Chapter 4
Role of Microsatellite Markers in Molecular Population Genetics of Fruit Flies with Emphasis on the *Bactrocera dorsalis* Invasion of Africa

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Abstract Microsatellites, also referred to as short tandem repeats (STRs) or simple sequence repeats (SSRs) are short sequences of tandem repeats of 1–6 bp in length in clusters of less than 150 bp flanked by sections of non-repetitive unique sequences that are scattered throughout the nuclear genome. These markers are co-dominant and hypervariable, revealing many alleles per locus; they are inherited in Mendelian fashion making them useful for detecting genetic variability within species. Once isolated and characterized, microsatellites can also be used in closely related taxa. Microsatellites can be amplified, even from highly degraded DNA, and are very simple to score. More importantly, these markers are highly polymorphic due to the plethora of variations in the repeat motifs. Several studies have endorsed microsatellite markers as an effective genetic tool to determine the historical distinctiveness of populations, and hence, the designation of species. Being highly polymorphic and selectively neutral, microsatellite markers offer a powerful genetic tool for investigating population structure, colonization processes, temporal and spatial population dynamics and evolutionary trends of insect pests. Furthermore, these markers have been successfully applied to different invasive fruit fly species to infer the evolutionary aspects underlying their invasive processes. Microsatellite markers have offered an analytical tool for the study of fruit fly invasion genetics as exemplified for the Mediterranean fruit fly, *Ceratitis capitata*. Herein, a detailed utility of microsatellite markers in inferring invasion histories of key fruit flies of economic importance is given, with a special focus on invasion into Africa of *Bactrocera dorsalis*.

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1 Introduction to the Use of Molecular Markers

In Africa, and sub-Saharan Africa in particular, the agricultural sector is the backbone of most economies in pursuit of sustainable development. Specifically, the horticultural sub-sector is a precious tool that is contributing to poverty alleviation by increasing food security and income generation for continued economic growth. However, this sub-sector is constrained by a number of biotic and abiotic factors. Amongst the former is infestation by tephritid fruit flies that are well recognised as a group of pests of economic significance (Ekesi and Billah 2007). Sub-Saharan Africa is the native home to about 915 fruit fly species out of which 299 species belong to the genera *Ceratitis* MacLeay, *Trirhithrum* Bezzi and *Dacus* Fabricius (White and Elson-Harris 1992). Due to the globalisation of trade, the emergent tourism industry, porous borders and poor phytosanitary expertise, the likelihood of inadvertent introduction and spread of exotic fruit fly species across the continent is escalating. Therefore, measures to strengthen the phytosanitary/quarantine infrastructure are of paramount importance to avert the establishment of alien invasive pests.

Knowledge of the genetic structure and geographical variability of invasive fruit fly species is a vital pre-requisite to implementing quarantine, control and eradication measures (Roderick and Navajas 2003; Malacrida et al. 2007). In the past, morphological characters were considered sufficient to describe species. However, morphological characters for species delineation have several limitations. The existence of homoplasy amongst characters and cryptic speciation in some insect families, as in the tephritid fruit flies, make species-level descriptions based on adult and larval morphology extremely difficult (Armstrong et al. 1997; De Meyer 1998; McPheron 2000). This has led taxonomists and quarantine officials to seek alternative ways to identify tephritid fruit flies, including the use of molecular markers (Sonvinco et al. 1996; Armstrong et al. 1997; Morrow et al. 2000). Indeed, biochemical (allozymes) and DNA molecular markers have been used to elucidate the variability in population structure of several tephritid species (Baruffi et al. 1995; Malacrida et al. 1996). In addition to this, molecular markers have advantages over biochemical tools and hence have become the current tool of choice. Molecular markers are site specific DNA sequences that are easily detected in the genome. These markers are neutral and can be utilised in a number of ways including, but not limited to: the analysis of genetic variability; to make inferences on population genetic structure; for DNA fingerprinting; for chromosome mapping; and for the identification and description of species. There are a number of molecular markers that have been developed and are in use: RAPDs, RFLPs, PCR-RFLPs, microsatellites, minisatellites and SNPs amongst others (Baruffi et al. 1995; Barr et al. 2006;

Malacrida et al. 1996; Khamis et al. 2008). The best molecular markers have a combination of the following properties: they should occur frequently in the genome; be co-dominant; be highly polymorphic; be vastly reproducible and transferable to many taxa; be cheap to develop and apply; and not be affected by environmental conditions.

In some frugivorous tephritid fruit fly species, diagnostic morphological characters for the identification of adult flies have been made available (Adsavakulchai et al. 1999; De Meyer 2005; Drew et al. 2006, 2008). However, inconsistencies in the limits of fruit fly species identification based on conventional adult morphological features together with overlapping host and geographical ranges, have profound effects on quarantine, management and biological studies of these species (Clarke et al. 2005). These limitations have led to the development and improvement of molecular tools for identification and classification of the fruit flies pest species, and for understanding their population structure (Armstrong et al. 1997; Malacrida et al. 1998; Han and McPheron 1997; Manni et al. 2015). Furthermore, because molecular markers are phenotypically neutral and resistant to environmental cues (unlike morphological characters) they can be used as a single reliable taxonomic tool. Several molecular markers are available to discriminate amongst tephritid fruit fly species. These markers have successfully been used to validate species (Khamis et al. 2012; Schutze et al. 2014), infer phylogenetic relationships (Boykin et al. 2014), verify intra-specific variation between populations (Bonizzoni et al. 2000; Baliraine et al. 2004) and trace the routes of invasion of pest fruit fly species (Aketarawong et al. 2007, 2014; Khamis et al. 2009).

2 Application of Microsatellites: Advantages and Limitations

Several studies have endorsed microsatellite markers as an effective genetic tool to determine the historical distinctiveness of populations, and hence, the designation of species (Hedrick et al. 2001; Wang et al. 2001). Microsatellites are short sequences of tandem repeats of 1–6 bp in length in clusters of less than 150 bp in length flanked by sections of non-repetitive unique sequences that are scattered throughout the nuclear genome and mostly associated with conserved loci containing coding regions (Loxdale and Lushai 1998). Microsatellites have also been referred to as short tandem repeats (STRs) (Edwards et al. 1991) or simple sequence repeats (SSRs) (Jacob et al. 1991). These markers are co-dominant and hypervariable, revealing many alleles per locus; they are inherited in Mendelian fashion making them useful for detecting genetic variability within species. Once these markers have been isolated and characterized, they may also be used in closely related taxa. Microsatellites can be amplified even from highly degraded DNA and are very simple to score (Bruford and Wayne 1993). More importantly, these markers are highly polymorphic due to the plethora of variations in the repeat motifs. In 1989, Weber and May developed a universal method for isolating microsatellites. The polymorphism of these markers was confirmed by Litt and Luty (1989) who detected allelic

variants amongst individuals using amplification of the $(TG)_n$ microsatellites in the human actin gene. Although the origin of microsatellite polymorphism is still debated, it is likely to be due to slipped-strand mispairing (Levinson and Gutman 1987), slippage events during DNA replication/repair/recombination (Schlötterer and Tautz 1992) or asymmetrical cross-over between sister chromatids (Innan et al. 1997).

Despite the uncertainty surrounding microsatellite evolution, they have been adopted widely and applied in many fields of study since their initial description by Hamada et al. (1982). Microsatellites markers are an invaluable method for genome mapping in many organisms (Schuler et al. 1996), and are also applicable in various fields ranging from ancient and forensic DNA studies, to population genetics and conservation/management of biological resources (Jarne and Lagoda 1996). Furthermore, microsatellites can be used for species identification, genetic tagging, breeding studies, reproductive biology, taxonomy, phylogenetic studies, disease diagnostics and genetic diversity studies (Abdul-Muneer 2014). Moreover, these markers offer a diagnostic tool that can differentiate between species not easily separated by morphological traits (Kinyanjui et al. 2016). The usefulness of microsatellite markers is evidenced by the rising numbers of mapped genes based on microsatellites (Schuler et al. 1996).

The wide applicability of microsatellite markers is associated with their many advantages. Microsatellite markers are robust and very informative compared with other markers such as RFLPs, RAPDs and AFLPs (He et al. 2003; Lee et al. 2004). Importantly, microsatellite markers are PCR-based and therefore require low quantities of DNA. The primer lengths and high annealing temperatures of microsatellite markers in genotyping guarantee their reproducibility. Once isolated, microsatellite markers can be cross amplified amongst closely related species (Baliraine et al. 2002). Furthermore, these markers can be multiplexed hence reducing the time and cost of analysis. Although microsatellites are a successful tool in genetics, they do have some drawbacks. Firstly, microsatellite markers need to be isolated *de novo* for most species being analysed for the first time; this isolation is expensive, laborious and time consuming (Zane et al. 2002). Secondly, the likelihood of null alleles occuring when using ancient or degraded DNA is very high, leading to difficulties in estimating allelic frequencies and heterozygosity (Kumar et al. 2009). Last but not least, homoplasy in some organisms is a common problem for the application of microsatellite markers in phylogenetics, leading to false identification of species descents (Estoup et al. 2002).

3 Microsatellite Markers: Isolation and Characterization

There are several methods that have been described for the isolation of microsatellite loci; the first was the *de novo* isolation described by Rassmann et al. (1991). This protocol has become the traditional method for microsatellite isolation and it involved identification of microsatellite-containing clones by colony hybridization

with their respective specific probes. Following subsequent advances in methodologies and DNA sequencing techniques, isolation of microsatellites can now be achieved either by: (i) Constructing and screening either enriched or non-enriched genomic libraries or by utilizing the products generated by other molecular markers or by the application of next-generation sequencing systems; (ii) Making use of the EST sequences already deposited in the public domain databases or sequencing PCR products generated by consensus/universal primers; and (iii) Testing the amplification potential of other microsatellite markers developed in other related species (i.e. cross-species amplification).

3.1 Isolation of Microsatellites from Enriched Genomic DNA Libraries

This can be achieved through selective hybridization methods where the genomic DNA undergoes fragmentation either by use of restriction enzymes, sonication or, less frequently, nebulisation (Senan et al. 2014). Fragments of DNA in the range of 300–700 bp in length are selected and ligated into a common vector. Ligation can be done directly or after ligation to specific adapters (Zane et al. 2002). The DNA is then denatured and subjected to enrichment by hybridization with either: (i) biotinylated oligos followed by capture of biotinylated hybrids (oligo-bound DNA fragments) in a vectrex-aridin matrix (Kandpal et al. 1994); (ii) oligonucleotides (oligos) bound to nylon membranes (Karagyozov et al. 1993; Chen et al. 1995; Edwards et al. 1996) (iii) 5' biotinylated repeat oligos and subsequent capture by biotinylated hybrids by streptavidin coated magnetic beads (Aketarawong et al. 2006; Khamis et al. 2008); (iv) biotinylated microsatellite-probe-streptavidin coated magnetic bead complex (White and Powell 1997). Screening for positive clones is achieved by means of southern hybridization using the probes mentioned above and after blotting the bacterial colonies onto nylon membranes. Colony transfer is done either by classical replica plating or by picking single colonies and ordering them in new arrayed plates. After successful identification of positive microsatellite-containing clones, specific primers are designed and PCR conditions are optimized to allow the amplification of each locus from different individuals of a population. The fragments are then amplified, cloned and sequenced directly and probed for the presence of microsatellites. The efficiency of this approach entirely depends on the specific binding of streptavidin coated beads to biotin.

Ostrander et al. (1992) and Paetkau (1999) described protocols that allow the selective amplification of microsatellites containing genomic DNA using very specific primers. This is known as the primer extension method and it relies on the construction of a primary genomic DNA library in a phagemid vector to recover the library as single stranded DNA which is then subjected to primer extension using repeat specific non-biotinylated oligos or 5'biotinylated oligos. Ostrander et al. (1992) further demonstrated primer extension steps that selectively generated only

double-stranded products from vectors containing the tandem repeats of interest, and then transformed them in to *E. coli* cells. Streptavidin coated magnetic beads were used to selectively pick out the 5'biotinylated hybrids and convert them in to double-stranded DNA via a second round of primer extension for transformation. Pandolfo (1992) described the ligation of a vectorette (i.e. a linker containing a non-complementary region) to restricted yeast artificial chromosome (YAC) DNA. Using microsatellite-specific primers and universal vector primers, the vectorette-ligated DNA could be amplified and the products cloned and sequenced to probe for the desired repeat loci.

There are plenty of enrichment protocols available but the selective hybridization stands out as it allows for enrichment and selection prior to cloning, thereby providing a faster and easier method to handle multiple samples (Senan et al. 2014; Glenn and Schable 2005). This method is very simple, reproducible and cost effective for isolating microsatellites.

3.2 Isolation of Microsatellites from Non-Enriched Genomic DNA Libraries

Golein et al. (2006) demonstrated that genomic DNA could be restricted, ligated in to vectors and transformed to generate a non-enriched genomic DNA library. The clones were then spotted on to gridded nylon filters and screened with radio -labelled microsatellite probes or subjected to enrichment with biotin labelled-probes-streptavidin and sequenced.

3.3 Other Methods for Isolation of Microsatellites

A number of other techniques have been used to isolate microsatellite loci. These include isolation from RAPDs which involves the blotting of RAPD products on to nitrocellulose membranes which are then screened, using digoxygenin-labelled probes, for positive clones that could be detected using autoradiography. Another technique was described by Zane et al. (2002) and is known as FIASCO (Fast isolation by AFLP of sequences containing repeats). In this protocol the AFLP bands were hybridized with biotinylated probes which were then selectively probed using streptavidin-coated beads followed by cloning and sequencing of the enriched DNA fragments, to generate new microsatellite markers. However, due to the labour intensive process required for the *de novo* isolation of microsatellites, and in view of recent advances in DNA sequencing technologies (e.g. next generation sequencing [NGS] and better bioinformatics), these methods represent powerful alternatives to conventional methods for isolation of microsatellite markers. Large amounts of data can be produced via NGS and screening can be done using bioinformatics

tools; this avoids the need for construction of microsatellite-enriched DNA libraries and provides a rapid approach for the large-scale generation of microsatellite loci. Reductions in sequencing costs will make the rapid identification of microsatellite markers even easier and cheaper. There are plenty of EST sequences that have been deposited in public domain databases (Rudd 2003). Several tools are available to mine for microsatellite loci and these include TROLL (Castelo et al. 2002), MISA (Thiel et al. 2003), SciRoKo (Kofler et al. 2007), Msatcommander (Faircloth 2008) amongst others. However, generation of these markers is limited to the availability of EST sequences, particularly if EST sequences are not deposited in publicly accessible domains/databases. Closely related individuals tend to have greater DNA conservation in their coding regions hence EST microsatellite markers cannot be used in differentiating such individuals since they show less polymorphism and are therefore less efficient (Gupta et al. 2003). Recently, the high-throughput genomic-sequencing technique has produced millions of base pairs and short fragment reads which can be screened using bioinformatics tools to identify primers that amplify a large number of polymorphic microsatellite loci (Abdelkrim et al. 2009).

4 Prospects for Tracing the Routes of Fruit Fly Invasions out of Africa and the Population Structure of Fruit Flies Using Microsatellite Markers: The Case of *Ceratitis* Species and *Bactrocera oleae* (Rossi)

Being highly polymorphic and selectively neutral, microsatellite markers offer a powerful genetic tool for investigating population structure, colonization processes, temporal and spatial population dynamics and evolutionary trends of insect pests (Wu et al. 2009). Furthermore, these markers have been successfully applied to different invasive fruit fly species to infer the evolutionary aspects underlying their invasive processes (Bonizzoni et al. 2001, 2004; Baliraine et al. 2004; Khamis et al. 2009). Due to their polymorphic nature these markers have also been utilized in the analyses of fruit fly population structure across different geographical areas, and in tracing the origins of adventive populations (Bonizzoni et al. 2000, 2001; Meixner et al. 2002). Moreover, these markers have proven to be useful for cross-species amplification to study the population structure of tephritid species when no previous genetic information was available (Baliraine et al. 2003, 2004; Shearman et al. 2006).

Globally, phytophagous members of the family Tephritidae are amongst the most important pests of fruits and vegetables. With more than 4000 species described, this family is the most diverse and contains 500 genera of which the four most economically important genera are: *Ceratitis*, *Bactrocera*, *Anastrepha* and *Rhagoletis* (White and Elson-Harris 1992). All the genera have native distribution ranges. For example, *Ceratitis* is an Afro-tropical genus, *Bactrocera* is mainly confined to the Oriental and Australasian regions, *Anastrepha* to South and Central America and

the West Indies, while *Rhagoletis* has representatives in the Americas, Europe and temperate Asia (White and Elson-Harris 1992). In addition to the polyphagous nature of some species belonging to this family, several are considered highly invasive; aided by globalization of trade and poor quarantine infrastructure in the invaded countries. In recent years a member of these genera have been reported outside their native ranges. The pattern and routes of invasions of these species is of paramount importance for their management hence governments and NRI's have mobilized extensive ecological and evolutionary genetic research on these invasive pest species (Aluja and Norrbom 2000).

Microsatellite markers have offered an analytical tool for the study of fruit fly invasion genetics as exemplified for the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Weidemann) (Diptera: Tephritidae) (Bonizzoni et al. 2000). *Ceratitis capitata* is a tephritid fruit fly of global economic significance (Malacrida et al. 2007). In the past century, this pest has spread from its native Afro-tropical range to several countries including the Mediterranean basin, parts of South and Central America, and Australia (Fletcher 1989). Bonizzoni et al. (2000) isolated 43 microsatellite markers of which they used ten to unravel polymorphisms amongst *C. capitata* samples from six geographical populations (Kenya, La Réunion, Madeira, South Italy, Greece and Peru) from its native and invaded ranges. These markers detected a decrease in the number of polymorphisms from tropical Africa to the Mediterranean basin and to South America. Comparison of the Kenyan population with other populations in the study showed that the Kenyan population had the highest average number of alleles per locus, of which many were at low frequency, and most were private confirming an African origin for *C. capitata*. These results were consistent with the colonization history of *C. capitata* and indicative of a hierarchical migration structure through Spain and subsequently along the Mediterranean basin to the East (Malacrida et al. 1998; Gomulski et al. 1998).

Furthermore, a number of studies have demonstrated the utility of cross amplification of microsatellite markers to closely related species for population genetic studies (Baliraine et al. 2002, 2004; Shearman et al. 2006). For instance, Baliraine et al. (2002) screened 24 microsatallite markers from *C. capitata* for cross species amplification in the Natal fruit fly, *Ceratitis rosa* Karsch, *Ceratitis fasciventris* (Bezzi) and the mango fruit fly, *Ceratitis cosyra* (Walker). The sequence analysis indicated that most *C. capitata*-based microsatellite markers were useful for population genetic studies in the various species tested, a fact that will facilitate the tracing of the geographical origin of adventive pest populations, and assessment of their invasive potential and risk (Baliraine et al. 2002). In a similar study, Baliraine et al. (2004) compared genetic variability data from *C. rosa* and *C. fasciventris* with those derived from *C. capitata* to determine the geographical origin of the *Ceratitis* species. The results from this study confirmed the hypothesis of an East African origin for *Ceratitis* species (De Meyer et al. 2002).

A study by Nardi et al. (2005) used microsatellite markers and mitochondrial sequences to examine the population structure and colonization history of the olive fruit fly, *Bactrocera oleae* (Rossi). Their study revealed that Africa, and not the Mediterranean, is the origin of *B. oleae* infesting cultivated olives, which is

supported by the significantly greater genetic diversity of microsatellite loci from samples collected in Africa compared with samples collected from the Mediterranean region. The results also indicated that the recent invasion of *B. oleae* in to the Americas most likely originated from the Mediterranean area.

5 Potential for Use of Microsatellite Markers to Infer Fruit Fly Invasion Histories: The Case of *Bactrocera dorsalis* (Hendel) in Africa

Dacine fruit flies of the genus *Bactrocera* Macquart (Diptera: Tephrtitidae) are also economically important fruit fly pests (White and Elson-Harris 1992). With life-history traits that include high mobility and dispersive powers, high reproductive rates and extreme polyphagy, *Bactrocera* species are well documented invaders and rank high on quarantine lists worldwide (European and Mediterranean Plant Protection Organization 2009). Several *Bactrocera* species have been introduced accidentally in to different parts of the world. This is due to the globalisation of horticultural trade and has had major economic consequences (Clarke et al. 2005; De Meyer et al. 2009). In 2003, a new fruit fly pest was detected in Kenya (Lux et al. 2003) soon after the completion of a programme of monthly fruit collections carried out between 1999 and 2003 (Copeland et al. 2004). The insect was described as *Bactrocera invadens* (Drew et al. 2005) and was rated as "a devastating quarantine pest" by the Inter-African Phytosanitary Council in 2005 (French 2005). Within two years of its detection in the coastal region of Kenya, the species was recorded in several other countries on the African mainland (Mwatawala et al. 2004; Drew et al. 2005; Ekesi and Billah 2007). It is now known to be present in tropical Africa from Senegal to Mozambique, as well as in the Comoro Islands in the Indian Ocean. Through integrative multidisciplinary research efforts this species has now been synonymised with the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Schutze et al. 2014).

Since this species was a new invader in the African continent, the timing and the pathway of its invasion were unknown. The fact that the first historical records of this pest in Africa were from the East coast may indicate that this area was the port of entry of *B. dorsalis* in Africa, but this hypothesis had not been tested. Moreover the native range was not well defined: it has been suggested that it ranges from Sri Lanka to the Southern Indian sub-continent from where the species may have invaded Africa (Mwatawala et al. 2004; Drew et al. 2005; Khamis et al. 2009). The detrimental effects of this invasive species stimulated several studies to define its ecological niche and invasion potential (Ekesi et al. 2006; De Meyer et al. 2009; Mwatawala et al. 2006). However, due to the 'novelty status' of this fruit fly as a dispersive invader, no data were available on its genetic diversity or on the degree of co-ancestry amongst African populations and the supposed native populations from Southern India and Sri Lanka. Consequently, no inferences based on genetic data

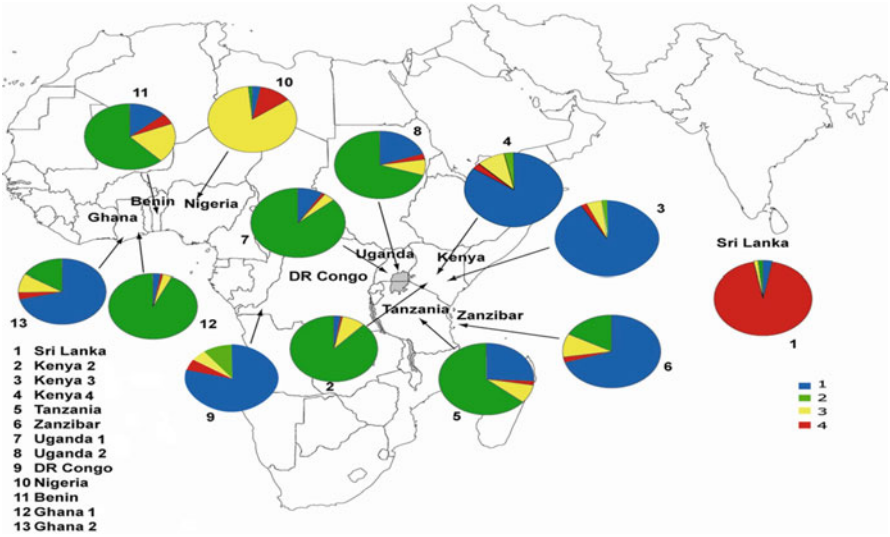

Fig. 4.1 Geographical representation of the clustering outcomes for 13 samples of *Bactrocera dorsalis*. The four colours represent the co-ancestry distribution of 351 individuals in four hypothetical clusters

were possible concerning the invasion route of *B. dorsalis*. To unravel the mystery surrounding this new invader, a set of 11 polymorphic microsatellite markers were isolated, characterised and utilised to evaluate the level of genetic diversity and the extent of common ancestry amongst several African populations collected across the actual invaded area in tropical Africa from East to West (Khamis et al. 2008; 2009).

Using these markers it was possible to successfully infer the dynamic aspects of the invasion of Africa by *B. dorsalis*, confirm its Asian origin, assess the diversity of African populations and its invasion routes in to Africa. The genetic data generated by microsatellite markers left no doubt that Sri Lanka was within the native range of *B. dorsalis* as the sample from there was characterized by all the genetic features expected in a large population from a native area; these included a large number of alleles coupled with a large number of private alleles occurring at high frequency. The Sri Lankan sample was also clearly genetically separate from the African samples and only a small percentage of genomes from Sri Lankan flies could be found in African flies (Fig. 4.1). From throughout the invaded range of East, Central and West Africa, the genetic data also suggested the presence of populations with relatively high levels of genetic diversity associated with limited geographic structure. Furthermore, although the invasion was a relatively recent event in Africa, there was no genetic footprint of bottlenecks although populations appeared large enough to maintain, especially in the West, a relatively large number of alleles with a low frequency. All these genetic features suggest a process of rapid population growth and expansion. The markers also identified genotypes that, when

Table 4.1 Average coefficient of ancestry obtained from a Structure run with K=4 for 351 individuals of *B. dorsalis* from 13 geographical regions

Area	Population	Clusters (K)			
		1	2	3	4
South Asia	Sri Lanka	0.030	0.015	0.014	**0.941**
East Africa	Kenya N	0.025	**0.880**	0.086	0.010
	Kenya K	**0.916**	0.016	0.049	0.019
	Kenya M	**0.847**	0.034	0.090	0.029
	Tanzania	**0.257**	**0.637**	0.085	0.020
	Zanzibar	**0.700**	**0.171**	**0.104**	0.025
	Uganda Ka	0.085	**0.864**	0.038	0.013
	Uganda Ki	**0.198**	**0.700**	0.075	0.027
Central Africa	DR Congo	**0.791**	**0.104**	0.053	0.053
West Africa	Nigeria	0.030	0.014	**0.837**	**0.119**
	Benin	**0.134**	**0.618**	**0.197**	0.051
	Ghana L	0.026	**0.930**	0.034	0.010
	Ghana M	**0.711**	**0.161**	0.092	0.035

Co-ancestry higher than 10 % of each population in a cluster is in *bold*

Khamis et al. (2009)

analysed using Structure 2.2 (a program that infers genetic clustering of populations using the Bayesian clustering algorithm; Pritchard et al. 2000) were present throughout Africa, i.e. some genotypes of East African flies could not be distinguished from West African flies (Fig. 4.1). Instead, the populations clustered into four genetic groups, three African clusters and the Sri Lankan cluster. Likewise, the genetic data demonstrated that the invasion and dispersal pattern of *B. dorsalis* in Africa was rapid and apparently chaotic, with the potential for multiple introductions as suggested from hypothetical outbreaks. Also analysis of the genotypes identified by the programs Structure 2.2 and GenClass 2.0 (which assigns or excludes reference populations as possible origins of individuals, on the basis of multi-locus genotypes) (Piry et al. 2004), allowed the main pathway of dispersal of *B. dorsalis* in to Africa to be inferred. Two results were found: (a) a higher or equal rate of co-ancestry of eastern and western flies in two African clusters (Fig. 4.1); (b) the major average assignment probability of eastern flies to the west than vice-versa (Table 4.1) (Khamis et al. 2009). These two results support the fact that the invasion of this pest began in East Africa. Based on their high values of co-ancestry, coastal regions of East Africa, where *B. dorsalis* was first found (i.e. Kenya and / or Tanzania), were consistently identified as the places from where the African invasion probably started. Another result was the major, although low, average assignment probability of East African flies to Sri Lanka compared with West Africa to Sri Lanka. These genetic data were consistent with the supposition that East Africa was the port of entry for *B. dorsalis*. Another very important aspect of the study concerned the Nigerian population of *B. dorsalis* which occupied its own cluster, suggesting that

this outbreak in the West arose at the same time as the other two outbreaks in the East.

6 Conclusion

This review, although focused on only a few species of tephritid fruit flies, provides evidence that microsatellite variation can play an important role in the study of fruit fly population dynamics. These markers enable researchers to scrutinize variability within populations and the rate of divergence amongst populations. The information gained provides insights into the population structure of tephritid species present in Africa, allowing inferences to be made concerning their source areas and invasion histories. Moreover, these population data are of paramount importance for implementation of eco-friendly sustainable management strategies against fruit flies. Establishing the origin of pest species has facilitated the identification and introduction of biological control agents (i.e. parasitoids) from the pest's native region, for use in Africa.

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