) have stressed the need to provide maximum genetic variability so that selection can operate after release to yield a population adapted to the new environment. Some studies (e.g. Peschken, 1972; Myers & Sabath, 1981) have provided evidence that adaptation occurs in biological control programmes and that an association exists between genetic diversity of a population and the ecological complexity of a habitat (Jones, 1980).

However, experimental fieldwork to test the theory concerning the importance of heterozygosity is lacking due to the difficulties in manipulating genetic diversity and population size in the field. The establishment of natural enemies for biological control projects offers a very good opportunity to determine the relationship between genetic diversity and the chances of establishment. Through field releases of low genetic

variability (isofemale lines) and high genetic variability *C. flavipes* populations against the stemborer *C. partellus,* it was found that the genetically diverse population showed higher colonization of stemborers than one of the isofemale lines but was not different from the other two isofemale lines. This suggests that genetic variability may not have been an important factor in the colonisation of *C. flavipes*. However, further studies are necessary to monitor the establishment and determine which genotypes adapt to a natural environment. The use of molecular tools like the DNA-based molecular markers mentioned in Chapter 1 and 6, in long term monitoring of *C. flavipes* on Zanzibar will provide greater insight into the importance of genetic diversity for colonization and establishment of *C. flavipes*. Moreover, we would learn more about the mating behaviour of the parasitoid in nature by examining the occurrence of hybridization between populations.

Future perspectives

Populations of parasitoids that were released in Zanzibar all originated from a rather limited area of central India. *Cotesia flavipes* is known to occur widely in Asia, from Nepal to Vietnam. It would seem likely that populations of *C flavipes* better adapted to conditions in Zanzibar could be found in this area. One suggestion could be to use climate matching tools to better target areas in Asia for collection of new genotypes of *C. flavipes* for releases in Zanzibar, and elsewhere in Africa.

Currently, the stemborer control methods practiced by farmers in Zanzibar consist almost exclusively of cultural practices such as residue management and manipulation of planting dates, intercropping and mechanical measures (Feijen *et al.*, 1988; van Keulen, 1990; Fowler, 1997). Many of the cultural control methods require education and labour (Arendse, 1990) and few farmers are aware of the effect of some cultural practices on stemborer attack (Arendse (1990). Moreover, it has been suggested that the effectiveness of cultural control is heavily dependent on the cooperation of neighbouring farmers (Seshu Reddy, 1998). The present extension service is weak and inadequately funded to carry out farmer education (Bruin & Meerman, 2001). Thus, at present, classical biological control is an option for reducing stemborer densities that does not require significant input from farmers.

However, although classical biological control is regarded as a promising approach to sustainable and cost-effective management of lepidopteran stemborers, it has limitations. Classical biological control of stemborers in annual grasses, such as in maize and sorghum, has proven to be only partially successful elsewhere in the world. In Kenya, where *C. flavipes* was released in 1993, stemborer densities have declined, but stemborer still cause serious damage to maize (Zhou *et al*, 2001). The following approaches to improve classical biological control of stemborers in Zanzibar can be suggested: -

The first approach could involve the release other exotic stemborer parasitoids that attack different host stages. Egg parasitoids such as *Trichogramma* spp. and *Telenomus* spp. have been reported to be important stemborer mortality factors in different parts of the world (Alba, 1991; El-Heneidy, 1991; Saroj, 2000; Kishore, 2000). In Zanzibar, little is known about the level of egg parasitism. As a first step, research should be conducted to determine the species composition and degree of mortality caused by egg parasitoids and if found to be low, egg parasitoids could be considered for introduction.

Exotic parasitoids of larvae and pupae could also be considered for introduction. *Sturmiopsis inferens* Townsend (Diptera: Tachinidae) (a larval or larval/pupal parasitoid from Asia) was found to be the predominant parasitoid of *C. partellus* larvae in India early in the season and was then superseded by *Cotesia* spp. at 30 to 40 days after sorghum emergence (Nwanze *et al.*, 1997). Chinwanda & Overholt (2001) speculated that the temporal variations in the pattern of larval parasitism by *Cotesia* spp. and *Sturmiopsis* spp. were due to their different host attack strategies. *Sturmiopsis* spp. larviposit mobile first instar maggots (planidia) at tunnel entrances (Smith *et al.*, 1993), which can move easily through the moist tunnel environment in young plants, while later in the season when stems get older and gradually dry out, the mobility of planidia may become reduced and they possibly desiccate before locating the host larvae. By contrast, *Cotesia* spp. which use an 'ingress and sting' strategy to attack stemborer larvae inside stemborer tunnels (Smith *et al.*, 1993), may be unable to locate host larvae in young stems early in the season as the stemborer tunnels would be clogged by wet and sticky frass. This, in turn, would hamper movement of female wasps as they look for host larvae in which to deposit their eggs. Later in the season, as stalks dry out, the wasps may be able to access host larvae in tunnels more easily. This putative partitioning of the niche would consequently reduce competition between the two parasitoids and make their effects complementary.

Xanthopimpla stemmator (Thunberg) (Hymenoptera: Ichneumonidae) is one of the most common pupal parasitoids of *C. partellus* in Asia (Rao & Ali, 1977; Hussein *et al.*, 1983; Duale & Nwanze, 1999) and has been found to parasitise up to 50% of the pupae of *C. partellus* in India (Rao & Ali, 1977). Host range studies conducted at ICIPE have demonstrated that *X. stemmator* is able to attack not only *C. partellus*, but also several native stemborers, including *C. orichalcociliellus*, *Sesamia calamistis*, *Busseola fusca* and *Eldana saccharina* (Gitau, 2002). It is likely that releases of this parasitoid would result into increased pupal mortality which at present is very low in Zanzibar (Niyibigira *et al.*, 2001).

Another biological control approach is augmentation of natural enemies. For instance, releases of *Trichogramma* spp. on sugarcane in the Philippines at a rate of 100 000 adult

parasitoids/ha resulted in 70.35% parasitism compared with 14.49% parasitism in untreated fields. Releases of parasitoids decreased borer infestations to 4.78% in treated fields compared with 77.7% in untreated fields (Alba, 1991). The effectiveness of these releases depended on synchronization with the appearance of the stemborer eggs. However, it is unlikely that this would be a feasible approach for Zanzibar due to the logistic difficulties (unavailability of mass-reared parasitoids, complexity of releases and inadequate extension services), the high costs compared to the low value of maize and sorghum and the fact that these cereals are not priority crops in Zanzibar.

Pathogens may also have potential use in an augmentation approach: - *Bacillus thuringiensis* (Bt), *Nosema marucae* and *Beauveria basianna* have been found to be effective at reducing stemborer infestation on maize and sorghum in the field in East Africa (Brownbridge, 1989; Maniania, 1991; 1992; 1993; Odindo *et al.*, 1991). As a result, plant damage was lower and a considerable yield increase could be obtained (Maniania *et al.*, 1994; Brownbridge, 1991; Odindo, 1990; Odindo *et al.*, 1990). *Metarhizium anisopliae* proved to be effective in laboratory studies (Maniania, 1991; 1992). The effectiveness of these pathogens depends on the correct timing in order to synchronize with the occurrence of susceptible stages (Bonhof *et al.*, 1997; Brownbridge, 1991). Several virulent strains of the above pathogens have been isolated but appropriate formulations have not been commercialized (Overholt, W.A. pers. comm.) and even if they were, it is unlikely that they would be affordable and widely available to the majority of small-scale farmers. Therefore, although these pathogens can be useful to supplement the current control methods of stemborers in Zanzibar, their cost and availability to farmers would have to be addressed. The feasibility of this option and its compatibility with the present stemborer control methods would also need to be evaluated under Zanzibar conditions.

Botanical formulations such as neem (*Azadirachta indica* A. Juss.) have been shown to be effective against eggs and early larval instars of stemborers (Ganguli *et al.*, 1997; 1998; Akbar *et al.*, 1999; Sharma *et. al.*, 1999; Shamshad *et. al.*, 1999). Moreover it was shown that neem extracts were non-toxic to *C. flavipes* and its parasitisation efficacy (Reddy & Srikanth, 1996) suggesting that it can be used in combination with biological control. Therefore, the use of neem extract can be useful to supplement the current control methods of stemborers, but their use may be limited by the proper timing of application, dosage rates, cost and availability to farmers. Studies to examine these issues and adoption by farmers could be carried out in Zanzibar.

Habitat management approaches, including intercropping maize or sorghum with other crops, may suppress stemborer populations (Mahadevan & Chelliah, 1986; Oloo & Ogedah, 1990; Skovgard & Pats, 1996), but no studies have been carried out in Zanzibar to recommend the best crop combination. Pats *et al* (1997) reported that

intercropping maize with cowpea was an effective way of reducing damage caused by *C. partellus* larvae migrating from neighbouring plants. This finding was confirmed by the reports that 30% of *C. partellus* oviposition in maize/sorghum/cowpea-intercropping systems was on cowpea, and the number of larvae reaching host plants from cowpea decreased with distance (Ampong-Nyarko *et. al.*, 1994a;b). Recent studies in Kenya have reported the effectiveness of controlling stemborers by intercropping maize with the non-host molasses grass, *Melinis minutiflora* (Khan *et al.*, 1997a,b, 2001). Field trials demonstrated that molasses grass, when intercropped with maize, repelled gravid stemborer females from ovipisiting on maize, resulting in significant reduction in stemborer infestation of the main crop. A significant increase in parasitism of stemborers by the larval parasitoid *C. sesamiae* was also observed. The use of molasses grasses has additional advantages: it serves as an effective cover crop, as well as providing a good fodder for livestock. Additionally, Khan *et al.* (1997b) found that napier grass (*Pinnesetum purpureum*) or Sudan grass (*Sorghum vulgare* var *sudanense*) could be planted as a border around maize fields to act as a trap plant for ovipositing borers. When combined with a repellent intercrop, this method has been called the 'pull-push' strategy (Khan *et al.* (1997a). This form of management could be useful for Zanzibari farmers as it provides additional income to those households that practice zero-grazing and therefore it warrants testing under Zanzibar conditions to examine its effectiveness to control stemborers and adoption by farmers. Results from these studies would also provide information on whether intercropping molasses grass enhances parasitism by the exotic parasitoid *C. flavipes* under Zanzibar conditions.

Habitat management may also influence the levels of predation. Studies on the levels of predation of cereal stemborers in Africa suggest that in some environments, predators cause heavy mortality to eggs and young larvae, but not to older life stages (reviewed in Bonhof *et al*, 1997, Bonhof, 1998; 2000; Oloo, 1989; Oloo & Ogeda, 1990). Of the predators identified, ants were frequently considered to be the most important. High variations in predation have been found between different regions which could explain the small impact of predators on stemborer mortality in some environments (Bonhof, 2000). Quantitative studies to determine the generational mortality due to predators may be warranted in Zanzibar. Such studies could examine the factors associated with high predation and whether those factors can be manipulated for improved pest management.

The use of host plant resistance has received little attention in Zanzibar (Feijen *et al.*, 1988) although some maize and sorghum genotypes were found to be resistant/tolerant to stemborer attack under on-station trials elsewhere in East Africa (Ampofo *et al.*, 1986; Seshu Reddy, 1985). A multiple borer resistance (MBR) population was developed using infestation of subtropical stemborer species. Marker-assisted selection is being used in two African countries (Kenya and Zimbabwe) to promote the transfer

of resistance into elite and adapted germplasm. In particular, the integration of biological control with host plant resistance could be a promising option for reducing stemborer populations especially in low-input subsistence farming systems (Nwanze & Nwilene, 1998) as is the case for Zanzibar.

Genetically modified maize with insecticidal properties is widely grown in different areas of the world, and is highly effective in eliminating damage due to stemborers (Rice & Plicher, 1998; Baute *et al.*, 2002; Costa *et al.*, 2002). In Africa, field trials using genetically modified insecticidal maize expressing the *Bacillus thuringiensis* (Bt) toxin are currently being conducted in Kenya, South Africa and Zimbabwe (Rensburg, 1999; Mugo *et al.*, 2001) and may eventually be adopted in Zanzibar. Under the Insect Resistant Maize for Africa (IRMA) project, the transfer of Bt-based resistance to adapted maize germplasm is being done initially in Kenya, but later in other African countries (Mugo *et al.*, 2001). It has been suggested that if genetically modified maize would be available to farmers at a reasonable cost, the benefits would include local and area-wide control of stemborers, improved timing and implementation of control and consequently, improved yields (Rice & Plicher, 1998; Baute *et al.*, 2002; Costa *et al.*, 2002). However, possible limitations to be considered include the possible development of stemborer resistance, affordability of seeds and variable yield performance (Rice & Plicher, 1998; Chaufaux *et al.*, 2001).

Lastly, considering the limitations of the various stemborer control options, the suppression of stemborer population to levels acceptable to farmers could be improved through the integration of biological control with other stemborer control measures mentioned above, in a biologically intensive integrated pest management (BIPM) strategy (Seshu Reddy, 1998). On-farm trials using selected control options among the existing ones in Zanzibar could be conducted under farmers' management conditions to examine their effectiveness and adoption by the farmers. Such field trials using BIPM menus were conducted in western Kenya and results showed that yield increases of maize and sorghum of up to 40% could be achieved (Saxena *et al.*, 1991).

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Summary

Maize and sorghum are important food crops for millions of people in sub-Saharan Africa. Damage caused by lepidopteran stemborers is one of the major constraints for increasing maize and sorghum production. Classical biological control of stemborers is an environmentally rational and viable strategy for decreasing losses due to stemborers. Genetic variability of released natural enemies has been proposed as one of the main causes for the failures in classical biological control. The aim of the study described here was to investigate the importance of genetic variability of the parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) to its establishment and performance as a classical biological control agent of lepidopteran stemborers.

In **Chapter 1**, an overview is given of the most important stemborer pests of maize and sorghum in East Africa, their biology, damage and the methods to control them. Genetic variability in parasitoid populations as an important factor for effectiveness of natural enemies and the genetic changes that may occur during the introduction and rearing of the natural enemies are reviewed. The research objectives are stated and thesis outline is presented.

Field surveys were conducted on two Zanzibar islands to determine the distribution and abundance of stemborer species and their associated indigenous parasitoids in maize and sorghum. The results are presented in **Chapter 2**. Three stemborer species were recorded, of which the exotic species *Chilo partellus* (Swinhoe) (Crambidae) was the most abundant, representing 75.3% of the stemborer populations. The other stemborer species were indigenous: *Chilo orichalcociliellus* Strand (Crambidae) and *Sesamia calamistis* Hampson (Noctuidae). The braconid *Cotesia sesamiae* Cameron was the most common parasitoid and accounted for 85.2% of parasitized larvae. Although ten larval and seven pupal parasitoids were recorded in Zanzibar, they were not able to reduce stemborer density to levels acceptable to farmers. Therefore, consideration for the introduction of exotic parasitoid species to supplement the stemborer mortality caused by the indigenous was needed.

In **Chapter 3,** the response of the introduced stemborer parasitoid *Cotesia flavipes* to host and host plant finding cues were measured over several generations to examine the effect of duration of laboratory rearing on parasitoid behaviour. Tests consisted of five genetically impoverished populations (isofemale lines) and a genetically diverse population. The response of *C. flavipes* to host- and host plant-associated odours was higher in early generations than in later generations, but the overall response of wasps from most populations remained stable regardless of the

genetic variability of the wasp population. There was also little change in response time to odours with duration in rearing.

Genetic variability at the sex locus of parasitoids may affect their population growth and hence their ability to optimally control pests. This is caused by a particular sex determination mechanism known as single locus complementary sex determination (sl-CSD). Under sl-CSD, unfertilized eggs develop as haploid males (hemizygotes) while fertilized eggs develop as diploid females if heterozygous at the sex locus, or as diploid males if homozygous. The production of diploid males reduces population growth because these males would normally develop into females. The presence of sl-CSD was studied in *Cotesia sesamiae* and its exotic congener *Cotesia flavipes* in **Chapter 4** and **5**. In Chapter 4, models were developed to predict the frequency of matched matings in populations with a number of sex alleles, different frequencies of sib mating and egg fertilization. The models showed that sl-CSD can be detected from brood sex ratios if the diploid male offspring survives. Field data were used to supplement the model predictions. It was shown that field sex ratio was highly female-biased, with broods in *C. flavipes* and *C. sesamiae* containing 79% and 75% of female offspring, respectively. The frequency distribution of sex ratio was unimodal with a peak at 70-95% instead of an expected bimodal distribution if sl-CSD with diploid male survival existed in these species. These results suggest that the presence of sl-CSD with diploid male mortality could not be excluded. Inbreeding crosses were conducted to deduce further evidence for the existence of sl-CSD in *C. flavipes* in **Chapter 5**. Sex ratio of *C. flavipes* did not differ among the within-line crosses, between-line crosses and crosses involving individuals from isofemale lines and a genetically diverse population. Brood sizes resulting from within-line crosses (matched matings) were not smaller than those from between-line crosses (unmatched matings) indicating that sl-CSD with diploid male mortality did not occur in within-line crosses. The observed proportion of males in within-line crosses differed significantly from the expected proportion of males if sl-CSD was present both under the assumptions that diploid males survived or that they died during development. Therefore, it was concluded that matched matings in *C. flavipes* did not result in the production of diploid males, and confirmed that sl-CSD was not the sex determination mechanism in this species. In addition, the rearing of *C. flavipes* under inbreeding conditions for at least 25 generations did not result in male-biased sex ratios suggesting that multilocus-CSD was also unlikely to occur in this species.

In **Chapter 6**, field studies to investigate the importance of genetic variability to the colonization and establishment of the introduced stemborer parasitoid *Cotesia flavipes* was carried out through the release of three genetically impoverished populations and one population of high genetic diversity on Unguja and Pemba islands of Zanzibar

The genetically diverse population showed higher colonization of stemborers than one of the isofemale lines, Karn8, but was not different from the other two isofemale lines. This suggests that genetic variability may not have been an important factor in the colonization of *C. flavipes*. A possible explanation could be that the climate of origin of the parasitioids in central India and the release areas in Zanzibar were similar, suggesting that all the parasitoids collected were pre-adapted to the conditions in Zanzibar. Colonization of *C. flavipes*, expressed in terms of parasitism, was higher on Pemba (16.7±3.7%) than on Unguja (6.1±1.1%). All parasitoid populations were recovered on both islands one year after the releases confirming their establishment. The use of DNA-based molecular markers in long term monitoring of *C. flavipes* will provide greater insight into the importance of genetic variability for colonization and establishment of *C. flavipes* on Zanzibar.

In the summarizing discussion, **Chapter 7,** the most important research findings on the importance of genetic variability of the parasitoid *Cotesia flavipes* to its establishment and performance are summarized and discussed in broader context. The main conclusion from this thesis is that genetic diversity exists in populations of natural enemies but it may not be an important factor for their colonization if the natural enemies are introduced in an area with a climate similar to the area of origin. Furthermore, the allelic diversity at the sex loci of *C. flavipes* may not affect its population growth both during laboratory rearing and after release in the field as no evidence for complementary sex determination was found in this braconid. It was also shown that during parasitoid rearing to increase their numbers before field-releases, behavioural responses to host finding cues in most populations remained stable over many generations regardless of the genetic variability of the wasp population. Suggestions for future research to improve the biological control of lepidopteran stemborers in maize and sorghum are presented.

Genetische variatie in *Cotesia flavipes* en zijn belang in de biologische bestrijding van stengelboorders (Lepidoptera)

Samenvatting

Maïs en sorghum vormen een belangrijke voedselbron voor miljoenen Afrikaanse mensen bezuiden de Sahara. Eén van de grootste schade veroorzakers aan de maïs en sorghum produktie zijn stengelboorders (Lepidoptera). Klassieke biologische bestrijding van stengelboorders is milieukundig gezien een rationele en geldige strategie om de verliezen veroorzaakt door de stengelboorders te verminderen. Genetische variatie in vrijgelaten natuurlijke vijanden wordt gezien als één van de hoofdoorzaken van het falen van klassieke biologische bestrijding. Het doel van het hier beschreven onderzoek is om het belang van genetische variatie in de parasitoïde *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) te onderzoeken bij zijn gebruik als klassiek biologisch bestrijdingsmiddel van stengelboorders.

In **Hoofdstuk 1** is een overzicht gegeven van de meest belangrijke stengelboorder soorten die in Oost-Afrika in maïs en sorghum voorkomen met hun biologie, de schade die ze veroorzaken en de bestrijdingsmethodes. Verder zal de genetische variatie in natuurlijk vijanden, die een belangrijke faktor vormt bij hun effectiviteit als plaagbestrijder, aan bod komen en zullen de genetische veranderingen die mogelijk op kunnen treden bij de introduktie en de kweek van natuurlijke vijanden besproken worden. Tevens worden hier de doelen van het onderzoek en de inhoud van het proefschrift gepresenteerd.

Op twee eilanden die deel uitmaken van Zanzibar zijn veldonderzoeken gedaan naar het voorkomen en de verspreiding van stengelboorders en hun bijbehorende inheemse parasitoïden in maïs en sorghum. De resultaten zijn in **Hoofdstuk 2** weergegeven. Er zijn drie soorten van de stengelboorder gevonden, waarvan de exotische soort *Chilo partellus* (Swinhoe) (Crambidae) met 75.3% het meeste voorkomt. De andere stengelboorder soorten zijn inheems: *Chilo orichalcociliellus* Strand (Crambidae) en *Sesamia calamistis* Hampson (Noctuidae). De meest voorkomende parasitoïde is de braconide *Cotesia sesamiae* Cameron, die in 85.2% van de geparasiteerde larven voorkomt. Ondanks het feit dat tien larvale en zeven pop parasitoïden in Zanzibar zijn gevonden, kon de dichtheid van de stengelboorder niet verminderd worden tot een voor de boeren acceptabel niveau. Om de sterfte onder de stengelboorders, die voornamelijk veroorzaakt wordt door de inheemse soorten, te vergroten, is het nodig de introduktie van exotische parasitoïden te overwegen.

In **Hoofdstuk 3** is het effect van langdurige laboratorium kweek op het gedrag van de stengelboorder parasitoïde *Cotesia flavipes* onderzocht. Hierbij is de respons van de geherintroduceerde parasitoïde op het vinden van de gastheer en de gastheer plant gemeten gedurende een aantal generaties. Vijf genetisch verzwakte populaties (homozygote lijnen) en één genetisch diverse populatie maakten deel uit van het onderzoek. De respons van *C. flavipes* op de geuren van de gastheer en de gastheer plant was hoger in eerdere generaties dan in latere generaties, maar de totale respons van de wepsen van de meeste populaties bleef gelijk waarbij de genetische variatie van de wespen populatie niet uitmaakte. De respons tijd op geuren vertoonde ook weinig verschil tussen de populaties met betrekking tot de duur dat de populaties in kweek waren geweest.

Genetische variatie in de sex locus van parasitoïden kan hun populatie groei beïnvloeden en daarmee ook hun vermogen om plagen te bestrijden. Dit wordt veroorzaakt door een bepaald sex bepalings mechanisme dat bekend is onder de naam single locus complementary sex determination (sl-CSD). Onder invloed van sl-CSD zullen onbevruchte eitjes zich ontwikkelen tot haploïde mannetjes (hemizygoten), terwijl bevruchte eitjes zich ontwikkelen tot diploïde vrouwtjes als de sex locus heterozygoot is, of als diploïde mannetjes als de sex locus homozygoot is. De produktie van diploïde mannetjes reduceert de populatie groei, omdat deze mannetjes zich normaal gesproken tot vrouwtjes zouden ontwikkelen. De aanwezigheid van sl-CSD is onderzocht in *Cotesia sesamniae* en zijn exotische soortgenoot *Cotesia flavipes* in **Hoofdstuk 4** en **5**. In **Hoofdstuk 4** zijn modellen ontwikkeld die de frequentie paringen tussen individuen met dezelfde allelen voorspellen in populaties met een aantal sex allelen en verschillende frequenties van broer-zus paringen en eibevruchting. De modellen lieten zien dat sl-CSD gedetecteerd kan worden in de sex ratio van broedsels als de diploïde mannelijke nakomelingen levensvatbaar zijn. Veld gegevens werden gebruikt om de voorspellingen van de modellen te valideren. De sex ratio in het veld bleek voornamelijk vrouwelijk te zijn. De broedsels van *C. flavipes* en *C. sesamiae* bestonden respektievelijk voor 79% en 75% uit vrouwelijke namkomelingen. De frequentie distributie van de sex ratio bleek unimodaal met een piek tussen 70 en 95% in plaats van de verwachte bimodale distributie indien sl-CSD in deze soorten zou voorkomen en de diploïde mannetjes zouden overleven. Deze resultaten suggereren dat de aanwezigheid van sl-CSD waarbij diploïde mannetjes sterven niet kan worden uitgesloten. In **Hoofdstuk 5** zijn broer-zus paringen uitgevoerd om meer bewijs te leveren voor de mogelijke aanwezigheid van sl-CSD in *C. flavipes*. De sex ratio in *C. flavipes* verschilde niet tussen kruisingen binnen één lijn, kruisingen tussen twee lijnen en kruisingen tussen individuen van een homozygote en een heterozygote lijn. De broedsel groottes van kruisingen binnen één lijn waren niet kleiner dan de broedsel groottes van kruisingen tussen twee lijnen. Dit impliceert dat sl-CSD waarbij diploïde

mannetjes sterven niet voorkomt in kruisingen binnen één lijn. De waargenomen hoeveelheid mannelijke nakomelingen in kruisingen binnen één lijn verschilde significant met de verwachte hoeveelheid mannelijke nakomelingen indien sl-CSD aanwezig zou zijn, zowel bij de aanname dat diploïde mannetjes zouden overleven als bij de aanname dat ze zouden sterven tijdens de ontwikkeling. Daarom kan geconcludeerd worden dat paringen tussen individuen met dezelfde allelen in *C. flavipes* niet resulteerden in de productie van diploïde mannetjes. Dit bevestigt de waarneming dat sl-CSD niet het sex bepalings mechanisme is in deze soort. Daarnaast resulteerde het intelen van *C. flavipes* gedurende minstens 25 generaties niet in een mannelijke sex ratio, wat suggereert dat multi-locus CSD ook waarschijnlijk niet voorkomt in deze soort.

In **Hoofdstuk 6** is met behulp van veldonderzoek het belang van genetische variatie onderzocht bij de kolonizatie en vestiging van de geïntroduceerde stengelboorder parasitoïde *C. flavipes*. Bij dit onderzoek werden drie genetisch verzwakte populaties (homozygoot) en één populatie met een hoge genetische variatie vrijgelaten op Unguja en Pemba, twee eilanden van Zanzibar.

De genetisch diverse populatie vertoonde een grotere kolonizatie van stengelboorders dan één van de homozygote lijnen, Kam 8, maar verschilde niet met de andere twee homozygote lijnen. Dit impliceert dat genetische variatie geen belangrijke rol speelt in de kolonizatie van *C. flavipes*. Een mogelijke verklaring voor de gelijke mate van kolonizatie zou kunnen zijn dat het klimaat van de plek waar de parasitoïden verzamelt zijn in centraal India en van het gebied waar de wespen zijn vrijgelaten in Zanzibar gelijk zijn. Hierdoor zouden de parasitoïden reeds aangepast kunnen zijn aan de klimaatomstandigheden in Zanzibar. De kolonizatie van *C. flavipes*, uitgedrukt in de mate van parasitisme, was groter op Pemba (16.7%±3.7%) dan op Unguja (6,1%±1,1%). Alle parasoïtoden populaties zijn één jaar na hun vrijlating op beide eilanden weer teruggevonden wat hun vestiging op beide eilanden bevestigt. Of genetische variatie voor de kolonizatie en vestiging van *C. flavipes* van belang is, zal blijken uit het op lange termijn onderzoeken van *C. flavipes* met gebruik making van op DNA gebaseerde moleculaire markers.

In **Hoofdstuk 7** worden de meest belangrijke onderzoeks resultaten over het belang van genetische variatie van de parasitoïde *C. flavipes* voor zijn vestiging en voorkomen samengevat en bediscusieerd. De hoofd conclusie van dit proefschrift is dat genetische diversiteit voorkomt in populaties van natuurlijke vijanden, maar dat het niet van belang is voor kolonizatie indien de natuurlijke vijanden worden geïntroduceerd in een gebied met hetzelfde klimaat als het gebied waar ze oorspronkelijk vandaan komen. Verder zou variatie in de allelen op de sex loci van *C. flavipes* geen invloed kunnen hebben op de populatie groei tijdens de kweek in het

laboratorium en de daaropvolgende vrijlating in het veld, omdat geen bewijs voor complementaire sex determinatie gevonden is in deze braconide. Gedurende de kweek van de parasitoïden om hun aantallen te vergroten voor de vrijlating in het veld bleven de gedrags reakties van de parasitoïden op gastheer zoek impulsen in de meeste populaties stabiel gedurende vele generaties. De genetische variatie van de wesp populaties had hier geen invloed op. Tot slot worden er nog suggesties gedaan voor toekomstig onderzoek om de biologische bestrijding van stengelboorders (Lepidoptera) in maïs en sorghum te verbeteren.

Resumé

Le maïs et le sorgho sont d'importantes cultures vivrières pour des millions d'habitants de l'Afrique Sub-Saharienne. Les dégats causés par les lepidoptères foreur du maïs sont un frein important à l'augmentation de la production de maïs et de sorgho. La lutte biologique classique contre les foreurs du mais est une méthode rationelle d' un point de vue environnemental et viable pour diminuer les pertes causées par les foreurs. La variabilité génétique des auxiliaires est souvent considérée comme l'une des cause d' échec les plus importantes en lutte biologique classique. Le but de ce travail est d'étudier l'importance de la variabilité génétique du parasitoïde Cotesia flavipes Cameron (Hymenoptera: Braconidae) pour son établissement et ses performances en tant qu'auxiliaire pour la lutte biologique contre les lepidoptères foreurs du mais.

Dans le **chapitre 1**, un inventaire est fait des espèces de foreurs ravageurs du maïs et du sorgho en Afrique comprenant des aspects de leur biologie, des dégats infligés et des méthodes de lutte. La variabilité génétique des populations de parasitoïdes comme facteur important de la qualité des auxiliaires et les changements génétiques qui peuvent apparaitre lors de l'introduction et l'élevage des auxiliaires sont traités de manière approfondie. Les objectifs de recherche sont présentés ainsi qu' une vue d'ensemble la thèse.

Des prélèvements aux champs ont été effectués dans deux îles du Zanzibar afin de déterminer la distribution et l'abondance des espèces de foreurs et des parasitoïdes indigènes qui leurs sont associés sur le maïs et le sorgho. Les resultats sont presentés au **chapitre 2**. Trois espèces de parasitoides ont été capturées. L'espèce exotique *Chilo partellus* (Swinhoe) (Crambidae) était la plus abondante, representant 75.3 % de la population totale des foreurs de maïs. Les autres espèces etaient indigènes: *Chilo orichalcociliellus* Strand (Crambidae) et *Sesamia calamistis* Hampson (Noctuidae). Le braconide le plus commun etait *Cotesia sesamiae* Cameron, étant présent dans 85.2% des larves parasitées. Bien qu'on aie enregistré à Zanzibar 10 espèces de parasitoïdes larvaires et sept espèces de parasitoides pupaires, ces parasitoïdes n'étaient pas capables de controller la populations des foreurs de maïs à des niveaux de densité acceptables pour les paysans. Ainsi donc il était nécessaire d'envisager l'introduction d'un parasitoïde exotique pour augmenter la mortalité causée par les parasitoïdes indigènes.

Dans le **chapitre 3**, on a evalué la réponse du parasitoïde du foreur introduit, *Cotesia flavipes*, aux odeurs de plantes et de leurs hôte. Ces tests ont été effectués sur plusieurs générations afin d'évaluer l'effet de la durée d'élevage en laboratoire sur le comportement du parasitoïde. Les tests ont été effectués sur cinq populations à faible

variabilité génétique (lignées iso-femelle,) et une population à forte variabilité génétique. La réponse de *C. flavipes* aux odeurs de plantes et de leurs hôtes était plus importante dans les premières générations que dans les générations ultérieures. Par contre la réponse moyenne des guêpes est restée la même quelque soit la variabilité génétique des populations. On a observé également une très faible variation du temps de réponse en fonction de la durée d'élevage en laboratoire.

La variabilité génétique au niveau du locus sexuel des parasitoïdes peut affecter la croissance de la population et donc la capacité des guêpes a controller les ravageurs de façon optimale. Ceci est causé par un certain mécanisme de détermination du sexe appelé détermination sexuelle complémentaire unilocus (*single locus complementary sex determination* ou sl-CSD). De par ce mécanisme, les oeufs non fertilisés forment des mâles haploïdes (hemizygotes) alors que les oeufs fertilisés forment des femelles si ils sont hétérozygotes au locus sexuel ou des mâles diploïdes s'ils sont homozygotes. La production de mâles diploïdes engendre une diminution de la croissance de la population car ces mâles auraient dû être des femelles. La présence de sl-CSD est étudée pour *Cotesia sesamiae* et son homologue exotique *Cotesia flavipes* aux **chapitres 4 et 5**. A **chapitre 4,** differents modèles ont été développés afin de prédire la fréquence d'accouplement entre individus ayant le même allèle au locus sexuel (*match mating*) dans des populations avec un certain nombre d'allèles sexuels, une certaine frequence de copulations entre frères et soeurs et different taux de fertilisation. Le modèle montre que la présence de sl-CSD peut être détectée en fonction du sexe-ratio au sein de la progéniture si du moins les mâles diploïdes survivent. Les données recueillies aux champs ont été utilisées pour compléter les prédictions du modèle. La proportion de femelles est plus grande que la proportion de mâles aux champs, atteignant respectivement 79% et 75% de la progéniture pour *C. flavipes* et *C. sesamiae*. La distribtion de la fréquence du sexe-ratio était unimodale, avec un pic arteignant 70-95% au lieu de la distribution bimodale escomptée dans le cas du sl-CSD avec survie des males diploides. Ces résultats suggèrent donc que l'existence de sl-CSD avec mortalité des males diploïdes ne peut être exclue. Au **chapitre 5** des croisements entre individus d'une même lignée ont été effectués afin de trouver d'autres indices de la présence de sl-CSD chez *C. flavipes*. Le sexe-ratio de *C. flavipes* est resté le même que les croisements soient effectués à l'intérieur d'une même lignée, entre lignées ou entre individus de populations génétiquement diverses et les lignées iso-femelles. La production de descendants issue des croisements au seins d'une même lignée n'étaient pas moins importante que celle issue des croisement entre lignées ce qui indique que le phénomène de sl-CSD avec mortalité des mâles diploïdes n'apparait pas lors de croisements au sein d'une lignée. La proportion observée de mâles dans les croisements d'individus issus d'une même lignée était significativement differente de la proportion escomptée de mâles si le phénomène de sl-CSD était présent que l'on assume la mortalité des mâles ou pas. En conséquence, on peut conclure que les

match mating chez *C. flavipes* n'engendrent pas la production de males diploïdes et que le phénomène de sl-CSD ne joue aucun role dans le mécanisme de determination du sexe de cette espèce. De plus, l'élevage de *C. flavipes* dans des conditions de consanguinité pendant au moins 25 générations, n'a pas provoqué une augmentation de la proportion de mâles, ce qui suggère que le multi-locus CSD n'existe probablement pas chez cette espèce.

Au **chapitre 6**, des tests aux champs ont été effectués pour évaluer l'importance de la variabilité génétique sur la capacité de colonisation du parasitoïde du foreur de mais *Cotesia flavipes*. Ces tests ont été réalisés par le lâcher de trois populations de *C. flavipes* à faible variabilité génétique (lignées iso-femelles) et d'une population à forte variabilité génétique dans les îles Unguja et Pemba au Zanzibar. La population à forte variabilité génétique était plus efficace que l'une des lignées iso-femelles, la Karn 8, pour parasiter les foreurs, mais on n'a pas constaté de differences avec les deux autres lignées iso-femelles. Ces résulats suggèrent que la variabilité génétique n'est probablement pas déterminante pour la colonisation de *C. flavipes*. Une des raisons que l'on peut évoquer est que le climat d'origine des parasitoïdes dans le centre de l'Inde, et celui des zones de lâchers au Zanzibar sont similaires, ce qui suggère que les parasitoïdes étaient préadaptés aux conditions du Zanzibar. La colonisation de *C. flavipes*, était plus importante à Pemba (16.7±3.7%) qu'à Unguja (6.1±1.1%). Toutes les populations de parasitoïdes ont été recapturées sur les deux îles un an après les lâchers, ce qui confirme leur établissement. L'utilisation de marqueurs moléculaires d'ADN dans le suivi de *C. flavipes* permettra une plus grande perception de l'importance de la variabilité génétique pour la colonisation et l'établissement des populations de *C. flavipes* au Zanzibar.

Au cours de la discussion, au **chapitre 7**, les plus importantes découvertes de ce travail de recherche sur l'importance de la variabilité génétique du parasitoïde *Cotesia flavipes* pour son établissement et ses performances sont résumées et discutées dans un contexte élargi. La principale conclusion de ce travail est que bien que la variabilité génétique existe au sein des populations d'auxiliaires, celle-ci n'est pas forcemment un facteur déterminant pour la colonisation si du moins le climat dans lequel les auxiliaires sont introduits est similaire au climat d'origine. De plus la diversité des allèles au locus sexuel de *C. flavipes* n'est probablement pas un frein à la croissance de sa population aussi bien pour les élevages en laboratoires qu'après le lâcher aux champs puisque aucune preuve de la présence de détermination sexuelle complémentaire (CSD) a été observée chez les braconides. Lors de l'élevage de masse en vu des lâchers, les réponses comportementales quant aux odeurs pour la recherche des hôtes sont restées stables sur plusieurs générations quelque soit la variabilité génétique des populations de guêpes. Des suggestions sont faites pour des recherches

futures en vu d'améliorer la lutte biologique contre les lépidoptères foreurs du maïs et du sorgho.

Curriculum vitae

Emmanuel Iyamulemye Niyibigira was born in Kalambi, Kisoro District in south west of Uganda on December 18, 1964. He attended Busengo and Rwanzu Primary Schools and between 1981-1987, he was at Kigezi College Butobere for his secondary school education ('O'- and 'A'-levels). In 1990, he graduated at Makerere University with B.Sc. majoring in Zoology and Biochemistry and the same year, he enrolled for a Postgraduate Diploma in Education specializing in teaching Biology and Chemistry.

After graduation he worked briefly as a secondary school teacher before joining Coffee Research Unit of Kawanda Agricultural Research Institute (KARI) under the Farming Systems Support project as a Research Trainee. In September 1992, he was employed in civil service as Research Entomologist under the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) and was posted to Animal Health Research Centre, Entebbe. Following the research department of MAAIF gaining autonomy and the inception of the National Agricultural Research Organisation (NARO), from 1994 to 1995 he worked under NARO with a duty station at the Livestock Health Research Institute (formerly EATRO).

Between August 1995 and January 1997, he pursued MSc. in Crop Science specializing in Crop Protection at Wageningen University, The Netherlands. He conducted his thesis research in the Laboratory of Entomology on biological control of storage pests on a topic entitled "*Competition and maternal age of Callosobruchus maculatus Fab.; pest of stored cowpea and their effect through egg size on the fitness of egg parasitoid Uscana lariophaga Steffan*". He was awarded MSc. with Distinction. After completion of MSc, he re-joined NARO and was deployed in Horticulture programme at KARI. He also worked briefly with Uganda Coffee Development Authority.

He returned to Wageningen University in August 1998 to start a PhD with a fellowship from The Netherlands Foundation for the Advancement of Tropical Research (WOTRO). This doctoral thesis is a result of the research he carried out in the Laboratory of Entomology at Wageningen University, The International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya (under a Dutch-funded project on biological control of cereal stemborers). He was affiliated to ICIPE as a Dissertation Research Internship Programme (DRIP) scholar. The fieldwork was conducted on Zanzibar islands of Tanzania in collaboration with the Zanzibar Plant Protection Department. As part of the research, he spent two months at The International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India.

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