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Population genetic structure of economically important Tortricidae (Lepidoptera) in South Africa: a comparative analysis

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Abstract

Comparative studies of the population genetic structures of agricultural pests can elucidate the factors by which their population levels are affected, which is useful for designing pest management programs. This approach was used to provide insight into the six Tortricidae of major economic importance in South Africa. The population genetic structure of the carnation worm *E. acerbella* and the false codling moth *T. leucotreta*, analyzed using amplified fragment length polymorphism (AFLP) analysis, is presented here for the first time. These results were compared with those obtained previously for the codling moth *Cydia pomonella*, the oriental fruit moth *Grapholita molesta*, the litchi moth *Cryptophlebia peltastica* and the macadamia nut borer *T. batrachopa*. Locally adapted populations were detected over local geographic areas for all species. No significant differences were found among population genetic structures as result of population history (whether native or introduced) although host range (whether oligophagous or polyphagous) had a small but significant effect. It is concluded that factors such as dispersal ability and agricultural practices have the most important effects on genetically structuring populations of the economically important Tortricidae in South Africa.

Keywords: *Thaumatotibia leucotreta*, *Epichoristodes acerbella*, dispersal, AFLP, geographic strains, host strains, genetic variation

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Introduction

Analysis of population genetic structure may provide insight into aspects such as the scale at which populations are structured, the development of host strains and the impact of environmental factors. These aspects are essential for understanding the ecology of insect agricultural pests since they are often implicit in the management of population levels. A comparative approach may be useful to determine the factors most influential in structuring populations of a taxon. Here, we use such an approach to investigate South African populations of economically important Tortricidae, a cosmopolitan family containing some of the most damaging insect agricultural pest species in the world (Horak & Brown, 1991).

Recently, the population genetic structure of four economically important Tortricidae in South Africa was investigated: the codling moth *C. pomonella* (Timm *et al*., 2006a), the

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litchi moth *Cryptophlebia peltastica* (Meyrick), the macadamia nut borer *Thaumatotibia batrachopa* (Meyrick) (Timm *et al.*, 2006b) and the oriental fruit moth *G. molesta* (Timm *et al.*, 2008). For all four species, the population genetic structure was unexpected but remarkably similar, with populations significantly structured not only at regional scale but also split into many isolated populations at local scales, such as farms or orchards. Little evidence was found to suggest that host strains of the four species had evolved in South Africa. Our aim with this study was to determine whether these patterns were also evident in the remaining two tortricid species of major economic importance in South Africa, the pear leafroller *Epichoristodes acerbella* (Walker) and the false codling moth *T. leucotreta* (Meyrick), and to use this information to further investigate which factors likely play a role in determining the population genetic structure of the Tortricidae in South Africa.

The economically important Tortricidae in South Africa are closely related and show similarities in their biology and ecology (Horak & Brown, 1991; Komai, 1999; Timm *et al.*, 2007). Tortricid larvae are typically concealed feeders, often boring into fruit and stems and spinning and rolling leaves in which they develop. The most marked differences among the economically important species in South Africa are their population history (whether native or introduced) and host range (whether oligophagous or polyphagous). A few Tortricidae, such as *C. pomonella* and *G. molesta*, are highly successful invaders and have become established throughout the world, causing heavy economic losses wherever they are found. Other species, such as *C. peltastica*, *T. batrachopa*, *T. leucotreta* and *E. acerbella*, which are native to sub-Saharan Africa and surrounding islands, have a more local distribution, which is restricted through strict quarantine practices (Myburgh & Basson, 1961; Oliver & Bolton, 1974; Quilici *et al.*, 1988; Newton, 1998). However, *E. acerbella* has become established in Europe as a pest of carnations (Allen, 1980; Van der Vrie, 1991). One of the factors contributing to the success of the Tortricidae is their host range. Species, such as *C. pomonella*, *G. molesta* and *T. batrachopa*, are limited to three or fewer hosts belonging to a single family in South Africa (Annecke & Moran, 1982; Blomefield, 1989; De Villiers, 2001), whereas others, such as *C. peltastica*, *E. acerbella* and *T. leucotreta*, are extremely polyphagous (Annecke & Moran, 1982; Wright, 1995; Newton, 1998). One of the most extreme examples of polyphagy is *T. leucotreta*, whose larval host range extends to at least 21 cultivated and 14 indigenous wild host plants and is, thus, one of the most economically important agricultural pests in Africa (Schwartz, 1981; Newton, 1998).

In the few studies available on Tortricidae population genetics, which have mainly focused on *C. pomonella*, it is evident that the choice of marker is an important factor to consider when designing such studies. DNA-based markers, such as mitochondrial genes (Meraner *et al.*, 2008) and microsatellites (Franck *et al.*, 2007; Fuentes-Contreras *et al.*, 2008), were unable to distinguish between populations, with one exception (Chen & Dorn, 2009). However, amplified fragment length polymorphism (AFLP) analysis was able to detect differences between populations, even over local scales (Timm *et al.*, 2006a; Thaler *et al.*, 2008). These results agree with studies of other taxa, where AFLP analysis has been shown typically to detect high levels of genetic variation among closely related populations, particularly over small spatial scales (Jakše *et al.*, 2001; Gaudeul *et al.*, 2004; Garoia *et al.*, 2007). Thus, to facilitate studies of variation and to enable direct comparison among South African Tortricidae previously studied, we selected AFLP analysis for analyzing *E. acerbella* and *T. leucotreta*.

Material and methods

Insect material

Insect specimens were obtained by collecting infested fruit and allowing the moths to emerge or by pheromone collection. *Epichoristodes acerbella* specimens ($n = 113$) were collected from seven regions in the Western Cape province, the main site of the deciduous and cut flower industries in South Africa, from commercial plantings of grapes, pears, *Protea* spp. and *Leucadendron* spp. (table 1, fig. 1). In analyses of host variation, only populations collected from grapes and pears were considered since limited numbers of individuals were collected from *Protea* spp. and *Leucadendron* spp. To assess variation over local scales, populations were collected from three sites in Stellenbosch ($n = 27$) and from 11 different farms in the Hex River Valley ($n = 45$; table 1). *Thaumatotibia leucotreta* specimens ($n = 163$) were collected from the Western Cape, Eastern Cape and Mpumalanga provinces, which are the principal areas of tropical and subtropical fruit production in South Africa (table 2, fig. 1). From the Western Cape, individuals ($n = 100$) were collected from six regions from citrus, pear, apple and plum orchards, as well as from acorns and pheromone traps. Populations were collected from citrus orchards in the Eastern Cape ($n = 19$) and citrus, litchi, macadamia, star fruit orchards and pheromone traps in Mpumalanga ($n = 45$). Due to limited population sizes collected from macadamias and star fruit, only individuals collected from citrus, pears, apples, plums, acorns and litchis were included to estimate variation among populations collected from different hosts. To obtain a measure of genetic variation over local scales, individuals were collected from a minimum of each of four different farms in Citrusdal ($n = 50$), Stellenbosch ($n = 24$), the Sundays River Valley ($n = 19$) and Nelspruit ($n = 26$; table 2). As outgroup, ten individuals obtained from Africa and maintained in the UK were used. Voucher material of specimens included for analysis was deposited in the museum of the Department of Conservation Ecology and Entomology, University of Stellenbosch.

DNA extraction

DNA was extracted from the head and legs of moths or, in rare instances, portions of the abdomens of larvae using a CTAB-based protocol (Reineke *et al.*, 1998).

AFLP analysis

The AFLP procedure was performed as originally described (Vos *et al.*, 1995), with minor modifications. Briefly, DNA was digested with the enzymes *Eco*RI and *Mse*I and pre-amplification of DNA templates was performed with primers containing no selective nucleotides. Selective amplifications were performed with ^{33}P-labelled *Eco*RI primers using five combinations of primer pairs, each containing three selective nucleotides. To test marker reproducibility, DNA was extracted from the same individual on different occasions and used to evaluate each selective primer pair

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Table 1. Collection details of *E. acerbella* specimens from South Africa used for AFLP analysis.

Province	Region	Farm	*n*	Host	Geographic coordinates
Western Cape	Elgin	Elgin Experimental Farm	4	Pears	34°09′S 19°02′E
		Oak Valley	4	Pears	34°09′S 19°03′E
	Jonkershoek	(Nature Reserve)	2	Unknown	33°59′S 18°57′E
	Stellenbosch	Timberlea	10	Pears	33°55′S 18°52′E
		Devon Valley	14	Vineyards, *Protea*, *Leucadendron*	33°54′S 18°50′E
		(Onderpapegaaiberg)	3	Unknown	33°56′S 18°52′E
	Hex River Valley	Bella Vista	4	Vineyards	33°28′S 19°40′E
		Boplaas	5	Vineyards	33°30′S 19°36′E
		Cairngorm	2	Vineyards	33°30′S 19°32′E
		De Vlei	8	Vineyards	33°20′S 19°41′E
		Idlewinds	4	Vineyards	33°30′S 19°34′E
		Kanetvlei	2	Vineyards	33°31′S 19°32′E
		Klipheuwel	6	Vineyards	33°30′S 19°31′E
		Moreson	4	Vineyards	33°28′S 19°37′E
		Ruimsig	4	Vineyards	33°29′S 19°37′E
		Somerlus	3	Vineyards	33°28′S 19°38′E
		Tesame	3	Vineyards	33°27′S 19°39′E
	Tulbagh	Roodezand	4	Vineyards	33°15′S 19°07′E
		Twee Jonge Gezellen	7	Pears	33°15′S 19°07′E
	Ceres	Paardekloof	14	Unknown	33°15′S 19°15′E
	Worcester	Protea	6	Unknown	33°36′S 19°24′E

Brackets indicate that specimens were collected from residential areas. Hosts are listed as unknown if pheromone traps associated with multiple hosts were used for collection.

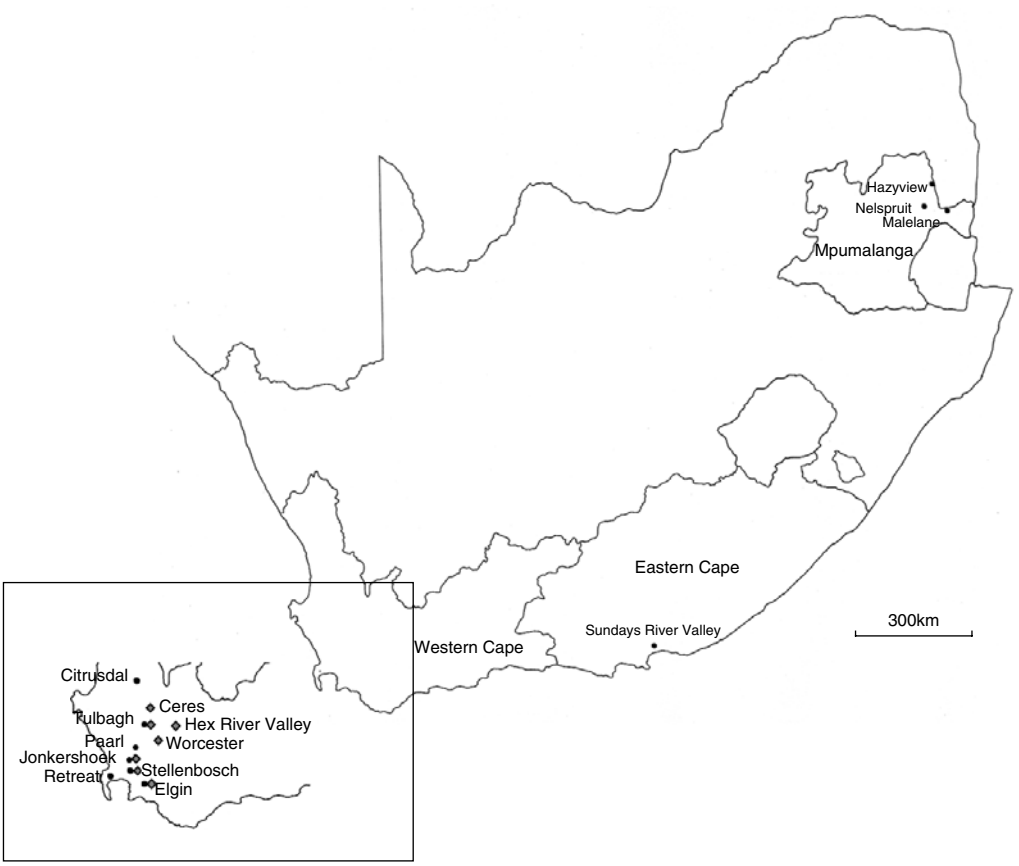

Fig. 1. Map of South Africa showing collection details of *E. acerbella* and *T. leucotreta* populations used for AFLP analysis (●, *T. leucotreta* collection sites; ◆, *E. acerbella* collection sites).

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Table 2. Collection details of *T. leucotreta* specimens from South Africa used for AFLP analysis.

Province	Region	Farm	*n*	Host (s)	Geographic coordinates
Western Cape	Citrusdal	Biesievlak	5	Citrus	33°56′S 18°51′E
		Boontjiesrivier	5	Citrus	32°34′S 19°01′E
		Hexrivier Blikhuis	5	Citrus	32°23′S 18°57′E
		Petersfield	5	Citrus	32°33′S 18°59′E
		Rivierplaas	5	Citrus	32°37′S 19°01′E
		Theerivier	15	Citrus, acorns	32°49′S 19°04′E
		Wilhelm Soete Trust	5	Citrus	32°43′S 19°03′E
	Jonkershoek	(Nature Reserve)	5	Unknown	33°59′S 18°57′E
	Stellenbosch	(University campus)	14	Acorns	33°56′S 18°52′E
		(Onderpapegaaiberg)	5	Unknown	33°56′S 18°52′E
		Timberlea	5	Pears	33°55′S 18°52′E
	Retreat	(Residential home)	5	Unknown	34°3′S 18°29′E
	Elgin	Bellevue Experimental Farm	9	Apples	34°8′S 19°01′E
	Tulbagh	Twee Jonge Gezellen	5	Plums	33°15′S 19°07′E
		Roodezand	2	Plums	33°15′S 19°07′E
	Paarl	(Residential home)	5	Unknown	33°45′S 18°59′E
Eastern Cape	Sundays River Valley	Woodridge	5	Citrus	33°28′S 25°41′E
		Penhill	5	Citrus	33°34′S 25°41′E
		Mfuleni	5	Citrus	33°27′S 25°32′E
		Carden	4	Citrus	33°28′S 25°40′E
Mpumalanga	Nelspruit	Institute for Tropical and Subtropical Crops (ITSC)	12	Citrus, litchis, macadamias, star fruit	30°58′S 25°26′E
		Friedenheim	5	Litchis	30°59′S 25°27′E
		Mataffin East	5	Litchis	30°56′S 25°27′E
		Halls Boschrand West	4	Litchis	30°57′S 25°26′E
	Hazyview	Rooigom	10	Litchis	31°4′S 25°02′E
	Malelane	Institute for Tropical and Subtropical Crops (ITSC)	5	Litchis	31°33′S 25°26′E
		Kaalrug	4	Litchis	

Brackets indicate that specimens were collected from residential areas. Hosts are listed as unknown if pheromone traps associated with multiple hosts were used for collection.

at least three times. Selective amplification products were electrophoresed on 6% (w/v) denaturing polyacrylamide gels at 60 W for 2–3 h. Gels were dried on Whatmann paper and exposed to Kodak Biomax x-ray films for visualization.

Statistical analysis

Due to the nature of AFLP markers and protocols used for analysis, fragments were assumed to be homologous if they were of the same size. In addition, a present fragment was assumed to be dominant to an absent fragment. In this manner, AFLP profiles were recorded manually in a binary matrix using '1' to denote fragment presence and '0' to denote fragment absence. The so-called band-based approach (Bonin *et al*., 2007), rather than analyses based on allele frequency, was generally used to analyse AFLP data. The band-based approach is more suitable for analysing dominant data since it reduces preliminary assumptions used to calculate allele frequencies. In addition, since analyses are conducted at an individual level, they are more suitable for small population sizes (Bonin *et al*., 2007), such as those used in the current study. For all calculations, populations were assumed to be in Hardy-Weinberg equilibrium unless otherwise stated.

Genetic diversity within each population was expressed using the Shannon-Weaver information index (*I*) (Shannon & Weaver, 1949), which does not assume that populations are in Hardy-Weinberg equilibrium but presupposes that estimates based on the numbers of bands that are present/absent are an estimate of genetic diversity (Whitkus *et al*., 1998). The Shannon-Weaver index was calculated as $I = -\sum p_i \log_2 p_i$ with p_i being the frequency of a given AFLP fragment (Lewontin, 1972) using POPGENE version 1.31 software for population genetic analysis (Yeh & Yang, 1997).

The partitioning of genetic variability within and among populations was determined using an AMOVA analysis based on a Euclidean distance metric (Huff *et al*., 1993), using the software GenAlEx version 6 (Peakall & Smouse, 2005) based on 1000 permutations. Among populations, variability based on AMOVA analysis was used to determine the Φ_{PT} estimator, which is analogous to F_{ST} and can be used as a measure of population differentiation for binary data. GenAlEx version 6 (Peakall & Smouse, 2005) was also used to conduct a Mantel test (Mantel, 1967) to evaluate correlations between the geographic origin of populations and their genetic distances, using pairwise genetic and geographic distance matrices (Smouse *et al*., 1986; Smouse & Long, 1992). The relationships between populations were determined based on pair-wise measures of genetic distance *D* (Nei, 1978; Lynch & Milligan, 1994) with 1000 replications, using the software AFLP-SURV (Vekemans *et al*., 2002) with default options. Subsequently, these genetic distances were used to construct unrooted neighbour-joining trees with Phylip version 3.6 software (Felsenstein, 2004). Relationships

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Table 3. Comparative population genetic parameters for six economically important species collected from South Africa, based on AFLP analysis.

	Origin	Host range	I	Φ_{PT}
G. molesta	Introduced	Oligophagous	0.2998	0.275
C. pomonella	Introduced	Oligophagous	0.3047	0.269
T. leucotreta	Native	Polyphagous	0.2742	0.147
T. batrachopa	Native	Oligophagous	0.3729	0.297
Cr. peltastica	Native	Polyphagous	0.3147	0.136
E. acerbella	Native	Polyphagous	0.2883	0.218

among individuals were viewed using the unweighted pair group means arithmetic (UPGMA), based on a dissimilarity matrix generated using Jaccard's general similarity coefficient (Sneath & Sokal, 1963), with MVSP Version 3.11c (Kovach, 1999). When using binary data, Gower's general similarity coefficient ($GGSc_{ij}$) is equivalent to Jaccard's coefficient, where $GGSc_{ij} = a/(a+b+c)$, where a is the number of bands shared by individuals i, and j, b is the number of bands present in i but not in j, and c is the number in j but not in i.

Population subdivision was also tested using the program STRUCTURE version 2.2 (Falush *et al*., 2007), which employs a model-based clustering algorithm to assign individuals probabilistically to populations (K) based on their AFLP banding profiles. AFLP data was coded as diploid individuals with missing data in one copy of all loci, according to the software manual recommendations for analysing dominant data. To estimate the number of populations, four independent runs were performed using the admixture model with an initial burn-in of 10,000 generations and 10,000 Markov Chain Monte Carlo repetitions and assuming correlated allele frequencies.

The amount and distribution of genetic variation was compared among *C. pomonella*, *G. molesta*, *C. peltastica*, *T. batrachopa*, *T. leucotreta* and *E. acerbella* using a Chi squared test. To determine whether the genetic parameters Φ_{PT} and I differed significantly between introduced and native species as well as between oligophagous and polyphagous species, single factor ANOVA analysis was used. Species were classified as oligophagous if the larvae were able to feed on hosts belonging to a single family or as polyphagous if hosts from more than one family were known to be utilized in South Africa (table 3).

Results

Gene flow among geographic regions

AFLP analysis using five primer combinations yielded a total of 228 fragments for analysis of *E. acerbella* populations and 323 fragments for analysis of *T. leucotreta* populations. Genetic diversity within the *E. acerbella* population in the Western Cape was calculated as $I = 0.288$. The degree of genetic diversity within the *T. leucotreta* population was similar to that of *E. acerbella*, with $I = 0.274$.

Despite the relatively high levels of genetic diversity found within total and regional populations, evidence of population differentiation in these scales was apparent in *E. acerbella* populations ($\Phi_{PT} = 0.218$, $P = 0.001$) as well as *T. leucotreta* populations ($\Phi_{PT} = 0.147$, $P = 0.001$) according to

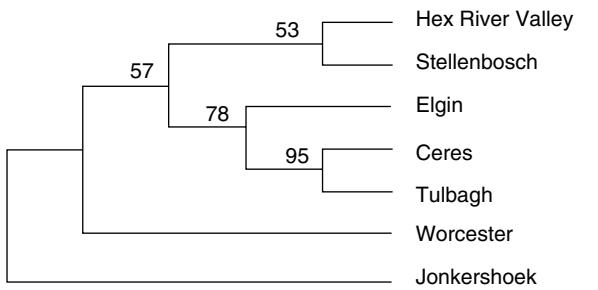

Fig. 2. Cluster analysis (unrooted neighbour joining) showing the relationships between *E. acerbella* populations collected from seven regions in the Western Cape, based on AFLP analysis. Only bootstraps above 50% are shown.

population differentiation values. For *E. acerbella* populations, cluster analysis (fig. 2) revealed that populations clustered broadly into four groups. The likelihood of the number of populations was also confirmed using the estimated natural logarithm (ln) of the probability of data in STRUCTURE, which increased substantially from $K = 1$ ($\ln = -9555.4$) to $K = 4$ ($\ln = -8822.5$) and remained relatively stable thereafter. The Hex River Valley and Stellenbosch *E. acerbella* populations appeared closely related with the Elgin, Ceres and Tulbagh populations forming a second group (fig. 2). Bayesian analyses confirmed that the population structure of *Thaumatotibia leucotreta* populations could best be described by $K = 2$ ($\ln = -17708.1$). A similar pattern of population clustering was observed using neighbour-joining analysis, where two main clusters were observed, the first containing five of the seven Western Cape populations and the second containing the remaining Western Cape populations as well as all populations from Mpumalanga and the Eastern Cape (fig. 3).

Gene flow among local populations

Analysis of molecular variance in *E. acerbella* populations indicated that, although the majority of genetic variability (78%, $P = 0.001$) could be attributed to variation within populations, significantly high proportions could be ascribed to variation among the seven regions sampled (5%, $P = 0.001$) as well as among different farms/sites (17%, $P = 0.001$) (table 4). Similar patterns were detected in *T. leucotreta* populations, where AMOVA analysis showed that 7% ($P = 0.001$) of the total variation could be attributed to variation among regions with a further 8% ($P = 0.001$) of the variation due to among farms/sites (table 4). The remaining variation (85%, $P = 0.001$) was due to individuals.

To further assess variation over local scales in *E. acerbella*, populations from three sites in Stellenbosch and 11 farms situated throughout the Hex River Valley were assessed. Evidence of population substructure was apparent in Stellenbosch populations, where 12% of the variation was attributed to variation among sites ($\Phi_{PT} = 0.121$, $P = 0.001$), as well as in Hex River Valley populations, where 22% of the variation was due to variation among farms ($\Phi_{PT} = 0.218$, $P = 0.001$; table 5). A similar experiment was conducted in *T. leucotreta*, using populations from seven farms in Citrusdal, three sites in Stellenbosch, three farms in the Sundays

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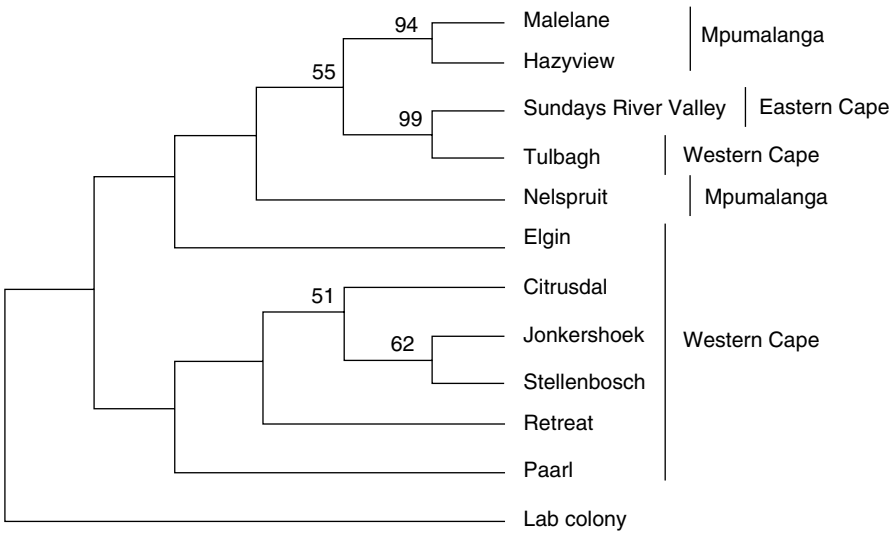

Fig. 3. UPGMA cluster analysis based on analysis of 323 AFLP fragments showing the relationships among *T. leucotreta* populations collected from three different provinces in South Africa, using a laboratory colony maintained in Britain as an outgroup.

Table 4. Analysis of molecular variance (AMOVA), based on AFLP analysis, for *E. acerbella* and *T. leucotreta* populations collected in South Africa.

	df	SS	MS	Est. Var.	Total Variance (%)
E. acerbella					
Among regions	6	427.128	71.188	1.303	5%
Among farms	14	618.122	44.152	4.752	17%
Within populations	92	1995.590	21.691	21.691	78%
Total	112	3040.841	–	27.747	100%
T. leucotreta					
Among regions	10	787.337	78.734	2.386	7%
Among farms	16	685.249	42.828	2.540	8%
Within populations	137	3913.584	28.566	28.566	85%
Total	163	5386.171	–	33.491	100%

River Valley and four farms in Nelspruit. In Citrusdal, 8% of the total variation was as result of differences among farms ($\Phi_{PT} = 0.076$, $P = 0.001$; table 5). These values were calculated as 5% in Stellenbosch populations ($\Phi_{PT} = 0.055$, $P = 0.001$), 9% in Sundays River Valley populations ($\Phi_{PT} = 0.088$, $P = 0.001$) and 15% in Nelspruit populations ($\Phi_{PT} = 0.151$, $P = 0.001$). For both *E. acerbella* and *T. leucotreta*, individuals that were collected from the same site/farm often appeared to be closely related using cluster analysis; and, in some instances, it was possible, more or less, to ascribe individuals to populations based on their AFLP profiles. For example, cluster analysis illustrating relationships among *E. acerbella* individuals collected from the Hex River Valley is shown in fig. 4. Mantel tests confirmed that there was a low but significant correlation between genetic and geographic matrices for both species ($r = 0.18$, $P = 0.001$ in *E. acerbella*; and $r = 0.093$, $P = 0.001$ in *T. leucotreta*).

Gene flow among host populations

Estimates of population structure based on host populations were small but significant for both *E. acerbella* and *T. leucotreta* populations. Five percent of the variation in *E. acerbella* populations was due to hosts ($\Phi_{PT} = 0.050$, $P = 0.001$), whereas 8% was due to hosts in *T. leucotreta* populations ($\Phi_{PT} = 0.079$, $P = 0.001$) (table 6). However, for both species, estimates of population variation were not significantly higher when populations from more hosts were included in analyses at regional and local scales. In addition, it was not possible to distinguish among populations collected from different hosts using UPGMA analysis at any geographic scale. Bayesian analyses confirmed that hosts populations of both *E. acerbella* and *T. leucotreta* were best described by a single cluster ($K = 1$, $\ln = -5912.7$ and $\ln = -16074.8$, respectively).

Comparison among species

The origin and host status for each of the six tortricid species are shown in table 3, along with estimates of Φ_{PT} and *I*. These parameters did not differ significantly at the species level when analyzed using a Chi squared test ($P = 1$ for both parameters). Statistical differences between Φ_{PT} and *I* values between native and introduced, as well as oligophagous

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Table 5. AMOVA analysis for populations of *E. acerbella* and *T. leucotreta* collected on a local scale (within regions).

	df	SS	MS	Est. Var.	Total %
E. acerbella					
Hex River Valley					
Among populations	10	414.172	41.417	5.446	22%
Within populations	34	663.383	19.511	19.511	78%
Total	44	1077.556		24.958	100%
Stellenbosch					
Among populations	2	95.986	47.993	3.062	12%
Within populations	25	554.621	22.185	22.185	88%
Total	27	650.607		25.247	100%
T. leucotreta					
Citrusdal					
Among populations	8	348.311	43.539	2.532	8%
Within populations	36	1111.600	30.878	30.878	92%
Total	44	1459.911		33.410	100%
Stellenbosch					
Among populations	2	86.425	43.213	1.796	5%
Within populations	21	648.200	30.867	30.867	95%
Total	23	734.625		32.662	100%
Nelspruit					
Among populations	3	175.431	58.477	5.042	15%
Within populations	22	623.800	28.355	28.355	85%
Total	25	799.231		33.396	100%
Sundays River Valley					
Among populations	3	101.682	33.894	2.244	9%
Within populations	15	348.950	23.263	23.263	91%
Total	18	450.632		25.508	100%

and polyphagous, species were investigated using single factor ANOVA analysis. Estimates of Φ_{PT} and I did not differ significantly when tested against population history ($P = 0.264$ and 0.769, respectively). However, when investigating the effects of host range, Φ_{PT} between oligophagous and polyphagous species were significantly different at less stringent levels ($P = 0.014$) although I did not differ significantly ($P = 0.275$).

Discussion

Gene flow among host populations

Little evidence was found to suggest that *E. acerbella* and *T. leucotreta* populations formed host strains, despite earlier suggestions that *T. leucotreta* races having different host preferences may exist (Ford, 1934; Omer-Cooper, 1939). Thus, none of the six tortricids of major economic importance South Africa showed evidence of developing strains specific to hosts. The practical implication of these results is that uncultivated hosts may maintain populations at times when fruit is unavailable in the orchard, confirming suggestions that the proximity of other susceptible cultivated or wild fruits has a considerable influence on, for example, the severity of *T. leucotreta* infestation (Gunn, 1921; Daiber, 1981; Anderson, 1986). In addition, since populations maintained on uncultivated hosts may affect the efficiency of chemical control and the development of insecticide resistance by maintaining reservoirs of susceptible populations, pest management programs should take into account the presence of alternative hosts. It should be emphasized, however, that the results found for these tortricids in South Africa may not necessarily apply to these species where they occur in other countries. For example, *C. pomonella* host strains have been found to be specific to apple, apricots and walnuts in central Europe (Thaler *et al*., 2008; Chen & Dorn, 2009).

Gene flow among geographic populations

All six tortricid species examined, including *E. acerbella* and *T. leucotreta*, showed evidence of being structured geographically. In addition, using AFLP analysis, it was possible to distinguish between some populations collected from the same region as well as, in certain instances, different farms or even orchards. Thus, it appears as if gene flow among populations of each of the six species is limited on a local scale. A number of factors may counteract the effects of gene flow and produce genetic structure over geographic scales in populations of phytophagous insect pests. Here, we consider the impact of host range, origin, dispersal ability, control practices and anthropogenic movement on the population genetic structure of the six tortricid species analyzed.

Host range

It seems instinctive to assume that polyphagous species will display relatively pronounced levels of population subdivision, given their relative abundance of hosts that may act as staging posts for dispersal and subsequent gene flow. However, estimates of population subdivision for polyphagous *C. peltastica*, *E. acerbella* and *T. leucotreta* were significantly different to oligophagous *C. pomonella*, *G. molesta* and *T. batrachopa* only at less stringent significance levels. These results seem to confirm studies, such as that by Peterson & Denno (1998), that diet breadth alone cannot be used to infer the population genetic structure of a species. Host range, thus, seems to have played a relatively small role in determining the population genetic structure of economically important Tortricidae in South Africa.

Historical effects

Historical effects, including the introduction of pest species to regions where they are not native, have long been known to affect population genetic structure. The tortricids *E. acerbella*, *T. leucotreta*, *C. peltastica* and *T. batrachopa* are native to southern Africa (Myburgh & Basson, 1961; Oliver & Bolton, 1974; Quilici *et al*., 1988; Newton, 1998). In contrast, *C. pomonella* was first reported in South Africa in 1885 (Lounsbury, 1898; Giliomee & Riedl, 1998), whereas *G. molesta* was first observed only in 1990, but may have been present earlier (Blomefield & Geertsema, 1990; Timm *et al*., 2008). Because of the recent introduction of *C. pomonella* and *G. molesta*, it might be expected that their populations are likely to be much less genetically diverse than the populations from which they are derived (Barrett & Kohn, 1991). However, both species displayed similar levels of diversity to that of native species. In addition, patterns of *C. pomonella* genetic structure in South Africa are comparable to that in its native range in Europe (Thaler *et al*., 2008). It is likely that both *C. pomonella* and *G. molesta*, which are known to be successful invaders, were introduced into South Africa multiple times (Blomefield & Geertsema, 1990; Giliomee & Riedl, 1998; Timm *et al*., 2008), which may have contributed to its genetic diversity (Sakai *et al*., 2001). Because of their

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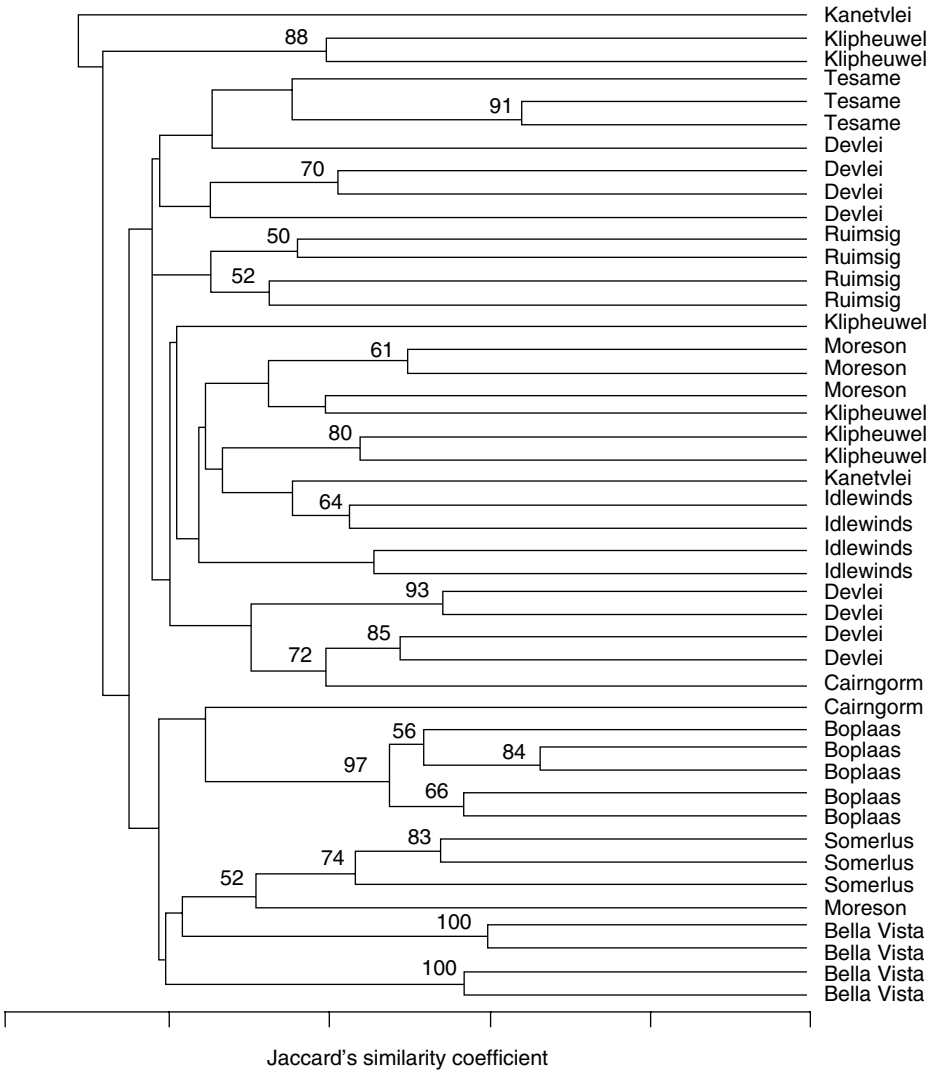

Fig. 4. Cluster analysis (UPGMA) showing the relationships between *E. acerbella* individuals collected from nine farms in the Hex River Valley, based on AFLP analysis. Bootstrap values above 50%, based on 1000 permutations, are indicated.

Table 6. Analysis of molecular variance of *E. acerbella* and *T. leucotreta* populations collected from various hosts.

	df	SS	MS	Est. Var.	Total Variance (%)
E. acerbella					
Among populations	1	71.302	71.302	1.344	5%
Within populations	77	1954.452	25.382	25.382	95%
Total	78	2025.754	–	26.726	100%
T. leucotreta					
Among populations	5	421.115	84.223	2.665	8%
Within populations	139	4323.037	31.101	31.101	92%
Total	144	4744.152	–	33.766	100%

recent history in South Africa, both species may not be in mutation-drift equilibrium, and it is possible that effects observed are more relevant to historical patterns of gene flow than to the current population dynamics (Avise, 1994; Bossart & Pashley Prowell, 1998). However, since the other four species analysed all produced similar patterns of gene flow and *C. pomonella* is known to display similar patterns in its native range (Thaler *et al.*, 2008; Chen & Dorn, 2009), it is likely that the results found in South Africa could be relevant elsewhere.

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Dispersal ability

Limited dispersal ability may be a distinguishing feature of the six Tortricidae analysed in South Africa. AFLP analysis was able to distinguish between closely situated populations of both *E. acerbella* and *T. leucotreta*, indicating that local strains have evolved within regions in these species. These results are similar to those produced for *C. pomonella* (Timm *et al*., 2006a; Thaler *et al*., 2008; Chen & Dorn, 2009), *C. peltastica* and *T. batrachopa* (Timm *et al*., 2006b) and *G. molesta* (Timm *et al*., 2008). Mark-recapture or flight-mill studies of *C. pomonella*, *G. molesta* and *T. leucotreta* have shown that, generally, individuals vary greatly in their dispersal capacity (Mani & Wildbolz, 1977; Sziraki, 1979; Vickers *et al*., 1985; Rothschild & Vickers, 1991; Schumacher *et al*., 1997a,b; Newton, 1998; Keil *et al*., 2001; Gu *et al*., 2006). Although individuals may undertake single long flights of up to several kilometres, the majority of individuals appear to be fairly sedentary. In *C. pomonella*, there is known to be a trade-off between mobility and fitness, which may favor sedentary individuals (Gu *et al*., 2006). Variation in dispersal has previously been explained in terms of the life history of tortricid moths in an orchard (Schumacher *et al*., 1997a; Timm *et al*., 2006a). If sufficient resources are readily available, it may be advantageous for moths to stay within the habitat or the orchard. However, individuals with the capacity for a longer flight range will be favoured when the food resource of the larvae fluctuates. It is most likely that such individuals, infesting newly-established orchards, cause subsequent patterns of variation since moths in newly established orchards appear to be more closely related to each other than to moths from established orchards. The sedentary nature of individuals, thus, may favour splitting into many local populations, aided by the effects of genetic drift.

Insecticide use

Conventional chemical treatments may also be responsible for producing locally adapted populations. This pattern was highlighted in *C. pomonella* populations in France, where populations were found to be mainly structured according to the history of insecticide applications (Franck *et al*., 2007). It may be likely that a similar effect is evident in South African populations, since chemical sprays form a major component of pest management programs against tortricids in South Africa (Newton, 1989; Blomefield, 1994, 1996; Hofmeyr & Pringle, 1998; Riedl *et al*., 1998; Blomefield & Barnes, 2000; Blomefield & du Plessis, 2000).

Anthropogenic movement

Anthropogenic movement of fruit or other plant material also may have affected the population genetic structure of the Tortricidae analysed (Higbee *et al*., 2001; Franck *et al*., 2007). This effect may be evident in the close relationship between *E. acerbella* populations collected from the Hex River Valley and Stellenbosch, as both regions are known for intensive wine grape production, which may have facilitated the exchange of plant material between regions. Patterns of population variation between regions in *T. leucotreta* may have been similarly affected by agricultural practices. For example, it has been suggested that the Western Cape population has its origin from material originally obtained from Mpumalanga province (Giliomee & Riedl, 1998), which could possibly explain the close relationships between the Elgin and Nelspruit populations.

Conclusion

The Tortricidae, of major economic importance in South Africa, appear to be structured genetically as a result of geography and, to a lesser extent, host use. Furthermore, populations are structured over local spatial scales, most likely as result of limited dispersal ability, insecticide use and anthropogenic movement. The aforementioned factors can be manipulated and, therefore, should be taken into account in a pest management program. For example, various pest management practices, such as the release of parasitoids or sterilized insects and the placement of insecticide treatments, are affected by limited dispersal ability. The results produced, thus, are useful for understanding the ecology of the Tortricidae of economic importance in South Africa, facilitating in turn an improvement of their eventual control.

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