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Research paper

Population genetic structure of the parasitic nematode *Spirocerca lupi* in South Africa

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ABSTRACT

Spirocerca lupi is a parasitic nematode of canids and occurs in most tropical and subtropical regions around the world. While its life cycle is well known, insight is lacking about its mating structure within-hosts, genetic variability and long-distance dispersal ability. These characteristics contribute significantly to the dynamics and spread of potential resistance genes, which impacts on the control of *S. lupi*. To evaluate the population structure and infer potential mating behaviour of *S. lupi*, we genotyped 130 samples at nine microsatellite loci from three geographical locations in South Africa, between 600 and 1000 km apart. These loci identified unique individuals with high levels of polymorphism suggesting that these are not newly established *S. lupi* populations in South Africa and that effective population sizes must be large. Population genetic analyses showed that populations are not very distinct, that worms within dogs are more similar to each other than random worms from each population, and that mating is at random within dogs. We can thus infer that the parasite is frequently transported over great distances. Even so, two genetically distinct populations could be identified. Relatedness of worms within dogs were significantly higher than between dogs and together with *F*-statistics suggests some non-random transmission of parasites between hosts. While mating is random within a host, parasites from a host are more likely to be related and hence an increase in homozygosity is seen. The implications of this genetic structure on parasite control are considered.

1. Introduction

The canine oesophageal worm, *Spirocerca lupi*, is a parasitic nematode that infects carnivores, predominately the domestic dog (Bailey, 1972; Soulsby, 1982). It is important because of the poor prognosis in the untreated final host (Oryan et al., 2008; van der Merwe et al., 2008) and because it occurs on all continents except Antarctica (Fitzsimmons, 1960; van der Merwe et al., 2008). Potential increases in prevalence (Mazaki-Tovi et al., 2002; Kok et al., 2011; Aroch et al., 2015) and distribution (Al-Sabi et al., 2014; Giannelli et al., 2014; Wright et al., 2016) add to the potential threat posed by *S. lupi*. Knowledge of *S. lupi*'s life cycle has been exploited to control the parasite (Bailey, 1972; van der Merwe et al., 2008). By augmenting this knowledge with a molecular population genetic approach, we can quantify transmission dynamics of the parasite further and predict how resistance may develop and spread (Criscione et al., 2010; Beesley et al., 2017).

Transmission of the parasite can be prevented at two points in its life cycle. The life cycle has been summarized by many (Bailey, 1972; Soulsby, 1982; van der Merwe et al., 2008) as follows — eggs of *S. lupi* are released in the faeces of the final host (canine). If these eggs are ingested by dung beetles that are the intermediate host, the eggs develop to the third larval stage. To continue the life cycle, infected beetles must be eaten by either the final host or a paratenic host (including lizards, poultry, various wild birds and small mammals). If a paratenic host eats the larvae, they will briefly excyst but then re-encyst. If the final host consumes an infected paratenic or intermediate host, larval development continues and is completed. The adult worms live within nodules in the oesophageal wall of the definitive host. Here they mate and females lay eggs in the oesophageal lumen that pass out in the host faeces. The lifecycle can thus be broken by picking up dog faeces, which severs the link provided by dung beetles (van der Merwe et al., 2008) and by not feeding raw remains of potential paratenic hosts to dogs, which eliminates the link that paratenic hosts play (Bailey, 1972).

Any single or combination of the hosts - intermediate, paratenic and final, can disperse the parasite; but how effective this dispersal is, and

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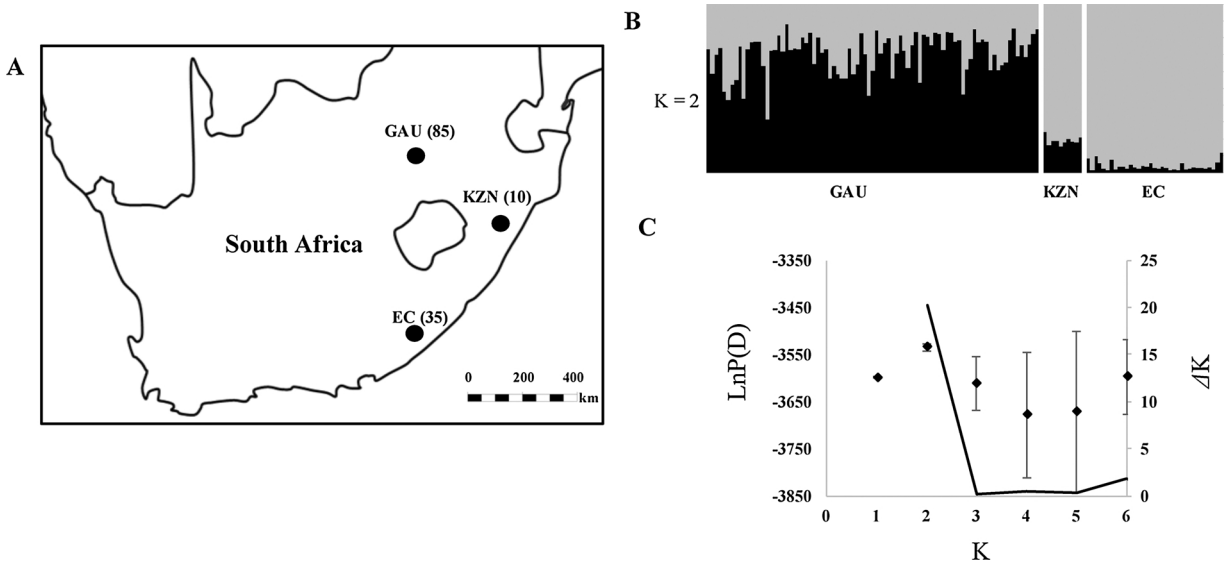

Fig. 1. Sampling locations and geographic structuring of *Spirocerca lupi* from South Africa. A) Map of South Africa indicating the sampling locations and number of worms per population GAU – Tshwane metropole in Gauteng, KZN – Durban in Kwa-Zulu Natal and EC – Grahamstown in the Eastern Cape. B) STRUCTURE plot of K = 2 C) Likelihood (LnP(D), filled circles and error bars) and Delta K (ΔK, solid line) plots.

over what distances they travel are unclear. In a study on *S. lupi* that included samples from a 25 km x 15 km area, the mitochondrial cox1 gene showed no geographical structure suggesting that dispersal is in excess of 10's of kms (de Waal et al., 2012). To quantify dispersal over longer ranges, samples need to be compared from more distant localities (Ebbs et al., 2016).

Any *S. lupi* population is divided into smaller populations confined to hosts, so-called infrapopulations (Margolis et al., 1982). While worms are not able to disperse by themselves, their hosts' dispersal and feeding behavior will determine the worms' population structure. Infrapopulations can be admixed when a host feeds on more than one infected intermediate or paratenic host or when a beetle consumes infected faeces from more than one infected dog. If in this way, eggs and/or larvae are sufficiently mixed during infection all infrapopulations would form one genetic population. On the other extreme, if eggs and/or larvae from different sources never mix, then each infrapopulation is a genetic unit called a deme. These are two extremes between which reality lies. As a result, the fraction of genetic variation between and within infrapopulations depends on the amount of mixing (Nadler, 1995; Hedrick, 2000; Paterson et al., 2000; Prugnolle et al., 2002; Théron et al., 2004; Criscione et al., 2005, 2010; Prugnolle et al., 2005a, b; Criscione and Blouin, 2006; Steinauer et al., 2010; de Waal et al., 2012). When there is limited mixing of infrapopulations, genetic drift will cause infrapopulations to become different from one another over time (Wright, 1931). Such genetic drift will increase differences between infrapopulations and will reduce the genetic variation within infrapopulations (Wright, 1931). The impact of genetic drift is more severe when populations are small (Wright, 1931) and *S. lupi* has small infrapopulations. The harmonic mean population size in the intermediate host is only 6 individuals at the most (du Toit et al., 2008; de Waal et al., 2012) and the mode is between 6 and 12 for the final host (Fitzsimmons, 1960). Random mating within the infrapopulation will result in a Hardy-Weinberg equilibrium within the infrapopulation. However, since members of the infrapopulation are related, such random mating will inevitably result in an excess of homozygosity at the population level.

de Waal et al. (2012) inferred frequent admixture would be required to explain the high mitochondrial diversity observed in infrapopulations. Since mitochondria are maternally inherited de Waal et al. (2012) only measured the female population's dynamics and could not reveal the degree of inbreeding in worms. Nuclear microsatellites can overcome these shortcomings of mitochondrial DNA.

Intermediate levels of admixture among small infrapopulations results in a mixture of matings between unrelated individuals on the one side and between relatives on the other (mixed mating system). The consequence of such inbreeding is that even rare recessive alleles can be homozygous and selection can favour such alleles (Wright, 1921). In the context of parasites, this means that even if resistance alleles are recessive, they can be selected for and increase rapidly (Criscione et al., 2010; Beesley et al., 2017). Co-dominant nuclear markers such as microsatellites can be used to quantify inbreeding as well as relatedness between worms.

To evaluate the population structure over a large spatial scale in South Africa and to provide insight about relatedness between worms between and within hosts, we did a population genetics study of 130 worms from 47 dogs from three populations that are between 600 and 1000 km apart using nine previously developed microsatellites (Reid et al., 2015).

2. Materials and methods

2.1. Sample collection, DNA extraction and microsatellite amplification

Veterinarians collected *S. lupi* samples from necropsies of naturally infected *Canis familiaris* which had passed away from complications of *S. lupi* infections in three populations in South Africa from 2005 to 2009. The populations are in three different provinces namely, Gauteng (Tshwane Metropole - 85 specimens), Kwa-Zulu Natal (Durban - 10 specimens) and the Eastern Cape (Grahamstown - 35 specimens) (Fig. 1A). In order to quantify within-dog variation, three worms from each dog were genotyped, where possible. We will refer to the three different regions as populations and to worms from different host dogs as infrapopulations.

We extracted DNA from tissue samples not exceeding 25 mg from each worm with the Qiagen DNeasy blood and tissue kit (Qiagen, California) following the manufacturers specifications. Nine microsatellite loci were amplified and genotyped in all specimens in a single diagnostic multiplex as described in Reid et al. (2015).

Microsatellite loci were genotyped on an ABI3500 at the sequencing facility of the University of Pretoria (South Africa). Twenty-two individuals were re-genotyped to estimate scoring error and to confirm the consistency of migration of individual alleles. We scored