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# Analysis of genome-wide SNPs based on 2b-RAD sequencing of pooled samples reveals signature of selection in different populations of *Haemonchus contortus*

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The parasitic nematode *Haemonchus contortus* is one of the world's most important parasites of small ruminants that causes significant economic losses to the livestock sector. The population structure and selection in its various strains are poorly understood. No study so far compared its different populations using genome-wide data. Here, we focused on different geographic populations of *H. contours* from China (Tibet, TB; Hubei, HB; Inner Mongolia, IM; Sichuan, SC), UK and Australia (AS), using genome-wide population-genomic approaches, to explore genetic diversity, population structure and selection. We first performed next-generation high-throughput 2b RAD pool sequencing using Illumina technology, and identified single-nucleotide polymorphisms (SNPs) in all the strains. We identified 75,187 SNPs for TB, 82,271 for HB, 82,420 for IM, 79,803 for SC, 83,504 for AS and 78,747 for UK strain. The SNPs revealed low-nucleotide diversity ($\pi$ = 0.0092–0.0133) within each strain, and a significant differentiation level (average $F_{st}$ = 0.34264) among them. Chinese populations TB and SC, along with the UK strain, were more divergent populations. Chinese populations IM and HB showed affinities to the Australian strain. We then analysed signature of selection and detected 44 (UK) and 03 (AS) private selective sweeps containing 49 and 05 genes, respectively. Finally, we performed the functional annotation of selective sweeps and proposed biological significance to signature of selection. Our data suggest that 2b-RAD pool sequencing can be used to assess the signature of selection in *H. contortus*.

**Keywords.** 2b-RAD sequencing; genome-wide SNP analysis; *Haemonchus contortus*; selective sweeps; signature of selection

## 1. Introduction

Parasites are the major health problem in human as well as in animals. They cause significant economic losses to the agriculture sector. The parasitic nematode *Haemonchus contortus* is the most important parasite species of livestock (Gasser and Samson-Himmelstjerna 2016). This parasite is a major threat to the profitable production of small ruminants worldwide. It is a blood feeder parasite which lives in the abomasum of cattle, sheep and goats (Besier *et al.* 2016; Guo *et al.* 2016; Hoberg and Zarlenga 2016). The larvae of this parasite start haemorrhages in hosts at very early i.e. 3rd day post infection (dpi), and they moult to stage-4 (L4) by 4th dpi (Rahman and Collins 1990). The L5 and adult worm

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may suck 30 μL blood daily (Emery *et al.* 2016). When the environmental conditions support the free-living larval stages, the grazing land becomes more contaminated by infective larvae, leading to high intensity of infection in grazing animals, and in such cases, a heavy burden of adult worms may occur in the hosts (Besier *et al.* 2016). A daily blood loss of 30 mL has also been reported in sheep (Albers and Le Jambre 1983). Severe and chronic infestations eventually result in death of animals. *H. contortus* is a versatile parasite which has a broad range (from livestock to wildlife) of host species (Emery *et al.* 2016), cosmopolitan geographic distribution (Brasil *et al.* 2012; Hoberg and Zarlenga 2016) and parasitic as well as free-living stages of its lifecycle (Peter and Chandrawathani 2005). New research directions towards *H. contortus*-associated microbiomes (Guo *et al.* 2016; El-Ashram and Suo 2017), and host resistance to this parasite species (Li *et al.* 2016) are also being explored. Its prevalence has also been reported from China (Wang *et al.* 2006, 2017; Liu *et al.* 2009; Ma *et al.* 2014; Yang *et al.* 2016). Despite its prime economic importance, not much has been known until recently about the molecular biology of *H. contortus* (Gasser *et al.* 2016).

Genetic diversity in parasites underpins the phenomena of antigenic variation and diversity, which help the parasite to avoid eradication by the host-immune system (Deitsch *et al.* 2009). According to the Red Queen hypothesis (Van Valen 1973), there is a co-evolutionary arms race between the host and parasite. To stay in this never-ending contest the parasite has to evolve accordingly. The three major components contributing to the outcome of this contest are the abiotic environment, host genetics and parasite genetics (Curtis *et al.* 2002). Maintenance of genetic diversity is of paramount importance in this race (Morran *et al.* 2011; Gibson and Fuentes 2015). Acquisition of genetic diversity also complicates the control measures for parasites such as drug treatment and vaccination. Therefore, it is important to appreciate the extent of genetic diversity across the parasite populations. The question, whether the populations are genetically isolated from each other, or there is extensive gene flow across them, has practical implications for parasite-control strategies. For example, high gene flow among the populations offers great opportunity for the spread of rare local drug-resistant alleles to other populations, whereas distinct population structuring will not allow the spread of local drug-resistant genotypes. Similarly, a vaccine providing immunity against multiple genotypes in one population may not be effective against distinct genotypes in other populations.

Population-genetic structure describes the patterns of genetic diversity within and among the populations. Understanding the distribution of genetic diversity across the parasite populations is essential to obtain insight into parasite's genetic response to selective pressures (Gilleard and Beech 2007). It is needed to expand the development of inclusive information about population-genetic structure at landscape to regional and global scales (Hoberg and Zarlenga 2016). Substantial amount of research using a variety of low-coverage genetic markers provides the basic information on genetic diversity and population structure of *H. contortus* across the globe (Hoekstra *et al.* 1997, 2000; Otsen *et al.* 2000a, 2000b; Troell *et al.* 2006; Redman *et al.* 2008; Silvestre *et al.* 2009; Chaudhry *et al.* 2015). In summary, the published literature shows that *H. contortus* has high levels of genetic diversity with substantial global and a low but discernible regional population structure within countries (Gilleard and Redman 2016). Low-coverage marker systems are of limited resolution, and the presence of null alleles for microsatellite markers (Hunt *et al.* 2008; Redman *et al.* 2008) may lead to false calculations of genetic distances (Dakin and Avise 2004; Chapuis and Estoup 2007). Current understanding of the population structure and genetic diversity of *H. contortus* is based on the studies of specific genes, or the application of low-coverage neutral-genetic markers (Gilleard and Redman 2016). So far, there has been no published study on genetic diversity of *H. contortus* which has compared the field populations and laboratory strains using genome-wide data. Only, the genome sequencing consortia of *H. contortus* (Laing *et al.* 2013; Schwarz *et al.* 2013) provide some insight into the genome-wide variation for laboratory strains. Recent innovations in the sequencing technologies together with improvements in *H. contortus* genomic resources, have made it feasible to apply more powerful genome-wide population-genomic approaches, for the analysis of genetic diversity, population structure and selection in various populations of this parasite (Gilleard 2013; Gilleard and Redman 2016).

The population-genomic approach (Black *et al.* 2001) provides basis for understanding the genome-wide and locus-specific effects of evolutionary process that leads to variations across genomes and populations (Luikart *et al.* 2003). Assaying single-nucleotide polymorphisms (SNPs) (Morin *et al.* 2004) across the loci is one of the critical assumptions of this approach. The various attributes associated with SNP, such as its relative abundance, genome-wide coverage, data quality and higher genotyping efficiency, make it the marker of choice for genome-wide studies. Whole genome scans for potential SNPs, and their allele frequencies across the population can reveal the genomic regions under selection (Schlotterer 2002; Luikart *et al.* 2003; Beaumont and Balding 2004). Variation in SNP allele frequencies is quantified by $F_{st}$ (Wright 1950, 1951) as a measure of population differentiation. The loci under selection show a higher than the expected value of $F_{st}$, and are identified as outliers (Lenormand *et al.* 1998; Black *et al.* 2001; Luikart *et al.* 2003). The $F_{st}$ calculations based on SNPs are more accurate than those based on microsatellites (Kalinowski 2002), and even can be obtained from a very small sample size (Willing *et al.* 2012). Combining multiple individuals from the same population in a pool for genotyping is effective and a valuable option in terms of cost, time and labour involved (Sham *et al.* 2002; Amaral *et al.*

2009; Rubin *et al.* 2010; Ferretti *et al.* 2013). Pool sequencing is generally applied for allele-frequency estimation (Druley *et al.* 2009) while statistical modifications to its genetic diversity estimators have also been proposed (Futschik and Schlotterer 2010; Perez-Enciso and Ferretti 2010). A recently developed genotyping by sequencing technique, 2b-RAD (Wang *et al.* 2012) is a powerful pool-sequencing method for genome-wide SNP discovery in populations (Seetharam and Stuart 2013; Guo *et al.* 2014; Tian *et al.* 2015; Palaiokostas *et al.* 2016; Pecoraro *et al.* 2016; Hernandez-Castro *et al.* 2017; Liu *et al.* 2017; Luo *et al.* 2017; Zhao *et al.* 2017).

*H. contortus* is ubiquitously distributed in China (Wang *et al.* 2017). Although it is the most economically important parasite of small ruminants in China, there is no detailed information on the population structure, genetic diversity and selection of *H. contortus* populations across the country (Yin *et al.* 2016). Only a few studies using low-coverage genetic markers provide some information on the population genetics of *H. contortus* in China (Yin *et al.* 2013, 2016; Zhang *et al.* 2016). In this study, we analysed four different geographic populations of *H. contours* from China, using a genome-wide population-genomic approach. We also compared the samples of Chinese populations with that of two available foreign populations of *H. contortus* (one from UK and one from Australia). We investigated the extent of genetic diversity and differentiation across populations. To this end, we first applied 2b RAD pool sequencing to discover thousands of SNPs in all the populations, and characterized them by describing their distribution. We then investigated the patterns of genetic diversity, analysed population structure and detected the signature of selection. Finally, we investigated the potential influence of all the genomic regions under selection, and proposed biological significance to signature of selection.

## 2. Materials and methods

### 2.1 *Collection of worms*

Adult worms of *H. contortus* from all the populations (Chinese, Australian and UK) were collected, identified and processed. Samples of Chinese-field populations (Hubei, HB; Sichuan, SC; Inner Mongolia, IM and Tibet, TB) were collected from the abomasa of sheep or goats from four different geographic locations: Gong'an, Hubei (HB); Liangshan, Sichuan (SC); Xilinhaote, Inner Mongolia (IM) and Nimu, Tibet (TB), in China. Adult worms were identified morphologically by light microscopy (supplementary figure 1). Molecular level identification of *H. contortus* was carried out by sequencing ITS-2 rDNA as described previously (Stevenson *et al.* 1995). Screening of SNPs at three conserved sites (24, 205 and 219) established the species of *H. contortus*. Originally obtained from Australia, the Haecon-5 strain (AS) of *H. contortus* was maintained for 2 years by serial passage in helminth-free goats (Preston *et al.* 2015), at Huazhong Agricultural University, China. Similarly, the UK strain of *H. contortus* (originally obtained from Moredun Research Institute, UK) was maintained in sheep for 6 years at Inner Mongolia Agricultural University, China.

### 2.2 *DNA extraction, library construction and pool sequencing*

For DNA isolation, 12 worms from each population were pooled. Total genomic DNA was extracted using a TIANamp Genomic DNA Kit (TIANGEN Biotech, Beijing, China). The material for 2b-RAD sequencing was prepared according to the standard protocol (Wang *et al.* 2012). Briefly, DNA samples were digested with BsaXI and verified on 1% agarose gel. Library-specific adaptors were ligated to digestion products, which were then polymerase chain reaction (PCR) amplified. The target band was purified from 2% agarose gel. Finally, sample-specific barcodes were added by PCR with platform-specific barcode-bearing primers. PCR products were purified via a QIA quick PCR purification kit. Pooled sequencing was carried out on an Illumina HiseqXTen platform.

### 2.3 *SNP calling*

Before SNP calling, the raw 2b-RAD sequencing data were filtered and processed. Initial cleaning was performed for adaptor removal by trimming the raw reads using the FastQC tool (*http://www.bioinformatics.babraham.ac.uk/projects/fastqc/*). The clean reads from each population were then aligned against the *H. contortus* reference genome draft assembly (Laing *et al.* 2013) using SOAP2 software (*http://soap.genomics.org.cn/soapaligner.html*). SNP calling was then carried out using software SAMtools (Li *et al.* 2009). For this study, SNP is defined as a nucleotide that is variable within *H. contortus'* genome (i.e. with at least one alternative allele different from the reference genome) in at least one population (Clement *et al.* 2013). SNPs which were common in at least two populations were categorized as shared SNPs, while those unique to a specific population were categorized as private SNPs. Based on alternative-allele frequency (AAF), SNPs were also classified into rare ($0<AAF \leq 0.2$), frequent ($AAF > 0.2<1$) and fixed ($AAF = 1$) categories. The SnpEff program (Cingolani *et al.* 2012) was used for annotation of SNPs and categorization of their coding effects (e.g. synonymous and non-synonymous). Genome assembly of *H. contortus* (*ftp://ftp.sanger.ac.uk/pub/pathogens/Haemonchus/contortus/genome/*) was used to build the SnpEff database. Effect calculations were then performed on it.

**Table 1.** Distribution of polymorphic sites and SNPs for all strains of *H. contortus*

| | TB | HB | IM | SC | AS | UK |
|---|---|---|---|---|---|---|
| 1 – Total number of polymorphic sites | 75,187 | 82,271 | 82,420 | 79,803 | 83,504 | 78,747 |
| 1.1 – Polybase substitution (% of total polymorphic sites) | 11,389 (15.15%) | 7676 (9.33%) | 6589 (7.99%) | 8155 (10.22%) | 5609 (6.72%) | 9318 (11.83%) |
| 2 – Total SNPs located on scaffolds | 86,750 | | | | | |
| 2.1 – Shared SNPs (% of total SNP) | 44,818 (51.66%) | | | | | |
| 2.1.1 – Rare (% of total SNP–% of total shared) | 9665 (11.14–21.56%) | | | | | |
| 2.1.2 – Frequent: (% of total SNP–% of total shared) | 32,274 (37.20–72.01%) | | | | | |
| 2.1.2.1 – Fixed (% of total SNP–% of total shared) | 2879 (3.32–6.42%) | | | | | |
| 2.2 – Private SNPs (% of total SNP) | 15,727 (18.13%) | 10,707 (12.34%) | 8995 (10.37%) | 6517 (7.51%) | 4532 (5.22%) | 22,520 (25.96%) |
| 2.2.1 – Rare (% of total SNP–% of total private) | 15,562 (17.94–98.95%) | 10,630 (12.25–99.28%) | 8928 (10.29–99.26%) | 6478 (7.47–99.40%) | 4532 (5.22–100.00%) | 22,477 (25.91–99.81%) |
| 2.2.2 – Frequent: (% of total SNP–% of total private) | 84 (0.10–0.53%) | 25 (0.03–0.23%) | 43 (0.05–0.48%) | 13 (0.01–0.20%) | 0 | 14 (0.02–0.06%) |
| 2.2.2.1 – Fixed (% of total SNP–% of total private) | 81 (0.09–0.52%) | 52 (0.06–0.49%) | 24 (0.03–0.27%) | 26 (0.03–0.40%) | 0 | 29 (0.03–0.13%) |

AAF, alternative allele frequency.

Rare SNPs correspond to $0<AAF \leq 0.2$, frequent correspond to $AAF > 0.2<1$ and fixed correspond to $AAF = 1$.

### 2.4 *Characterization of genetic variability*

Nucleotide diversity was assessed using nucleotide-diversity estimators π and Watterson's $\theta$ by implementing in the PoPoolation toolbox (Kofler *et al.* 2011a) in 50 kb windows. Genetic differentiation was evaluated by calculating the fixation index ($F_{st}$) in PoPoolation2 software (Kofler *et al.* 2011b). The population structure was analysed in STRUCTURE software V2.3.4 (Pritchard *et al.* 2000). With two foreign populations (AS and UK) of *H. contortus*, the overall level of homozygosity of Chinese populations (CN) was assessed (AS *vs* CN and UK *vs* CN) by pooled heterozygosity (Hp) in 100 kb windows. The Hp scores were log-transformed and regions with an Hp score of >2 were considered as having a high level of similarity between the strains.

**Table 2.** Summary of nucleotide diversity for the genome-wide SNPs in all strains of *H. contortus*

| Populations | Nwin | Nsites | π | TH |
|---|---|---|---|---|
| TB | 75,815 | 4.5467 | 0.0110 | 0.0179 |
| HB | 75,300 | 5.2761 | 0.0123 | 0.0213 |
| IM | 74,497 | 5.2608 | 0.0133 | 0.0221 |
| SC | 74,681 | 3.9318 | 0.0104 | 0.0180 |
| AS | 72,947 | 4.1361 | 0.0123 | 0.0179 |
| UK | 72,723 | 4.1827 | 0.0092 | 0.0182 |

Nwin is the number of 50 kb windows; Nsites is the average (min–max) number of polymorphic sites per windows in the strains; π is the average (min–max) π estimator of nucleotide diversity per window in the strains; TH is the average (min–max) Watterson's $\theta$ estimator of nucleotide diversity per window in the strains.

### 2.5 *Selective-sweep analysis*

Genomic regions under selection were screened by using the Pool-hmm program (Boitard *et al.* 2013) which detects the selective sweeps based on allele-frequency spectrum (AFS) within sliding windows along the genome (Boitard *et al.* 2012). This model assumes that each SNP belongs to one of the three possible hidden states: (i) selection, (ii) neutral and (iii) intermediate, in association with swept sites. A selective sweep is detected if the hidden state 'selection' is inferred for a window of sites. The AFS is expected to be skewed in regions under selection. Hidden states parameter, 'per-site probability $q$' value ($q = 10^{-10}$) was used. The confidence index was calculated for each specific selective sweep as the maximum of $-\log(1 - q_i)$ over the window, where $q_i$ is the posterior probability of hidden state 'selection' given after simulations. Functional annotations of transcripts in private (non-overlapping) selective sweeps were analysed by interrogating the WormBase database.

**Table 3.** Pairwise population differentiation ($F_{st}$) among six *H. contortus* populations

| | HB | TB | IM | SC | AS | UK |
|---|---|---|---|---|---|---|
| HB | | | | | | |
| TB | 0.3668 | | | | | |
| IM | 0.2933 | 0.3532 | | | | |
| SC | 0.3182 | 0.3038 | 0.2921 | | | |
| AS | 0.2774 | 0.3665 | 0.275 | 0.2989 | | |
| UK | 0.3843 | 0.4537 | 0.3725 | 0.4156 | 0.3683 | |

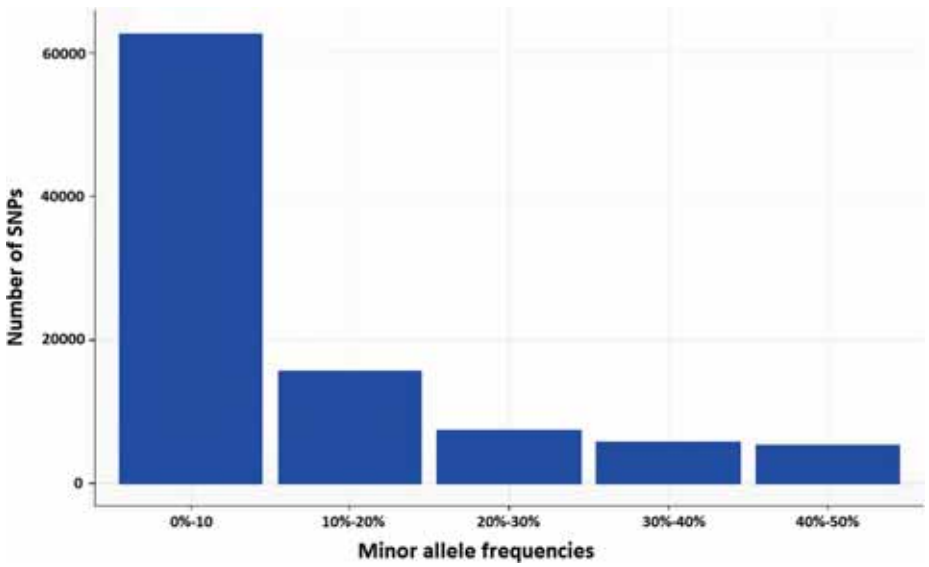


**Figure 1.** Distribution of SNP MAF. SNPs were separated into five categories with the range of 10% according to their MAF level.

### 2.6 *Statistical analysis*

Principal component analysis (PCA) and heatmap of population structure based on pairwise $F_{st}$ values were accomplished using the web tool ClustVis (Metsalu and Vilo 2015).

## 3. Results

### 3.1 *SNP description and characterization*

To apply a population-genomic approach, we generated genome-wide SNP data from 2b-RAD sequencing for each strain. After filtering, processing and alignment of raw reads, SNP calling for each strain was carried out separately (table 1). A total of 75,187 polymorphic sites for TB, 82,271 for HB, 82,420 for IM, 79,803 for SC, 83,504 for AS and 78,747 for the UK strain were identified. The frequency of multiple alleles was recorded as 15.15 for TB, 9.33 for HB, 7.99 for IM, 10.22 for SC, 6.72 for AS and 11.83 for the UK strain. SNPs were characterized into private, shared, rare, frequent and fixed categories (Clement *et al.* 2013). Based on these criteria, a total of 86,750 SNPs were identified, among which 44,818 (51.66%) were shared while 15,727 (18.13%) were private to TB, 10,707 (12.34%) to HB, 8995 (10.37%) to IM, 6517 (7.51%) to SC, 4532 (5.22%) to AS and 22,520 (25.96%) to the UK strain. Majority (70.01%) of shared SNPs were frequent while private SNPs belonged mainly to the rare category in all the strains. None of the private SNP was observed in frequent and fixed categories in AS. The predicted SNPs were then used for evaluating the distribution of minor allele frequencies (MAF). Approximately 60,000 SNPs have an estimated MAF $\leq$ 10% while the rest of the SNPs have MAF $>$ 10% (figure 1). The overall trend of strain-specific mutations reflected from private SNP data was as follows: UK > TB > HB > IM > SC > AS. Among the total predicted SNPs, 44,453 were annotated by using the SnpEff program. Out of these, 8.45% were synonymous SNPs while the rest of the SNPs were non-synonymous (supplementary table 1).

### 3.2 *Nucleotide diversity and population differentiation*

To assess the genetic diversity, we calculated nucleotide-diversity estimators $\pi$ and Watterson's $\theta$, which showed

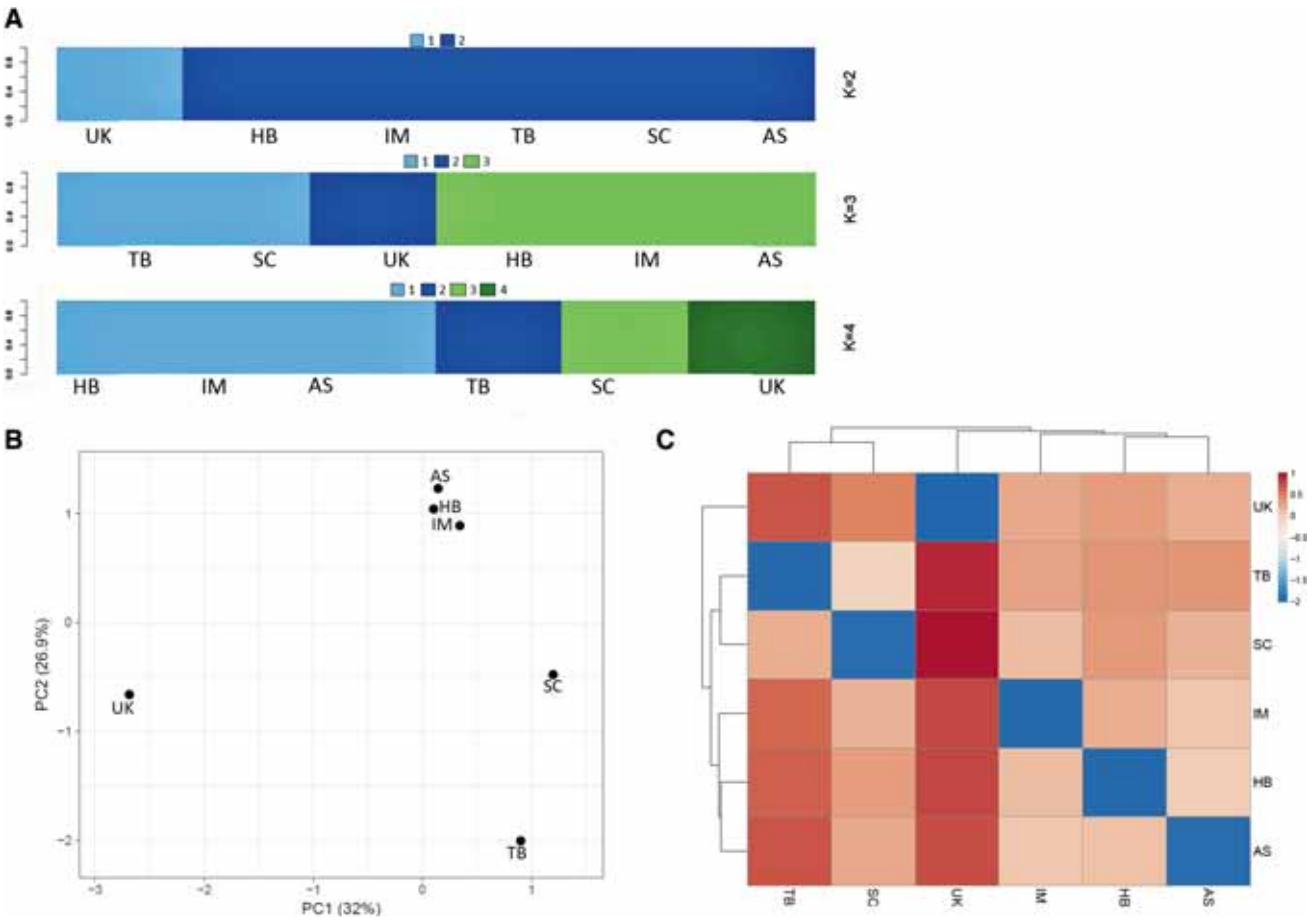


**Figure 2.** Population structure analysis of *H. contortus*. (**A)** Genetic structure analysis by using STRUCTURE software. UK strain was grouped separately each time for different *K* values (2, 3 and 4). (**B)** PCA for population structure. UK strain was clustered very far than rest of the strains. (**C)** Heatmap of population structure.

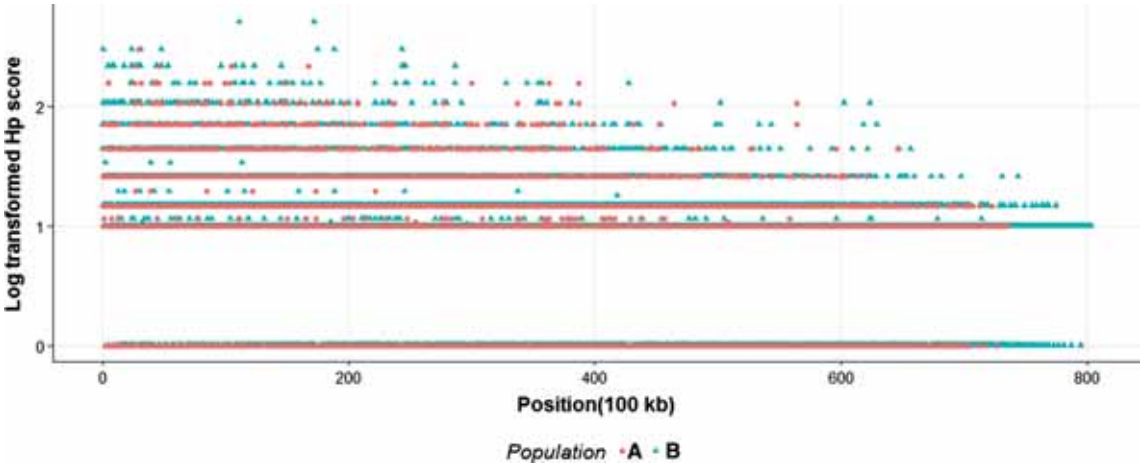


**Figure 3.** Genome-wide comparison of pooled heterozygosity (Hp) in various strains of *H. contortus*. UK *vs* CN (**A**) and AS *vs* CN (**B**).

lower-genetic variation within different populations (table 2). The lowest π value (0.0092) was recorded for UK population. To determine the degree of population differentiation among the populations the $F_{st}$ values were calculated (table 3). Based on the fixation index a significant differentiation was observed among different populations. Highest $F_{st}$ values were recorded in comparison of the UK population with CN populations. It was 0.4537 between the UK and TB while 0.4156 between the UK and SC. The average $F_{st}$ score was 0.34264.

The population structure was investigated using STRUCTURE, PCA and heatmap. In STRUCTURE analysis, at different values of *K* (2, 3 and 4) the UK strain was always found in a separate cluster (figure 2A). At a *K* value of 4, all the populations were divided into four groups: group 1 (UK), group 2 (SC), group 3 (TB) and group 4 (AS, IM and HB). This division was also supported by PCA (figure 2B). In PCA, the two axes (i.e. 26.9 + 32%) accounted for 58.9% of the variation. The same clustering was shown by heatmap (figure 2C). From all three analyses of population structure the UK, TB and SC were more divergent populations. Chinese populations IM and HB were clustered with AS. Overall genetic affinity of Chinese strains (CN) with two other foreign strains was further examined (UK *vs* CN and AS *vs* CN) by log-transformed Hp values (figure 3). UK *vs* CN comparison showed less homozygosity than AS *vs* CN comparison. Although both the foreign strains were from two different continents but still AS showed similarities to Chinese populations in STRUCTURE, PCA and Hp analyses as compared with the UK strain.

### 3.3 *Identification of selective sweeps*

To further explore the genetic background of strains we analysed the genomic regions for selection and investigated the potential influence of selection in different populations of *H. contortus*. In total, we detected 03 and 44 private selective sweeps across the genome in the AS and UK strains, respectively (table 4). No selective sweep was detected in CN. There was no shared selective sweep between the strains. The length of private selective sweeps ranged 49–2606 nucleotides.

### 3.4 *Functional annotation of private selective sweeps*

To identify which adaptive traits and molecular functions were targeted by selection, we investigated the predicted private selective sweeps for the presence of genes. We found 05 and 49 genes laying within or near the private selective sweeps in the AS and UK strains, respectively (supplementary table 2a and b). Interrogating gene ontology (GO) and performing enrichment analysis of the respective transcripts revealed several biological processes and molecular functions associated with predicted private selective sweeps (supplementary table 3a and b). We classified all the biological processes into 13 major areas under a combined graph for the AS and UK strains (figure 4). Although all the transcripts correspond to private selective sweeps we observed common biological areas along with strain specific. Regulation, reproduction-development, apoptosis and locomotion were common areas under selection in both the strains. However, macromolecule modification, nervous system, cell signalling and ion transport, metabolic processes, cell fate determination, reverse transcription, oxidation–reduction and protein–protein interactions were specific to the UK strain. RNA interference was specific to AS (supplementary table 3b) and in the combined graph we classified it under the area of regulation. Some gene products were found uncharacterized (10 for the UK and 03 for AS) and we were unable to investigate their biological functions.

The molecular pathways containing enzymes in regions under selection were then investigated. Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, 43 pathways (supplementary table 4) were found for enzymes in the UK strain. Various metabolic processes, signalling pathways, cancer, viral infections, biosynthesis of long-chain

**Table 4.** Selective-sweep regions and number of corresponding genes for the AS and UK strains

| Scaffold | Start | End | Length | CI |
|---|---|---|---|---|
| | | **AS** | | |
| 337 | 120,965 | 121,906 | 942 | 0.217596933 |
| 552 | 102,040 | 102,995 | 956 | 0.215107783 |
| 1293 | 47,718 | 49,059 | 1342 | 0.208067108 |
| | | **UK** | | |
| 1 | 659,492 | 660,209 | 718 | 0.218633258 |
| 27 | 6621 | 6896 | 276 | 0.231986191 |
| 17 | 325,785 | 326,995 | 1211 | 0.236644272 |
| 239 | 166,608 | 167,410 | 803 | 0.261668753 |
| 30 | 378,345 | 379,251 | 907 | 0.229402957 |
| 69 | 258,698 | 259,597 | 900 | 0.218991656 |
| 50 | 74,935 | 75,432 | 498 | 0.222639155 |
| 55 | 200,502 | 202,957 | 2456 | 0.251540642 |
| 62 | 35,202 | 35,466 | 265 | 0.232520146 |
| 79 | 230,269 | 231,591 | 1323 | 0.230292143 |
| 85 | 213,824 | 215,891 | 2068 | 0.226891267 |
| 136 | 23,627 | 23,807 | 181 | 0.238828946 |
| 158 | 62,555 | 62,603 | 49 | 0.249549768 |
| 160 | 179,974 | 180,811 | 838 | 0.218934451 |
| 228 | 175,548 | 175,722 | 175 | 0.23634598 |
| 235 | 50,879 | 51,559 | 681 | 0.211584041 |
| 236 | 61,959 | 62,502 | 544 | 0.217525408 |
| 240 | 117,687 | 117,979 | 293 | 0.230707293 |
| 250 | 113,279 | 115,733 | 2455 | 0.244994586 |
| 285 | 111,106 | 113,711 | 2606 | 0.259063592 |
| 347 | 158,378 | 160,335 | 1958 | 0.274687989 |
| 3344 | 290 | 1563 | 1274 | – |
| 429 | 30,706 | 30,931 | 226 | 0.234830564 |
| 431 | 80,394 | 80,858 | 465 | 0.221011883 |
| 436 | 70,752 | 71,235 | 484 | 0.228297147 |
| 452 | 147,189 | 147,488 | 300 | 0.229888225 |
| 470 | 113,124 | 113,565 | 442 | 0.221902933 |
| 501 | 28,453 | 29,730 | 1278 | 0.234340447 |
| 584 | 116,138 | 117,943 | 1806 | 0.25967784 |
| 629 | 106,656 | 106,803 | 148 | 0.238632079 |
| 637 | 24,133 | 25,465 | 1333 | 0.256016864 |
| 712 | 24,403 | 25,386 | 984 | 0.250989891 |
| 743 | 101,196 | 101,718 | 523 | 0.245275429 |
| 789 | 10,513 | 10,702 | 190 | 0.237364441 |
| 923 | 85,959 | 87,286 | 1328 | 0.246364412 |
| 1445 | 48,206 | 48,732 | 527 | 0.218024111 |
| 984 | 74,740 | 75,034 | 295 | 0.230223255 |
| 994 | 18,902 | 19,756 | 855 | 0.260649452 |
| 994 | 24,344 | 25,867 | 1524 | 0.210389891 |
| 1102 | 46,528 | 47,020 | 493 | 0.220002785 |
| 2090 | 29,554 | 30,118 | 565 | 0.230774267 |
| 1299 | 13,344 | 13,896 | 553 | 0.214825233 |
| 1459 | 6221 | 6753 | 533 | 0.217782628 |
| 1461 | 66,163 | 67,337 | 1175 | 0.225783615 |

Confidence index (CI) was calculated as the maximum of $-\log(1-q)$ over the window, where $q$ is the posterior probability of hidden state 'selection'.

fatty acids, biosynthesis of antibiotics, antifolate resistance, axon guidance and immunity-related pathways are some common examples. No molecular pathway containing enzyme was found in private selective sweep of AS.

## 4. Discussion

The population structure, genetic diversity and selection among the different strains of *H. contortus* are poorly understood. In this study we applied a population-genomic approach, analysed genomic features, explored the population-genetic structure and detected the signature of selection in different populations of *H. contortus*. By mapping 2b-RAD reads from next-generation pool sequencing to the reference genome of *H. contortus* (Laing *et al.* 2013), we first identified thousands of SNPs in all the populations. About half of the SNPs were shared among all the populations indicating common polymorphic loci. Majority (72.01%) of the shared SNPs were frequent. Highest quantity of private SNPs (26% of total SNPs) was observed in the UK strain, of which 99.8% were rare. This indicates that the majority of specific polymorphic loci belong to the UK strain, which is in favour of its divergent nature in the context of its geographic background. The lowest number of private SNPs (5%) was recorded in AS, and all of these were rare. Different numbers of private SNPs were found in Chinese populations ranging from 7.5 to 18%. Overall, 99% of private SNPs in all the strains were rare which indicates segregating polymorphism across the strains. Distribution of MAF confirms the estimates of rare SNPs. Intra-population genetic diversity was low in all the strains while lowest $\pi$ value was recorded for the UK strain. Low estimates of genetic diversity are in agreement with those of previous study (Luo *et al.* 2017). These are in contrast with that of some other studies (Redman *et al.* 2008; Silvestre *et al.* 2009; Yin *et al.* 2013, 2016; Zhang *et al.* 2016), which reported a high level of genetic diversity in *H. contortus*. This may be due to our small sample size, as a large population size is linked to high levels of genetic diversity within *H. contortus* populations (Gilleard and Redman 2016). Another explanation for this scenario could be the difference in approaches. Genotyping with low-coverage genetic markers resulted in high levels of genetic diversity while high-throughput genome-wide genotyping by a sequencing approach gave low levels of genetic diversity. Some naturally associated biases with RAD-sequencing (e.g. restriction fragment and site heterozygosity bias) may lead to allele dropout (Davey *et al.* 2013; Puritz *et al.* 2014). This can also be a putative reason for low levels of genetic diversity relative to previous studies.

Under neutrality the two nucleotide-diversity estimators $\pi$ and Watterson's $\theta$ should be equal (Tajima 1989), however, differences were observed in both the values. In the case of UK strain, Watterson's $\theta$ value was about two times larger than the $\pi$ value, which reflects the selective pressure. Being originally adapted to warmer climates, *H. contortus* has to overwinter inside the host in colder regions (e.g. Europe), and very few larvae thus survive on pastures during winter (Waller *et al.* 2004; Sargison *et al.* 2007; Falzon *et al.* 2014). This represents a population bottleneck (Gilleard and Redman 2016). A significant $F_{st}$ differentiation was observed among the different

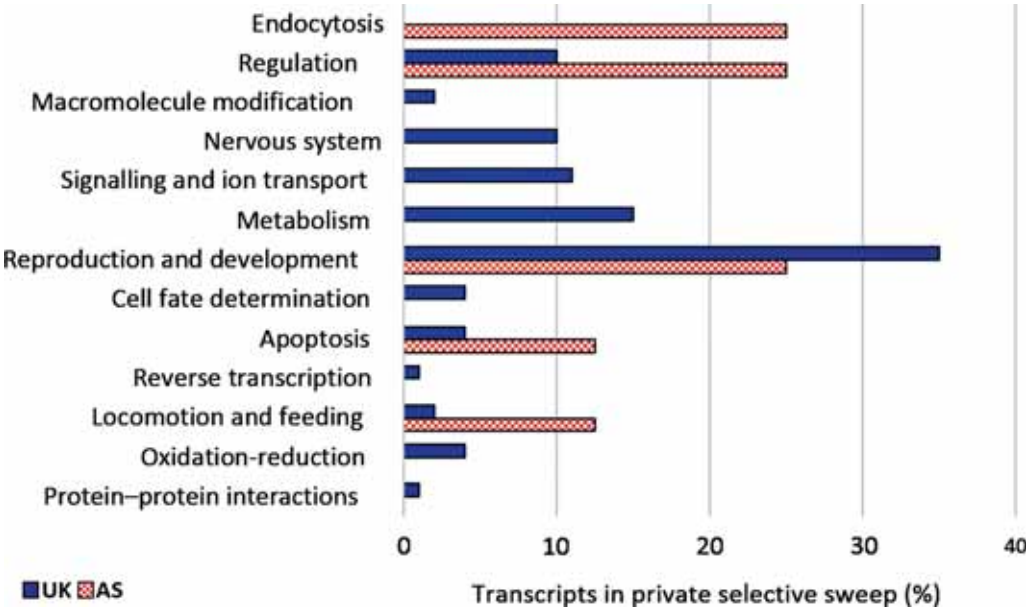


**Figure 4.** Distribution of biological processes found under selection for the AS and UK strains based on their GO terms.

strains, which is in agreement with the previous study (Luo *et al.* 2017). Highest $F_{st}$ scores were observed in pairwise comparisons of the UK strain with others. Previous studies using low-coverage genetic markers reported low genetic differentiation and high gene flow among the different populations of *H. contortus* in China, with no geographical sub-structuring (Yin *et al.* 2013, 2016; Zhang *et al.* 2016). In this study, strong population sub-structuring was observed. Chinese populations TB and SC along with the UK strain were more divergent populations. Chinese populations IM and HB showed affinities to the Australian strain. The UK strain showed a different line of selection from SNPs, genetic diversity and population structure analyses. The high numbers of selective sweeps in the UK strain may indicate a high selective pressure on its genome. GO terms and enrichment analysis revealed some common areas under selection in the UK and AS strains despite the fact that transcripts were belonging to different private selective sweeps. A study (Clement *et al.* 2013) on another helminthic parasite *Schistosoma mansoni* has also reported some common areas under selection in private selective sweeps of two different strains. Among functional areas specific to the UK strain, nervous system, cell signalling and ion transport are the well-known drug targets (Cully *et al.* 1994; Arena *et al.* 1995; Krause *et al.* 1998; Adelsberger *et al.* 2000; Zheng *et al.* 2002; Mounsey *et al.* 2007; Nakatani *et al.* 2016; Chen *et al.* 2017). Reverse transcription in eukaryotes is associated with telomerase and retrotransposons. ROS production and oxidative stress are important for ivermectin (IVM)-induced cell death (Sharmeen *et al.* 2010), interaction with signalling molecules (Ray *et al.* 2012) and neurodegeneration (Shukla *et al.* 2011). Maintenance of redox homoeostasis is of paramount importance for parasites (Salinas 2013) and has been found associated with drug resistance in bacteria (Kaakoush *et al.* 2009). Various genes or traits falling under these areas were targeted by selection in the UK strain. Their putative role in the context of its geographic background can further be explored.

A large-scaled genome-wide comparison of SNPs in combination with population and functional analyses enabled us here to assess genetic diversity and signature of selection in *H. contortus*. However, population-genetic structure of a parasite is shaped by many factors, both inside and outside of the host (Gilleard and Redman 2016), so a representative sampling of all these factors is a major limitation to completely decipher the genetic structure. May be some of the detected genes were identified because of their genomic proximity with truly selected genes. 2b-RAD is a reduced-representation genotyping by sequencing method and thus has some limitations too. A large representative sampling, based on various variables affecting genetic structure of *H. contortus* population, followed by high-throughput pool sequencing, is expected to provide deeper insights into the evolutionary history, genetic structure and selection in this parasite at a global scale.

## 5. Conclusions

In conclusion, our work provides one of the first examples of a comparative genomic approach to assess the genetic diversity, population structure and selection in different strains of *H. contortus*. Low within population-genetic diversity and significant population differentiation were observed across all the populations. All the analyses showed that the UK strain is under high selective pressure with 44 private selective sweeps and 49 genes. Our findings provide further insights into the genetic diversity and population structure of *H. contortus*.

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