POPULATION GENETICS OF THE MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* IN THE WESTERN CAPE PROVINCE, SOUTH AFRICA: INVASION POTENTIAL AND DISPERSAL ABILITY

by
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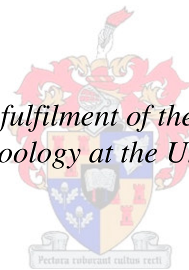

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ABSTRACT

The Mediterranean fruit fly (medfly), *Ceratitis capitata*, is a highly invasive species throughout the world and considered as one of the most successful agricultural and economical pests. The increase of global trade in fruit and human travel combined with the biology of the medfly has allowed the species to spread from its proposed Afrotropical origin, to a number of locations throughout the world. In the Western Cape various control strategies have been implemented to control medfly populations, including insecticides and more environmentally-friendly techniques such as the Sterile Insect Technique (SIT). In order to be effective, however, an SIT program requires some knowledge of the population structure and the movement of individuals between pest-occupied sites. The identification of sites from which re-invasion is most likely to occur and knowledge regarding the primary routes through which pests are likely to re-establish are critical to ensure successful SIT programmes. To provide this important information to SIT and area-wide pest control programs in South Africa, sampling at two different spatial scales (regional- and fine-scale) in South Africa was undertaken. Regional scale sampling was done at 13 locations in the Western Cape and fine scale sampling was done at 13 locations within the Ceres-valley. All individuals were genotyped at 11 polymorphic microsatellite markers and selected individuals from the regional scale were sequenced for the mitochondrial gene COI. Our results show that populations at regional- and fine-scale in the Western Cape are characterized by high levels of genetic diversity ($H_{\mathrm{Eregional}}$ = 0.805; H_{Efine} = 0.803). Little or weak population differentiation was detected at the regional- and fine-scales, suggesting overall high levels of gene flow among sampling locations. These findings were supported by coalescent based methods indicating sufficient levels of gene flow to prevent population differentiation between neighbouring (200 m) and distant (350 km) populations. However, natural dispersal in *C. capitata* has been shown to rarely exceed 10 km. As such, high levels of gene flow between distant populations are more likely the result of human-mediated dispersal, linked to the movement of fresh produce within South Africa. This high level of gene flow has important implications for pest management practices, as my results suggests that

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area-wide pest management should be undertaken at a regional scale, rather than on a farm or valley scale. My results are placed within a management framework, and I argue for more stringent control when fruit are transported within South Africa. Of particular interest for future studies is the investigation of gene flow at broader spatial scales (i.e. the whole of South Africa) and a comparison of the genetic diversity, population differentiation and gene flow patterns of *C. capitata* with that of *Ceratitis rosa* will be important to establish a successful pest management strategy in South Africa.

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OPSOMMING

Die Mediterreense vrugtevlieg (medvlieg), *Ceratitis capitata*, is 'n indringerspesie wêreldwyd en word beskou as een van die mees suksesvolle ekonomiese en landbou peste. Die medvlieg het 'n Afrotropiese oorsprong, maar die toename in wêreldwye handel en reis, gekombineer met die biologie van die medvlieg het gelei tot die verspreiding van die spesie na 'n groot aantal bestemmings regoor die wêreld. Die Wes-Kaap provinsie van Suid-Afrika implementeer tans verskeie strategieë om medvlieg bevolkings te beheer. Hierdie strategieë sluit in die gebruik van plaagdoders sowel as meer omgewingsvriendelike tegnieke soos die Steriele Insektegniek (SIT). Om 'n effektiewe SIT program te implementeer vereis basiese kennis ten opsigte van die genetiese struktuur van die bevolking sowel as van die beweging van individue tussen verskillende pes-voorkomsgebiede. Die identifisering van areas van waar herkolonisering mees waarskynlik kan plaasvind en kennis in verband met die primêre roetes waardeur pes spesies hervestig, is van kritiese belang om 'n suksesvolle SIT program te verseker. Medvlieg individue is op twee verskillende ruimtelike skale (streeks- en plaaslike-skaal) versamel om die nodige inligting aan SIT en area-wye pes beheer programme in Suid-Afrika te verskaf. Streeks-skaal individue is by 13 lokaliteite regoor die Wes-Kaap versamel en plaaslike-skaal individue by 13 lokaliteite in die Ceres-vallei. Alle versamelde individue is vir 11 polimorfiese mikrosatelliet merkers gegenotipeer en DNS volgordebepaling van geselekteerde individue vanuit die streek-skaal is gedoen vir die mitochondriale geen COI. My resultate toon dat bevolkings op beide skale gekarakteriseer word deur hoë vlakke van genetiese diversiteit (H_{Estreeks} = 0.805; $H_{\mathrm{Eplaaslik}}$ = 0.803) en geen of swak bevolkings differensiasie. Hierdie resultate dui daarop dat daar hoë vlakke van geenvloei tussen bevolkings is. Hierdie bevindinge word verder ondersteun deur metodes gebaseer op die statistiese eienskappe van die genealogiese verhouding tussen allele onder sekere mutasie en demografiese modelle, wat voldoende vlakke van geenvloei aandui tussen nabye (200 m) sowel as verafgeleë (350 km) bevolkings om bevolkings differensiasie te verhoed. Natuurlike beweging in *C. capitata* is egter selde meer as 10 km, sodanig kan die hoë vlakke van geenvloei toegeskryf word aan die

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verspreiding van individue met menslike hulp, spesifiek in die vervoer van varsprodukte in Suid-Afrika. Hierdie hoë vlak van geenvloei het verreikende implikasies vir pes beheer praktyke, omdat my resultate voorstel dat area-wye pes beheer onderneem moet word op ‘n streeks-skaal eerder as op ‘n plaas-tot-plaas of vallei wye area. Ek plaas my resultate in ‘n bestuursraamwerk, waarin ek streng beheer van vrugtevervoer in Suid-Afrika beklemtoon. Verdere navorsing moet fokus op die ondersoek van geenvloei op ‘n landswye skaal (hele Suid-Afrika) in *C. capitata*, sowel as die vergelyking van die genetiese diversiteit, bevolkings differensiasie en geenvloei patrone van *C. capitata* met die van *Ceratitis rosa* om ‘n suksesvolle pes beheer strategie vir Suid-Afrika te formuleer.

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DEDICATION

For my parents.

For unconditional love, neverending support and faith in my abilities.

Thank you.

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CHAPTER 1

GENERAL INTRODUCTION

The genus *Ceratitis* MacLeay, 1892, consists of 89 species divided into 6 subgenera (De Meyer 2005). The Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) (Diptera: Tephritidae) is not only a very successful invasive species around the world (Malacrida *et al*. 2007) but also a very important economical and agricultural pest (Reyes & Ochando 2004; De Meyer *et al*. 2008). The increase in global fruit trade and human travel (Villablanca *et al*. 1998) combined with the biology of the Mediterranean fruit fly (medfly) has facilitated the spread of the species from its proposed Afrotropical origin (De Meyer *et al*. 2002), to a number of locations around the world including Australia, North and South America and the Mediterranean basin in the last 200 years (White & Elson-Harris 1992) (Fig. 1.1). Several life history characteristics further contribute to the success of *C. capitata* as a global invader; these are a polyphagous life-history (Malacrida 1992), rapid growth and population reproductive potential (Lance & McInnis 2005) and tolerance to a relatively wide range of climatic conditions (Nyamukondiwa & Terblanche 2009).

In Africa, approximately 150 different host plants are used for oviposition by female flies (De Meyer *et al*. 2002; see Appendix 1). The life cycle of the medfly is similar to that of other Diptera and the entire life cycle takes between 30 and 40 days to complete depending on temperature (see Appendix 2). Flies could therefore have eight or more generations per year under favourable conditions. Females oviposits their eggs under the skin of ripening fruit and can lay as many as 500 eggs in a lifetime. Eggs hatch after about two days and larvae start eating and tunnelling through the fruit (Christenson & Foote 1960). Larvae go through three instars within 6-10 days in optimum conditions (at 25°C–26°C) and will then leave the fruit to pupate in the soil (minimum of 6-13 days at 24°C -26°C). Adults emerge and will become sexually mature within a minimum of five days (Christenson & Foote 1960).

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The fruit industry in South Africa is a large role player in the countries' economy both in terms of annual income and job creation. Specifically, as of 2000, the export of fruit was estimated to provide South Africa with approximately R6 billion in income and 450 000 jobs (Barnes 2000). Fruit flies have a significant impact on fruit production and pose a two-fold risk. First, they make fruit unmarketable owing to direct and visible damage. Secondly, the presence of fruit flies in fruit consignments for export are invariably banned from entering the country to which it is exported (Barnes 2000). The management of an established pest that requires continuous control measures is more costly than the once-off eradication of a pest species (Siebert & Cooper 1995). For deciduous fruit alone, South Africa suffers a loss of around R3.5 million per year because fruit flies reduce crop yields (Barnes 2000). The Western Cape Province (South Africa) spends around R17 million annually on the control of fruit flies on citrus and deciduous fruit. Due to the economic impact of these fruit flies, research addressing their behaviour and physiology has been increasing and *C. capitata* has been identified as a priority species to be targeted in programs for detection and suppression (Gasperi *et al*. 2002; Bonizzoni *et al*. 2004).

South Africa currently implements four different methods to control fruit fly populations in orchards. First, a bait application technique (BAT) that uses food baits combined with a pesticide (such as Malathion, Dipterex or Rogor), sprayed on a localized spot in the tree canopy away from fruit to attract and kill adult flies (Ekesi & Billah 2007). Secondly, bait stations that work on the same principle as BAT, but the food bait and pesticide mix are placed in a container or trap, where exposure to the insecticidal bait is localised that retains the flies (Ekesi & Billah 2007). Thirdly, full-cover sprays that uses insecticides to spray the whole orchard at specified time intervals. Because of the negative impact that pesticides and insecticides have on the environment and human health, techniques that are more environmentally friendly are preferred. One such technique, the Sterile Insect Technique

(SIT), is a commonly applied technique for insect population control worldwide (Hendrichs *et al*. 2002) including in South Africa. This method is based on matings between mass-reared sterile males and wild females that aims to decrease population numbers to a threshold from which the population is unable to recover (Bonizzoni *et al*. 2002). Although SIT has been shown to be successful for some insects including tsetse flies (Diptera: *Glossina* spp.) and screw worms (Diptera: *Cochliomyia*), other taxa have proved more challenging to control by this method. For example, a range of problems have limited SIT success in other organisms such as codling moth (Lepidoptera: Tortricida, *Cydia pomonella*) and the Mediterranean fruit fly (Lance & McInnis 2005). Key problems include difficulties involved with mass-rearing of individuals, complicated and long life cycles and also the level of damage a pest can cause even after sterilization (Economopoulos 1992; Lance & McInnis 2005). Other major problems include producing quality laboratory-reared flies that are capable of surviving under natural conditions, and being mating compatible and competitive with wild flies (Meats *et al*. 2004; Terblanche & Chown 2007; Weldon *et al*. 2010). In *C. capitata* these difficulties include the re-mating of females (Bonizzoni *et al*. 2002), because the sperm of sterile males are at a fitness disadvantage to wild males (Bonizzoni *et al*. 2002).

In general fruit flies show an aggregated distribution (Meats *et al*. 2006) and can be found in high concentrations close to the point of release (Meats *et al*. 2006). When releasing sterile males into a wild population, it is considered best practice if the whole target area is treated simultaneously, hence recent calls for the adoption of an area-wide strategy in pest management (Klassen 2005). However, to be effective some *a priori* knowledge is assumed regarding the population structure, movement patterns and spatial network of population connections. Moreover, identification of sites from which re-invasion is most likely to occur and knowledge regarding the primary routes through which pests are likely to re-establish are critical aspects of an area wide management plan. To this end, determining gene flow among

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populations can help ensure that population control measures are successful and that pest control resources (e.g. baiting stations) are allocated efficiently.

Gene flow constitutes the movement of propagules between populations; these propagules can be seed or pollen in plants or individuals (eggs, larvae, pupae) in animals (Segelbacher *et al*. 2010). If an organism has limited dispersal ability, this will decrease the amount of gene flow between different populations, which may lead to lower genetic diversity and higher levels of inbreeding (Forde *et al*. 2004; Suni & Gordon 2010). The long-term persistence of populations is often dependent on gene flow to counteract population processes such as genetic drift and to maintain genetic variation (Segelbacher *et al*. 2010). Gene flow, especially in pest species, is an important consideration because it influences a variety of aspects important for pest management. These include adaptation of the organism to local environmental conditions and the development of resistance to insecticides (Kourti 2004); both will complicate the managing of these populations. Kourti (2004) showed that *C. capitata* populations, although geographically separated, show relatively high levels of gene flow, but also show geographic differentiation worldwide which is fuelled by selection and genetic drift.

If an organism can disperse rapidly, the sterile individuals might leave the target area before mating (Meats & Smallridge 2007). Barry *et al*. (2002) recaptured Mediterranean fruit flies at four different distances from a release point and showed that very few flies were caught more than 360 m away from the point of release. Mediterranean fruit fly can travel on average 21 km in a lifetime (Sharp & Chambers 1976; Meats & Smallridge 2007). For optimal population control through SIT, released flies should thoroughly blanket an area, and some

degree of overlap between adjacent release points should be achieved. Gaps in SIT coverage could be detrimental to effective population control.

The movement of individuals between populations can be measured using different approaches including direct and indirect methods. The dispersal of *C. capitata* has in the past been predominantly investigated using mark-and-recapture methods (Barry *et al*. 2002) which is a direct method, although not without its limitations. For example, this method may be biased because sampling is spatially restricted (Koenig *et al*. 1996) and there might be dispersal in life stages which are not sampled (Wilson *et al*. 2004). In contrast, indirect approaches which typically make use of the genetic structure of a species are averaged over many generations (Bohonak 1999). Some studies have shown that estimates of gene flow can be directly compared to direct estimates of dispersal (Peterson & Denno 1998; Bohonak 1999). However, Kourti (2004) found that estimates of gene flow for medfly calculated using genetic tools were higher than the estimates generated in mark-and-recapture studies (direct method). A number of reasons exists as to why dispersal may not always be considered equal to estimates of gene flow; these include sampling at spatial scales unfit to answer the question at hand, failure of individuals immigrating into a population to be reproductively successful, small sample sizes, migration not occurring at constant rates over time and non-equilibrium conditions (Caccone 1985; Caccone & Sbordoni 1987; Grant & Little 1992).

Early studies investigating the genetic structure of *C. capitata* populations used random amplified polymorphic DNA (RAPD) (Baruffi *et al*. 1995), enzyme electrophoresis (Gasperi *et al*. 1991; Kourti 2004), multilocus enzyme electrophoresis (Baruffi *et al*. 1995; Malacrida *et al*. 1998), polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) (Barr 2009), sequence data from nuclear introns (Gomulski *et al*. 1998) and

mitochondrial markers (Meixner *et al*. 2002). These markers have shown genetic differences between the natural medfly populations in Afrotropical regions and the newly introduced populations. Specifically, genetic studies across large geographical scales suggested that *C. capitata* populations can be divided into three groups: the ancestral populations from sub-Saharan Africa, populations from the Mediterranean Basin and lastly, populations from Latin America and the Pacific (Malacrida *et al*. 1998; Gasperi *et al*. 2002). In some cases there is a clear isolation by distance pattern and in others some of the variability from the ancestral populations is still present, characteristic of a recent range expansion or multiple introductions to the area. Studies employing microsatellite markers (Haymer *et al*. 1997; Gasperi *et al*. 2002; Bonizzoni *et al*. 2001, 2004) which are typically informative when investigating population structure (Zhang & Hewitt 2003), have supported these previous findings.

Recent studies have found that combining nuclear and mitochondrial data are more informative in population genetic studies than using these markers separately (Zhang & Hewitt 2003; Heckel *et al*. 2005; Bryja *et al*. 2010; Bock *et al*. 2010). In the study of population structure, the markers chosen have to satisfy certain requirements. These include not being under selection or dependent on different life-stages and also that the marker is scored easily (Bailiraine *et al*. 2003). Microsatellites comply with these requirements and are abundant in the genome, express high levels of polymorphism and are easily isolated in the laboratory (Bruford & Wayne 1993). Microsatellites have also been used frequently in the investigation of colonization at different spatial scales (Bonizzoni *et al*. 2000, 2001; Meixner *et al*. 2002; Baliraine 2003) and the identification of the origin of a species (Bonizzoni *et al*. 2000, 2001, 2004; Gasperi *et al*. 2002; Meixner *et al*. 2002). Mitochondrial DNA markers have slower mutation rates than microsatellites and can therefore be used to infer historical processes that shaped evolutionary processes (Wang 2010). Over the last 20 years the amount of genetic information available on *C. capitata* has increased dramatically. Furthermore, the

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tools available for the analysis of genetic data has grown rapidly and have become very powerful for the quantification of gene flow (Gasperi *et al*. 1991; Baruffi *et al*. 1995; Malacrida *et al*. 1998; Kourti 2004; Meats & Smallridge 2007; Stratikopoulus *et al*. 2008).

Study Aims

The broad aims of this study were to estimate gene flow and possible routes of movement in *C. capitata* within the Western Cape to complement current SIT and area-wide pest control programs. Population genetic knowledge and information on the ability of *C. capitata* to move were investigated using fine- and regional-scale gene flow between populations. The specific null hypotheses that are tested in this project are (1) There is no genetic differentiation among 13 different populations of *C. capitata* across the Western Cape at a regional scale or at a local (fine) scale in the Ceres-valley and (2) there is no gene flow between different populations of *C. capitata* at a regional scale in the Western Cape or at a local (fine) scale in the Ceres-valley.

The results of this study are discussed in the context of pest management in South Africa. The information presented is used to make informed decisions about how wide an area-wide SIT program should be conducted (small-scale, regional, national), and thus, inform management plans for the control and eradication of this pest species at local to regional scales in South Africa.

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CHAPTER 2

POPULATION GENETICS OF THE MEDITERRANEAN FRUIT FLY *CERATITIS CAPITATA* IN THE WESTERN CAPE PROVINCE, SOUTH AFRICA AT DIFFERENT SPATIAL SCALES

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Introduction

Through globalization and increased economic trade, species are frequently transported outside of their natural ranges (Wilson *et al*. 2009). Some of these species fail to establish and reproduce. However, an increasing number of species overcome these barriers and become invasive (Blackburn *et al*. 2011). The impact of invasive species can be wide-ranging, from direct impacts on natural biodiversity and resources to affecting human-well being and agriculture. A case in hand concerns the Mediterranean fruit fly, *C. capitata* Weidemann (Diptera: Tephritidae), a successful invader and one of the most economically destructive pest species worldwide (Reyes & Ochando 2004; Malacrida *et al*. 2007; De Meyer *et al*. 2008). This species has, through fruit production and associated trade-related transport, spread from its native Afrotropical range to several of the main fruit-producing regions across the world including Australia, North and South America and the Mediterranean basin (White & Elson-Harris 1992). To this end, *C. capitata* fits the notion that insect species (other than biological control species) are mainly introduced through accidental (non-intentional) transportation (Lockwood *et al*. 2005).

Several factors contribute to the successful and wide-spread introductions of *C. capitata*. Amongst these is the species' polyphagous life-history (Malacrida 1992); specifically, the Mediterranean fruit fly (medfly) utilizes more than 150 different host plant species for oviposition in Africa alone (De Meyer *et al*. 2002), many of which are of commercial value. *Ceratitis capitata* can also withstand somewhat higher temperatures (critical thermal maxima (CT_{max}) 42.4-43.0 °C) than, for example, *Ceratitis rosa* (CT_{max} = 41.8-42.4 °C) in South Africa (Nyamukondiwa & Terblanche 2009). Furthermore, Nyamukondiwa *et al*. (2010)

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showed that plasticity of low temperature tolerance in *C. capitata* may contribute to their invasion potential.

Within South Africa, deciduous fruit is mainly produced in the Western Cape (Barnes & Venter 2006) with the bulk of production originating from the Ceres-valley (McDonald & Punt 2001). Control of medfly populations predominantly relies on insecticide use which is problematic not only for human health but also detrimental to the environment (the Cape Floristic Region, which spans the Western Cape region, is recognized as a Biodiversity Hotspot; Myers *et al*. 2000). As such, more effective, environmentally friendly techniques are increasing in popularity. Foremost amongst these is the Sterile Insect Technique (SIT) (Hendrichs *et al*. 2002). SIT involves the release of mass-reared sterile males that mate with wild females and thereby decrease population numbers to a threshold from which populations are unable to return (Bonizzoni *et al*. 2002).

Successful control of pest species relies heavily on accurate information regarding the movement of individuals as well as the effective population sizes for different populations and regions (Estoup & Guillemaud 2010). These types of information can be obtained through direct methods, including mark-and-recapture studies, or through indirect methods, such as gene flow, estimated using molecular information (Slatkin 1985; Koenig *et al*. 1996; Roderick 1996; Bohonak 1999; Broquet & Petit 2009). Several recent studies have indicated that direct methods may significantly under-estimate population sizes and migration (see e.g. Kourti 2004; Katzner et al. 2011). Misinformation hampers successful control efforts as it frequently leads to inaccurate estimates of the minimum size for area-wide pest management or funding necessary to complete programmes (Barclay *et al*. 2011). It is not surprising, therefore, that molecular tools are increasingly being used in pest management to investigate genetic

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structure and dispersal between groups of individuals or populations (Behura 2006, Rollins *et al*. 2009).

Efforts to understand movement patterns and control of *C. capitata* globally have incorporated information from a wide array of molecular markers (see Gasperi *et al*. 1991, 2002; Baruffi *et al*. 1995; Haymer *et al*. 1997; Malacrida *et al*. 1998; Gomulski *et al*. 1998; Bonizzoni *et al*. 2001; Meixner *et al*. 2002; Kourti 2004; Barr 2009; Alaoui *et al*. 2010) . A combination of mitochondrial and nuclear markers (primarily information from microsatellite markers) provide information regarding population structure and gene flow patterns (see Zhang & Hewitt 2003; Heckel *et al*. 2005; Bryja *et al*. 2010; Bock *et al*. 2010). In this study I add to this growing body of literature by using a combination of molecular sequence (mitochondrial cytochrome oxidase subunit I) and microsatellite data to document the spatial distribution of genetic variation in the medfly across the Western Cape region at different spatial scales (regional-and local-(fine) scale). The first null hypothesis I test is that there is no genetic differentiation among different populations of *C. capitata* at a regional scale across the Western Cape or at a fine scale in the Ceres valley. The second null hypothesis I test is that there is no gene flow between different populations of *C. capitata* on a regional- or fine-scale.

Materials and Methods

Sampling sites and fly collection

The sampling regime was designed to capture genetic diversity at two different spatial scales. To this end *C. capitata* individuals from 13 locations in the Western Cape, representing regional scale sampling and 13 locations in the Ceres-valley representing fine scale sampling

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were collected (Fig. 2.1). In the Ceres-valley, sampling was designed to provide localities at intervals of increasing geographic distance to enable pairwise genetic comparisons (Fig. 2.1). While it is clear that sampling localities are not necessarily equal to populations in the strict sense of the word (i.e. closed breeding units), however, for ease the term population is used here as interchangeable with sampling locality. The seasonal abundance of *C. capitata* peaks in the months between March and May. Bucket traps (Chempac, Paarl, South Africa) were put up in different fruit orchards and geo-referenced using a hand-held GPS. Traps were dry baited with a three-component attractant, Biolure 3C (Chempac) consisting of putrecine, ammonium acetate and trimethylamine. Traps were collected every two weeks and flies caught in different traps were regarded as being separate populations and stored in 100% ethanol. Flies were identified and sexed under a stereomicroscope. Flies were inspected to make sure that they were not part of a Sterile Insect Technique program. Flies from the SIT program are covered in fluorescent dye that accumulates in the head suture before release and are easily identified under a UV light. Even in ethanol storage for up to a week, trials verified that SIT flies can be reliably distinguished from wild flies. DNA was extracted from whole flies using a DNeasy ® tissue kit (QIAGEN Inc.).

Mitochondrial DNA amplification and sequencing

A portion of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified for five individuals randomly selected out of each population to cover the entire Western Cape (Table 2.1). The primers C1-J-2183 (5'-CAACATTTATTTTGATTTTTTGG-3') and TL2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3') (Simon *et al*. 1994) were used. The 30 µL PCR reaction contained 2 µL of DNA, 3µL of 10x Buffer, 3µL of 25mM $MgCl_2$, 3µL of 1 mM dNTPs, 1 µM of each primer and 250u of Super-Therm *taq* DNA polymerase (JMR holding, Kent, USA). PCR conditions were: 5 min of initial denaturation at 96 ºC, 30 cycles

of 30 s of denaturation at 96 ºC, 30 s of annealing at 52 ºC, 50 s of extension at 72 ºC and 5 min of final elongation at 72 ºC. Amplification products were run on a 1% agarose gel to verify successful amplification. Sequencing cocktails were cleaned using Centrisep spin columns (Princeton Separations) and sequencing reactions were performed using BigDye chemistry (Applied Biosystems, Foster City, California, USA) and analyzed on an ABI 3170 automated sequencer (Applied Biosystems, Foster City, California, USA).

Mitochondrial DNA analysis

DNA sequences were aligned and edited in GENEIOUS Pro™ 5.0 software (Biomatters Ltd, New Zealand). We calculated the number of haplotypes (N_h), haplotype diversity (h) (the probability that two haplotypes from the same sample are different when randomly chosen) and nucleotide diversity (π) (an indication of the level of polymorphism in the population; defined by the average number of pairwise nucleotide differences per site between two sequences (Nei 1987)) (ARLEQUIN v3.5.1.2; Excoffier & Lischer 2010). To test for selective neutrality I used Fu's F-statistic (Fu 1997) (ARLEQUIN v3.5.1.2; Excoffier & Lischer 2010) which show that under neutral expectation the number of segregating sites are correlated with the average number of nucleotide differences. A parsimony haplotype network was used to investigate the relationship between COI haplotypes with a 95% connection limit (TCS v1.21; Clement *et al*. 2000).

Overall (all populations considered together) and population pairwise Φ_{ST} values were calculated using 1000 permutations (ARLEQUIN v3.5.2.1; Excoffier & Lischer 2010). I tested for isolation by distance (IBD) using a Mantel test (Mantel 1967) with 1000 permutations by testing for a correlation between genetic distance and geographic distance (ARLEQUIN v3.5.2.1; Excoffier & Lischer 2010). The minimum straight line distance (i.e.

as-the-crow-flies) between the GPS coordinates of sampling sites were taken as the geographic distance.

Microsatellite genotyping

A total of 385 *C. capitata* samples were collected from the Western Cape (for regional sampling) and an additional 382 samples from the Ceres-valley (for the local scale sampling). These samples were genotyped for 12 microsatellite markers obtained from previously published studies (Table 2.2). Forward primers were 5'-labelled with one of four fluorophores (6-FAM, HEX, VIC or NED) and microsatellite loci were pooled for amplification if there was no signal inhibition during amplification. A Multiplex PCR Kit (Qiagen Inc.) with a final PCR reaction volume of 10µl consisting of 6µL of 2x Qiagen Multiplex Master Mix, 2µl of primer mix (2mM), 1µl water and 2µl of template DNA (30 ng) were used. PCR conditions were: 15 min of initial denaturation at 95 ºC, 35 cycles of 30 s of denaturation at 94 ºC, 90 s of annealing at 55 ºC, 50 s of extension at 72 ºC and 10 min of final elongation at 60 ºC. PCR products (1 ml diluted (1/80)) were combined with 15 ml of deionized formamide and 0.2 ml of GS500LIZ size standard (Applied Biosystems, Foster City, California, USA). Samples were genotyped on an ABI 3130 Automated Sequencer (Applied Biosystems, Foster City, California, USA) and alleles were scored using GENEMAPPER v3.7 (Applied Biosystems, Foster City, California, USA). A positive control was also run to check that all plates were read consistently.

Microsatellite DNA analysis

Microsatellite loci were tested for departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium using 10 000 permutations in GENEPOP (Raymond & Rousset 1995;

Rousset 2008). Significance levels were adjusted using False Discovery Rates (QVALUE; Storey 2002). To quantify the efficiency of each microsatellite and the genetic structure similarity over loci I used a multivariate coinertia analysis (MCOA; Laloë *et al*. 2007; Berthouly *et al*. 2010) conducted in R v2.12 software (Ihaka & Gentleman 1996; R Development Core Team 2011). Three parameters were calculated, the variance of genetic diversity within each locus, the similarity (Cos^2, a correlation between tables of individual scores of loci and a synthetic similarly ranked variable) and the typological value (TV, the contribution of the markers compared to the reference typology). The marker Medflymic88 was found to be monomorphic and was subsequently excluded from further analyses (Table 2.2).

Levels of genetic diversity were assessed by computing basic statistics for 11 microsatellite loci. The number of alleles (N_A), expected heterozygosity (H_E, expected allele frequencies given under Hardy-Weinberg equilibrium) as well as observed heterozygosity (H_O, actual heterozygosity measured in a population) were calculated for each microsatellite locus and for each location (GENETIX v4.05.2; Belkhir *et al.* 2004; GenAlEx 6.4; Peakall & Smouse 2006). Allelic richness (A_R), which is a measure of genetic diversity independent of sample size, was calculated in FSTAT v2.9.3.2 (Goudet 2002). The inbreeding coefficient (F_{IS}, proportion of variance in a population contained in an individual) was calculated in GENETIX v4.05.2 (Belkhir *et al.* 2004) with 10 000 permutations to assess deviations from the null hypothesis of no inbreeding ($F_{IS} = 0$).

To assess the degree of population differentiation (at both regional and local (fine) scale), I used three complementary approaches: F_{ST} and two Bayesian clustering methods with and without spatial information. First, pairwise F_{ST} values (a measure of the genetic variance in a subpopulation compared to the total genetic variance in the entire population) and overall F_{ST}

values were calculated in ARLEQUIN v3.5.1.2 (Excoffier & Lischer 2010). Second, STRUCTURE v2.3.3 (Pritchard *et al*. 2000; Falush *et al*. 2003) were run to assign the multilocus genotypes of individuals to populations without any prior spatial information. To estimate the number of clusters (K), I ran 10 independent runs for each K value varying between 1 and 20. A burn-in period of 1 000 000 followed by 1 000 000 Markov Chain Monte Carlo (MCMC) permutations were run to allow statistical parameters to reach stability. To determine the true number of populations (K) in the dataset the method described by Evanno *et al*. (2005) was used. The method utilizes the second order rate of change in the log probability of "successive" K values. The STRUCTURE output was visualized using DISTRUCT v1.1 (Rosenberg 2004). Thirdly, TESS v2.3 (Chen *et al*. 2007; Durand *et al*. 2009) which has an option to implement an admixture model that uses spatial coordinates of populations as prior information, were used to detect spatial genetic structure. The number of clusters (K) were estimated running 10 independent runs for each K value varying between 2 and 20, with 120 000 MCMC iterations discarding 20 000 iterations as burn-in. The optimal number of clusters (K) was chosen based on the deviance information criterion (DIC) stabilizing. The runs were averaged in CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007) and visualized in DISTRUCT v1.1 (Rosenberg 2004).

Spatial genetic structure was investigated using two different approaches. First, a Mantel test (Mantel 1967) was used to assess the significance of the association between geographic and genetic distance (ARLEQUIN v3.5.1.2; Excoffier & Lischer 2010). Secondly, in SPAGEDI v1.3 (Hardy & Vekemans 2002) I characterised the spatial genetic structure of sampled populations using global F-statistics and pairwise F_{ST} (Weir & Cockerham 1984). The standard error of F_{ST} computation were estimated using 10 000 permutations. The results were visualized by plotting the F_{ST} values against the spatial distance between sampled populations.

Gene flow was assessed using two different approaches. First, MIGRATE-N v3.2.15 (Beerli & Felsenstein 2001; Beerli 2006, Beerli 2009) was used to estimate the migration rates between all populations simultaneously. Bayesian inference was used, with the starting parameters of both migration rates and theta based on F_{ST}-calculations. Assuming a constant mutation rate over all loci, the starting tree was an UPGMA tree generated from the data and uniform priors were used for both migration rate (bound between 0 and 5000) and theta (bound between 0 and 1000). Several short runs were performed, with different random seeds to refine search parameters and to ensure convergence and adequate chain swapping. For the final run I sampled 500 000 genealogies, discarding 10 000 genealogies (burn-in) using one long chain and four short chains with static heating (1, 1.5, 3, 10 000) and a swapping interval of one. Secondly, the probability of assigning an individual to any of the populations was estimtated using GENECLASS v2.0 (Piry *et al*. 2004). The probability values were calculated using Bayesian inference, following the method described by Rannala & Mountain (1997), which is able to identify recent migrants when there is low population divergence between source-sink-populations. Monte Carlo resampling with 10 000 simulated individuals was used to infer the significance of the assignments (probability threshold = 0.05).

Results

Mitochondrial DNA analysis

An 800-bp segment of the COI gene was sequenced for a total of 65 individuals from the Western Cape (Table 2.1). The final alignment contained 49 polymorphic sites of which 30 were parsimony informative. I identified 46 distinct haplotypes. Haplotype diversity was notably high and ranged between 0.900 (Citrusdal and Stellenbosch) and 1.000 (Barrydale,

Calitzdorp, Ceres, Clanwilliam, Ladismith, Lutzville, Porterville, Robertson, Simondium, Tulbagh, Wellington), with an overall value for the region of 0.983 (Table 2.1). Nucleotide diversity ranged between 0.003 (Clanwilliam) and 0.009 (Ceres) with an overall value of 0.007 (which is comparable to other *Ceratitis* spp.; see e.g. Virgilio *et al*. 2008) in the Western Cape (Table 2.1). Fu's F-statistic was highly negative and significant (FS = -25.380; $p < 0.001$) indicating a deviation from equilibrium (possibly due to an excess of heterozygotes as would be expected under population expansion).

The haplotype network showed no clear spatial pattern of genetic variation across the Western Cape (Fig. 2.2). Rather, the structure appeared almost random. This was supported by *F*-statistics where variation was overwhelmingly partitioned within populations with only 3.1% of the variation accounted for by the between-site component ($\Phi_{ST} = 0.031$, $p = 0.123$). Pairwise Φ_{ST} values between sampling sites were low and non-significant (Table 2.3) after correction for multiple testing using False Discovery Rates. No correlation between genetic and geographic distances were found (regression coefficient = 0.169, $p = 0.841$) indicating the absence of isolation by distance.

Microsatellite DNA analysis

All populations deviated from HWE (genotype frequencies differ from ideal population (random mating, no drift, mutation or migration)) with relatively high levels of inbreeding indicative of non-random mating (Table 2.4 andTable 2.6) and showed an excess of homozygotes (more homozygotes than expected under HWE). No linkage disequilibrium was observed among the 11 polymorphic microsatellite markers (Table 2.2) and they were therefore considered as independent. Although all markers included here displayed acceptable levels of variation (the number of alleles (N_A) for the marker ranged between 8 and 28

(Ccmic9, Medflymic128) (Table 2.2), not all markers contributed equally to the overall topology (Fig 2.3). Specifically, the variance and typological value for one of the markers (dccap6) was notably higher than for any of the other markers. Observed and expected heterozygosity values for each of the markers are given in Table 2.2.

Regional scale

A total of 195 alleles were detected with all of the sampling localities having private alleles (N_{AP}) except for Lutzville and Citrusdal (Table 2.4). Genetic diversity as indicated by mean expected heterozygosity (H_E) for *C. capitata* in the Western Cape was high (0.803), ranging between 0.787 (Citrusdal) and 0.822 (Calitzdorp) (Table 2.4). Allelic richness (A_R) ranged from 9.211 (Simondium) to 10.456 (Citrusdal) (mean = 9.586, based on minimum of 21 individuals; Table 2.4).

Pairwise F_{ST} values were used to quantify the genetic structure between populations. Similarly to the mtDNA findings, almost all of the comparisons were not significant after corrections for multiple testing using False Discovery Rates (Table 2.5). The highest level of genetic differentiation ($F_{ST} = 0.019$) was between Tulbagh and Citrusdal. The overall F_{ST} value 0.006 ($p = 0.002$), indicate weak but significant population differentiation (possibly due to the few significant pairwise comparisons).

The investigation of population differentiation using two different Bayesian clustering approaches gave similar results. STRUCTURE indicated no population differentiation (Fig. 2.4 (a) and (b)). Evanno's method for estimating the optimal number of clusters (*K*) cannot

calculate a ΔK value at $K = 1$, as it uses the second order rate of change. Examination of the log of the posterior probability of the data [ln P(D)] for each K value revealed the highest ln P(D) value at $K = 1$, an indication of no population differentiation. Similarly, analyses in TESS cannot return $K = 1$ and indicated $K = 2$ as the optimal number of clusters. However, the majority of individuals (88%) had more than 70% membership in only one cluster, confirming the presence of a panmictic population pattern (Fig. 2.5 (a) and (b)). Concordant results were found when investigating isolation by distance (IBD) patterns by estimates of correlation between genetic distance and geographic distance ($r = 0.000011$, $p = 0.128$) and using global F-statistics in SPAGEDI (Fig. 2.7). Both analyses indicated no pattern of IBD.

The detection of migrants in GENECLASS showed low overall assignment success with only 16.4% (64 out of 385 individuals were assigned at $p > 0.05$) being assigned to specific populations. Failed assignment of individuals probably occurred because of the panmictic nature of *C. capitata* populations in the Western Cape. Coalescent-based estimates of the number of immigrants per generation (m) for all populations simultaneously calculated in MIGRATE ranged between 0.70 and 2.06. In most cases, gene flow values among localities at the regional scale was above 1 ($m > 1$), which is sufficient to prevent population differentiation. However, a more critical evaluation of gene flow values are neccessary as several of the parameters did not stabilize or converge to zero, possibly indicating a flat parameter space. Also, the large number of parameters calculated in the full migration model, may reduce the statistical power of the analysis.

Fine scale

From the Ceres-valley a total of 190 alleles were detected with all of the sampling localities having private alleles (N_{AP}) except for Ceres 7 (Table 2.6). Average genetic diversity (H_E) for *C. capitata* in the Ceres-valley was 0.805, ranging between 0.793 (Ceres 5) and 0.816 (Ceres 6) for sampling localities (Table 2.6). Allelic richness (A_R) ranged from 9.273 (Ceres 12) to 10.122 (Ceres 2) (mean = 9.586, based on minimum of 19 individuals; Table 2.6).

The overall F_{ST} value was 0.004 ($p = 0.182$), indicating no significant population differentiation. All of the pairwise F_{ST} values for sampling localities in the Ceres-valley were not significant after corrections for multiple testing using False Discovery Rates (Table 2.7). Both the Mantel test and the global F-statistics did not indicate any pattern of isolation by distance ($r = 0.000002$, $p = 0.45$; Fig. 2.8).

Concordant results for the population differentiation were found in the Ceres-valley, with STRUCTURE (Fig. 2.8 (a) and (b)) and TESS (Fig. 2.9 (a) and (b)). The optimal number of clusters (K) was calculated in the same way as for the regional sampling. For the STRUCTURE results I found the highest K at one, indicating no population differentiation. TESS results showed $K = 2$ as the most likely number of clusters, but TESS cannot calculate number of clusters at $K = 1$ and most (90%) of the individuals had more than 70% membership in only one cluster, indicating no population differentiation.

Detection of migrants in the Ceres-valley had low overall assignment success about (9.2%, 35 out of 382 individuals were assigned at $p > 0.05$). Failed assignment of individuals probably

occurred because of the lack of population genetic structure (panmictic population). Coalescent-based estimates of the number of immigrants per generation (*m*) ranged between 16.17 and 26.26, an indication of high gene flow sufficient to prevent differentiation between sampled populations.

Discussion

The population genetics of introduced species has been widely studied utilizing molecular tools and Bayesian approaches. In this study I characterized the genetic diversity, population genetic structure and population connectivity of *C. capitata* at a regional scale (Western Cape) and at a fine scale in a key fruit production area (Ceres-valley). Several interesting and perhaps also unexpected findings emerged. First, *C. capitata* is characterized by exceptionally high levels of genetic diversity at both local and regional scales. Secondly, medflies form a panmictic population at local and regional scales with no significant population structure detected at either scale in my sampling. These major findings are discussed in detail below.

Genetic diversity

Overall, my genetic diversity estimates for *C. capitata* in the Western Cape and also in the Ceres-valley are high (H_{Eceres} = 0.805, H_{Ewc} = 0.803), but comparable to estimates from other studies for South Africa (H_{E} = 0.857, Bonizzoni *et al*. 2004; H_{E} = 0.775, Baliraine *et al*. 2004) and other African populations, specifically Kenya (H_{E} = 0.750, Gasperi *et al*. 2002; H_{E} = 0.896, Bonizzoni *et al*. 2004; H_{E} = 0.795 Baliraine *et al*. 2004). The genetic diversity found in *C. capitata* in the Western Cape is higher than values reported for other *Ceratitis* spp. in South Africa and elsewhere (*Ceratitis rosa* and *Ceratitis fasciventris*, Baliraine *et al*. 2004),

which may simply reflect a bias in sampling effort, or alternatively, might indicate higher population sizes of *C. capitata* in South Africa. Also, the diversity values found for *C. capitata* in South Africa are notably higher than those reported for this species from other invaded regions of the world such as the island of Réunion (H_E = 0.660, Gasperi *et al*. 2002), Australia (Western, H_E = 0.385-0.606, South, H_E = 0.239-0.429, Bonizzoni *et al*. 2004), the Mediterranean Basin (H_E = 0.484-0.633, Bonizzoni *et al*. 2004) and Hawaii (H_E = 0.454-0.653, Bonizzoni *et al*. 2004). Higher haplotype diversity for medfly populations in African countries and also in South Africa may reflect the importance of propagule pressure and the effect of multiple introductions on the genetic diversity. For example, medfly may easily spread from Kenya to other African countries (including South Africa) perhaps through dispersal mediated by humans, thereby invading these countriesmultiple times (i.e. high propagule pressure). However, the number of introductions to countries such as Australia and Hawaii may be limited because of stricter quarantine control, coupled with relative geographic isolation, and as such, result in lower genetic diversity values (fewer introductions equates to lower genetic diversity). Multiple and continuous introductions in the establishment of invasive or pest species are very important and have shown to be a common occurance (Kolbe *et al*. 2004; Bossdorf *et al*. 2005; Roman *et al*. 2007; Darling *et al*. 2008; Facon *et al*. 2008). In addition to the possible multiple, continued introductions, the high genetic diversity found in *C. capitata* in South Africa can also be due to South Africa, specifically the Western Cape being part of *C. capitata*’s native range (De Meyer *et al*. 2002), large effective population sizes, high mutation rate or numerous other behavioural, physiological and morphological adaptive traits (for a review see Yuval & Hendrichs 2000; see also discussions in Chapuis *et al*. 2011). This high genetic diversity reservoir provides elevated evolutionary potential, and perhaps also the potential for phenotypic plasticity (Lavergne & Molofsky 2007; Novak 2007), therby increasing their ability to colonize other suitable habitats.

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Genetic structure

In addition to high levels of genetic diversity, I found no genetic differentiation among populations in the Western Cape or the Ceres-valley. Bonizzoni *et al*. (2004) similarly found genetic homogeneity and a lack of spatial population differentiation in *C. capitata* populations in the coastal regions of Australia from Perth northwards. They ascribed the lack of population differentiation to the subtropical climate and a continuous distribution of a wide range of suitable host plants, a situation similar to that in the fruit production areas of the Western Cape. In contrast, Alaoui *et al*. (2010) found population genetic structure in Moroccan *C. capitata* populations in endemic Argan forests, predominantly driven by their occurrence at different altitudes. They ascribed the population structure to rapid changes of environmental conditions across altitude zones compared to latitudinal changes; as such, climate factors may be a strong selective force (see e.g. De Meyer *et al*. 2008, Nyamukondiwa *et al*. 2010; Basson *et al*. 2011)**.**

All populations sampled from both the Ceres-valley and from the Western Cape showed slight levels of inbreeding (F_{ISwc} ranged between 0.138-0.260; F_{ISceres} ranged between 0.185-0.260) which indicates that non-random mating is occuring despite the high levels of gene flow and may well expalin the deviations we see from HWE.

Dispersal

Many investigations have focused on the dispersal of *C. capitata*, which is not surprising since the species is not only a significant economic pest but also widespread. Although limited structure and higher levels of connectivity may have been expected for the Ceres-valley (maximum distance between sampling localities is 17 km), this was an unexpected finding for my regional investigation as sampling sites are geographically more distant with

up to 350 km apart (Lutzville (CV) and Calitzdorp (CD)). Sharp & Chambers (1976) showed that *C. capitata* can fly a maximum distance of 7-8 km (within 200 min), whereas most individuals flew between 1-3 km within 2-3 hours. Similarly, Meats & Smallridge (2007) indicated dispersal in *C. capitata* of between 0.5-9.5 km through natural dispersal (i.e. mainly flight); but that a very small percentage of individuals are likely to do so (90% of released individuals remain within 400-700m from release point, leptokurtic pattern).

The distribution of suitable host plants (both wild and cultivated) in the Western Cape and in the Ceres-valley are probably able to facilitate the movement of *C. capitata* using their natural dispersal ability, but are interspersed with areas of unfavourable habitat. These long distance dispersal events postulated for *C. capitata* in the Western Cape are therefore more likely the result of human-mediated dispersal. My suggestion of jump dispersal is further supported by the absence of isolation by distance, which is the pattern that I would have expected if dispersal was primarily through natural means. The most likely vectors of human-mediated dispersal include fruit consignments, the movement of nursery material and also individual movement of fruit between different locations throughout the province (Tolley *et al*. 2007; Hulme 2009; Estoup & Guillemaud 2010). My genetic results further indicate population expansions for fruit flies in the Western Cape and the coalescent-based migration rates indicated that there are high levels of gene flow between populations in the Ceres-valley at a fine scale and sufficient amounts of gene flow in the at the regional in the Western Cape scale to prevent population differentiation.

Future directions

The effective population size of *C. capitata*, like many other pest species, may be extremely large. A recent study on the Australian plague locust, *Chortoicetes terminifera*, showed that a large population size can affect the level of genetic differentiation between populations,

hampering the levels of gene flow between them (Chapuis *et al*. 2011). Other confounding effects on the detection of population differentiation include low numbers of microsatellites utilized as simulation studies have shown that statistical power can be improved by increasing the numbers of markers used (Chapuis *et al*. 2011; Marko & Hart 2011). However, most genetic structure studies on pest species to date, sample comparable numbers of individuals (20-40) and use between eight and 10 microsatellites (Baliraine *et al*. 2004; Torriani *et al*. 2010; Virgilio *et al*. 2010).

Of particular interest for future studies is investigating the effective population size of *C. capitata* and the influence it has on population connectivity, as well as more intensive sampling of the species range throughout South Africa. Furthermore, another species of economic concern in South Africa is *C. rosa* (Natal fruit fly), a polyphagous congener of *C. capitata* which has high invasion potential. For example, on Réunion island *C. capitata* was outcompteted by *C. rosa* (White *et al*. 2001; Duyck & Quilici 2002; Duyck *et al*. 2004). Therefore, comparisons of genetic diversity, population differentiation and gene flow patterns between *C. capitata* and *C. rosa* may be useful for determining the relative population dynamics of these species in South Africa.

Ceratitis capitata is of major economic importance in South Africa and has high genetic diversity and no population genetic differentiation at a regional (Western Cape) or fine (Ceres-valley) scale. The lack of genetic differentiation between populations is most likely due to human-mediated dispersal and multiple introductions between distant populations. This high level of gene flow has an important implication for pest management practices as most rely on the release of sterilized insects and/or insecticide treatments which are affected by the dispersal of *C. capitata*. If these results are a reliable indication of movement patterns and population structure, one potential implication of this work is that it suggests area-wide pest

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management should perhaps be undertaken at a regional scale, rather than on a farm or valley basis, as is presently the case (Hendrichs *et al*. 2007). The results produced here are thus useful for better understanding the ecology of *C. capitata*, and in turn should facilitate improved area-wide pest management programs for sustainable crop production**.**

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CHAPTER 3

GENERAL DISCUSSION

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The fruit industry in South Africa provides employment to at least several hundred thousand of people and makes a considerable contribution to the country's' annual income. The effect that fruit flies, specifically *Ceratitis capitata,* have on fruit production in South Africa is profound. My results show that *C. capitata* populations in the Western Cape (regional scale) and in the Ceres-valley (fine scale) have high genetic diversity and weak or no population differentiation. Furthermore, I hypothesize that the lack of differentiation is probably due to human-mediated dispersal (jump dispersal) between more distant populations. This high level of gene flow has important implications for pest management strategies as the whole Western Cape should potentially be considered a management unit. This management unit needs to encompass wild host areas, home gardens and multi-owner fruit orchards to prevent fruit flies from taking refuge and recolonizing areas under control (Manrakhan & Addison 2007).

Human-mediated movement of *C. capitata* over larger spatial scales in South Africa needs investigation as no quarantine strategies are in operation within the boundaries of the country. Furthermore, De Meyer *et al*. (2008) made use of two different correlative ecological niche modelling techniques (GARP and PCA) to show that *C. capitata* may have a tolerance to a wider range of climatic conditions than another fruit fly, *C. rosa*. *Ceratitis rosa* is restricted to areas with high rainfall, specifically the eastern coastal parts of the country. *Ceratitis rosa* is able to outcompete *C. capitata* in regions where they co-occur and environmental conditions are favourable for the survival and development of the former, as is the case on Réunion island (De Meyer *et al*. 2008). Concern exists that successful suppression of *C. capitata* in the Western Cape will simply result in the

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succession of *C. rosa*. Therefore, being able to compare the genetic variation and population structure between different *C. rosa* and *C. capitata* populations across South Africa is imperative for understanding their population dynamics and developing a successful area wide pest management strategy for South Africa.

Furthermore, one of the most important factors in prohibiting further introductions of invasive pest species is the ability to identify the species with ease. As most quarantine workers are not trained taxonomists, and some fruit fly species are hard to identify in mature or immature stages, a quick and easy-to-use technique is high in demand.

Moreover, for the management of *C. capitata* in the Western Cape, the application of insecticides needs to be undertaken on a large scale (Western Cape), with timed aerial sprays over large areas. In addition, a set of overarching guidelines for fruit producers seems critical. With the undesirable effects that insecticides have on the environment, effective biological control such as the mass release of sterilized insects needs to be investigated; a campaign that drives such an initiative would enhance the chances of effective population suppression, and perhaps also increased the probability of local eradication(s).

The Sterile Insect Technique (SIT) is strongly influenced by the dispersal capability of the released individuals and the ability of mated wild females to immigrate into the area

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being treated by SIT (Meats *et al*. 2003). A model system for a successful area wide pest management strategy involving SIT requires a small area, ideally isolated, and surrounded by a buffer zone to prevent recolonization from adjacent sites (Knipling 1955, Barclay *et al*. 2011). Collectively, successful wild female reproduction potential needs to be minimized combined with a decrease in population numbers.

Most pest species have high effective population sizes. Recently Chapuis *et al*. (2011) highlighted the effect of extremely large effective population sizes on population differentiation estimates. One way to overcome these limitations is to make use of Approximate Bayesian Computations (ABC). ABC makes use of simulations of the evolution of DNA sequences under complex population models. Combinations of simulations, the estimation of effective population sizes and an increased number of microsatellite loci are needed to further understand population differentiation in *C. capitata*. Also, since I only sampled a limited number of individuals (although more than other studies with individuals from South Africa. For example, Baliraine *et al*. 2004 sampled only 9 individuals from Somerset West), in light of the assumed high effective population size, even higher numbers of individuals might need to be analyzed per population. However, preliminary randomization analyses of expected heterozygosity as a function of individuals reaches an asymptote around nine to ten individuals. This analysis suggests that more individuals are not required to give the study greater discriminating power. Instead the microsatellite markers used in this study where highly polymorphic, but had discrepancies in their discriminating power. As such, more markers may need to be included in future studies of *C. capitata* population genetics.

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The diversity of fruit flies (Diptera: Tephritidae) in the Western Cape has to date not been thoroughly documented in different orchards or agro-ecological zones. The importance of knowledge about local diversity can shed light on inter-specific competition, mis-identification in traps and also help to favourably modify trapping protocols in order to monitor and implement control programmes successfully. Additionally, knowledge of indigenous Tephritidae in Fynbos and Renosterveld, and their potential interactions with agricultural areas is sparse. Related studies show that some species, especially native species, are not able to survive solely on cultivated crops and depend on natural habitats for their survival (Szinicz *et al*. 2005). Hence, investigating the diversity of fruit flies in orchards and agro-ecological landscapes are important to understand the interactions between fruit flies found in their natural habitat and those found in habitat transformed by agriculture.

Finally, information gained through research regarding the diversity of fruit fly species in the Western Cape (orchards and agro ecological), techniques for easy identification and population genetic structure will provide invaluable insight to help create a conceptual model for an area-wide pest management strategy. Ultimately, the conceptual model can be used in practical applications to improve management and intervention strategies.

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Zhang D-X, Hewitt GM (2003) Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology* **12**:563-584.

FIGURES

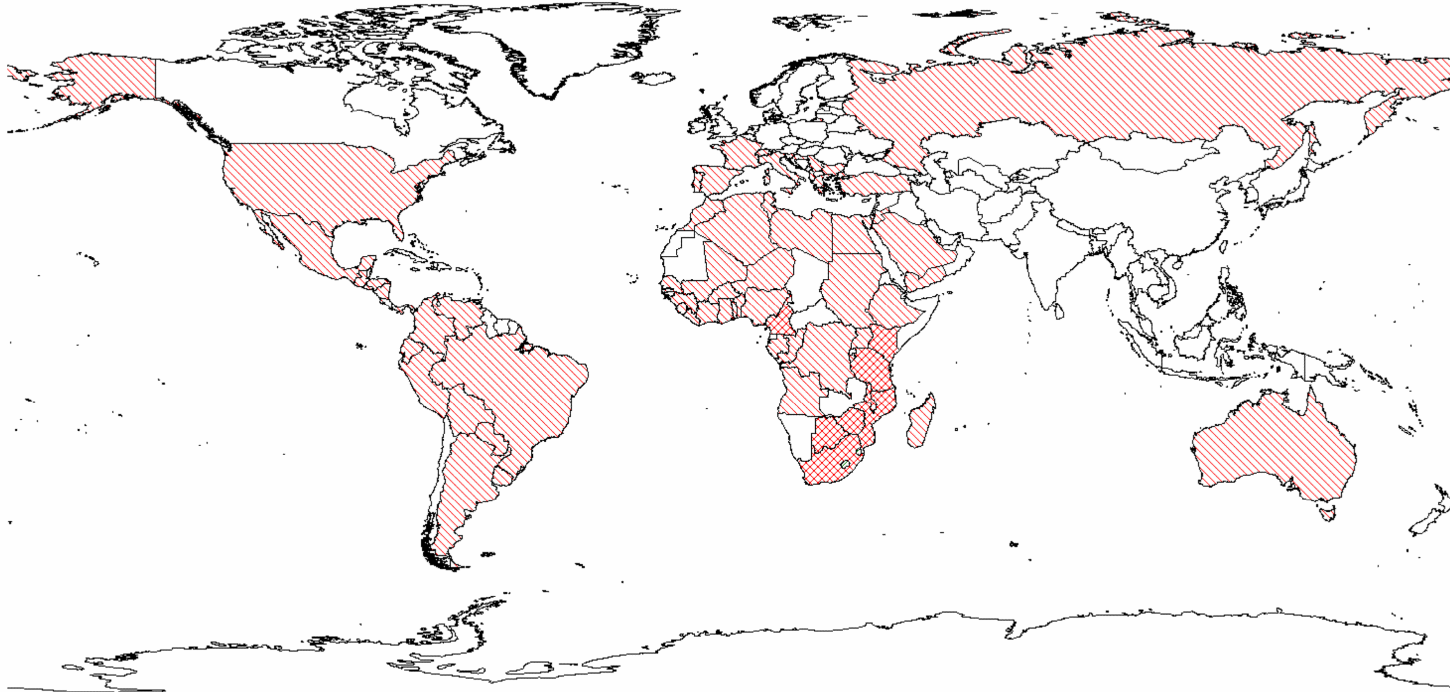

Figure 1.1: The distribution of *Ceratitis capitata* (red upward diagonal stripes) and countries where both species are present are indicated by red crossed hashes (Source: EPPO (2006, see www.eppo.org) and M. De Meyer, pers. comm.).

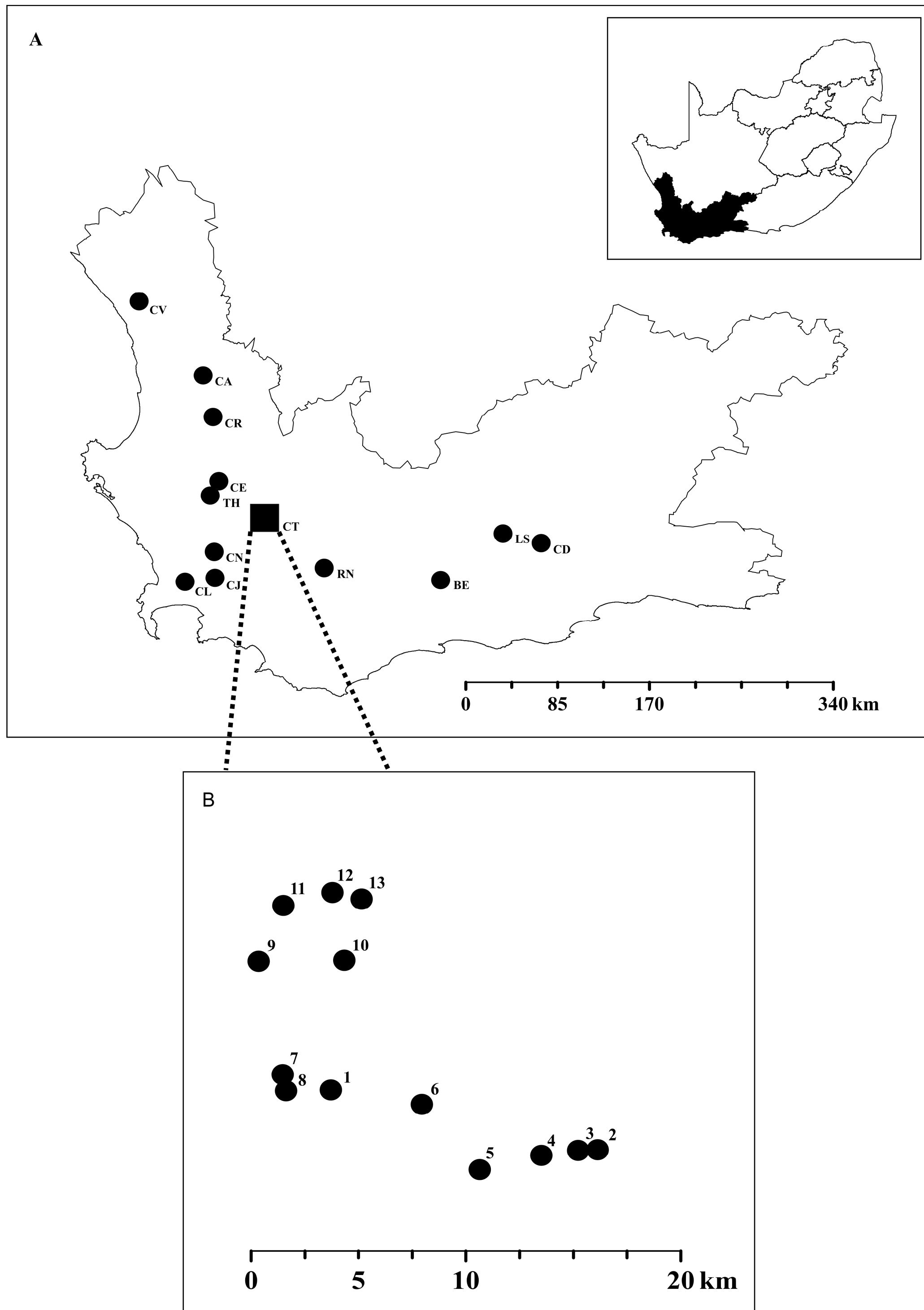

Figure 2.1: Sampling sites for *Ceratitis capitata* in (A) the Western Cape (regional scale), South Africa (CV, Lutzville; CA, Clanwilliam; CR, Citrusdal; CE, Porterville; TH, Tulbagh; CT, Ceres; CN, Wellington, CJ, Simondium; CL, Stellenbosch; RN, Robertson; BE, Barrydale; LS, Ladismith; CD, Calitzdorp and (B) the Ceres-valley (fine scale).

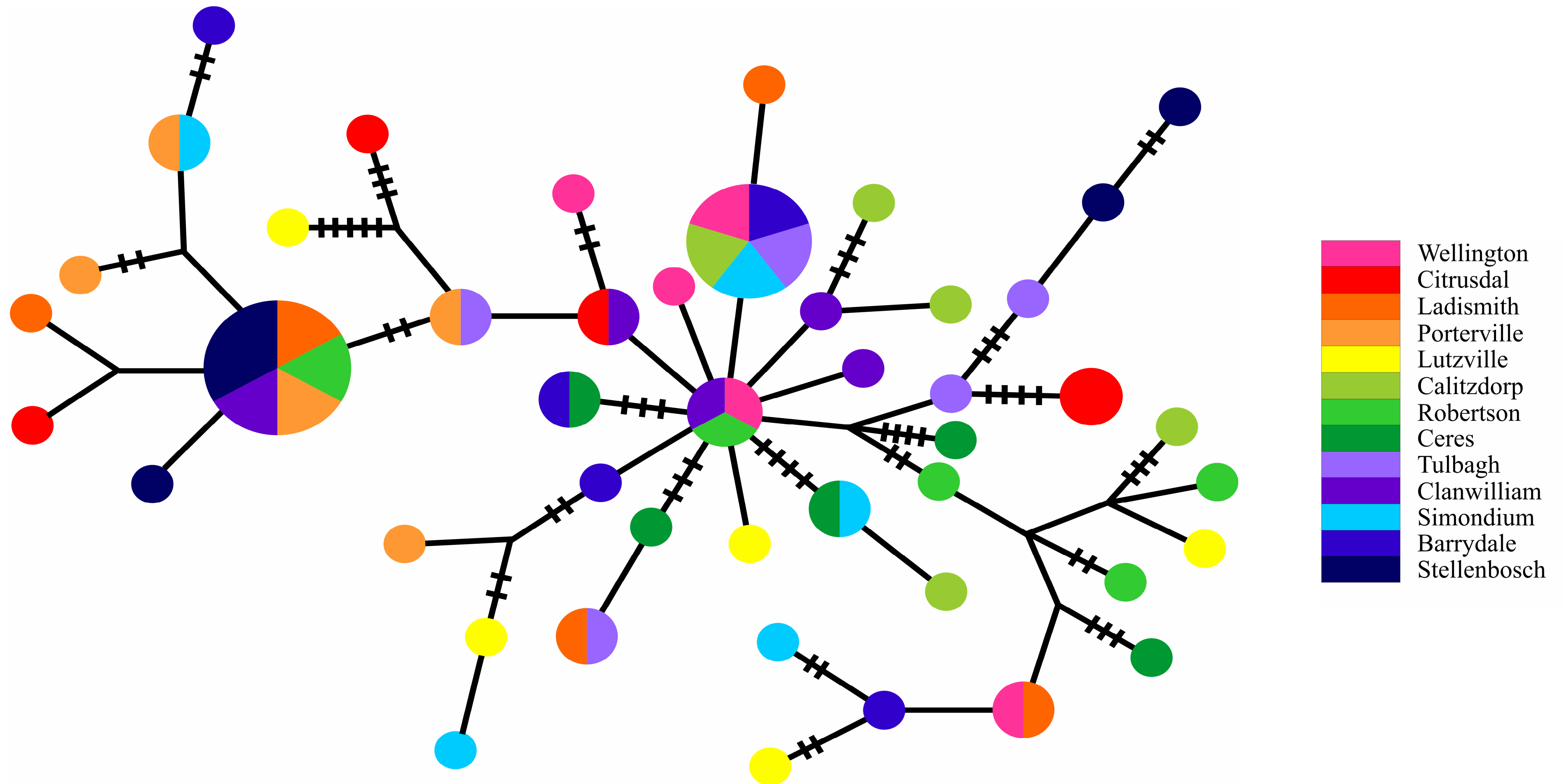

Figure 2.2: The parsimony haplotype network for *Ceratitis capitata*. The size of the pie charts are representative of the number of individuals that possess that haplotype. The small pie charts show haplotypes with a frequency of one individual. Every connecting line represents a mutational step of one between the different haplotypes. The perpendicular lines indicate additional mutational steps.

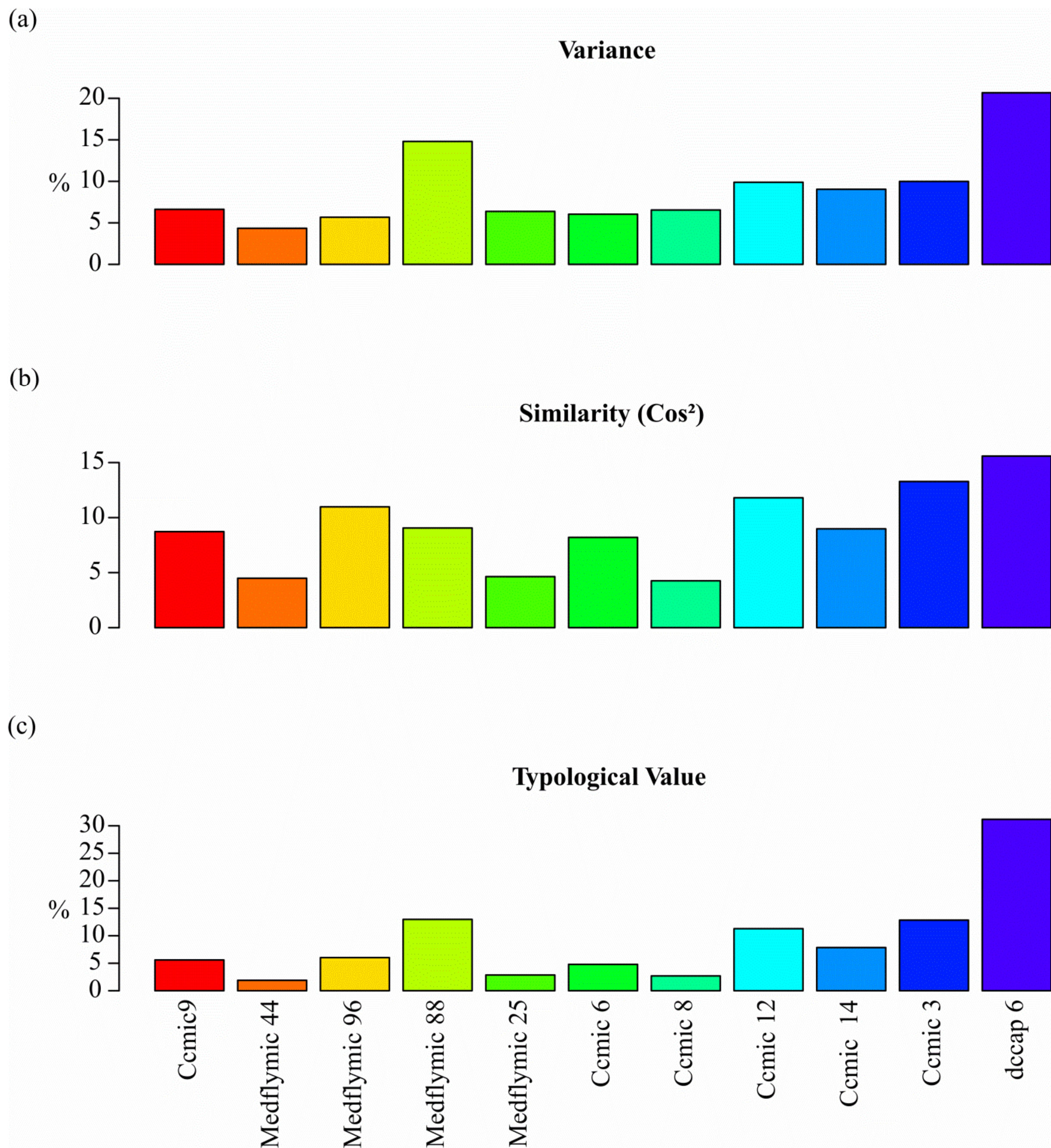

Figure 2.3: Distribution of values across 11 microsatellite loci for *Ceratitis capitata* from the first axis of the multivariate coinertia analysis (MCOA), (a) variance; (b) Cos^2; (c) TV.

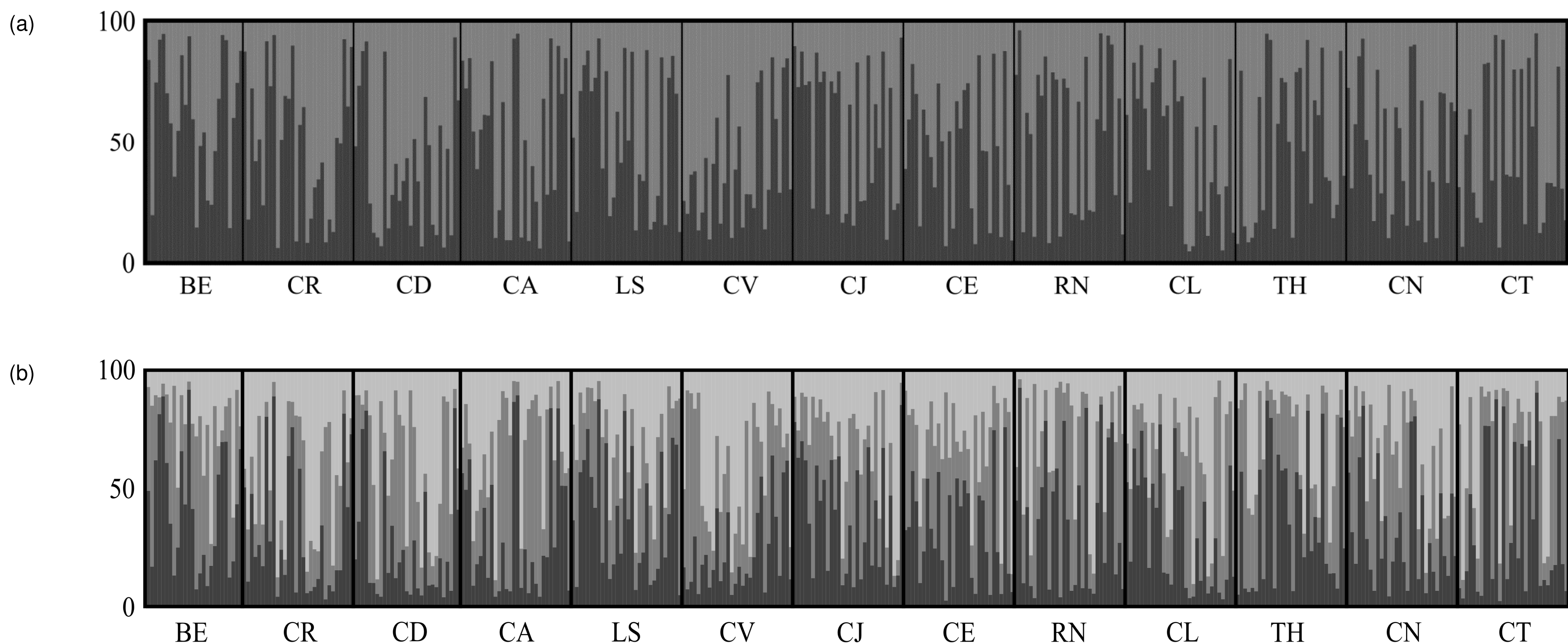

Figure 2.4: Analysis of 385 *Ceratitis capitata* individuals from 13 populations in the Western Cape using the Bayesian model based method implemented in the program STRUCTURE (Pritchard *et al*. 2000). Each individual is indicated with a vertical line, the different shades of grey represent the individual's estimated percentage membership to the K clusters. Genetic population structure is shown for $K = 2$ (a) and $K = 3$ (b).

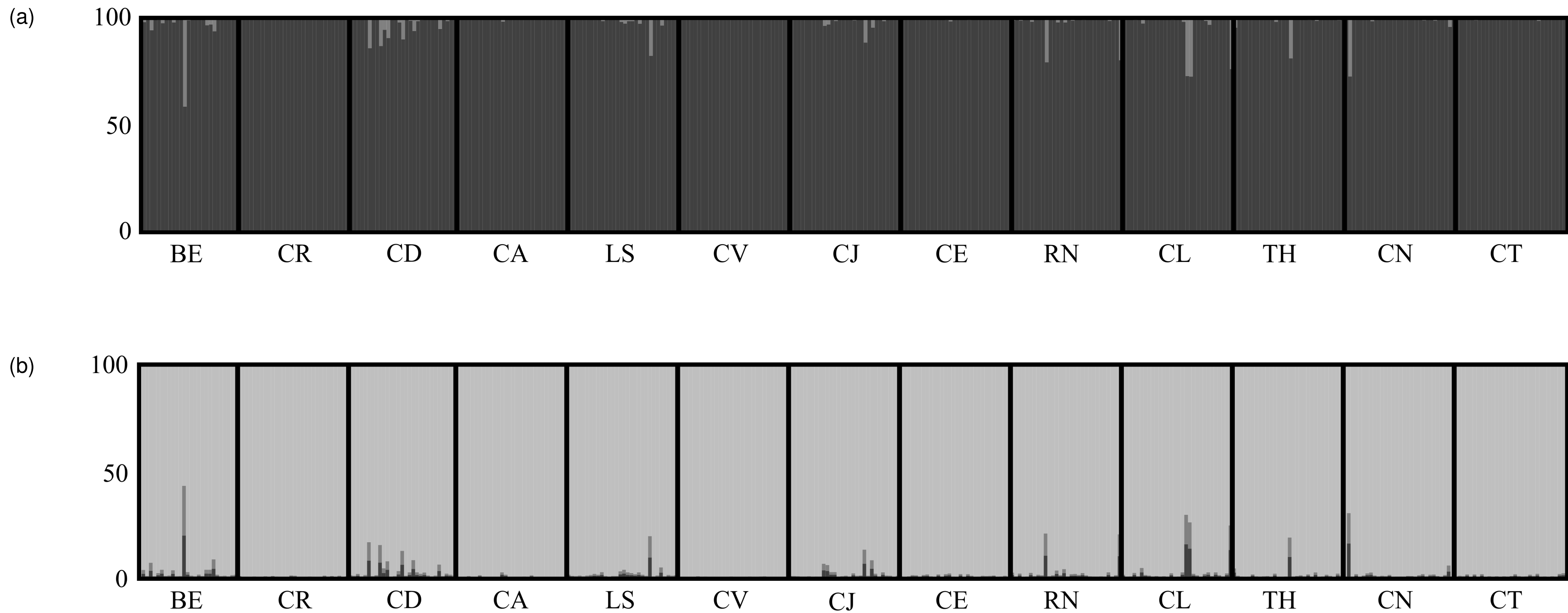

Figure 2.5: Analysis of 385 *Ceratitis capitata* individuals from 13 populations in the Western Cape using prior spatial information and the Bayesian model based method implemented in the program TESS (Chen *et al*. 2007; Durand *et al*. 2009). Each individual is indicated with a vertical line, the different shades of grey represent the individual's estimated percentage membership to the *K* clusters. Genetic population structure is shown for *K* = 2 (a) and *K* = 3 (b).

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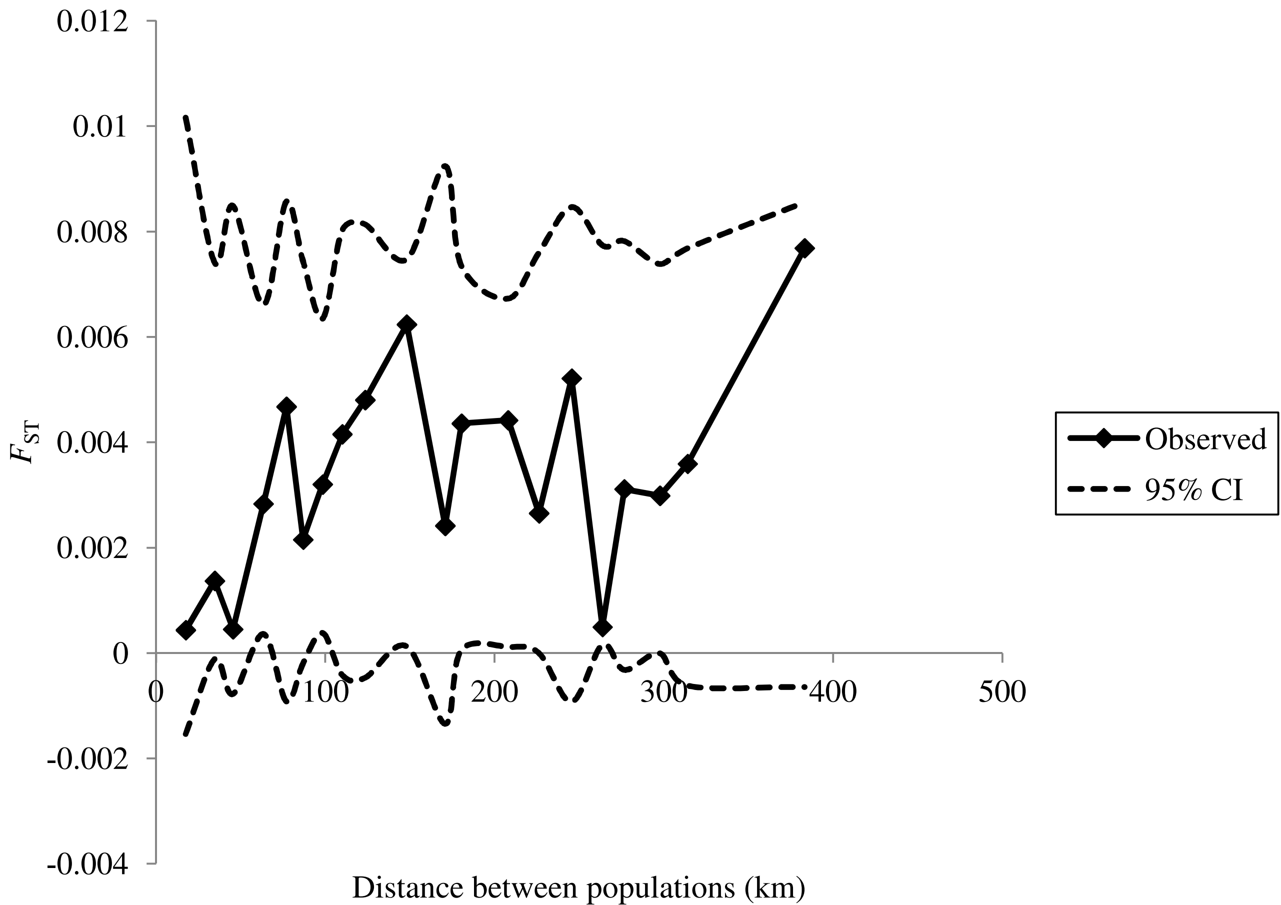

Figure 2.6: The spatial genetic structure within the 13 *Ceratitis capitata* populations sampled within the Western Cape. The solid line represents the mean of the multilocus pairwise F_{ST} values within each distance class and dashed lines represent the 95% confidence intervals of the null distributions obtained from 1000 random permutations.

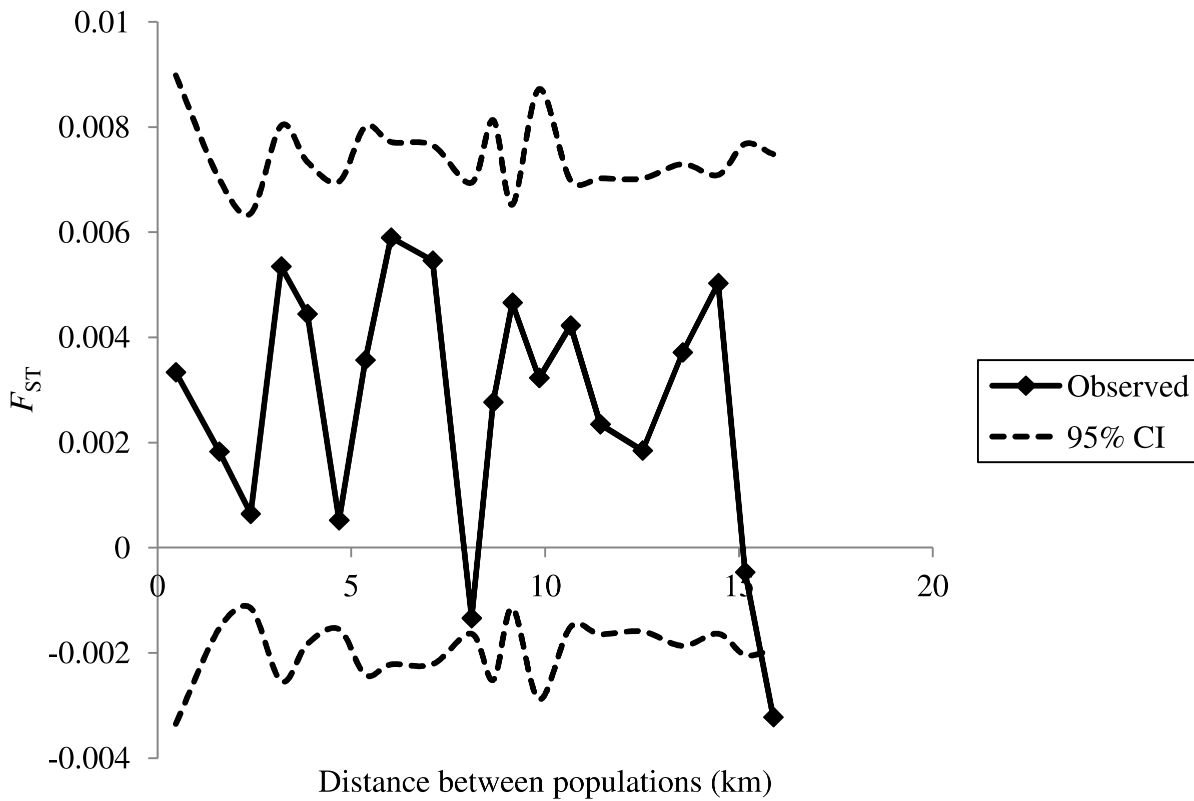

Figure 2.7: The spatial genetic structure within the 13 *Ceratitis capitata* populations within the Ceres-valley. The solid line represents the mean of the multilocus pairwise F_{ST} values within each distance class and dashed lines represent the 95% confidence intervals of the null distributions obtained from 1000 random permutations.

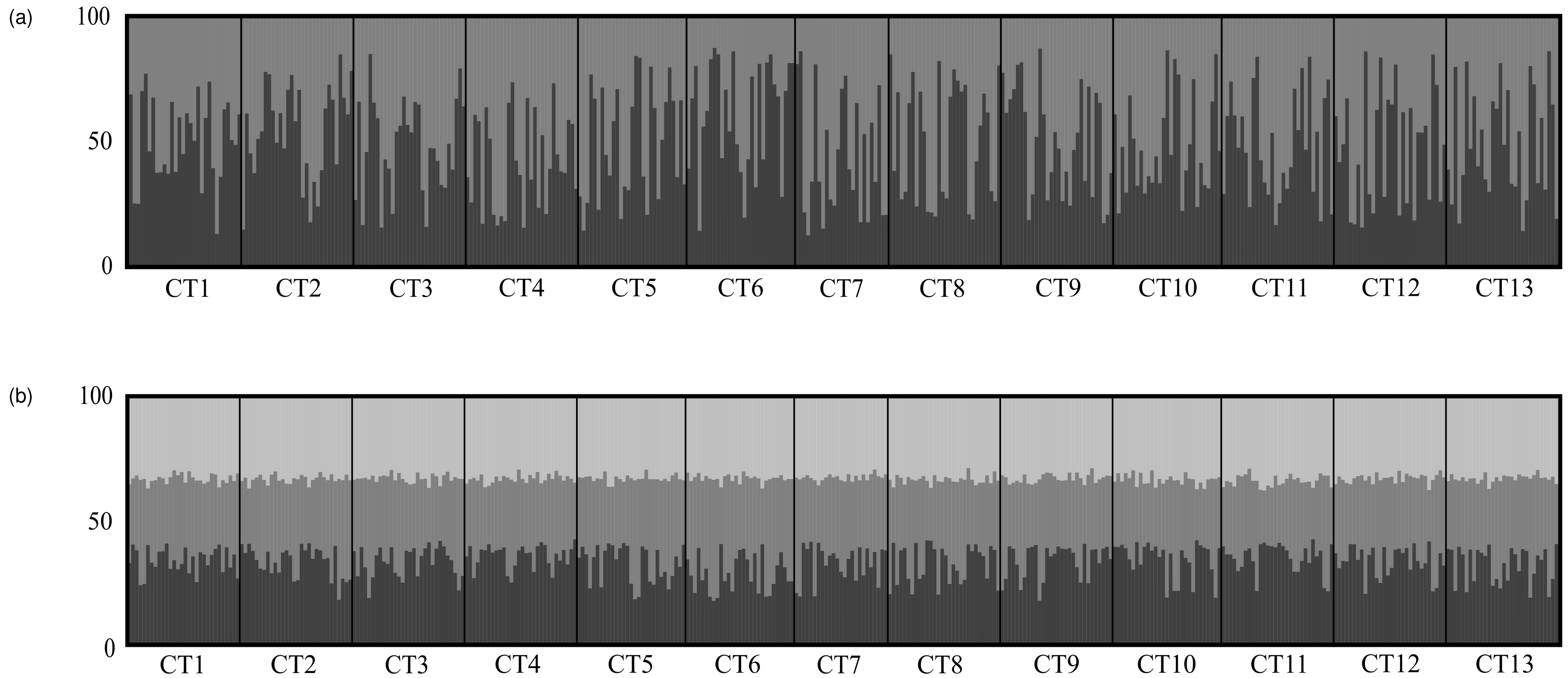

Figure 2.8: Analysis of 382 *Ceratitis capitata* individuals from 13 populations in the Ceres-valley using the Bayesian model based method implemented in the program STRUCTURE (Pritchard *et al*. 2000). Each individual is indicated with a vertical line, the different shades of grey represent the individual's estimated percentage membership to the *K* clusters. Genetic population structure is shown for $K = 2$ (a) and $K = 3$ (b).

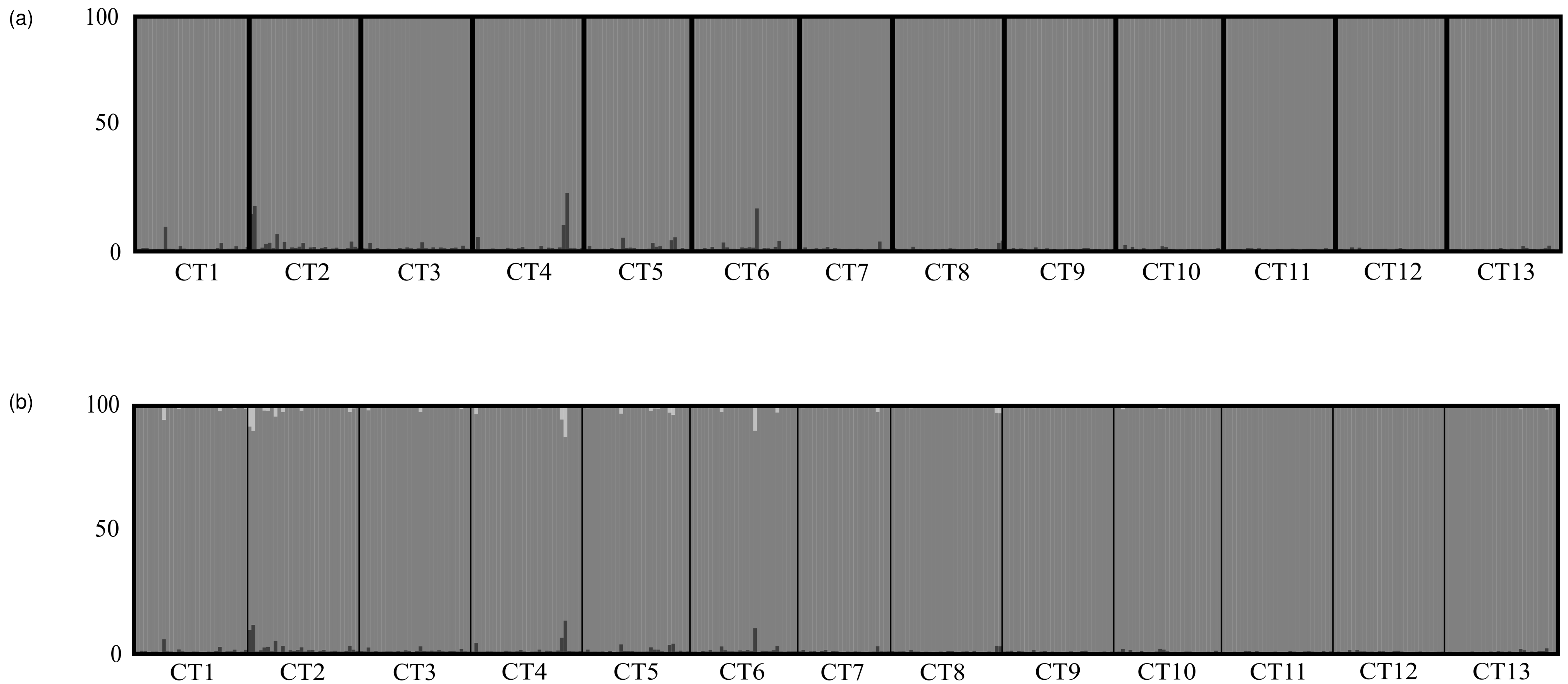

Figure 2.9: Analysis of 382 *Ceratitis capitata* individuals from 13 populations in the Ceres-valley using prior spatial information and the Bayesian model based method implemented in the program TESS (Chen *et al*. 2007; Durand *et al*. 2009). Each individual is indicated with a vertical line, the different shades of grey represent the individual's estimated percentage membership to the K clusters. Genetic population structure is shown for $K = 2$ (a) and $K = 3$ (b).

TABLES

Table 2.1: Sampling locations of *Ceratitis capitata* in the Western Cape and genetic diversity indices for the mitochondrial COI gene with N, sample size; N_h, the number of haplotypes; *h*, the haplotype diversity; π, nucleotide diversity and Fu's F-statistic with corresponding significance value (p) (± = standard error).

Location	ID	N	N_h	*h*	π	Fu's FS	p
Barrydale	BE	5	5	1.000±0.127	0.007±0.005	-1.139	0.119
Calitzdorp	CD	5	5	1.000±0.127	0.008±0.005	-0.964	0.140
Ceres	CT	5	5	1.000±0.113	0.009±0.006	-0.792	0.186
Citrusdal	CR	5	4	0.900±0.161	0.009±0.006	0.980	0.590
Clanwilliam	CA	5	5	1.000±0.1267	0.003±0.002	-2.517	0.015
Ladismith	LS	5	5	1.000±0.1267	0.008±0.005	-0.964	0.155
Lutzville	CV	5	5	1.000±0.1267	0.0085±0.006	-0.875	0.187
Porterville	CE	5	5	1.000±0.1267	0.007±0.005	-1.223	0.109
Robertson	RN	5	5	1.000±0.1267	0.005±0.004	-1.593	0.068
Simondium	CJ	5	5	1.000±0.1267	0.008±0.005	-0.918	0.156
Stellenbosch	CL	5	4	0.900±0.161	0.005±0.004	0.212	0.435
Tulbagh	TH	5	5	1.000±0.127	0.006±0.004	-1.445	0.072
Wellington	CN	5	5	1.000±0.127	0.004±0.003	-2.238	0.021
Total		65	46	0.980±0.007	0.007±0.004	-25.380	0.000

Table 2.2: Summary of the characteristics of microsatellite markers used in the analyses of *Ceratitis capitata*. The locus name, repeat motif, multiplex composition, annealing temperature (T_A) as well as allele size range (bp) is given. I also present the number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosity per locus.

Locus name	Repeat motif	Multiplex	Label	Size range (bp)	T_A (^{0}C)	N_A	H_O	H_E
Ccmic9[1]	(GA)9TA(GA)5TAGG(GA)2TA(GA)6TAGATA(GA)13	2	VIC	102-167	55	28	0.5572	0.8641
Ccmic6[1]	(TG)18	2	NED	68-117	55	24	0.3691	0.8986
Ccmic8[1]	(TG)2GG(TG)5CG(TG)5	1	PET	111-143	55	13	0.731	0.8191
Ccmic12[1]	(CA)14AA(CA)3AA(CA)3	2	6FAM	71-118	55	22	0.5733	0.8949
Ccmic14[1]	(CA)10CCAA(CA)2	1	VIC	66-87	55	8	0.7709	0.7857
Ccmic3[1]	(TG)11	2	PET	66-93	55	15	0.5401	0.8425
dccap6[2]	(AT)2AG(AT)4(AC)2(AT)2(AC)3	2	6FAM	212-245	55	13	0.4676	0.5732
Medflymic44[3]	(TG)13	1	6FAM	149-202	55	25	0.7514	0.8275
Medflymic96[3]	(TG)11(CAA)3	2	PET	159-237	55	27	0.8517	0.89
Medflymic88[3*]	A8/(GAA)3	1	VIC	179-239	55	2	0	0
Medflymic128[3]	(TG)4TA(TG)14	1	NED	163-220	55	28	0.8719	0.9107
Medflymic25[3]	(TA)9	1	NED	248-295	55	19	0.6412	0.7622

[1] Bonizzoni *et al*. 2000; [2] Casey & Burnell 2001; [3] Stratikopoulus *et al*. 2008
* Medflymic88, not polymorphic, not included in further analysis

Table 2.3: Pairwise Φ_{ST} values calculated in ARLEQUIN for COI for the 13 *Ceratitis capitata* populations in the Western Cape.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Barrydale	0												
2 Citrusdal	0.059	0											
3 Calitzdorp	-0.036	0.073	0										
4 Clanwilliam	-0.081	0.082	-0.061	0									
5 Ladismith	-0.104	-0.019	-0.026	-0.092	0								
6 Lutzville	-0.049	0.000	0.018	-0.013	-0.031	0							
7 Simondium	-0.131	0.064	-0.076	-0.036	-0.055	-0.034	0						
8 Porterville	0.016	0.059	0.162	0.123	-0.046	0.098	0.007	0					
9 Robertson	0.038	-0.013	0.051	0.042	-0.021	-0.043	0.020	0.108	0				
10 Stellenbosch	0.152	0.141	0.239	0.198	0.011	0.162	0.140	-0.034	0.150	0			
11 Tulbagh	-0.016	0.079	-0.005	-0.003	-0.051	-0.027	-0.016	0.158	0.038	0.157	0		
12 Wellington	-0.066	0.131	-0.004	-0.045	-0.004	-0.047	-0.026	0.228	0.039	0.302	-0.013	0	
13 Ceres	-0.061	0.074	-0.043	0.012	-0.024	-0.029	-0.106	0.090	0.022	0.186	-0.055	-0.004	0

Table 2.4: The locations of *Ceratitis capitata* sampling in the Western Cape with sample size (N), number of alleles (N_A), number of private alleles (N_{AP}), allelic richness (A_R, based on a minimum of 21 individuals), expected (H_E) and observed (H_O) heterozygosity (± = standard error) and the inbreeding coefficient (F_{IS}).

Location	ID	N	N_A	N_{AP}	A_R	H_E	H_O	F_{IS}
Barrydale	BE	26	107	1	9.434	0.812±0.096	0.658±0.215	0.209
Citrusdal	CR	30	112	0	9.282	0.788±0.119	0.593±0.201	0.264
Calitzdorp	CD	29	126	2	10.456	0.822±0.090	0.632±0.216	0.248
Clanwilliam	CA	30	113	3	9.449	0.793±0.097	0.628±0.168	0.225
Ladismith	LS	30	116	3	9.610	0.803±0.089	0.672±0.174	0.180
Lutzville	CV	30	109	0	9.211	0.793±0.088	0.697±0.198	0.138
Simondium	CJ	30	114	3	9.493	0.802±0.096	0.632±0.197	0.228
Porterville	CE	30	125	2	10.429	0.808±0.101	0.640±0.199	0.226
Robertson	RN	30	117	2	9.738	0.802±0.100	0.634±0.180	0.227
Stellenbosch	CL	30	118	4	9.792	0.810±0.088	0.641±0.189	0.225
Tulbagh	TH	30	120	4	9.922	0.802±0.104	0.637±0.185	0.222
Wellington	CN	30	119	3	10.023	0.805±0.104	0.675±0.217	0.179
Ceres	CT	30	115	2	9.664	0.808±0.096	0.658±0.124	0.203
Total		385	195	29	9.088	0.803	0.646	0.213

Table 2.5: Pairwise F_{ST} values (Weir & Cockerham 1984) calculated from 11 polymorphic microsatellite markers for *Ceratitis capitata* populations in the Western Cape.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Barrydale	0												
2 Citrusdal	0.002	0											
3 Calitzdorp	0.004	0.008	0										
4 Clanwilliam	0.009	0.005	0.008	0									
5 Ladismith	-0.002	0.002	0.000	0.009	0								
6 Lutzville	**0.014***	0.005	0.006	0.005	0.001	0							
7 Simondium	-0.001	0.008	0.005	0.007	-0.002	**0.010***	0						
8 Porterville	0.001	0.002	-0.003	0.002	-0.003	0.001	0.003	0					
9 Robertson	-0.001	0.005	0.005	**0.011***	0.001	**0.014***	0.007	0.005	0				
10 Stellenbosch	0.000	0.009	**0.011***	0.008	0.007	**0.013***	0.006	0.010	0.005	0			
11 Tulbagh	0.004	**0.019***	0.003	**0.016***	0.004	**0.012***	0.007	0.005	**0.014***	0.009	0		
12 Wellington	-0.005	0.006	0.002	**0.012***	-0.004	0.006	0.008	0.001	0.001	0.002	0.005	0	
13 Ceres	0.003	**0.016***	0.003	**0.017***	0.002	**0.013***	0.008	0.003	0.009	**0.016***	0.000	0.001	0

* Statistical significance at q < 0.05 after FDR correction

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Table 2.6: The locations of *Ceratitis capitata* sampling in the Ceres-valley with sample size (N), number of alleles (N_A), number of private alleles (N_{AP}), allelic richness (A_R, based on a minimum of 19 individuals), expected (H_E) and observed (H_O) heterozygosity (± = standard error) and the inbreeding coefficient (F_{IS}).

Location	ID	N	N_A	N_{AP}	A_R	H_E	H_O	F_{IS}
Ceres 1	CT1	30	123	4	9.847	0.807±0.102	0.671±0.135	0.185
Ceres 2	CT2	30	126	2	10.122	0.812±0.087	0.634±0.184	0.236
Ceres 3	CT3	30	123	2	9.879	0.805±0.109	0.662±0.179	0.194
Ceres 4	CT4	30	116	1	9.427	0.803±0.100	0.634±0.215	0.227
Ceres 5	CT5	29	113	2	9.340	0.793±0.095	0.654±0.192	0.193
Ceres 6	CT6	29	123	3	9.926	0.816±0.079	0.660±0.167	0.209
Ceres 7	CT7	25	110	0	9.537	0.798±0.097	0.643±0.171	0.215
Ceres 8	CT8	30	115	2	9.287	0.808±0.096	0.656±0.127	0.205
Ceres 9	CT9	30	116	2	9.497	0.813±0.099	0.665±0.155	0.199
Ceres 10	CT10	29	118	3	9.631	0.800±0.087	0.665±0.176	0.187
Ceres 11	CT11	30	116	2	9.510	0.808±0.081	0.651±0.168	0.211
Ceres 12	CT12	30	114	1	9.273	0.811±0.108	0.617±0.144	0.256
Ceres 13	CT13	30	113	3	9.342	0.797±0.094	0.638±0.182	0.217
Total		382	190	27	9.586	0.805	0.650	0.210

Table 2.7: Pairwise F_{ST} values (Weir & Cockerham 1984) calculated from 11 polymorphic microsatellite markers for *Ceratitis capitata* populations in the Ceres-valley.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Ceres1	0												
2 Ceres2	0.002	0											
3 Ceres3	-0.004	-0.004	0										
4 Ceres4	-0.005	0.002	-0.001	0									
5 Ceres5	0.007	0.003	-0.002	0.008	0								
6 Ceres6	0.004	0.002	0.002	0.012	0.008	0							
7 Ceres7	0.000	0.004	-0.001	0.000	0.005	0.009	0						
8 Ceres8	0.001	0.000	-0.001	0.006	0.010	0.007	0.008	0					
9 Ceres9	-0.003	-0.003	-0.003	0.006	0.005	0.004	0.004	0.002	0				
10 Ceres10	0.001	0.007	0.003	0.009	0.010	0.013	0.013	0.007	0.004	0			
11 Ceres11	-0.004	-0.002	-0.001	0.004	0.016	0.008	0.006	0.008	0.000	0.015	0		
12 Ceres12	0.002	0.000	-0.003	0.002	0.011	0.005	0.007	0.011	0.005	0.006	0.007	0	
13 Ceres13	0.000	0.004	-0.001	0.008	0.002	0.009	0.003	-0.002	-0.001	-0.002	0.009	0.004	0

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APPENDIX 1: List of hosts (including those that are heavily infested, occasionally infested, rarely infested and where infestation needs to be confirmed) used by the Mediterranean fruit fly (*Ceratitis capitata*) in Southern Africa. The list is adapted from White & Elson-Harris 1992; CABI 15 July 2011 (see www.cabi.org).

Acokanthera sp. (Toxicophlaea). *A. longiflora* Stapf, Bushman's-poison.

Actinidia chinensis, kiwi fruit.

Anacardium occidentale, cashew.

Ananas comosus (L.) Merrill (*A. sativus* Schult.), pineapple.

Annona cherimola Mill., cherimoya.

Annona glabra L., pondapple, alligator apple.

Annona muricata L., soursop, guan -bana.

Annona reticulata L., bullocks-heart, custard apple, anona.

Annona squamosa L., sugar apple, sweetsop.

Arbutus unedo L., strawberry madrone.

Arenga pinnata (Wurmb) Merrill, (*A. saccharifera* Labill.), gomuti, sugar palm.

Argania spinosa (L.) Skells (*A. sideroxylon* Roem. & Schult.), hardwood evergreen tree, Morocco ironwood.

Argemone mexicana L., Mexican prickle poppy, cardosanta, cardo.

Artabotrys hexapetalus (L.f.) Bhand. (*A. uncinatus* (Lam.) Merrill; *A. odoratissimus* R. Br.), fragrant tailgrape, climbing ylang-ylang.

Artocarpus altilis (Parkins.) Fosb. (*A. incisus* L.F.; *A. communis* Forst.), breadfruit.

Asimina obovata (Willd.) Nash, bigflower pawpaw.

Asimina parviflora (Michx.) Dunal, smallflower pawpaw.

Asimina pygmaea (Bartr.) Dunal, sprawling pawpaw.

Asimina reticulata Schuttlw. ex Chapm., seminoletea pawpaw, common pawpaw.

Asimina triloba (L.) Dunal, papaw, pawpaw.

Asparagus densiflorus (Kunth) Jessop 'Sprengeri', Sprenger asparagus.

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Atropa belladonna L., belladonna.

Averrhoa carambola L., carambola, star-fruit.

Berberis holstii Engl., barberry.

Blighia sapida K König (*Cupania sapida* (K König) Voigt), akee.

Brucea ferruginea L'Her. (*B. antidysenterica* Lam.).

Bumelia lycioides (L.) Pers., buckthorn bumelia.

Bumelia tenax (L.) Willd., tough bumelia, buckthorn.

Butia sp., butia palm. *B. capitata* (Mart.) Becc. (*Cocos capitata* Mart.), jelly palm, Brazilian butia palm, pindo palm.

Calophyllum inophyllum L., indiapoon beauty-leaf, kamani, Alexander laurel.

Cananga odorata (Lam.) Hook. f. & T. Thoms., ylang-ylang.

Capparis citrifolia Lam., caper.

Capsicum annuum L. (*C. frutescens* auct.; *C. baccatum* Vell.), Conoides Group: red pepper; Grossum Group: bell pepper, sweet pepper; Cerasiforme Group: cherry pepper; Longum Group: cayenne pepper, chili, long red.

Carica papaya L., papaya, papaw.

Carica quercifolia Solms, dwarf papaya, oakleaf papaya.

Carissa bispinosa (L.) Desf. ex Brenan (*C. arduina* Lam.; *C. acuminata* A. DC.), hedge thorn.

Carissa carandas L., karanda carissa.

Carissa edulis, Egyptian carissa.

Carissa grandiflora (E.H. Mey.) A. DC., Natal plum, carissa.

Carissa macrocarpa, Natal plum.

Casimiroa edulis Llave, white sapote, casimiroa, Mexican apple.

Cestrum sp., cestrum, jessamine: *C. nocturnum* L., night-jessamine, Chinese inkberry, night-blooming jasmine.

Chrysophyllum africanum A. DC. (*C. argyrophyllum* Hiern), African star apple.

Chrysophyllum cainito L., cainito, star apple.

Chrysobalanus ellipticus Soland. ex Sabine.

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Chrysobalanus icaco L., icaco coco plum, gopher apple, gopher plum.

Chrysophyllum oliviforme L., satin leaf, star apple, caimitillo, damson plum (Jamaica).

Chrysophyllum polynesicum Hillebr., chrysophyllum.

Chrysophyllum viridifolium Wood & Franks.

Citharexylum fruticosum L. (*C. cinereum* L.), Florida fiddlewood.

X *Citrofortunella mitis* (Blanco) J. Ingram and H.E. Moore (*Citrus mitis* Blanco), calamondin, Panama orange.

Citrus aurantiifolia (Christm.) Swingle, lime.

Citrus aurantium L., sour orange, Seville orange, bitter-sweet orange; (*C. myrtifolia* Raf.), myrtle-leaf orange.

Citrullus lanatus (Thunb.) Matsum. & Nakai (*C. vulgaris* Schrad.), watermelon.

Citrus limetta, sweet lime.

Citrus limon (L.) Burm. f., lemon, except 'Eureka', 'Lisbon', and 'Villa Franca' cultivars (smooth-skinned sour lemon).

Citrus x *limonia* Osbeck (*C. taitensis* Risso), lemon, lemandarin.

Citrus maxima (Burm.) Merrill (*C. grandis* (L.) Osbeck; *C. decumana* (L.) L.), pummelo, pomelmous shaddock, Pernambuco.

Citrus medica L., citron.

Citrus x *nobilis* Lour., king orange, tangor.

Citrus x *paradisi* Macfady, grapefruit, pomelo.

Citrus reticulata Blanco (*C. deliciosa* Ten.; *C. nobilis* Andr. var. *deliciosa* Ten.), mandarin orange, tangerine.

Citrus sinensis (L.) Osbeck 'Valencia' and 'Parson Brown' and 'Lue Gim Gong' , Malta orange, Lambs summer orange.

Citrus x *tangelo,* tangelo.

Clausena lansium (Lour.) Skeels (*C. punctata* (Sonn.) Rehd. & E.H. Wils.; *C. wampi* (Blanco) D. Oliver), Chinese wampee, wampi.

Clintonia umbellulata (Michx.) Morong, spreckled beadlily.

Coccoloba uvifera (L.) L., sea grape.

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Cotoneaster adpressus Bois. var. *praecox* Bois. & Berthault (*C. praecox* (Bois & Berthault) Hort. Vilm. - Andr. ex Meuniss.), early creeping cotoneaster.

Coffea sp. coffee.

Crataegus azarolus L., azarole hawthorn, haw.

Crinum asiaticum L. (*C. sinicum* Roxb. ex Herb.) St. John's lily.

Cucumis dipsaceus C.G. Ehrenb. ex Spach, hedgehog, teaselgourd, wild cucumber.

Cucumis melo L., Cantalupensis Group: cantaloupe; Inodorns Group: casaba melon; Reticulatus Group: muskmelon.

Cucumis sativus L., cucumber.

Cucurbita maxima Duchesne, winter squash, hubbard squash.

Cucurbita moschata (Duchesne) Poir., cushaw pumpkin, Canada and winter crookneck pumpkin.

Cucurbita pepo L., pumpkin, vegetable marrow.

Cydonia oblonga Mill. (*C vulgaris* Pers.), quince, mannela.

Cyphomandra crassicaulis (Cav.) Sendtn., tree tomato.

Diospyros sp., persimmon.

Dovyalis caffra (Hook. f. & Harv.) Warb. (*Aberia caffra* Hook. f. & Harv.), kei-apple, umkokolo.

Dovyalis hebecarpa (G. Gardn.) Warb., kitembilla, Ceylon gooseberry.

Ekebergia capensis Sparrm., dog plum.

Eriobotrya japonica (Thunb.) Lindl. (*Photinia japonica* Thunb.), loquat, Malta plum.

Eugenia brasiliensis Lam. (*Stenocalyx brasiliensis* Berg), Brazil eugenia, Brazilian plum, Spanish cherry.

Eugenia uniflora L. (*E. michelii* Lam.; *Stenocalyx michelii* Berg), Surinam cherry, pitanga, Brazil cherry, cayenne cherry, Florida cherry, French cherry.

Euphorbia lathyris L., caper euphorbia, gopher apple.

Euphoria longan (Lour.) Steud. (*Nephelium longan* Lour.; *Dimocarpus longan* Lour.), longan, dragon's eye.

Feijoa sellowiana O. Berg, feijoa guavasteen, pineapple guava.

Ficus carica L., common fig, lemon fig.

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Flacourtia indica (Burm. f.) Merrill (*F. ramontchi* L'Her.), ramontchi, governor's plum.

Fortunella crassifolia Swingle, meiwa kumquat.

Fortunella japonica (Thunb.) Swingle (*Citrus japonica* Thunb.), round and marumi kumquat.

Fortunella margarita (Lour.) Swingle, Eustis limequat, megami kumquat.

Fragaria x *ananassa* Duchesne (*Fragaria chilonensis* (L.) Duchesne x *F. virginiana* Duchesne), cultivated or garden strawberry.

Garcinia livingstonei, imbe.

Garcinia mangostana L., mangosteen.

Garcinia xanthochymus Hook. f. ex T. Anderson, garcinia, gourka.

Gardenia sp., gardenia.

Glycosmis pentaphylla (Retz.) Correa, glycomis, Malay glycosmis.

Gossypium sp., cotton.

Harpephyllum caffrum Bernh. ex C.F. Krauss, Kafir plum.

Hevea brasiliensis (Willd. ex A. Juss.) Mull. Arg., para rubber tree, caoutchoue, Brazil rubber.

Homalocladium platycladum (F.J. Muell.) L.H. Bailey, ribbon bush.

Ilex vomitoria Ait., Carolina holly, yaupon.

Juglans sp., walnut.

Juglans hindsii (Jeps.) Jeps.

Landolphia sp., gumvine.

Latania loddigesii Mart. (*L. glaucophylla* Hort. ex Baker), blue palm.

Litchi chinensis Sonn. (*Nephelium litchi* Camb.), litchi, lychee.

Lycium carolinanum Walt., Carolina wolfberry, boxthorn.

Lycium chinense Mill. (*L. carnosum* Hort.), (L. campanulatum Drege ex Dun.), boxthorn.

Lycium europaeum L., European wolfberry.

Lycium horridum Thunb., African buckthorn.

Lycopersicon esculentum Mill., tomato.

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Maclura pomifera (Raf.) C.K. Schneid. (*M. aurantiaca* Nutt.; *Toxylon pomifera* Raf.), osage orange.

Malpighia glabra L., Barbados cherry, acerola, huesito.

Malpighia punicifolia L., Barbados cherry, acerola.

Malus domsetica, Apple.

Malus pumila Mill. (*M. communis* Poir.; *Pyrus pumila* (Mill.) C. Koch), common apple.

Mammea americana L., mamey, mammee apple.

Mangifera indica L., mango.

Manilkara zapota (L.) Van Royen (*Achras zapota* L.; *Sapota achras* Mill.; *M. zapotilla* (Jacq.) Gilly), sapodilla.

Marrubium vulgare L., common hoarhound.

Mastichodendron foetidissimum (Jacq.) Lam. (*Sideroxylon mastichodendron* Jacq.; *Sideroxylon foetidissimum* Jacq.), mastic, jungle plum, ironwood.

Melicoccus bijugatus Jacq. (*Melicocca bijuga* L.), mamoncillo, mamon, Spanish lime, genip.

Melothria pendula L., creeping cucumber.

Mespilus germanica L., medlar.

Mimusops caffra E.H. Mey. ex A. DC., Kafir bulletwood.

Mimusops elengi L., elengi tree, pogada, West Indian medlar, elengi bulletwood.

Mimusops kirkii Bak.

Mimusops sp., milkwood.

Momordica balsamina L., balsam apple.

Monstera deliciosa Liebm. (*Philodendron pertusum* Kunth & Bouche), ceriman.

Morus sp., mulberry.

Muntingia calabura, calabur

Murraya paniculata (L.) Jacq. (*M. exotica* L.), orange-jessamine, mock orange.

Musa acuminata Colla (*M. cavendishii* Lamb. ex Paxt.; *M. nana* auct.; *M. chinensis* Sweet), dwarf banana, Chinese banana.

Musa x *paradisiaca* L. (*M.* x *sapientum* L.), common banana, plantain.

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Myrciaria cauliflora, jaboticaba.

Myrciaria edulis (Vell.) Skeels (*Eugenia edulis* Vell.), willow-leaved eugenia.

Noronhia emarginata (Lam.) Thouars ex Hook., Madagascar olive, noronhia, Chinese plum.

Nyssa ogeche Bartr., ex Marsh., ogeche lime, ogechi plum, ogeechee tupelo.

Nyssa sylvatica Marsh., sour gum.

Nyssa sylvatica Marsh. var. biflora (Walt.) Sarg., black gum, swamp black tupelo.

Ochrosia elliptica Labill., ochrosia.

Olea europaea L., common olive.

Opuntia sp., prickly pear. *O. humifusa* (Raf.) Raf. (*O. compressa* (Salisb.) Macbr.; *O. opuntia* (L.) Karst.; *O. rafinesquei* Engelm.; *O. mesacantha* Rafin.), *O. humifusa* Raf. 'Variegata', *O. dilleni* Haw., *O. tuna* (L.) Mill.(*O. humilis* Haw.; *O. horrida* Salm-Dyck ex DC.), *O. polyantha* Haw., *O. vulgaris* Mill. (*O. monocantha* (Willd.) Haw.; *O. nana* DC.).

Opuntia ficus-indica (L.) Mill. (*O. engelmanni* Salm-Dyck; *O. megacantha* Salm-Dyck; *O. occidentalis* Engelm. & Bigel.), Indian fig, spineless cactus.

Parmentiera aceleata, cuachilote.

Passiflora edulis Sims, purple granadilla, lilikoi, passion fruit.

Passiflora incarnata L., wild passion flower, maypop.

Passiflora laurifolia L., yellow granadilla, water lemon, Jamaica honeysuckle.

Passiflora lingularis Juss., sweet granadilla.

Passiflora mollissima (HBK) L.H.Bailey, lilikoi, soft-leaf passion flower.

Passiflora quadrangularis L. (*P. macrocarpa* M.T. Mast.), giant granadilla.

Passiflora sp., passion flower, *P. caerulea* L., blue-crown passion flower, *P. foetida* L., tagua passion flower.

Peponia mackennii Naud., wild cucurbit.

Pereskia aculeata Mill. (*P. pereskia* (L.) Karst.), Barbados gooseberry.

Persea americana Mill. (*P. gratissima* C.F. Gaertn.), avocado, alligator pear.

Phaseolus limensis Macfady., lima bean.

Phaseolus lunatus L., sieva bean, cibet bean.

Phaseolus vulgaris L., kidney bean, haricot, string bean.

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Phoenix dactylifera L., date palm.

Phyllantus acidus (L.) Skeels, otaheite gooseberry, leafflower, Ceylon gooseberry.

Physalis peruviana L., cape gooseberry, poha.

Pimenta dioica (L.) Merrill (P. officinalis Lindl.), allspice, pimenta.

Pleiogynium cerasiferum (F.J. Muell.) R. Parker (*P. solandri* (Benth.) Engl.; *Spondias solandri* Benth.), burdekin plum.

Podocarpus elongatus (Ait.) L'Her. ex Pers., yellowood, fern podocarpus.

Poncirus trifoliata (L.) Raf., trifoliate orange.

Pouteria campechiana (HBK) Baehni (*Lucuma nervosa* A. DC.; *L. rivicoa* Gaertn. f.), canistel incuma, egg fruit.

Pouteria sapota (Jacq.) H.E. Moore & Stern (*Calocarpum sapota* (Jacq.) Merrill; *C. mammosum* auct.; *Lucuma mammosa* auct.), sapote.

Pouteria vuridis, green sapote.

Prunus americana Marsh., native plum, American plum.

Prunus armeniaca L. (*Armeniaca vulgaris* Lam.), apricot.

Prunus cerasus L. (*Cerasus caproniana* DC.), sour cherry.

Prunus domestica L., garden plum, *Prunus domestica* L. spp. *insitita* (L.) Schneid., damson,bullace.

Prunus dulcis (Mill.) D.A. Webb (*P. amygdalus* Batsch; *P. communis* (L.) Arcang.) almond.

Prunus japonica Thunb., Chinese bushcherry, plum.

Prunus persica (L.) Batsch. (*Amygdalus persica* L.; Mill.), *Persica vulgaris* (Mill.), peach.

Prunus persica (L.) Batsch. var. *nucipersica* (Suckow) C.K. Schneid. (*P. persica* (L.) Batsch. var. nectarina (Ait. f.) Maxim.), nectarine.

Prunus salicina Lindl., Japanese plum.

Prunus umbellata Ell., wild plum, flatwoods plum.

Psidium cattleianum Sab. (*P. littorale* Raddi var. *longipes* (O. Berg.) Fosb.), strawberry guava, cattley guava, waiawi.

Psidium guajava L., guava.

Psidium guineense Swartz, Brazilian guava.

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Psidium littorale Raddi var. *littorale* (*P. littorale* Raddi var. *lucidum* (Degener) Fosb.), yellow cattley guava.

Punica granatum L., pomegranate.

Putranjiva roxburghii Wallich, wild olive, India amulet plant.

Pyracantha coccinea M.J. Roem. 'Lalandei', laland firethorn.

Pyrus communis L., common pear.

Pyrus x *leconte* Rehd., leconte pear.

Ribes sp., currant, gooseberry.

Robinia sp., locust.

Rosa sp., rose.

Royena pallens Thunb. (*R. pubescens* Willd.), pale-branched royena.

Rubus sp., blackberry, youngberry.

Salix sp., willow leaf.

Santalum album L., sandalwood, white sandalwood.

Santalum freycinetianum Gaudich. (*S. paniculatum* Hook. & Arn.), beach sandalwood.

Scaevola plumieri (L.) Vahl, goodenia beechberry.

Schinus molle L., California pepper tree (blossoms).

Sechium edule (Jacq.) Swartz (*Chayota edulis* Jacq.); chayote, christophine.

Selenicereus pteranthus (Link & Otto) Britt. & Rose (*Cereus nycticalus* Link; *Cereus pteranthus* Link ex Dietr.), cactus.

Serenoa repens (Bartr.) Small (*S. serrulata* (Michx.) Nichols; *Brahea serrulata* H. Wendl.; *Chamerops serrulata* Michx.; *Sabal serrulatum* Shult. f.), saw palmetto.

Severinia buxifolia (Poir.) Ten. (*Atalantia buxifolia* (Poir.) D. Oliver; *Triphasia monophylla* DC.), Chinese box orange.

Sideroxylon inerme L., ironwood.

Smilax beyrichii Kunth, *Smilax sandwicensis* Kunth, smilax.

Solanum aculeatissimum Jacq., solanum, Sodoms apple, nightshade.

Solanum capsicastrum Link ex Schauer, solanum cherry, false Jerusalem cherry.

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Solanum carolinense L., Caroline horse nettle.

Solanum incanum L. (*S. coagulans* Forssk.).

Solanum melanocerasum All. (*S. nigrum* L. var. *guineense* L.), solanberry, black nightshade, garden huckleberry.

Solanum melongena L. var. *esculentum* Nees, garden eggplant.

Solanum pseudocapsicum L., Jerusalem cherry.

Solanum sodomeum L., apple of Sodom.

Sorbus sp., mountain ash.

Spondias cytherea Sonn. (*S. dulcis* G. Forst.), ambarella, otaheite apple, vi-apple.

Spondias mombin L. (*S. axillaris* Roxb.; *S. lutea* L.), yellow mombin, Spanish plum, hog plum, jobo, cajamerin.

Spondias purpurea, red mombin.

Spondias sp., West Indian plum.

Strychnos atherstonei Harv., cape teak.

Strychnos pungens Solered., poison nut, wild orange.

Syagrus campestris (Mart.) H. Wendl. (*Cocos campestris* Mart.), field syagrus palm.

Syzygium cumini (L.) Skeels (*S. jambolana* (Lam.) DC. *Eugenia cumini* (L.) Druce; *E. jambolana* Lam.), jambolan, Java palm.

Syzygium jambos (L.) Alston (Eugenia jambos L.; *Caryophyllus jambos* Stokes), rose apple, jambos, Malabar plum.

Syzygium malaccense (L.) Merrill & L.M. Perry (*Eugenia malaccensis* L.; *Jambosa malaccensis* (L.) DC.; *Caryophyllus malaccensis* Stokes), ohia, Malay apple, pomerack, mountain apple.

Syzygium samarangense (Blume) Merrill & L.M. Perry (*Eugenia javanica* Lam.).water apple

Teclea trichocarpa (Engl.) Engl.

Terminalia catappa L., tropical almond, false kamani, winged kamani.

Terminalia chebula Retz., black myrobalan, chebula terminalia.

Terminalia pallida Brandis, terminalia.

Theobroma cacao L., cacao, cocoa.

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Thevetia peruviana (Pers.) K. Schum. (*T. neriifolia* A. Juss. ex Steud.; *Cerbera thevetia* L.), yellow oleander, be-still, lucky nut thevetia.

Vaccinium cereum Forst. f., blueberry.

Vangueria edulis Vahl (*V. madagascariensis* J.F. Gmel.), vangueria.

Vangueria infausta Burchell, wild medlar, vangueria.

Vicia faba L., broad bean, horse bean.

Vitis lambrusca L., fox grape, Isabella grape.

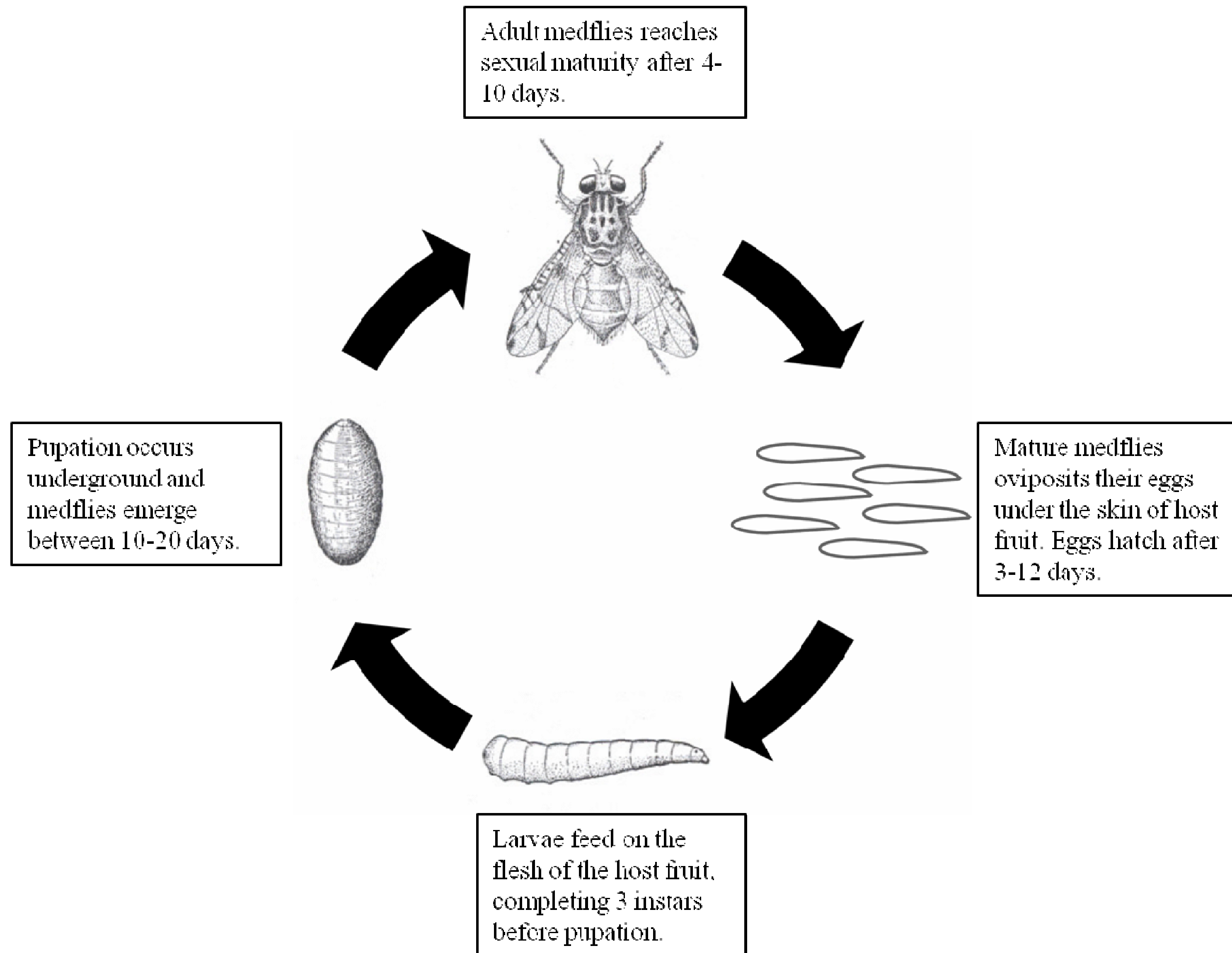
Vitis sp. (hybrid), beacon grape.

Vitis vinifera L., wine grape, European grape.

Wikstroemia phillyreifolia Gray, wikstroemia.

Ziziphus jujuba Mill. (*Z. vulgaris* Lam., *Z. sativa* Gaertn.), jujube, Chinese date.

Ziziphus mauritiana, Indian jujube.



APPENDIX 2: Schematic representation of the life-cycle of the Mediterranean fruit fly (*Ceratitis capitata*). Drawings from Skaife (1953).