Chapter 3

Spirocerca lupi population genetics

3.1. ABSTRACT

Spirocerca lupi is a nematode that parasitizes canid species across the world, causing the disease called spirocercosis. Although many studies have been conducted on the morphology and life cycle, very little work has been done on the genetic diversity and the population structure of this parasitic nematode. Microsatellite markers were used in this study to determine the genetic variation of *S. lupi* nematodes from three geographical locations in South Africa, namely KwaZulu-Natal (Durban), Eastern Cape (Grahamstown) and Gauteng (Tshwane Metropole). The analyses performed suggested low levels of genetic variation between the three locations and potentially two genetic clusters. Genetic variation was studied between and within individual hosts (dogs), where high levels of heterozygosity were detected. There are two hypotheses of how this genetic variation is generated: this could be attributed to the life cycle characteristics of this nematode where either intermediate hosts (dung beetles) carrying genetically diverse larvae contribute to further genetic diversity in the definitive host, or due to definitive hosts consuming many intermediate/ paratenic hosts, however further studies are required to resolve this. Studies such as this one, which assess genetic diversity in parasitic populations by applying molecular techniques, can be used to further understand disease progression or transmission. The findings can be used to implement management strategies to control the spread of disease caused by parasitic species.

Keywords: genetic variation, microsatellites, population genetic structure, *Spirocerca lupi*

3.2. INTRODUCTION

Microevolutionary processes in parasitic organisms can be better understood by determining population genetic structure (Hudson *et al.* 1992; Nadler 1995). By determining what factors contribute to the genetic structure that arises within a population, more information can be gained on the epidemiology. Epidemiology of parasitic diseases improves our understanding of the role that parasites play in disease causation through the use of molecular and analytical tools that track parasite movement and relate their spread to environmental factors (Lymbery & Thompson 2012). Genetic epidemiology is a field of study that uses genetic tools to study the dynamics of a disease in a population.

Genetic variation in parasites is studied to gain more information on their ecology and evolutionary potential so that some insights can be gained on how to reduce the risk of infection and prevent the spread of rare alleles that are associated with resistance to anthelminthic drugs (Blouin *et al.* 1995). Unlike parasitic nematodes of plants and insects, gastrointestinal nematodes have relatively high levels of diversity and gene flow (Blouin *et al.* 1992). Thus they have the genetic potential to respond to chemical attacks and the ability to spread resistance alleles.

Transmission dynamics of parasitic diseases are determined by the dispersal of parasites within and among hosts (Lymbery & Thompson 2012). Genetic structuring shows the number of alleles that are exchanged between populations, which has a marked consequence on the genetic make-up of individuals in a population (Balloux & Lugon-Moulin 2002). Novel genetic variants can be created through the processes of recombination, mutation and gene flow (Brandt *et al.* 2007). Genetic diversity and its structure within and among populations is determined primarily by the effective sizes of the populations and the rates of gene flow among them (Blouin *et al.* 1995).

Large effective population sizes (N_e) are likely to result in high within-population diversity (Blouin *et al.* 1992; Blouin *et al.* 1995; Redman *et al.* 2008). Small isolated populations are likely to experience genetic drift, since inbreeding causes the fixation of alleles and reduces genetic diversity (Roos *et al.* 2004). This in turn affects the evolutionary potential of a population since deleterious mutations can become fixed (Balloux & Lugon-Moulin 2002). High gene flow prevents local adaptation by homogenising populations (Brandt *et al.* 2007) and a substantially high amount of gene flow will prevent distinct genetic differentiation among subpopulations (Nadler 1995). According to isolation by distance (Slatkin 1993), populations that are in close proximity to each other are genetically more similar than those that are more distant (Balloux & Lugon-Moulin 2002). Thus if a species occupies a large geographic area, local differentiation can occur without the presence of any geographic barriers (Belanger *et al.* 2011). This is important to note when management strategies are implemented because the extent to which populations are genetically isolated from each other needs to be understood.

Mating systems are known to affect levels of genetic diversity within populations along with other evolutionary processes (Charlesworth 2003). It is expected that mating systems are largely affected by transmission dynamics and the distribution of parasites among hosts (Criscione & Blouin 2006). Thus the life cycle of a parasite will strongly influence mating behaviour. In order to control a disease effectively, it is important to understand the routes by which nematodes are transmitted and also their mechanisms of dispersal through the different stages in the life cycle (Lymbery & Thompson 2012). In most parasites parthenogenesis or asexual reproduction occurs (Huyse *et al.* 2005), resulting in a more inbred population. However, if intermediate hosts carry a diverse array of unique parasite genotypes (Rauch *et al.* 2005), then the definitive host is expected to consume many infested intermediate hosts, thereby acquiring a highly

diverse mixture of parasitic genotypes. This will ultimately reduce the possibility of mating between genetically identical individuals (inbreeding), resulting in a more outbred population.

Host movement is another important factor that determines gene flow (Blouin *et al.* 1999; Criscione *et al.* 2005). It is assumed that the most mobile host will control gene flow in a parasite that has a complex life cycle (Prugnolle *et al.* 2005). Highly vagile hosts are known to contribute to a lower population structure than those that are less vagile (Nadler 1995). Genetic structure is influenced by both intermediate and definitive host mobility in parasites that have an indirect lifecycle (Lymbery & Thompson 2012). Therefore, the more hosts there are, the higher the chances of dispersal of the parasite and an increased opportunity for mixing of parasite offspring (Gorton *et al.* 2012; Nadler 1995).

Very few studies have been conducted thus far on the population genetic variation and transmission dynamics of *S. lupi* (De Waal et al. 2012), a parasitic nematode that causes the disease called spirocercosis, mainly in canid species (Van der Merwe *et al.* 2008). The nematode is known to occur most commonly in regions with a warm, tropical climate (Lobetti 2011). For over a decade now, the number of clinical cases presented has increased worldwide (Kok *et al.* 2011) and early detection is not possible due to diagnostic limitations (Dvir *et al.* 2010). The levels of infestation vary amongst dogs in different countries and relate mostly to their age, size, breed and behaviour.

The life-cycle of this nematode begins when intermediate hosts such as coprophagous beetles ingest larvae that are excreted in faeces of infested hosts (Du Toit *et al.* 2008). Hosts are infested by ingestion of a coprophagous beetle or other paratenic host that harbours the larvae (Van der Merwe *et al.* 2008). When the nematode develops into an adult worm it reproduces sexually within the infested host. This species of nematode is of particular concern in dogs, where it causes the formation of oesophageal nodules, which often become cancerous and may be fatal. Dogs show symptoms such as vomiting, regurgitation and dyspnoea (Last & Smith 2007) due to obstruction of the oesophagus by the nodule. Symptoms present very late in the infestation stage when treatment is considered ineffective because the parasite has already completed its life-cycle in the host and caused irreversible damage.

Little is known about the genetic variability in *S. lupi* populations. Previously, a low amount of genetic variation was found in the *cox1* gene among *S. lupi* individuals from different geographic areas; two haplotypes were identified in samples from Africa, Asia and Europe (Traversa *et al.* 2007). A recent study, however, also targeting the *cox1* gene (De Waal *et al.* 2012), identified eleven haplotypes in the Tshwane Metropole (Gauteng, South Africa) alone, indicating that a high amount of genetic variation is present within one geographical area. Most of the genetic variation found could be explained by differences between worms sharing the same host.

In order to resolve the extent to which genetic diversity exists within *S. lupi* populations, fine-scale genetic analyses are required with high-resolution genetic markers to provide a broader perspective on the dispersal and distribution of the nematode. Thus, population genetic analyses will be conducted across different geographical locations. Gauteng, Eastern Cape and Kwazulu-Natal have previously been reported as focal areas that have a high incidence of *S. lupi* infestation in dogs (Du Toit *et al.* 2008; Lobetti 2000). By using genetic approaches we can address questions pertaining to the gene flow between these locations (Figure 3.1a) and also determine the degree to which the populations are isolated or connected (panmictic) either due to geographical overlap or migration (Waples & Gaggiotti 2006). The variation will also be compared between and within hosts (Figure 3.1b) so that more insight can be gained on the transmission dynamics. The calculation of F_{IS} values will provide an indication of the level of inbreeding within the populations. F_{ST} (fixation index) values will measure the level of population differentiation due to genetic structure (Balloux & Lugon-Moulin 2002). The values of F_{ST} may range from zero to one, where zero indicates that populations are interbreeding (panmixia) and where one indicates that populations are highly differentiated. A population is classified as panmictic when every individual has an equal chance of mating with each other (Gorton *et al.* 2012) and disruption of panmixia results in genetic structuring.

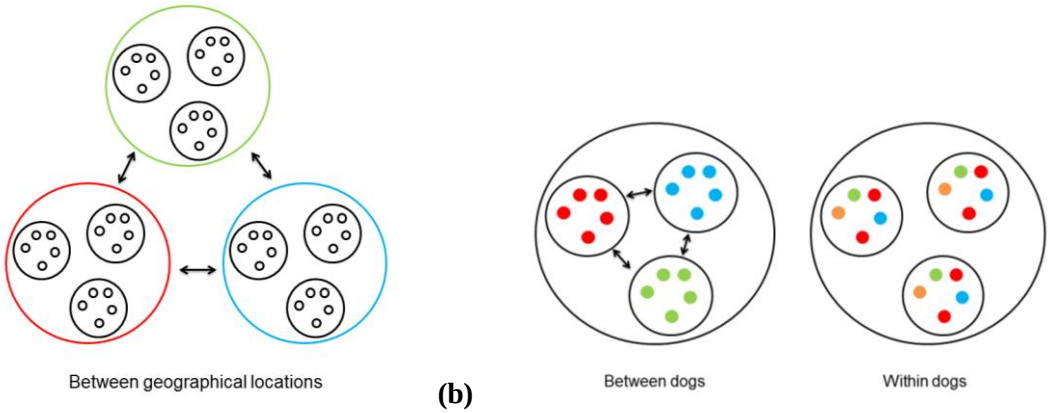


Figure 3.1: Diagrams indicating (a) variation between geographical locations and (b) variation between and within dogs.

The De Waal *et al.* (2012) study on the mitochondrial *cox1* gene, sampled worms from the Tshwane Metropole that had a range of approximately 6,298 km^2. In this study, further insight will be gained on the long range dispersal of the nematode by analysing the variation across three geographical locations. The dispersal pattern may also indicate whether the variation detected is due either to host or parasite movement. It has been observed that *S. lupi* worms have small population sizes within individual hosts, with numbers of worms per nodule ranging from one to ten. The study by De Waal *et al.* (2012) showed that the population size is stable and has not been expanding. Despite the fact that small populations are more likely to undergo genetic drift (Roos *et al.* 2004), the mitochondrial DNA data suggested a high amount of variation (De Waal *et al.* 2012), observed as a result of mixed offspring originating from different infrapopulations. More insight

can be gained on mating patterns using microsatellites, since they provide a multi-locus approach, whereas mitochondrial DNA provides information based on a single locus.

Thus, the main aim of this study was to assess population genetic variation in the parasitic nematode *Spirocerca lupi* in primary hosts from South Africa, using nine polymorphic microsatellite loci, which were developed previously (reported in chapter two). Firstly, the spatial genetic variation of *S. lupi* individuals from South Africa will be determined by taking into account the geographical locations from which the samples were obtained; and secondly, the genetic variation within and between definitive hosts will be assessed.

3.3. MATERIALS AND METHODS

3.3.1. *Sample collection and DNA extraction*

DNA extraction from *S. lupi* worms was performed as described in Chapter 2. A maximum of three worms were sampled per dog in order to evaluate relationships within dogs. A total of 130 *S. lupi* individuals were genotyped (see Chapter 2) at nine microsatellite loci. Worms were sampled opportunistically from three geographical locations in South Africa (Figure 3.2) namely, KwaZulu-Natal (Durban: 10), Eastern Cape (Grahamstown: 35) and Gauteng (Tshwane Metropole: 85) between 2005 and 2012. These samples are distinct of the de Waal *et al.* (2012) study and none of the samples used in this study were used in previous studies.

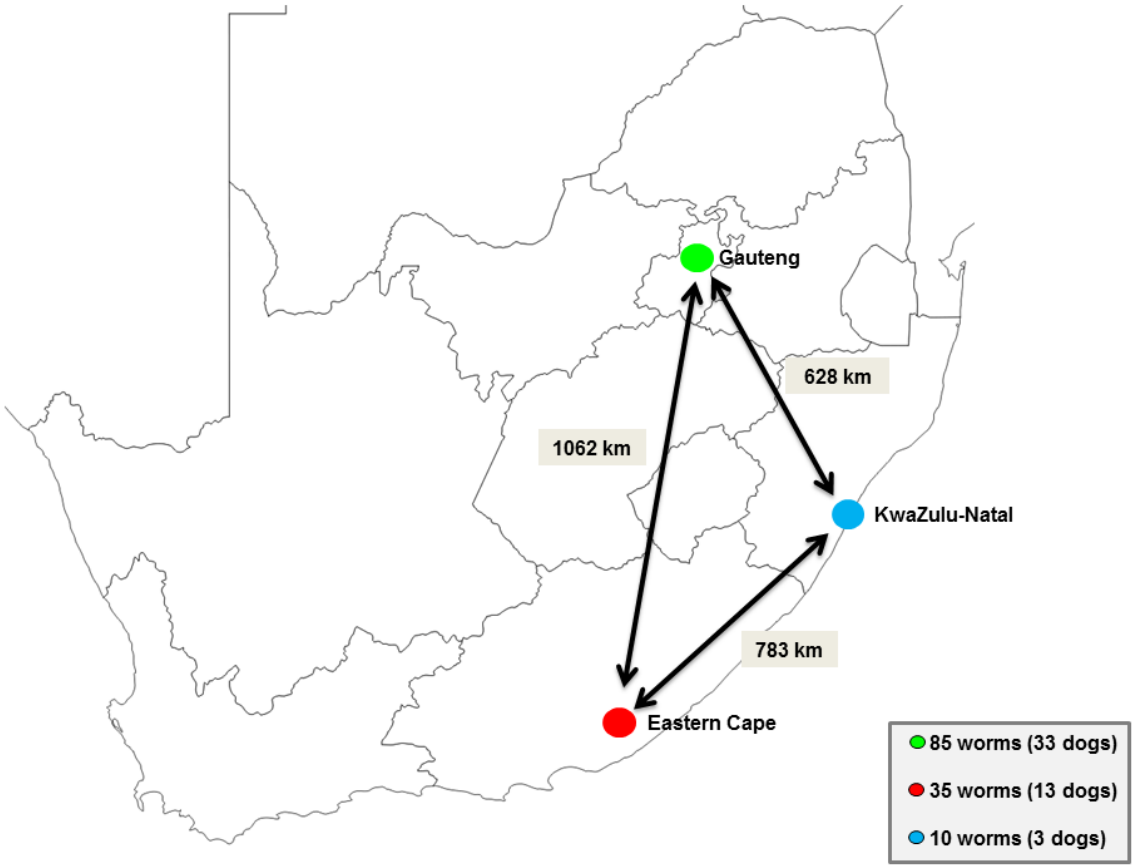


Figure 3.2: Map of South Africa showing the various locations from where *S. lupi* samples were obtained. The number of worms that were genotyped for each location is indicated together with the number of dogs from which they were obtained. Distances between locations are indicated on the map.

3.3.2. Data analysis

The number of alleles, expected heterozygosity (H_E), observed heterozygosity (H_O) and Weir and Cockerham's inbreeding coefficient (F_{IS}) were calculated by location and over all locations with Genetix version 4.05.2. (Belkhir *et al.* 1996-2004). The allele sizes found in the different geographical locations were plotted on a graph drawn in the program R version 3.0.2 using the Graphics package (RCoreTeam 2013).

Genetic differentiation between the geographical locations, measured by F_{ST} values, was calculated using Arlequin v 3.5.1 (Excoffier & Lischer 2010). Genetic differentiation (pairwise estimates of F_{ST}) was calculated by analysing each location as a distinct subpopulation. Arlequin v 3.5.1 (Excoffier & Lischer 2010) was also used to conduct Analysis of Molecular Variance (AMOVA) to determine population differentiation at various levels. Data were defined as "microsatellite" to implement the stepwise mutation model into the analysis. The variation between and within dogs, where each individual dog was specified as a population, while still keeping initial geographical groupings, was tested.

The program STUCTURE v 2.3.4 (Pritchard *et al.* 2000) was used to assess population structure by using a Bayesian approach to infer the most likely number of populations. The program uses individual multi-locus genotypes to infer clusters of individuals that minimize Hardy Weinberg and linkage disequilibria. An admixture model, with sampling locations as prior and correlated allele frequencies was used. Runs for K=1 to 6 were carried out each with 1 000 000 Markov Chain Monte Carlo (MCMC) iterations allowing a burn-in period of 100 000 iterations. Default values were maintained for all other parameters.

The program STUCTURE HARVESTER (Earl & von Holdt 2012) was used to apply the Evanno method (Evanno *et al.* 2005) to detect the value of K, the number of genetic groups, that best fit the data. The signal strength of the posterior probability was determined using ΔK.

3.4. RESULTS

Calculations for individual locations across all loci for the number of alleles, observed and expected heterozygosities and F_{IS} values are indicated in Table 3.1. F_{IS} values ranged from -0.134 to 0.187 across all locations and from -0.328 to 0.308 when measured within each geographical location.

Table 3.1: Summary of standard population genetics analyses for each location. 'Total' values are calculated per locus over all samples. N_A: number of alleles, H_E: expected heterozygosity, H_O: observed heterozygosity and F_{IS}: inbreeding coefficient. The last row indicates the mean values calculated over all the loci.

	Total				**Durban (n=10)**				**Grahamstown (n=35)**				**Tshwane Metropole (n=85)**			
Locus	**N_A**	**H_E**	**H_O**	**F_{IS}**	**N_A**	**H_E**	**H_O**	**F_{IS}**	**N_A**	**H_E**	**H_O**	**F_{IS}**	**N_A**	**H_E**	**H_O**	**F_{IS}**
SL02	9	0.682	0.653	0.047	3	0.555	0.750	-0.292	5	0.567	0.559	0.029	9	0.717	0.683	0.053
SL04	6	0.471	0.465	0.017	4	0.512	0.444	0.190	4	0.506	0.543	-0.058	6	0.447	0.434	0.035
SL06	8	0.794	0.648	0.187	4	0.510	0.700	-0.326	7	0.741	0.618	0.180	8	0.793	0.655	0.180
SL10	10	0.726	0.602	0.175	4	0.415	0.400	0.089	6	0.663	0.706	-0.050	9	0.761	0.583	0.239
SL13	9	0.675	0.726	-0.072	5	0.664	0.750	-0.063	5	0.673	0.697	-0.021	9	0.673	0.735	-0.086
SL14	18	0.833	0.857	-0.026	8	0.846	1.000	-0.125	7	0.723	0.882	-0.206	17	0.831	0.831	0.006
SL15	13	0.559	0.472	0.159	4	0.377	0.444	-0.123	5	0.209	0.226	-0.063	12	0.668	0.565	0.160
SL17	9	0.772	0.632	0.185	5	0.675	0.500	0.308	4	0.719	0.516	0.297	9	0.772	0.691	0.111
SL18	7	0.777	0.884	-0.134	4	0.655	0.900	-0.328	6	0.749	0.914	-0.206	7	0.783	0.869	-0.104
All loci	9.889	0.699	0.660	-	4.556	0.579	0.654	-	5.444	0.617	0.629	-	9.556	0.716	0.672	-

Most of the alleles observed in the samples from Durban and Grahamstown were observed in the samples from the Tshwane Metropole. Three alleles, at locus SL10, SL14 and SL15, were detected only in worms from Grahamstown (Figure 3.3).

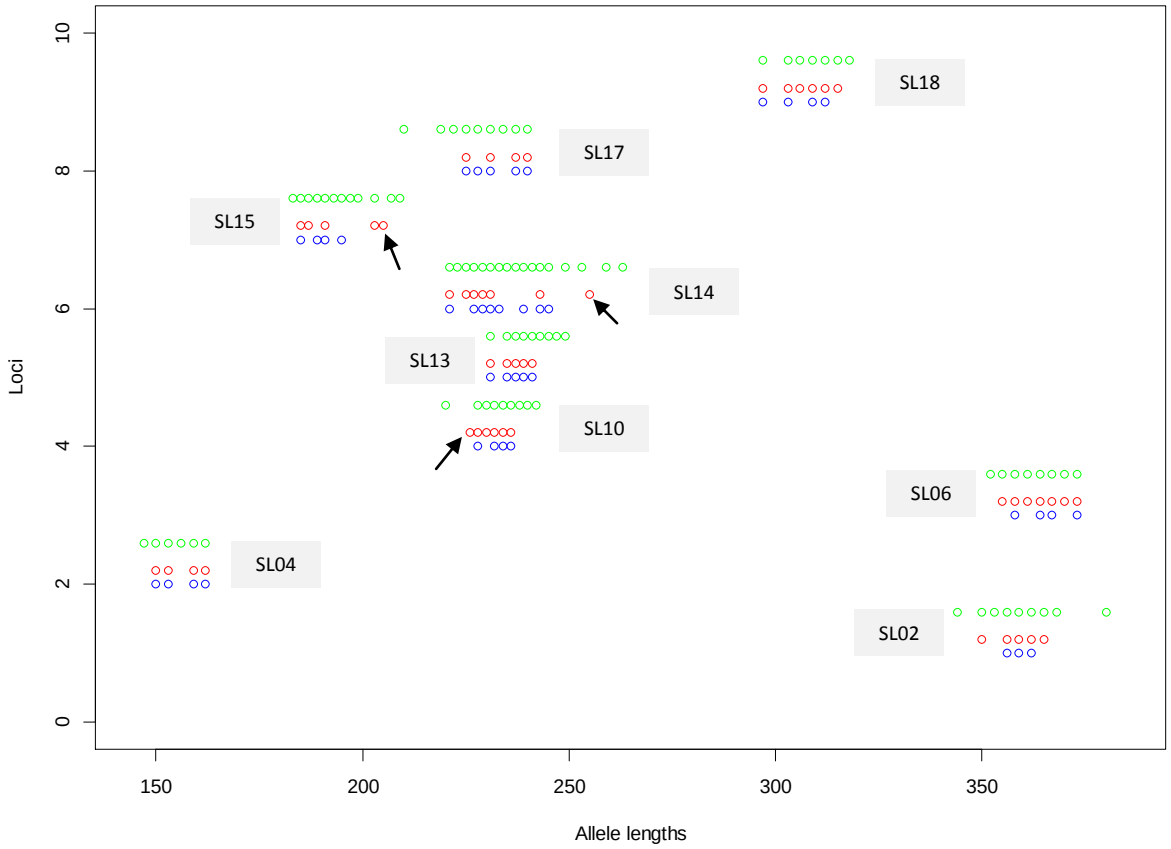


Figure 3.3: Plot showing the allele lengths at nine loci for each different location: Durban (blue), Grahamstown (red) and the Tshwane Metropole (green). The arrows indicate unique alleles.

Estimates of the genetic differentiation between the three geographical locations were calculated as 0.038, 0.045 and 0.035 (Figure 3.4). P-values indicated that all the F_{ST} values were highly significant.

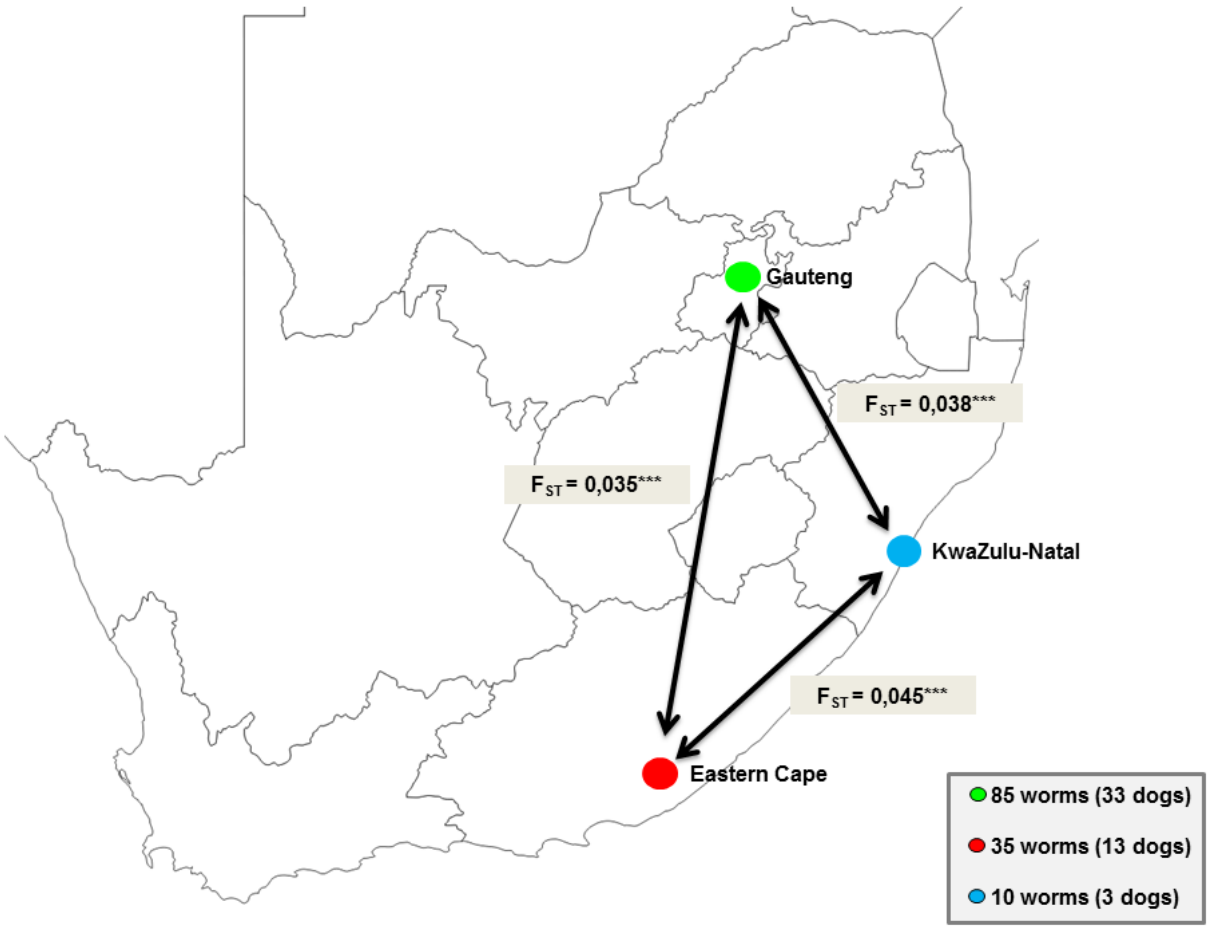


Figure 3.4: Estimates of genetic differentiation between the three geographical locations measured by F_{ST}. ***P-value < 0.001

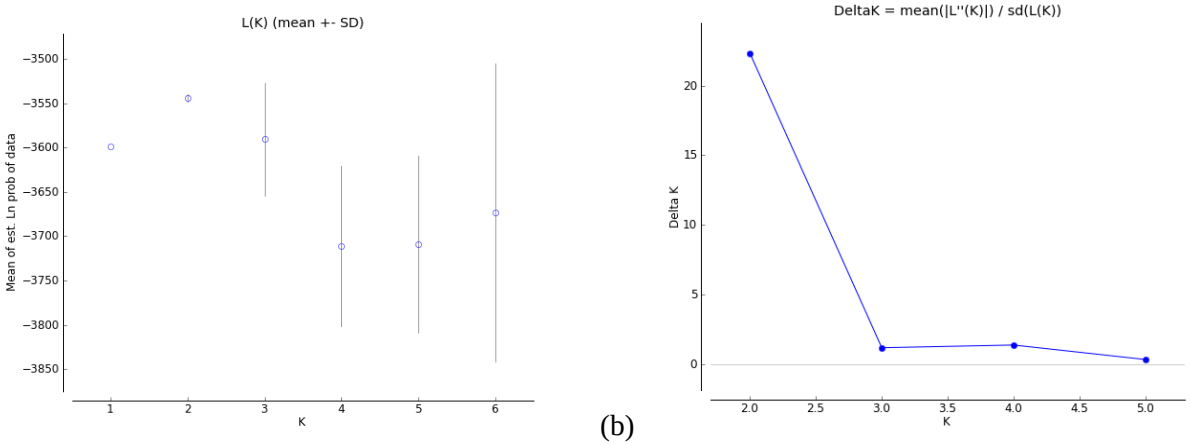
An analysis of molecular variance revealed that the majority of genetic variance was explained by variation within individual worms (87.03%) and that the proportion of variance between geographical locations (3.78%) was less than the variance between dogs within geographical locations (11.28%). The F_{IS} value was not significant when calculated between individual dogs and the percentage of variation was negative (-2.09%). However, F_{CT}, F_{SC} and F_{IT} fixation indices were highly significant (Table 3.2).

Table 3.2: Results of an AMOVA analysis indicating the genetic variation between and within dogs by taking geographical locations into consideration

Source of variation	d.f.	Variance components	Percentage of variation	Fixation indices
Between geographical locations	2	0.070 Va	3.78	F_{CT}: 0.038***
Between dogs within geographical locations	46	0.210 Vb	11.28	F_{SC}: 0.117***
Between worms within dogs	81	-0.039 Vc	-2.09	F_{IS}: -0.025^{ns}
Within individual worms	130	1.619 Vd	87.03	F_{IT}: 0.130***

*****P-value < 0.001. F_{CT}: the variance between groups relative to the total variance, F_{SC}: the variance between subpopulations within groups, F_{IS}: Inbreeding Coefficient, the mean reduction in heterozygosity of an individual due to non-random mating within a subpopulation, F_{IT}: Overall Fixation Index, the mean reduction in heterozygosity of an individual relative to the total population.**

Since ΔK shows the rate of change of the log probability between the different assumed numbers of populations, the highest value (the largest change of ΔK) indicates the most likely number of populations. The likelihood values and ΔK indicated that the most likely number of populations is two (Figure 3.5).



(a) (b)

Figure 3.5: (a) Plot of the mean likelihood L (K) and variance per K value from STRUCTURE on a dataset of 130 individuals genotyped for nine polymorphic microsatellite loci. (b) A plot based on the Evanno method (Evanno *et al.* 2005) to detect the number of K groups that best fit the data.

The Bayesian clustering analysis also suggested the existence of two genetic clusters (Figure 3.6). The first cluster, denoted in green, occurs primarily in the Durban and Grahamstown sampling sites, with limited introgression being noted at these sites. The second cluster, denoted in red, predominates in the Tshwane Metropole, where higher levels of introgression are observed.

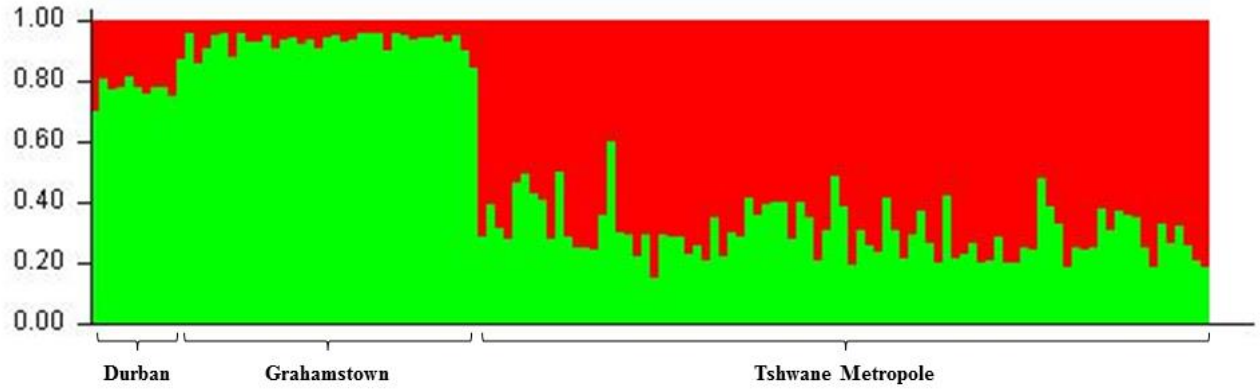


Figure 3.6: Population genetic structure of 130 *S. lupi* nematodes from different geographical locations based on genotype data from nine microsatellite loci. Each bar represents an individual in the population in the following order: Durban (10), Grahamstown (35) and the Tshwane Metropole (85). Red and green coloured segments indicate an individual's membership in each cluster.

3.5. DISCUSSION

Despite all the information available on the developmental process and infestation mechanisms of *S. lupi*, very little knowledge is available of this species' population genetics or evolutionary history. Population genetics is aimed at determining the processes that underlie the distribution of genetic variation within and among populations of a particular species (Lymbery & Thompson 2012). In this study, we expand the knowledge base of this nematode by inferring genetic variation and population structure of *S. lupi* populations using microsatellite data. Early studies using mitochondrial DNA as markers suggested that high levels of genetic diversity and panmixia were characteristic of populations of helminth parasites (Blouin *et al.* 1992). However, later studies predicted that the genetic structure of parasitic populations had high levels of inbreeding, low intra-population genetic variability (Sivasundar & Hey 2003) and large genetic differentiation among individuals from different geographical locations or hosts (Otranto *et al.* 2005). Numerous studies have been performed on parasitic nematodes and the results either support or refute these hypotheses. In many cases it has been shown that parasites have a varied range of gene diversity and structure that is influenced by the life histories of both the parasites and their hosts.

The nine loci that were used in the analysis of *S. lupi* nematodes were polymorphic in all subpopulations and a high frequency of shared alleles was observed. Three alleles that were not found in any of the other locations were detected in nematodes sampled from Grahamstown. The supposedly low F_{ST} values obtained when comparing individuals from different geographical locations suggests that allele frequencies within each population are similar (Holsinger & Weir 2009). This may indicate that dogs and beetle intermediate hosts move around sufficiently to cause substantial amounts of gene flow between the populations. This

exchange of alleles between populations will generate new polymorphism and will result in an increase in local effective population size since random changes in allele frequencies are resisted (Balloux & Lugon-Moulin 2002). Gene flow will also introduce novel or lost genetic variation into the population (Brandt *et al.* 2007). The amount of gene flow between geographical locations determines the level of population structure and may be limited by habitat barriers, distance or life-history traits of the species (Belanger *et al.* 2011).

When the final host deposits faeces in an area, it is likely that the eggs it contains are related parasites. If these parasites remain in close proximity, a clumped transmission pattern will be maintained through to definitive hosts over several generations (Steinauer *et al.* 2010). These parasites within a single definitive host at one time will represent the infrapopulation, which will constitute a parasite deme (breeding unit) (Criscione & Blouin 2006; Lymbery & Thompson 2012). Through this process local scale genetic structure will emerge and in extreme cases will result in inbreeding (Nadler 1995), thus reducing the effective infrapopulation size (Criscione *et al.* 2005; Steinauer *et al.* 2010). Although it is suggested that most parasites are likely to be inbred, the low F_{IS} values and the significant F_{ST} values detected in this study do not support a clumped transmission pattern, which indicates that at one or several points in the life cycle of *S. lupi*, there are opportunities for outbreeding.

The low F_{IS} values that were detected overall suggest an excess of heterozygotes at these loci (Criscione *et al.* 2007; de Meeûs *et al.* 2007; Rauch *et al.* 2005). This is contrary to what was found in a study on the canine heartworm, *Dirofilaria immitis,* which had moderately structured populations with a lower than expected heterozygosity (Belanger *et al.* 2011). The low level of inbreeding detected at the microsatellite loci clarifies the study conducted on mitochondrial data (De Waal *et al.* 2012) and supports the hypothesis that each dog host contains a highly diverse genetic mixture of parasitic nematodes.

Having an intermediate host in the life cycle of a nematode promotes high genetic diversity and well-mixed infrapopulations (Criscione & Blouin 2006). The only way for the nematodes to disperse between sampling sites is through host movement, thus *S. lupi* must depend largely on dung beetles and dogs for dispersal. Dung beetle members of the genus *Onthophagus*, are known to be suitable host species for the *S.lupi* nematode (Du Toit *et al.* 2012). This genus of beetles are thought to have originated in Africa and have spread across all major continents (Emlen *et al.* 2005; Monaghan *et al.* 2007), thus indicating that they are capable of widespread dispersal (Fincher *et al.* 1983; Sole & Scholtz 2010). Dung beetles are known to fly long distances in search of dung (Sakai & Inoue 1999) and since parasite dispersal is determined by the most mobile host (Prugnolle *et al.* 2005; Rauch *et al.* 2005), the dung beetles will be able to transfer the parasite across a wide geographical range.

The life cycle of this parasite has important influences on mating behaviour. It can be hypothesised that like trematode snails, *S. lupi* nematodes have intermediate hosts in their life cycles as an inbreeding avoidance

mechanism (mating with unrelated individuals) (Criscione *et al.* 2005; Rauch *et al.* 2005). Intermediate hosts may accumulate several distinct larval genotypes by consuming eggs from different definitive hosts from many different locations over a period of time (Criscione & Blouin 2006; Rauch *et al.* 2005). Thus the outcrossing rates would increase, and the possibility that mating occurs between genetically similar individuals is decreased (Steinauer *et al.* 2010). These different individuals will then reproduce within the definitive host once ingested and will produce genetically varied offspring (Rauch *et al.* 2005).

An alternative hypothesis could be that definitive hosts ingest many intermediate hosts that contain genetically diverse larvae, thus allowing more opportunity for mixing and dispersal of parasite genotypes (Criscione & Blouin 2006). This has been illustrated in the trematode *Schistosoma mansoni* where its snail intermediate host carried an average of 1.1 unique genotypes whereas its rat definitive host carried an average of 34 unique genotypes (Theron *et al.* 2004), indicating that multiple parasite genotypes had been transmitted to the definitive host via numerous infected intermediate hosts. Sampling of intermediate hosts would thus be of great benefit for future studies to determine whether mixing is occurring at only one specific stage of the parasite life cycle or at all potential stages of dispersal (Criscione & Blouin 2006).

In both of the above scenarios, the component population will function as a deme since offspring are well mixed (Criscione & Blouin 2006).The component population refers to all the adult parasites among a host population at a given place and time (Bush *et al.* 1997). Thus, in *S. lupi* it appears that the component population is behaving as the parasite deme since a high amount of diversity was found within definitive hosts.

Since *S. lupi* has fairly small infrapopulation sizes (De Waal *et al.* 2012), worms have limited mating partners. If the worms within individual hosts were siblings, a high amount of inbreeding would be observed (Criscione & Blouin 2006), however this is not the case. A high amount of genetic diversity must exist within definitive hosts for there to be low levels of inbreeding. A small infrapopulation size may be an adaptive strategy for parasites. Assuming that parasites can detect each other's presence, they will modify their growth strategy so as not to kill the host by overexploitation (Parker *et al.* 2003). Since parasites must compete for limited resources provided by the host, increased competition can result in overexploitation of the host, thus causing an increased risk of transmission failure (Ebert & Herre 1996; Parker *et al.* 2003; Prugnolle *et al.* 2002). On the other hand, greater host exploitation is likely to increase transmission but decrease host survival (Galvani 2003), thus limiting the time available for parasites to reproduce. The limited numbers of adult worms found in hosts of *S. lupi* may suggest that this parasite prefers to maximise its own chances of survival by causing relatively minimal damage to the host, however extensive tests will need to be conducted in order to further validate this suggestion.

Usually, F_{ST} values between 0 and 0.05 indicate little genetic differentiation, however such low values should not be considered as negligible (Balloux & Lugon-Moulin 2002). F_{ST} values between the locations were highly significant, thus indicating that a low level of structuring is in fact present in Durban, Grahamstown and the Tshwane Metropole. Populations can become genetically differentiated over time as a result of genetic drift, when a species occupies a large territory, even if there are no natural barriers in the landscape (Belanger *et al.* 2011; Holsinger & Weir 2009). In this study, the small sample sizes may have reduced the chance of obtaining higher F_{ST} values, or it may be that the level of admixture is insufficient to cause homogenisation of the parasite populations among dogs (De Waal *et al.* 2012).

The high amount of genetic variance that was detected with the AMOVA analysis within individual worms (87.03%) is consistent with that found in the nematode *Trichostrongylus tenius* where 98.5% of variation was observed within individuals (Johnson *et al.* 2006). When the *cox1* gene was used as a marker for this nematode, three quarters of the variation found was due to worms within dogs and the rest was due to variation of worms between dogs (De Waal *et al.* 2012). If the same levels of variation are found within dogs as between dogs, this will provide additional support to the hypothesis that beetles carrying genetically diverse nematode populations may infect a single dog thus allowing several nematodes to enter the dog and reproduce, thereby contributing to the high amount of variation that is observed.

When comparing genetic variation between dogs in this study, a negative and non-significant F_{IS} value was obtained. This could have resulted from the small population size that was sampled; however it more likely indicates that little relatedness exists between worms within a dog. The Wahlund effect may have also been produced because the nematodes analysed within dogs came from different locations, however were grouped as a single population. When the heterozygosity was measured in chapter 2, a deficit was also observed, as in other nematodes (Deter *et al.* 2009; Wasimuddin *et al.* 2012). However when the populations were split according to location, each population was in Hardy-Weinberg equilibrium, which confirms the suggestion in chapter 2 that the Wahlund effect played a role in the high number of null alleles that were observed.

The Bayesian clustering method infers populations by assigning individuals to populations (Pritchard *et al.* 2000). The clustering analysis supported the observation that many individuals from different locations share alleles; however two clusters were detected, thus indicating that spatial genetic structure does exist. This result provides additional support that the observed heterozygote deficit is in fact due to the Wahlund effect, which may suggest that the spread of the parasitic nematode can be spatially limited. If this is the case then in a small population, genetic drift will play a role in the population structure. The assignment of individuals to their respective clusters did not seem to correlate with a specific geographical location, as was also observed when studying genetic variability in the cotton pest, *Rotylenchulus reniformis* (Leach *et al.* 2012). In order to assign individuals to a given sub-population, a minimum number of thirty individuals are required to calculate allelic frequencies (de Meeûs *et al.* 2007). Thus, a third cluster may be observed if more samples

become available for Durban and Grahamstown, which both have relatively small sample sizes compared to the Tshwane Metropole. Additional microsatellite markers may also need to be included to get a stronger signal of population differentiation, which will allow us to further elucidate the spatial range of nematode dispersal and the scale at which populations function (Mallez *et al.* 2013).

The low amount of genetic variation that was detected in an earlier study between worms from Africa, Europe and Asia (Traversa *et al.* 2007) may have been due to a technical limitation, owing to the fact that the *cox1* gene is a mitochondrial gene and represents a single locus. Mitochondrial genes are maternally inherited and are thus not co-dominant and provide limited information. Another possible explanation is that the small sample sizes (Africa: n=4, Europe: n=6, Asia: n=10) may have not been adequate to make inferences about variation between locations.

A substantial amount of genetic differentiation can have major consequences when interpreting and comparing experimental studies (Redman *et al.* 2008). A high genetic diversity with low population structuring implies that nematodes may be able to respond to a chemical attack very easily by evolving genes for disease resistance. This can be seen as a mechanism of adaptation in a parasitic species' since it will allow populations of nematodes to adapt easily to unfavourable situations or environmental conditions, which will lead to an increased distribution of the species (Leach *et al.* 2012). Vertebrate parasites are known to live in unpredictable environments and thus each generation has to be able to adapt to a new host with a different set of immune defenses (Johnson *et al.* 2010). Thus it is a beneficial strategy of these parasites to have a strong evolutionary potential (Prugnolle *et al.* 2005). By having an increased genetic variation among offspring, the chances of survival in an unknown environment are optimised (Johnson *et al.* 2010).

3.6. CONCLUSION

The data presented in this study suggests that high levels of gene flow exist between populations of *S. lupi* that parasitise dogs in South Africa, thus there is a great opportunity for the spread of rare alleles that confer resistance to anthelminthic drugs. *S. lupi* individuals do in fact have a high amount of genetic variation in individual hosts as well as between different hosts, with high levels of heterozygosity, which correlates with the results found when studying the mitochondrial *cox1* gene on samples from the Tshwane Metropole. High genetic variation is critical for the survival of a species, since it allows the species to adapt to constantly changing environments (Grant 1994). The high amount of genetic diversity that was detected within hosts indicates that low levels of inbreeding exist within *S. lupi* and the life cycle characteristics of the nematode provide support for this finding. However, the role of intermediate hosts in contributing to nematode dispersal within and between dogs, and ultimately genetic structure, must be explored further. This will help to answer the question of whether variation exists because the definitive host ingests a variety of different intermediate hosts that contain infective larvae, or whether the definitive host ingests one type of

intermediate host that contains high amounts of variation in the larvae that it carries. There is an indication that genetic subdivisions exist between geographical locations, however, a larger number of samples are required from the individual locations to study the effects that gene flow has had across the three locations. More in-depth population genetics studies on parasites from a wider range of geographical locations can be performed and additional microsatellite markers could be developed. However, it seems more advantageous to study more types of samples from the different life stages of the nematode, i.e. eggs, larvae and worms in order to further elucidate the mechanisms of dispersal of *S. lupi*. It would also be of added value to include paratenic hosts since they may be a key factor in contributing to genetic variation.

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