## Title: Landscape genetics approach for onchocerciasis control with the parasite (*Onchocerca volvulus*)and the vector (*Simulium damnosum*) mitochondrial data from the transition region of Ghana

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## Abstract

*The word limit (background, methods, results and conclusions) is 350 words; currently, 393 words*

**Background:** Population genetics is crucial for understanding the transmission dynamics of diseases like onchocerciasis. The population genetic estimates are the product of biological processes influenced by the ecological features, particularly onchocerciasis, a filarial disease transmitted by the bites of blackflies with a specific ecological niche. Here, we have used a landscape genetics framework to understand the relationship of the environmental features to the population genetic estimates of both the parasite (*Onchocerca volvulus*) and the vector (*Simulium damnosum*) population. We do this for the parasites and the vectors sampled from the transition ecological region of Ghana, where the transmission has persisted despite almost half a century of onchocerciasis control efforts.

**Methods:** We analysed mitochondrial data from 163 parasites and 93 vectors collected from 15 communities and four breeding sites, respectively. We generated the population genetic estimates and tested if the environment and climate variables could explain the genetic distance between sampling locations. We transformed the significant environmental variables into resistance surfaces to understand the vector and the parasite geneflow. Further, we generated a baseline microfilarial prevalence map from the point estimates of pre-intervention microfilarial prevalence from 47 locations in the study area and compared it with the resistance surfaces.

**Results:** We found that the resistance surface derived from elevation (r = 0.793, p = 0.005) and the soil moisture (r = 0.507, p = 0.002) was significantly associated with the genetic distance of the parasite. Similarly, for the vector populations, the resistance surfaces derived from the soil moisture (r = 0.788, p = 0.0417) and precipitation (r = 0.835, p = 0.0417) were significant. The correlation of the baseline prevalence map was stronger with the parasite resistance surface map than with the vector resistance surface map. The central parts of the transition region were conducive for both the parasite and the vector geneflow with a high baseline onchocerciasis prevalence.

**Conclusions:** We present a framework to incorporate environmental data into the genetic data for onchocerciasis. Here, we were able to identify areas with higher suitability of the parasite and the vector geneflow, which might help us gain deeper insights into the concept of transmission zones for onchocerciasis. Furthermore, this framework is translatable to any other endemic areas and could be used to match the scale of the intervention units with natural transmission zones and thus might make mass administration of ivermectin effective for onchocerciasis elimination.

**Key words:** onchocerciasis, *Onchocerciasis volvulus*, *Simulium damnosum*, population genetics, disease ecology, landscape genetics, transmission zones, persistence of transmission, transition ecological region of Ghana

## Background

Onchocerciasis is a neglected filarial disease transmitted by the bites of blackflies and occurs predominantly in Africa and some parts of South America (Hill et al., 2019). Upon infection, the human host exhibits a range of chronic clinical manifestations such as severe skin itching, skin depigmentation, blindness and epilepsy or nodding syndrome in children (Basáñez et al., 2006; Colebunders et al., 2019). Onchocerciasis is an age-old infection with a huge socio-economic impact in the poorest of the poor nations of the world (Cupp et al., 2011; Dunn et al., 2015). Therefore, control of onchocerciasis has been a public health priority and has progressed through several stages since the commencement of the onchocerciasis control program (OCP) with black fly larviciding in 1975 (Boatin, 2008). Vector control was later complemented with the annual mass drug administration with ivermectin (MDAi) in 1987 (Richards et al., 2001). With OCP ending in 2002, semi-annual MDAi in most of the hyper- and meso-endemic villages has been the sole strategy to control onchocerciasis (Noma et al., 2002). MDAi has led to a significant reduction in the onchocerciasis transmission in the majority of the onchocerciasis endemic foci (with elimination in south American foci, Mali, Senegal), and therefore, the onchocerciasis elimination is now the primary goal (Lakwo et al., 2020; Tekle et al., 2016). Nevertheless, there are instances of persistence of onchocerciasis transmission despite repeated MDAi in some foci, which thwart the target of onchocerciasis elimination (Abong et al., 2021; Awadzi, Boakye, et al., 2004; Basáñez et al., 2006; P. H. Lamberton et al., 2014).

*Simulium damnosum*, the primary vector for the disease, has a specific ecological niche, where *Simulium* larvae need fast-flowing rivers with high oxygen saturation (Cheke et al., 2015, 2017). The narrow range of ecological suitability of blackflies leads to spatial heterogeneity in the prevalence and transmission of onchocerciasis where areas of varying endemicity are in close proximity to each other (Cromwell et al., 2021; Shrestha et al., 2022; Zouré et al., 2014). In addition, there is a spatial variation in treatment, i.e. not all communities (particularly hypoendemic communities) undergo MDAi. These untreated but low-endemic communities might act as a source of infection in the areas where onchocerciasis is controlled with MDAi. This cross-transmission is usually facilitated by the migration of either infected human hosts or infected vectors, or both, as suggested by some modelling studies (Hedtke et al., 2020; Vos et al., 2021). The migration of the parasites via humans has been linked to recrudescence of onchocerciasis in previously eliminated foci of Burkina Faso (Koala et al., 2017; Nikièma et al., 2018). Similarly, failure to achieve elimination of onchocerciasis in West Africa with OCP was attributed to rapid insecticide resistance due to high vector geneflow (Cupp et al., 2011). However, disease control programs have historically focused on government administrative units as the unit of intervention which has led to a situation where treatment decisions are being made without much consideration of where transmission is actually occurring, i.e. the transmission zones.

Transmission zones can be defined as a geographical unit where the disease transmission occurs via locally breeding vectors and forms the basis of biological intervention units (African Programme for Onchocerciasis Control & World Health Organization, 2010). It is crucial to understand transmission zones to ensure that the intervention focus is at the correct scale. The control of onchocerciasis transmission depends on prioritising the limited resources to the most essential areas. The way forward to achieving elimination goals is to align intervention units as close as possible to the natural transmission zones. However, delineating a transmission zone is challenging, and several tools have been deployed to understand transmission zones.

We can gain some insights into the transmission zones based on prevalence mapping, where point prevalence data are interpolated spatially (O’Hanlon et al., 2016; Zouré et al., 2014). However, this is a static map and ignores the 'innate' connectivity between locations; therefore, prevalence map alone is insufficient for distinguishing if the locations belong to different transmission zones. The persistence of transmission is usually facilitated by the migration of pathogens which is challenging to quantify and thus, are rarely incorporated into prevalence mapping. Population genetics has been used to infer the movement of the pathogen where movement can be indirectly measured by the genetic relatedness of samples across locations (Crawford et al., 2019; Hedtke et al., 2020; Small et al., 2019). Genetic relatedness gives us an idea about how common the samples are based on the genetic traits they share, which might be the result of the movement of the fraction of the study population from one location to another.

Population genetics has been used for quite a while to study the transmission dynamics of onchocerciasis (Adler et al., 2010; Agatsuma, 1987; Charalambous et al., 2005; Choi et al., 2016; Doyle et al., 2017; Hedtke et al., 2020). Population genetics can be used to quantify genetic relatedness between the samples, infer demographic history (e.g. host switching), the evolution of epidemiologically relevant traits like resistance, identify the origin population of the study samples etc. (Archie et al., 2009). However, we are not able to get a complete spatial picture of transmission processes of parasitic diseases with population genetics alone. The dispersal and thus, the geneflow of the parasites and the vectors are a subject of influence by the environmental features of the landscape. Since transmission zone is a spatial concept, population genetics estimates alone are not sufficient to gain insights without incorporating the spatial information and environmental data. Ecological variables can be incorporated into population genetics with the help of landscape genetics approach.

Landscape genetics combine population genetics, landscape ecology and spatial analytical techniques to explicitly quantify the effects of landscape on evolutionary processes like gene-flow, drift, and selection (Balkenhol, 2016). These techniques have traditionally been used in the field of species conservation, but have several potential implications for understanding the epidemiology of diseases and their control and elimination (Hemming-Schroeder et al., 2020; Lo et al., 2017; Saarman et al., 2018; Schwabl et al., 2017). With landscape genetics, we are able to add a spatial dimension to the utility of genetic data in understanding the disease processes, which is well suited to understanding onchocerciasis transmission zones. Spatial information can be added in the form of sample geographic coordinates, remote sensing satellite data of different environmental and climate variables such as elevation, slope, distance to the water bodies, mean annual temperature, mean annual precipitation etc. This allows us to understand how the physical environment influences the population genetic structure of the parasite and the vectors.

Landscape genetics involves a series of steps on how it could be used to infer transmission zones (Schwabl et al., 2017). First, we need to measure the spatial pattern of genetic differentiation of the parasite and the vector population. Second, we can use those parameters of genetic differentiation to see which environmental features might govern the spatial pattern of genetic differentiation. Third, we can transform the most important environmental maps to resistance surface maps based on the genetic connectivity optimisation algorithms. Resistance surface maps quantify the resistance of environmental features to a geneflow of the study population (Hemming-Schroeder et al., 2018; Peterman, 2018). Resistance maps can be used to simulate the pattern of gene flow, which gives us an idea about the migration routes of the parasites and the vectors and thus, the transmission zones (B. H. McRae et al., 2008).

We have implemented this technique to infer about onchocerciasis transmission in the transition ecological region of Ghana where there is persistence of onchocerciasis transmission despite onchocerciasis interventions for almost half a century. Onchocerciasis control started as a vector control in the transition region of Ghana as early as 1974 (Walsh et al., 1979), and ivermectin has been distributed for more than three decades (Alley et al., 1994). The region comprises three river basins viz. Black Volta/Tombe, Pru and the Daka river basins which were considered transmission zones. Initial population genetics analysis of the parasite samples from these rivers suggested a lack of isolation by distance (IBD) i.e. they were genetically homogeneous (Crawford et al., 2019). This suggests cross-transmission of onchocerciasis across river basins, the initially proposed transmission zones and a likely reason for the persistence of onchocerciasis. Not only were the samples collected at a greater distance similar, but some locations were geographically nearer but genetically isolated. This remains unexplained, and environmental factors might likely play a role in resulting to such patterns.

We have incorporated environmental data to the parasite genetic data and additional vector samples sequenced from the transition ecological regions with an objective to: i. determine ecological factors affecting the spatial variation in the parasite and the vector population genetic estimates; ii. infer patterns and routes of gene-flow for the parasite and the vector populations. The underlying hypothesis is that the genetic relatedness between parasites and vectors in different geographical locations allows us to quantify gene flow. For gene flow between these locations, there should be the movement of parasites and the vectors in space which can be inferred with the help of resistance surface maps. This will help us not necessarily to delineate transmission zones but infer about them. Further, we have compared the resistance surface maps with the baseline microfilarial prevalence maps and discussed the immediate implications of the pipeline developed to aid elimination goals.

## Methods

### Sampling locations

A total of 163 *Onchocerca volvulus* samples were analysed, isolated from 97 individuals living with onchocerciasis belonging to 15 communities in the ecological transition region of Ghana. Adjacently, 93 vector samples were sequenced from four different locations in the same region. The sampling locations belonged to the historically classified three river basins on the transition region of Ghana, viz. Black Volta, Pru and Daka. The sampling locations for the vector population were chosen as the representative communities for their respective river basins (Gyan, 2020). Most of the parasite samples were collected in 2010–2012 (Crawford et al., 2019) whereas most of the vector samples were collected from 2013–2015 by Osei-Atweneboana’s group (Gyan, 2020). Ethics approvals for sampling parasites from people is reported in Crawford et al. (2019).

A bounding box formed based on the convex hull boundary with a buffer of 35 km around the sampling locations was used for the landscape analysis. The dimension for the bounding box was 293.68×129.38 km (an area of 37,995.59 km2). Geographic coordinates for all the communities were used to calculate the pairwise geographic distance between the communities (Table 1). We merged the communities near each other (less than 5 km) and used the centroid of the geospatial coordinates of the communities in closed proximity for the merged communities. This brought the number of parasite sampling locations down to 11 but increased the sample size per community (Figure 1).

There is a mixed ecology of the Savannah ecotype on the north and the forest ecotype in the south and thus, called the transitional ecological region of Ghana (Gyan, 2020; Klutse et al., 2014). There is the presence of the Volta Lake between the Pru and the Daka river basin and Bui national park on the west. The elevation ranges from 70–525 m from the sea level, and mean annual temperature and mean annual precipitation ranges from 24–29°C and 1077–1355 mm, respectively (Farr et al., 2007; Fick & Hijmans, 2017). We chose this area for the study as there is ongoing persistence of onchocerciasis transmission despite decades of control efforts (Osei-Atweneboana et al., 2007; Otabil et al., 2019; Yaméogo, 2008). The onchocerciasis control program has been running for almost half a century now in the transition region of Ghana, with vector control initiated in 1974 as a part of the Onchocerciasis Control Program (OCP) (Biritwum et al., 2021). The use of ivermectin to control onchocerciasis in Ghana commenced in the Pru and Black Volta in 1987 (Biritwum et al., 2021; Borsboom et al., 2003). Asubende (ASU) was among the first communities to receive ivermectin during the early clinical trials of ivermectin (Alley et al., 1994). Consequently, there have also been reports of suboptimal response in these areas (Awadzi, Attah, et al., 2004; Awadzi, Boakye, et al., 2004; Osei-Atweneboana et al., 2011).

Map

Description automatically generated

**Figure 1. The spatial context of the sampling locations of the *Onchocerca volvulus* and *Simulium damnosum* in the transition region of Ghana.** Geographic coordinates are represented as the circle for parasites and square for vectors, and their sizes correspond to the number of parasite samples from the respective locations. The communities are represented with community codes. The river lines and the administrative borders are shown along with the water body, which is Lake Volta. The inset map shows the map of Africa and Ghana with the bounding box for our study area. More information about sampling locations and the number of samples are present in Table 1.

### Genetic data

The details on the genetic data generation and the parasite samples are available in Crawford et al (2019). For bioinformatics, the raw sequence reads were trimmed using the *Trimmomatic* (Bolger et al., 2014) and then mapped to the *O. volvulus* (NC\_001861) mitochondrial reference genome for the parasites and to custom the *S. damnosum* mitochondrial reference genome (assembled by SMH) for the vectors using *BWA-MEM* (Li, 2013). The mapping files were sorted and converted to *.bam* files using the *SAMtools* (Li et al., 2009), and variants were called using *GATK UnifiedGenotyper* (McKenna et al., 2010) and more recent *GATK HaplotypeCaller* (Poplin et al., 2017) for the parasite and the vector data respectively, and also using *freebayes* (Garrison & Marth, 2012). We filtered the variants with indels, missing regions, and non-bi-allelic sites using *VCFtools* (Danecek et al., 2011). Finally, mitochondrial SNP data with 189 SNPs each from 163 *O. volvulus* samples and 632 SNPs each 93 *S. damnosum* were used for the landscape genetics analysis.

### Prevalence data

Prevalence data based on the skin microfilarial test were obtained from the Expanded Special Project for Elimination of Neglected tropical diseases (ESPEN) database (ESPEN, 2020). Prevalence data collected for the initial mapping of the disease, i.e. before the intervention, were used to create a baseline prevalence map. Most of them were collected before 2001. Prevalence data that fell within the study area bounding box was used to analyse and interpolate the prevalence values, accounting for spatial correlation and the environmental variables. Prevalence data with duplicate observations were removed, and those data collected at different years, but the same location was merged, and the average prevalence was used. There were 47 unique locations with prevalence data that fell within the area and was used for the geospatial mapping of prevalence.

### Environmental data

There is an inherent subjectivity to the ecological requirements of vector and parasite distribution. We tried to reduce this by including all the environmental variables relevant to vector breeding sites, parasite distribution and disease ecology. We compiled different continuous environmental rasters which were ecologically relevant to the onchocerciasis based on the published literature, both field experiments (Cheke et al., 2017; Opoku, 2006) and geospatial modelling studies (Barro & Oyana, 2012; Cheke et al., 2015; Cromwell et al., 2021; O’Hanlon et al., 2016; Shrestha et al., 2022). Environmental variables like distance to the nearest river, soil moisture, elevation, slope, temperature, and precipitation, essential for onchocerciasis disease ecology, were used for the analysis (Barro & Oyana, 2012; Cromwell et al., 2021; Shrestha et al., 2022). In addition, the spatial distribution of onchocerciasis is highly contingent on the vector habitat and the ecological preference for their biting and breeding activities. The dispersal capacity of the *Simulium* vector is generally as high as around 27 km and is dependent on the vegetation type and time of the year (WHO, 2020). Therefore, we included vegetation and seasonality related variables into our analysis. In addition to environmental variables, we also included some socio-economic aspects of the study area—for example, the human population density to consider the availability of human hosts for disease transmission. We used the environmental variables from the corresponding year to account for the differences in the time period when the samples or data were collected. For prevalence data, environmental variables before 2001 were used, and similarly, for the *O. volvulus* and *S. damnosum*, environmental variables from 2010–2012 and 2013–2015 were used respectively as per the data availability. Our starting set of environmental and socio-economic datasets consisted of 32 continuous environmental rasters at a spatial resolution of 1 km from publicly available repositories via Earth Engine (Table S1) (Gorelick et al., 2017).

#### Selection of the environmental variables

A rigorous variable selection approach was used to select the most pertinent variables for both parasites and the vectors. For the analysis of the prevalence data, we extracted the values for the sample locations from the environmental and sociodemographic rasters using the *raster* package in R (version 4.1.0) (Hijmans et al., 2015; R Core Team, 2021). Similarly, for testing the association of the landscape factors to the genetic differentiation or gene flow between the populations, between site characteristics are crucial (Balkenhol, 2016; Hemming-Schroeder et al., 2020). Thus, we estimated the average of the values encountered by a pairwise straight path between each sampling site for all the environmental and sociodemographic variables.

We used principal component analysis for the variable selection from a multidimensional dataset to identify the variables that contributed most to the variance in the sampling sites (Figure S1, S3). For different categories of environmental variables, we generated the pairwise correlation matrix to identify the correlated variables. We included only those variables where Pearson's correlation coefficient was less than (Hemming-Schroeder et al., 2020). For the group of correlated variables, we selected only one variable based on the contribution score of each environmental variable for total variance in PCA analysis and considering the ease of interpretability of the variables. After carrying out the initial selection of variables from each category, a similar correlation analysis was done for the selected variables from all the categories combined.

A suite of five environmental variables was selected for the landscape genetics analysis, and a group of eight environmental variables was selected for the analysis and interpolation of the prevalence data. For the *Simulium* landscape genetics, three out of four locations were also present in parasite sampling locations. Thus, we did not carry a separate variable selection step for the *Simulium* data. The environmental variables selected for the parasite sampling locations were also used for the vector landscape genetics for easier comparison between the vector and the parasite landscape genetics. The five environmental variables selected for the landscape genetic analysis were elevation, isothermality, soil moisture, flow accumulation and annual precipitation. Similarly, for the analysis of the prevalence data, the land surface temperature at night, temperature seasonality, minimum temperature of the coldest month, soil moisture, annual precipitation, slope, distance to the nearest river and prevalence of improved housing were selected.

### Population genetic analysis

We carried out unsupervised -means clustering analysis and haplotype network analysis for the parasite and the vector samples using *adegenet* package (Jombart, 2008) and PopART (Leigh & Bryant, 2015), respectively. We used this to explore the clustering pattern of the samples and identify any outliers. We tried to infer the optimal number of (groups) for the population by looking at the Bayesian Information Criterion (BIC) values. We filtered some of the outlier vector samples separated largely from the cluster of other samples. Then, we carried out the Discriminant Analysis of the Principal Components (DAPC) by assigning samples to their respective communities. DAPC is sensitive to the number of principal components retained. Therefore, we performed stratified cross validated DAPC by varying the number of principal components using *xvalDapc* function in the *adegenet* package. We calculated the membership probability of each sample, communities, and the posterior correct assignment probability for the communities.

We calculated the summary statistics for the genetic data like as number of alleles and observed gene diversity and the pairwise measure of genetic differentiation () using the *Hierfstat* package (Goudet, 2005). Similarly, mean allelic richness and number of haplotypes were calculated using *PopGenReport* and *haplotypes* package, respectively (Adamack & Gruber, 2014; Aktas, 2020). Pairwise matrix was adjusted for finite populations by linearising it with the equation as suggested by (Rousset, 1997; Saarman et al., 2018; Slatkin, 1995).

### Landscape genetic analysis

Landscape genetics analysis helps us understand how landscape features influence spatial genetic variation. The simplest starting model is the isolation by distance model, where we test if there is a correlation between the pairwise genetic distance and the pairwise straight path geographic distance between the sampling sites (Manel & Holderegger, 2013; Schwabl et al., 2017).

#### Isolation by distance

Isolation by distance test was done by calculating the pairwise Euclidean distance between the geographic coordinates of the sampling sites using the *graph4lg* package and comparing it with the pairwise linearized genetic differentiation between sites (Savary et al., 2021). Geographic coordinates were converted to Universal Transverse Mercator (UTM) projection, a two-dimensional cartesian coordinate referencing system (CRS) which is accurate while performing distance-related operations on spatial objects (Diggle, 2019). The CRS used in our analysis for all the spatial objects was: epsg-32630 (+proj=utm +zone=30 +datum=WGS84 +units=m +no\_defs). We performed the Mantel tests between the geographic distance and the genetic distance matrix with *vegan* package, and the significance of the correlation was calculated based on 10000 permutations (Oksanen et al., 2013).

#### Cost distances

We generally calculate cost distances to see the effect of landscapes on genetic differentiation. Cost distances reflect both the geographic distance and the hypothesized effect of the intervening landscape features between the sampling sites on the dispersal of the organism of interest (B. H. McRae, 2006; Schwabl et al., 2017). Cost distances are calculated based on the resistance surface maps. Each pixel in resistance surface maps is assigned a value of resistance reflecting the extent to which the landscape feature on that the pixel impedes or facilitates the movement or connectivity of the populations of interest at different locations (Peterman, 2018; Spear et al., 2010).

Cost distances are of different types. One of the simplest forms of cost distances is the least cost distance based on the path of the least resistance an organism follows in traversing from one location to the other. However, biological organisms, blackflies and humans in this case, do not always follow a single path of the least resistance. To incorporate the multiple paths an organism might follow, there are other distance metrics like circuit distance and commute distance. This avoids the assumption that the organism has complete knowledge of the landscape and the potential paths, and thus, might be suitable for multi-generational gene flow (Adriaensen et al., 2003; Bauder et al., 2021). In addition, circuit and commute distances consider multiple pairwise connections and might be suitable for multi dependent dispersal systems in a landscape of continuous resistance (Schwabl et al., 2017).

Circuit distance is based on the circuit theory and has been used in chemical, social, neural networks (reviewed in McRae et al., (2008)), and more recently to model connectivity in heterogeneous landscapes (Hemming-Schroeder et al., 2018, 2020; B. McRae et al., 2016; B. H. McRae et al., 2008). This is based on the fact that current, voltage and resistance in an electrical circuit demonstrate a good relationship with a random walk (B. H. McRae et al., 2008). Therefore, the current density obtained using this algorithm can be used to measure connectivity or isolation between locations and identify essential elements for movement (corridors) in a landscape. Commute distance is an alternative distance metric that incorporates multiple paths and correlates well with circuit distance.

Commute distance represents the random walk commute time between two locations, the number of edges traversed during movement from one location to the destination location and returning to the starting point on the resistance surface (van Etten, 2017). Commute distance is proposed to be more computationally efficient than calculating circuit distance in R (Peterman, 2018). However, we calculated circuit distance using the recent version of Circuitscape implemented in Julia, which was faster than optimising via commute distance (Kimberly R. Hall et al., 2021). In addition, connectivity maps were generated using Circuitscape. Therefore, we used circuit distance to optimise the resistance surfaces and test environmental variables' effect on genetic differentiation.

#### Resistance surface maps

There are different methods to parameterise resistance surfaces. First, through the assignment of the hypothesized resistance effect of the landscape features based on published literature and expert opinions. This trial-and-error process explores resistance in limited parameter space (Peterman, 2018). The other is the exhaustive search and optimisation method, where resistance surface parameters are explored to maximize the association between the pairwise genetic distance and the cost distance (Graves et al., 2013; Peterman, 2018; Wang et al., 2009). ResistanceGA is one of the optimisation methods based on a genetic algorithm that offers eight transformations of ricker and monomolecular functions to a continuous surface. The following equation gives the ricker and monomolecular transformation function:

Ricker transformation:

Monomolecular transformation:

ResistanceGA searches for the best combination of transformation function, magnitude, and shape parameter. It provides a framework for optimising resistance surfaces from an environmental raster surface without any prior assumptions about the contribution of those surfaces on the resistance (Peterman, 2018) and therefore, provides an unbiased representation of the resistance surface based on the genetic data.

Five different environmental variables selected for the landscape genetic analysis were used to optimise the resistance surface maps. Linearised pairwise genetic distance was used as the response parameter. The cost distance calculated from the transformed resistance surfaces is used as a predictor to find the best model that explains the genetic distance. A linear mixed-effects model with a maximum likelihood population effect (MLPE) is fitted to the data (Clarke et al., 2002; Fukuda et al., 2022). We optimised single surfaces of environmental variables and used log-likelihood as the objective function for the MLPE model. Four replicates of 1000 iterations each were run with the optimisation set to stop after 50 generations of no improvement. We set the maximum allowable resistance value to be 100 during the optimisation process for easier rescaling and comparison of the resistance values of different environmental variables.

#### Isolation by resistance

Each replicate of the resistance surface obtained via the optimisation process was tested using the circuit distance matrix obtained from those resistance surfaces. There are different methods available for assessing the correlation between the genetic distance matrix and the circuit distance obtained from the resistance surfaces of each environmental variable (Saarman et al., 2018). Partial Mantel tests are standard in landscape genetics for testing the association of resistance surfaces to genetic differentiation (Manel et al., 2003). The partial Mantel test assesses the correlation between two different distance matrices conditioned on a third matrix (Oksanen et al., 2013). We used the partial Mantel test to assess the correlation between the genetic distance matrix and the pairwise circuit distance matrix accounting for the geographical distance matrix. Partial mantel has been dominant in landscape genetics analysis but are high in type I error rates with spurious correlations (Cushman & Landguth, 2010). Therefore, we used mixed matrix regression with randomization (MMRR) as a confirmatory test.

The MMRR was performed using the *lgMMRR* function in the *PopGenReport* package based on Wang's, (2013) method. MMRR also gives us the effect of the resistance surface on the genetic differentiation accounting for the geographic distances. To avoid spurious correlations, we took a conservative approach, and the resistance surfaces were deemed significantly associated with the genetic distance only if both the partial mantel and MMRR tests were statistically significant (De Castro et al., 2016; Saarman et al., 2018). Significance for both the partial mantel and MMRR were assessed based on 10,000 permutations.

#### Composite resistance surface maps

As landscape features and environmental gradients do not exist in isolation, the environmental resistance surfaces significantly associated with the genetic distance matrix were manually combined to form a composite resistance surface map. They were rescaled from 0 to 1, where the maximum resistance value among all the significant surfaces was considered as 1, preserving the relative contribution of each optimised surface to the composite resistance map. The composite resistance map was obtained by multiplying the rescaled significant resistance surfaces described in Schwabi et al. (2017). The composite resistance surfaces were used for the connectivity mapping via Circuitscape.

### Mapping prevalence data

The mean of the posterior prevalence was obtained from the pre-intervention microfilariae prevalence data using the Bayesian approach with Integrated Nested Laplace Approximation (INLA) (Moraga et al., 2015; Rue et al., 2009). The number of positive cases out of the total number of people tested in a location was assumed to follow a binomial distribution. The prevalence was modelled with different environmental variables and a spatial random effect with a zero-mean Gaussian process following a Matérn covariance function. The Matérn field is represented with a finite element mesh formed of triangles around the sampling locations and adding vertices over the prediction region. Multiple triangulation meshes with different parameters for cut off and length of triangles inside and outside the boundary were tested for model fit and the computational cost. We created a triangulation mesh with a 3 km cut off; the maximum length of triangles inside and outside the boundary was set to 10 km and 100 km, respectively. Finally, we fitted the model and assessed the relationship of environmental variables with the prevalence data. The details of fitting a spatial model to the prevalence data for geospatial mapping are available in Shrestha et al. (2022).

The prediction of the posterior prevalence was made at 2 km resolution considering the high computational cost of prediction on a lower resolution. A bivariate map of posterior mean prevalence was plotted with the composite resistance surface maps to visualise areas of varying prevalence and resistance. Correlation coefficient measures were calculated between the mean prevalence map and vector and the parasite composite resistance surface maps to test the association between them. We also generated bivariate moving window correlation measures, their significance, and Moran's I measure of spatial autocorrelation to measure the correlation between two spatial processes (Goslee & Urban, 2007).

## Results

We carried out unsupervised -means clustering analysis and visualised the haplotype network for both the parasite and the vector mitochondrial data separately to observe if there were any inherent clusters and if there were any outlier samples. We chose the minimum number of principal components that explained highest cumulative variance. The number of principal components retained for the clustering analysis of parasite and the vector were 80 and 45 respectively. We chose the number of optimal clusters based on the BIC scores i.e. for the parasite data and for the vector data as the decline in BIC saturated beyond these values (Figure S5). Clustering and the haplotype network analysis on the *Simulium* data indicated the presence of the outlier groups which were removed from the downstream analysis. Group 6 and Group 10 were distant from the other clusters in the Linear Discriminant (LD) space and in the haplotype network analysis as well (Figure S6). Therefore, they were deemed to be outliers and removed from the downstream analysis while all the *O. volvulus* samples were considered for the analysis.

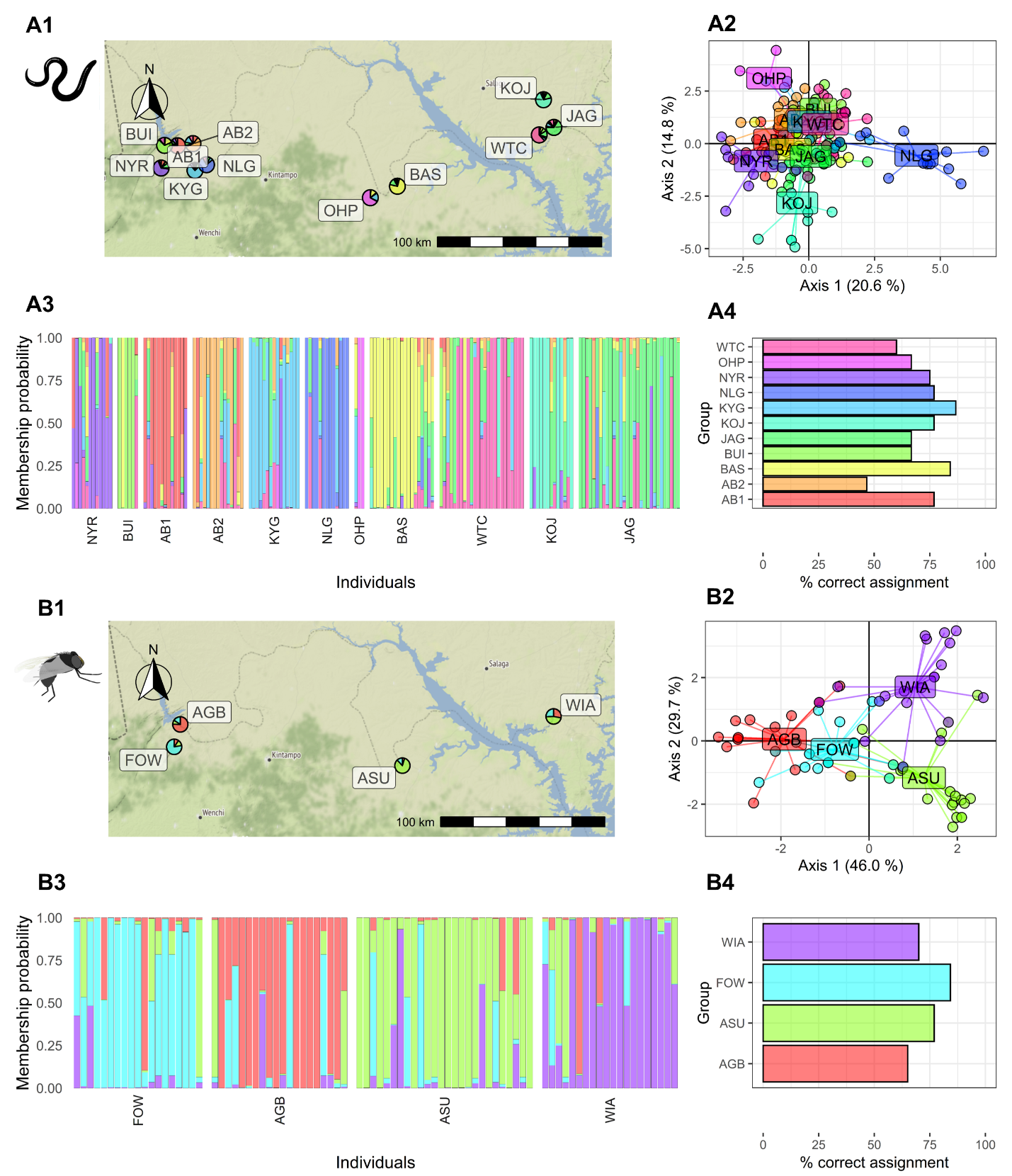
### Population genetic analysis

For the parasite samples, number of alleles and the number of haplotypes corresponded to the sample size of the population, while the mean allelic richness and the gene diversity correlated with each other (Table 1). The number of alleles were the highest for Jagbengbendo and Wiae/Takumdo/Chabbon (258) while the mean allelic richness (1.083) and gene diversity (0.055) was the highest for Bui. Across sites, the parasite's number of alleles averaged 234.18 (±5.001 SE), mean allelic richness averaged 1.071 (±0.003 SE), gene diversity averaged 0.047(±0.002 SE), and the number of haplotypes average 14.27 (±2.13 SE). For the vector samples, all the population genetic statistics, number of alleles, mean allelic richness and gene diversity were the highest for the Agborlekame/Agbelekame (1) despite having sample size lesser than Asubende and equal to Wiae. The average number of alleles for the parasite populations across sites was 941 (±15.54 SE), mean allelic richness averaged 1.438 (±0.023 SE), gene diversity and the number of haplotypes averaged 0.091 (±0.006 SE) and 18.5 (±1.708 SE) respectively.

**Table 1. Geographic coordinates of the sampling sites along with their, river basin, site code, sample size and population genetics summary statistics.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Samples** | **River basin** | **Site name** | **Site code** | **Number of samples (n)** | **Longitude** | **Latitude** | **Number of alleles** | **Mean allelic richness** | **Gene diversity** | **Number of haplotypes** |
| Parasites | Black volta | Agborlekame/Agbelekame (1) | AB1 | 13 | -2.21 | 8.23 | 227 | 1.0623 | 0.0415 | 13 |
| Agbelekame (2) | AB2 | 15 | -2.12 | 8.25 | 227 | 1.0579 | 0.0386 | 14 |
| Bui | BUI | 6 | -2.28 | 8.24 | 216 | 1.0825 | 0.0550 | 6 |
| Kyingakrom | KYG | 15 | -2.11 | 8.10 | 240 | 1.0747 | 0.0498 | 15 |
| New Longoro | NLG | 13 | -2.05 | 8.13 | 236 | 1.0790 | 0.0526 | 13 |
| Nyire | NYR | 12 | -2.30 | 8.11 | 236 | 1.0758 | 0.0505 | 12 |
| Daka | Wiae Chabbon\* | W/TAK/CHA | 14 | 8.28 | -0.21 | 258 | 1.0699 | 0.0466 | 24 |
| Takumdo\* | 2 | 8.28 | -0.19 |
| Wiae\* | 9 | 8.32 | -0.22 |
| Jagbengbendo | JAG | 30 | -0.13 | 8.33 | 258 | 1.0637 | 0.0425 | 28 |
| Kojoboni | KOJ | 13 | -0.18 | 8.49 | 237 | 1.0804 | 0.0536 | 12 |
| Pru | Baaya\* | B/ASU/SEN | 1 | 8.00 | -1.02 | 240 | 1.0693 | 0.0462 | 17 |
| Asubende\* | 16 | 8.02 | -0.96 |
| Senyase\* | 2 | 8.02 | -1.00 |
| Ohiampe | OHP | 3 | -1.14 | 7.95 | 201 | 1.0635 | 0.0423 | 3 |
| Vectors | Black volta | Agborlekame/Agbelekame (1) | AGB | 20 | -2.211 | 8.242 | 972 | 1.4964 | 0.1035 | 17 |
| Fawoman-Banda | FOW | 19 | -2.245 | 8.12 | 928 | 1.4388 | 0.0869 | 15 |
| Pru | Asubende | ASU | 26 | -0.981 | 8.017 | 961 | 1.4248 | 0.0981 | 23 |
| Volta | Wiae | WIA | 20 | -0.144 | 8.286 | 904 | 1.3905 | 0.0773 | 19 |
| \* Communities who were within the geographic distance of 5 km and were thus merged and the centroid was taken as the geospatial coordinate for the merged community. | | | | | | | | | | |

We performed the stratified cross-validated DAPC for the parasite and the vector data optimising the number of principal components to be retained which was 72 and 40 respectively. DAPC for the parasite genetic showed overlap between the clusters of the communities with exception of few communities like OHP and NLG (Figure 2). Looking at the individual membership probability of the samples, it is shared among different communities and the average % of correct assignment is 71.21% (±11.45% SD) for the parasites. For the parasites from some communities like KYG and BAS, the percentage of correct assignment was great than 80%. For the parasites from some communities like BUI and AB2, parasites were incorrectly assigned to other communities. Similarly, for vectors, DAPC showed less overlap between the clusters of the communities. There were shared membership probabilities for few samples within the communities. The average % correct assignment was similar (74.03%±8.36% SD, ) compared to the parasite samples. For communities like FOW and ASU, the % correct assignment was 84.21% and 76.92%, where FOW had the highest re-assignment probability.

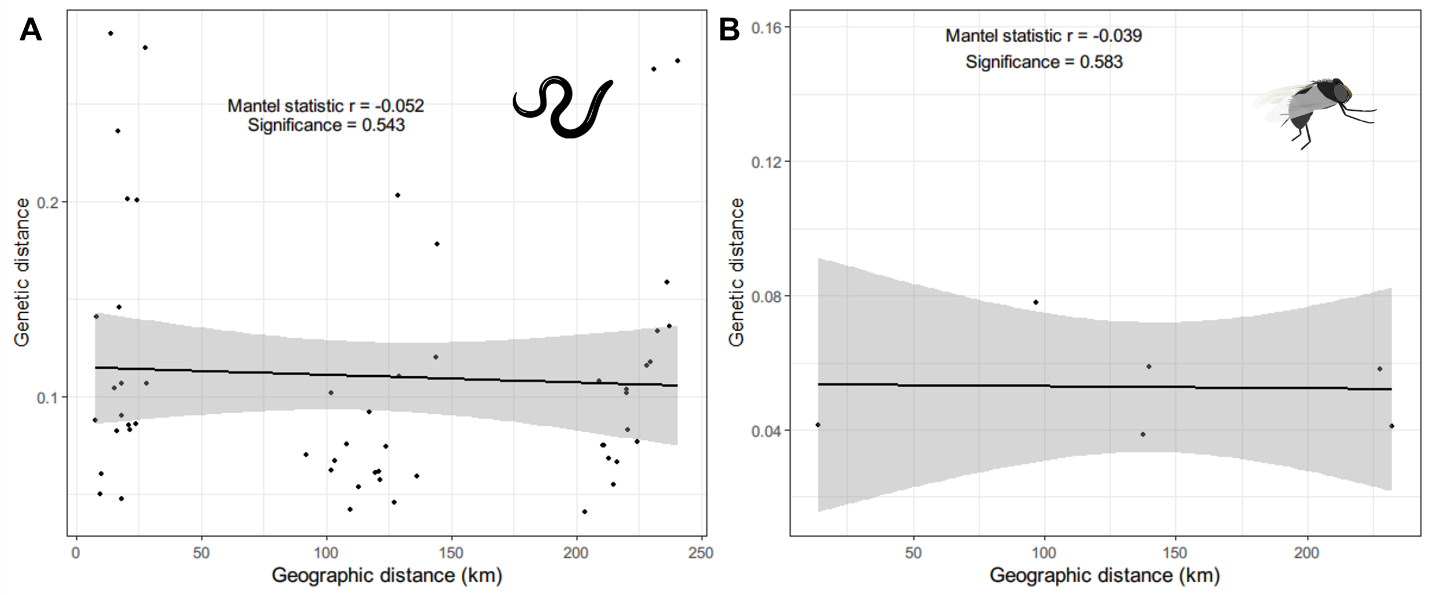


**Figure 2. Discriminant analysis of the principal components (DAPC) analysis for the parasite and the vector sample with respect to sampled 11 and 4 communities respectively in the transition region of Ghana.** The pie chart on the map (**A1, B1**) indicates community level of membership probability. The DAPC analysis showing the community clusters (**A2, B2**) and the individual level membership probability (**A3, B3**) with each block representing communities. The percentage of the samples assigned correctly to their respective communities are shown for both the parasites (**A4**) and the vectors (**B4**). Community codes: BAS: , WTC: ; all other community codes are presented in Table 1.

### Landscape genetic analysis

#### Isolation by distance

Euclidean distance matrix was calculated between the sample locations and linearised pairwise was used as a genetic distance to test the isolation by distance (IBD). The Euclidean geographic distance between locations ranged from 2.2 km to 240.39 km, and for the vectors it ranged from 14 km to 232 km. Note that, for the parasite sampling locations, six communities which were less than 5km apart were merged to two communities. The geographic distance for the parasites averaged 117.73 km (±11.50 SE; range: 7.86–240.43 km), and the genetic distance averaged 0.11 (±0.009 SE; range: 0.041–0.286). Similarly for the vectors, the geographic distance for the parasites averaged 141.40 (±33.61 SE), and the genetic distance averaged 0.056 (±0.007 SE; range: 0.04–0.084). The Mantel test for testing IBD indicated a poor correlation between the genetic distance and the geographic distance for both the parasite (Mantel's r = -0.052; p = 0.543) and the vector data (Mantel's r = -0.039; p = 0.583) (Figure 3).



**Figure 3. The relationship between the genetic (linearised ) and the Euclidean geographic distance**. The isolation by distance was tested by Mantel test and the significance and the strength of relationship is shown.

#### Resistance surface optimisation and testing

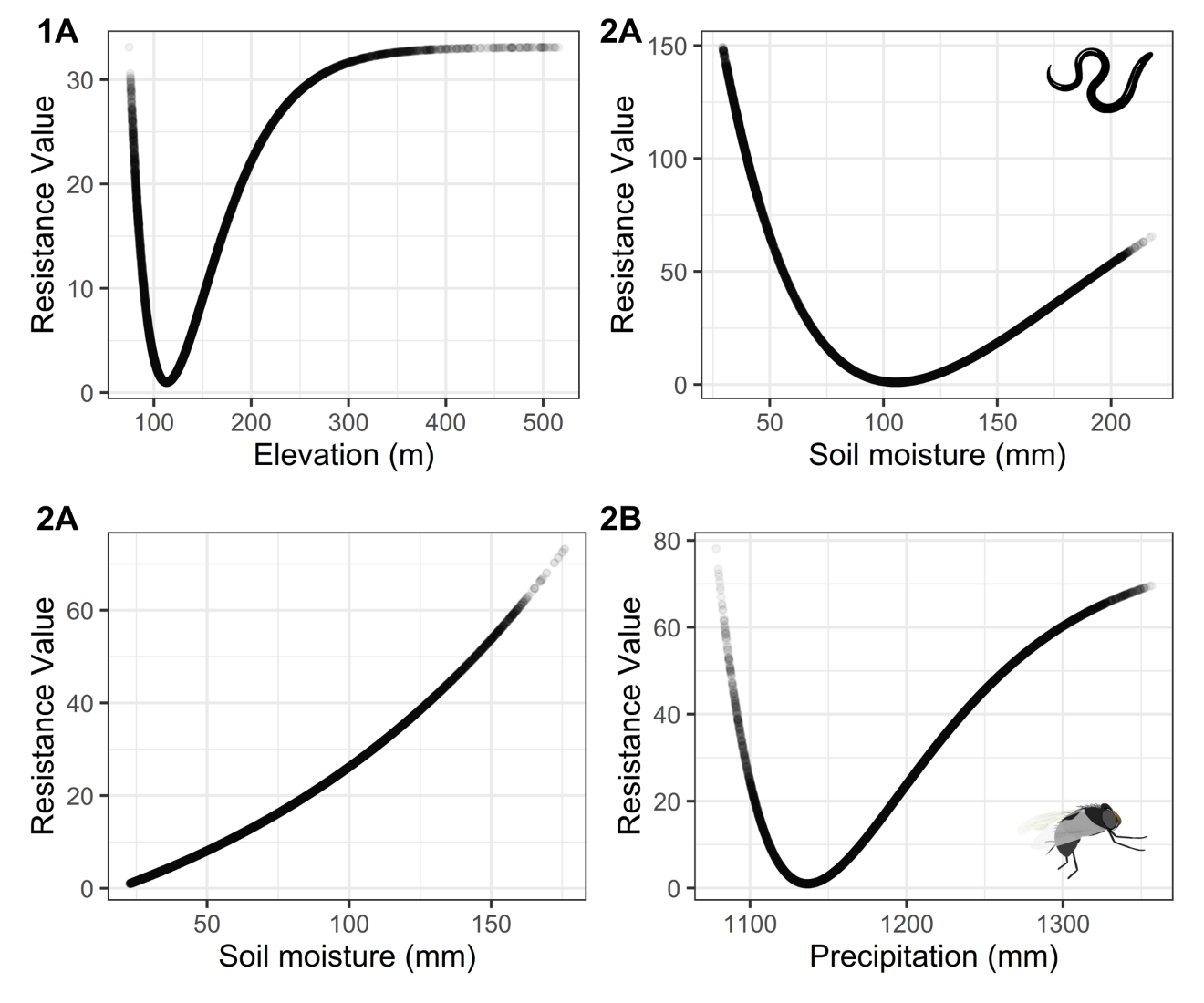
We tested for 5 different environmental surfaces to observe if they can explain the genetic differentiation in the parasite and vector population. We performed four replicates of optimisation for 1000 (default) iterations each and chose the best surface i.e., with the most significance (lowest p-value). For the parasites we found that the inverse ricker transformation for both the elevation (r = 0.793, p = 0.005) and the soil moisture (r = 0.507, p = 0.002) to be significant (Table 2). The inverse reverse monomolecular transformation for elevation and inverse monomolecular transformation for the soil moisture were significant as well but the level of significance was lower compared to the chosen resistance surfaces. Therefore, inverse ricker transformation surfaces for the elevation and soil moisture were used for the preparation of the composite resistance surface map for the parasite data.

In both the environmental layers, inverse ricker transformation was significant i.e., the shape with high resistance in the low and high environmental values, and low resistance in moderate range of environmental values, but the scale parameters were different. The shape was same for both the transformations i.e., there was high resistance for lower values of elevation of soil moisture whereas the resistance was the lowest (<30%) for 90–150 m for elevation and 60–190 mm for the soil moisture (Figure 5). A composite resistance surface map was prepared which showed high resistance around the western parts of transition Ghana which is characterised by areas with very low soil moisture (national parks in the west) and high elevation. The areas around lake Volta were also shown to have high resistance. Similarly, gene flow map suggests that there is relatively less movement/geneflow of the parasites from the northwestern part of the study area (Figure 6). There is a uniform signal of movement around the black volta basin clusters, there are areas in central parts of transition region of Ghana characterised with high gene-flow, showing the potential route for the movement of the parasites.

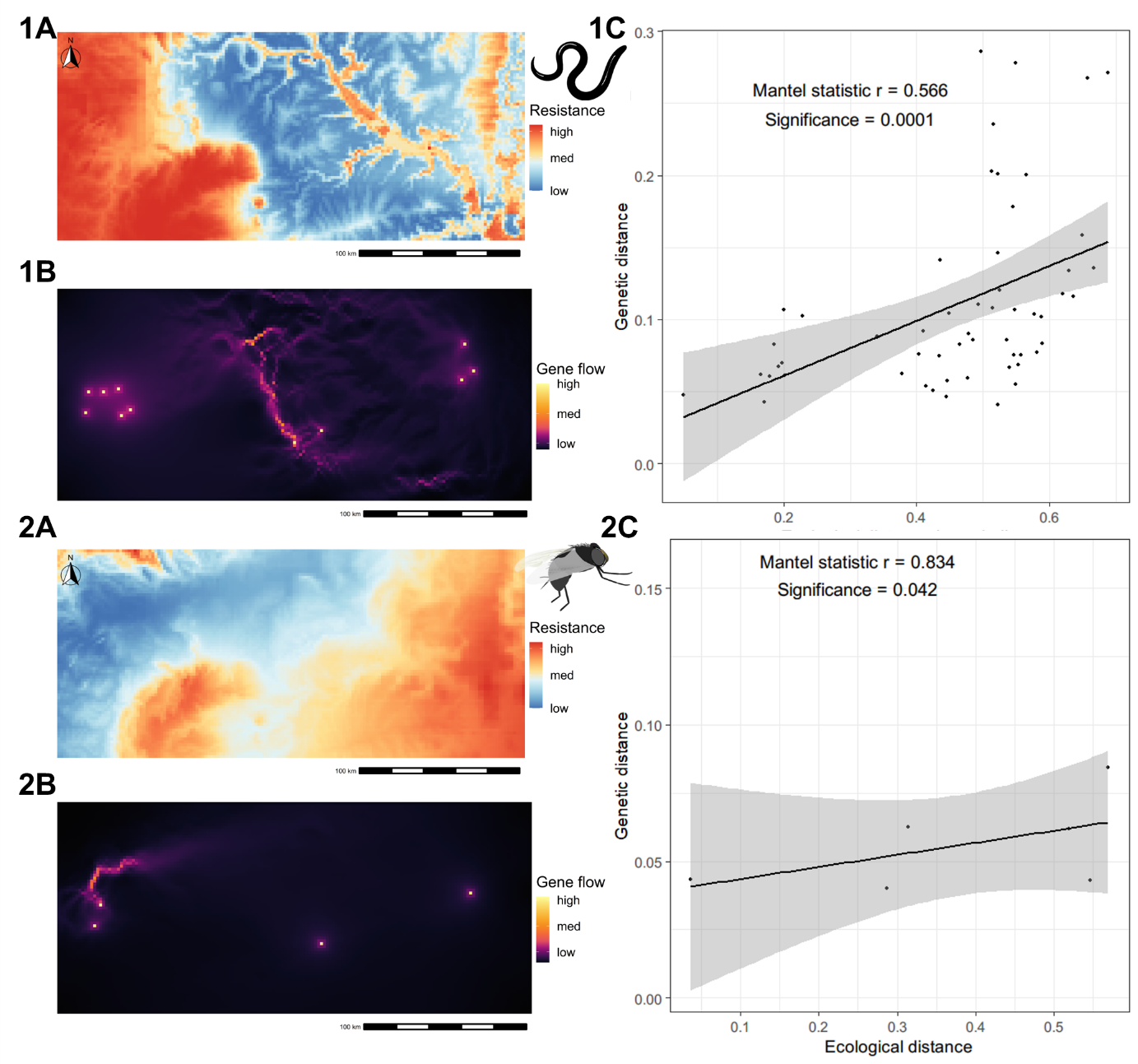
Similarly for the vector genetic data, resistance surfaces obtained from the soil moisture (r = 0.788, p = 0.0417) and precipitation (r = 0.835, p = 0.0417) were significant with inverse reverse monomolecular and inverse ricker transformation respectively. The lowest resistance (<30% of the maximum resistance) for vector gene flow were in the areas with soil moisture 22–90 mm and the precipitation of 110cm–120 cm. These two resistance surfaces were rescaled and merged to create a composite resistance surface like for the parasite data. The composite resistance surface for the vectors revealed that there was particularly low resistance along the western and northwestern areas of the study area and moderate level of resistance in the central areas. The current density map showed high level of geneflow around the communities in the black volta basin.

**Table 2. Transformation of environmental surfaces into resistance surfaces with an optimisation function available in *ResistanceGA*.** The strength and the direction of association of the resistance surface to the genetic distance is tested with the partial Mantel test and Multiple Matrix Regression with Randomisation (MMRR). The bold transformations are the selected resistance surfaces with the asterisks (\*) representing the significance of the coefficients.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Organism** | **Covariates** | # **replicates** | **Optimisation parameter for resistance surfaces** | | | **Genetic distance ~ resistance distance + geographic distance** | | | | | |
| **partial Mantel** | | **MMMR** | | | |
| **Equation** | **Shape** | **Max** | **r** | **p** |  | **p** |  | **p** |
| **Parasites (*O. volvulus*)** | **Elevation** | **2** | **Inverse Ricker** | **0.873** | **100.000** | **0.793** | **0.0002\*\*\*** | **-0.00038** | **0.008\*** | **0.022** | **0.0046\*\*** |
|  | 2 | Inverse-Reverse Monomolecular | 5.046 | 99.996 | 0.745 | 0.0002\*\*\* | -0.00084 | 0.009\* | 0.046 | 0.0074\* |
| Isothermality | 3 | Inverse-Reverse Ricker | 3.439 | 99.996 | 0.391 | 0.0640 | -0.00035 | 0.131 | 0.004 | 0.2242 |
|  | 1 | Ricker | 0.936 | 99.999 | 0.337 | 0.1324 | -0.00029 | 0.140 | 0.007 | 0.2748 |
| **Soil moisture** | **2** | **Inverse Ricker** | **4.031** | **99.997** | **0.507** | **0.0002\*\*\*** | **-0.00017** | **0.264** | **0.002** | **0.022\*** |
|  | 2 | Inverse Monomolecular | 0.500 | 99.922 | 0.489 | 0.0135\* | -0.00004 | 0.742 | 0.003 | 0.022\* |
| Flow accumulation | 4 | Inverse Monomolecular | 0.500 | 99.998 | 0.120 | 0.4380 | -0.00010 | 0.560 | 0.000 | 0.8181 |
| Precipitation | 4 | Inverse Ricker | 5.000 | 99.976 | 0.439 | 0.1155 | -0.00012 | 0.424 | 0.007 | 0.1364 |
| **Vectors (*S. damnosum*)** | Elevation | 3 | Inverse Monomolecular | 0.500 | 99.835 | 0.804 | 0.0833 | -0.00015 | 0.323 | 0.003 | 0.1229 |
|  | 1 | Inverse Ricker | 2.873 | 99.998 | 0.777 | 0.0833 | -0.00017 | 0.284 | 0.002 | 0.1229 |
| Isothermality | 4 | Inverse Ricker | 3.678 | 100.000 | 0.647 | 0.1250 | -0.00009 | 0.453 | 0.004 | 0.2960 |
| **Soil moisture** | **4** | **Inverse-Reverse Monomolecular** | **7.723** | **100.000** | **0.788** | **0.0417\*** | **-0.00016** | **0.202** | **0.002** | **0.042\*** |
| Flow accumulation | 3 | Inverse Ricker | 3.570 | 99.964 | 0.569 | 0.1250 | -0.00019 | 0.250 | 0.001 | 0.2503 |
|  | 1 | Ricker | 0.500 | 100.000 | 0.678 | 0.0833 | -0.00020 | 0.334 | 0.039 | 0.3721 |
| **Precipitation** | **4** | **Inverse Ricker** | **2.096** | **99.984** | **0.835** | **0.0417\*** | **-0.00018** | **0.161** | **0.002** | **0.0418\*** |
| \*: p < 0.05, \*\*: p < 0.005, \*\*\* p < 0.0005 | | | | | | | | | | | |



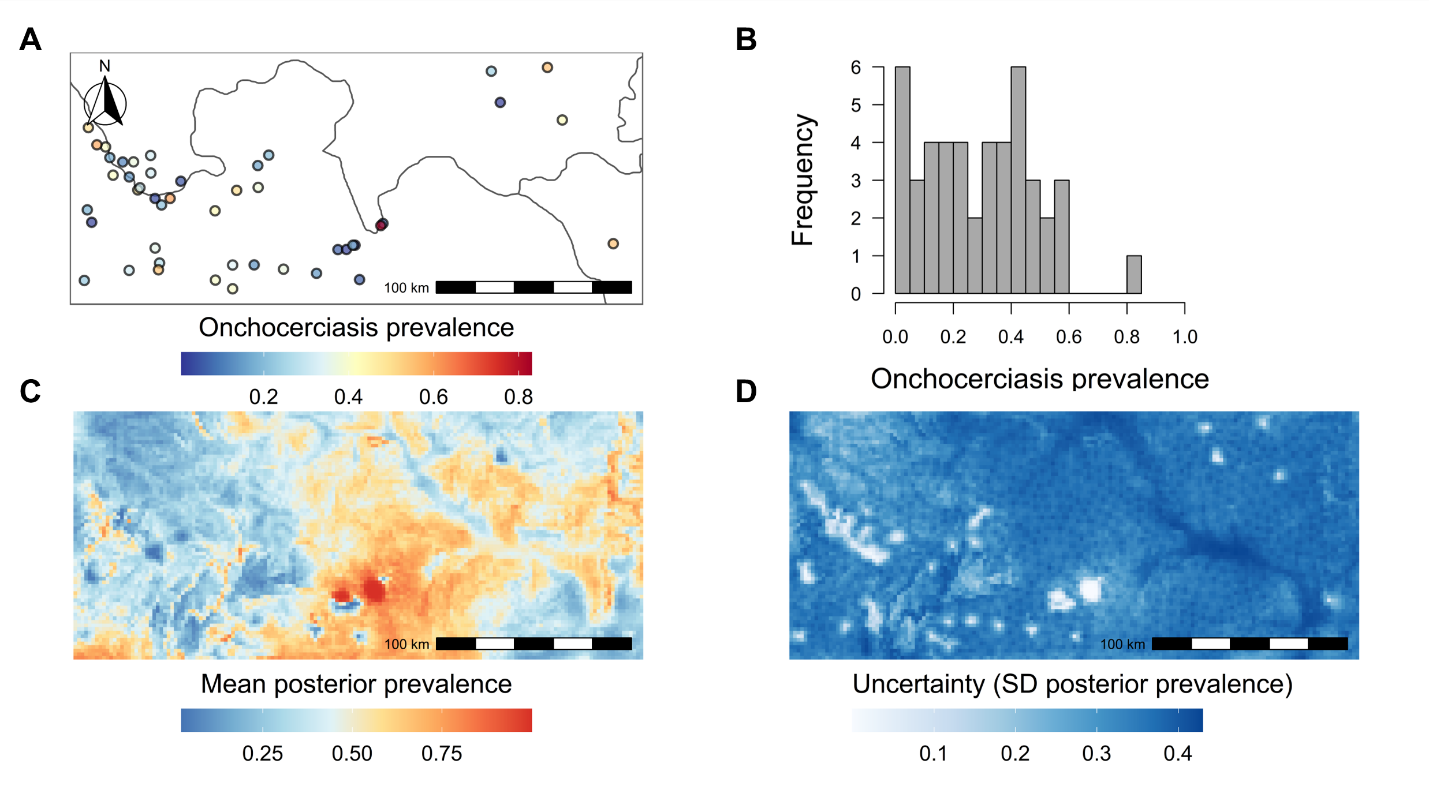
**Figure 5. Transformation functions for the significant environmental covariates.** The figure shows the relationship between the environmental variables with the resistance against gene flow of the *O.* *volvulus* (**1A, 1B**) and *S. damnosum* (**2A, 2B**).



**Figure 6. Composite resistance surface maps prepared from the significant environmental variables along with the gene flow map obtained based on the composite resistance surface map and its relationship with the observed genetic distance.** The resistance surface maps (**1A, 2A**) indicate the ease of movement for the parasite and the vector, and the gene flow map (**1B, 2B**) is obtained based on it with areas highlighted yellow showing the potential routes of movement/gene flow of the organism of interest. The relationship between the circuit distance (cost distance obtained based on the resistance surface) and the genetic distance (**1C, 2C**) is shown.

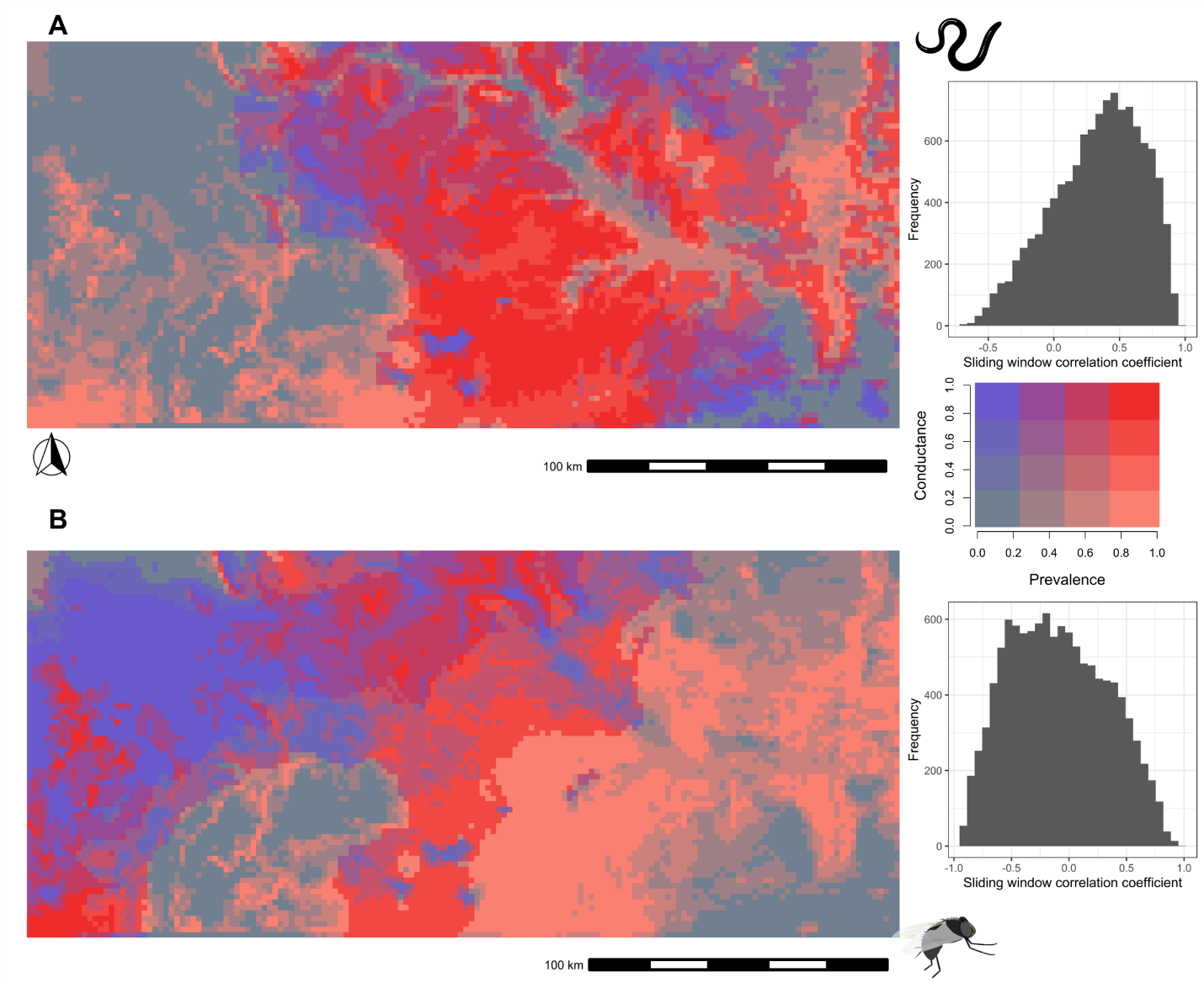
### Prevalence mapping and bivariate maps

Input microfilarial prevalence data ranged from 0.65% to 82.95% and the mean microfilarial prevalence was 29.01% (± 19.31% SD). Most of the data were from the eastern and south-central parts of the study area and there were about five data points from the western parts (Figure 7). The geostatistical interpolated map of baseline microfilarial prevalence based on environmental data shows that the prevalence is higher particularly in the south central Asubende region, the central and eastern areas of the transition Ghana. The prevalence is relatively low in the western areas of transition Ghana with scattered areas of high prevalence in the western parts of transition region of Ghana. The uncertainty map shows that the uncertainty was relatively lower in the sample locations with varying level of uncertainties in the interpolated areas. Based on the regression coefficients, the soil moisture (mean coefficient: 0.043, 95% BCI: 0.004–0.084) and slope (mean coefficient: 2.126, 95% BCI: 0.032–4.338) had a significant positive association with the microfilarial prevalence while the temperature seasonality (mean coefficient: -0.022, 95% BCI: -0.044–-0.001) had a significant negative association with the microfilarial prevalence (Supplementary Table 2). The range of the microfilarial prevalence map was estimated to be 4.4 km (95% BCI: 1.67–7.88 km).



**Figure 7. Mapping baseline prevalence of *O. volvulus* infection in the transition region of Ghana.** Pre-intervention point microfilarial prevalence data () was used to estimate the baseline prevalence of *O. volvulus* infection in the transition region of Ghana. The histogram of the pre-intervention microfilarial prevalence data used in the model and the uncertainty associated with the prevalence map are shown.

The bivariate map for the parasite shows that the area of high parasite conductance and high prevalence are in the central parts of the transition region of Ghana (Figure 8). There is a good correlation between the parasite's composite conductance surface and the *O. volvulus* infection prevalence map with majority (57.34%) of the sliding window correlation coefficients greater than 0.3. Therefore, the areas with high parasite conductance are also the areas of high *O. volvulus* infection prevalence and vice-versa. However, for the vector bivariate map there are quite a substantial portion of areas with high conductance but low prevalence, particularly around the northwestern region of the study area. The correlation between conductance map of vector and the microfilarial infection prevalence is not as strong as the correlation for the parasite counterpart. Only 21.24% of the sliding window correlation coefficients are greater than 0.3. The area of high vector conductance and high prevalence are in the central parts as well as the southwestern parts of the study area. There are areas in north-western parts of the study area, where there is high conductance but low prevalence.



**Figure 8. Bivariate map created using composite conductance surfaces and the onchocerciasis prevalence map.** Top row shows the bivariate map for the parasite (**A**) and the bottom row (**B**) for the vector. The legend for the bivariate map is shown on the right where red color indicates area with high prevalence and high conductance whereas blue color indicates areas with high conductance but low prevalence. The histogram on the right of the respective map shows the frequency of sliding window correlation coefficient between the conductance surface and the prevalence map.

## Discussion

We used a landscape genetics framework to identify the ecological factors influencing the *S. damnosum* and *O. volvulus* population structure and infer potential spatial patterns of the vector and the parasite geneflow and thus, the onchocerciasis transmission. A suite of environmental, climate and socio-demographic variables was considered. We compared the output of landscape genetics, the resistance surface maps, with the baseline microfilarial prevalence map, which could be informative for the control and elimination of onchocerciasis in transition ecological region of Ghana. We sequenced the parasite and vector samples from the onchocerciasis endemic communities and vector breeding sites, respectively from the transition ecological region of Ghana. We did the population genetic analysis for the parasite and the vector samples and compared the population genetics estimates in a spatial context. Population genetic estimates have been discussed by Crawford et al., (2019) and Gyan, (2020), which suggest that both the parasite and the vector population were genetically homogeneous. We used the same sequence data for the parasite samples, whereas the data for the vectors were re-sequenced and re-analysed. Further, we did not observe any isolation by distance (IBD) for both the parasite and the vector populations at the scale of the transition region of Ghana, even though IBD was reported at the country level when comparing the parasite population from Ghana, Mali and Cote de' Ivoire in Crawford et al., (2019). This suggests that the geneflow of the parasite and the vector populations were not restricted by geographic distance in the transition ecological region of Ghana.

Historically, the transition ecological region of Ghana has been considered three river basins viz. Black Volta/Tombe, Pru, and Daka river basins were thought to be independent transmission zones. However, the analysis of the genetic data suggests that the transmission zone in the transition ecological region of Ghana spans across multiple river basins. This might be a case of one big transmission zone where both parasites and the vectors are relatively freely moving from one place to the other and have been alluded to be a Greater Volta river basin, which includes Lake Volta and its' several tributaries (Sam Armoo, *pers. comm.*). This would not be surprising given the ability of vectors to fly in the range of hundreds of km, particularly when assisted by seasonal winds (Baker et al., 1990; Garms et al., 1979; WHO, 2020). However, it is essential to note that despite being geographically near, some locations had high genetic separation, i.e., low genetic connectivity between locations and vice-versa.

With the assumption that environmental factors influence the vector and the parasite geneflow, we looked at different environmental variables that might influence the genetic connectivity using the landscape genetics framework. Using an optimisation algorithm, we created resistance surfaces from selected environmental features and tested if the resistance distance obtained from the corresponding environmental resistance surfaces is associated with the genetic distance. For the parasite population, resistance surfaces obtained from the elevation and soil moisture were significantly associated with the genetic distance. The resistance for the geneflow was low in the areas of elevation around the range of 90–150 m and, similarly, in the areas with soil moisture, 60–190 mm. This roughly corresponds to the reported range of elevation (95–142 m), which was hypothesized to be suitable for onchocerciasis for geospatial modelling study of onchocerciasis in Ghana (Barro & Oyana, 2012). The high resistance for the parasites in low soil moisture areas could be due to the un-arability of the land and, thus, the lack of human hosts. Soil moisture is reported to be an important environmental feature influencing the occurrence of onchocerciasis in several other studies (Cromwell et al., 2021; Shrestha et al., 2022). Similarly, high soil moisture areas might also not be suitable for onchocerciasis as those were around lake Volta with non-flowing water and generally unsuitable for vector breeding. Lake Volta is one of the biggest artificial lakes in the world. The lakes formed by river dams have been reported to affect the vector breeding sites decreasing onchocerciasis transmission (Katabarwa et al., 2020; Post et al., 2013; Zarroug et al., 2019).

Similarly, the resistance surface derived from soil moisture was also significantly associated with vector population genetics. However, the transformation parameter differed from the resistance surface obtained for the parasite population. The resistance was low for areas with low soil moisture 22–90 mm (vs high resistance for the parasite population in areas with low soil moisture), and the resistance increased almost linearly with the increase in soil moisture. This suggests that vector population geneflow occurred with relatively low resistance in areas with low soil moisture, unlike the parasite geneflow. The possible explanation for this could be the absence of human hosts in areas of low soil moisture around the western areas of the transition ecological zone of Ghana. Some of those areas are a part of Bui national park, where there might be black flies but are particularly characterised by sparse human density.

Furthermore, in a study by Doyle et al., (2016) which discriminated the *O. volvulus* and *O. ochengi* larvae from blackflies collected in these regions, the proportion of *O. volvulus* larvae from flies were lower in western communities compared to the communities in the central and the eastern parts of the transition ecological region of Ghana. It was speculated that the spatial difference in the proportion of *O. ochengi* larvae was due to the seasonal increase (in the dry season) in the cattle population in the north-western regions. Therefore, the presence of *O. ochengi* in high proportion in these regions might have impacted the vectorial capacity for the *O. volvulus* as a result of saturation of the vectors with *O. ochengi* (Renz et al., 1982; Wahl et al., 1998), which might be a possible reason for high resistance for the *O. volvulus* populations but a low resistance for the vector populations.

The resistance surface derived from the mean annual precipitation was also significantly associated with the vector population genetic distance. The resistance was low in the areas with mean annual precipitation around 110–120 cm, and the resistance increased as the precipitation increased above 120 cm. While low precipitation decreases the river-flow, an essential feature for the breeding sites of the *S. damnosum*, a hefty rainfall was also reported not to be favourable for *Simulium* breeding in a study done in Ghana (Otabil et al., 2020). In a year-long longitudinal study, Otabil et al. (2020) found that heavy rainfall correlated with the decrease in the relative abundance of *Simulium*. Other studies also report a similar relationship between precipitation and the vector abundance, conducted in Nigeria (Ubachukwu & Anya, 2006) and Sudan (Zarroug et al., 2016). The possible reason behind the unfavorability of the high precipitation to vector abundance is that the heavy downpour might overflow the river banks, sweep away the *Simulium* larvae, and prove to be detrimental to the developmental stages in their lifecycle.

The connectivity analysis using the composite resistance surface maps derived from the significant resistance surfaces allowed us to identify likely geneflow patterns between sites and potential movement routes. The resistance distance obtained based on the connectivity analysis correlated well with the genetic distance. The connectivity map for the parasites showed that the parasite geneflow was high in the central parts of the transition ecological region of Ghana, around communities from the Pru river basin. Similarly, for the vector population, the intensity of the vector geneflow was higher in the western parts around the communities from the Black Volta-Tombe river basin. There were no clear pathways for the vector geneflow, which might be because of the lack of sampling sites. Nevertheless, we can use connectivity analysis as an exploratory tool to identify potential spatial patterns of gene flow of the parasite and vector populations and also hypothesize the source-sink dynamics.

The pre-intervention microfilarial prevalence data analysis showed that environmental features like soil moisture, temperature seasonality and slope were significantly associated with the prevalence in the transition ecological region of Ghana. The prevalence was positively associated with slope and soil moisture. Areas with high slopes usually comprise fast-flowing rivers which are essential for breeding vector populations. Similarly, soil moisture was also identified to be significant in an analysis of the Ethiopian *O. volvulus* nodule prevalence data, where areas with high soil moisture are arable land are usually inhabited by people and are exposed more to vector bites (Adeleke et al., 2010; Opoku, 2006; Shrestha et al., 2022). However, as the landscape genetics analysis suggests, very high soil moisture might also not be favourable for the vectors and the parasites.

Temperature seasonality was negatively associated with prevalence and is an important factor. The areas with relatively stable temperatures in a favourable range throughout the year might be suitable for onchocerciasis transmission. Conversely, the areas with low temperature and more fluctuations in temperature might not be favourable for *Simulium* (Cheke et al., 2015; Renz, 1987). Further, the significant relationship of microfilarial prevalence to the temperature seasonality highlights the potential effect of global warming and alterations in annual temperature patterns on the distribution of onchocerciasis. Finally, it is worth noting that the distance to the community was not significantly associated with the prevalence. This might be because almost all the communities surveyed for prevalence were near to the river (less than 10 km).

We analysed prevalence data because prevalence data are often the basis of MDAi and reflect human parasite population distribution. We used the baseline microfilarial prevalence because population genetic estimates also indicate a population's demographic history (past events). Therefore, comparing demographic history with the baseline prevalence would make more sense. Prevalence mapping revealed that the onchocerciasis prevalence was high, particularly in the central and some areas in the eastern parts of the transition ecological region of Ghana. We also compared the resistance surface for the parasites and the vectors with the microfilarial prevalence map as a bivariate fusion map. Unsurprisingly, areas in the central parts (Pru river basins) had low resistance for both the vector and the parasite populations. Despite being among the first communities targeted for both the vector control initially and MDAi later, the onchocerciasis transmission has persisted for quite a long time in the Pru river basin. The baseline prevalence in these areas was greater than 75% and returned to this level even after vector control (Alley et al., 1994; Lamberton et al., 2015).

We calculated the sliding window correlation coefficient between each resistance surface and the microfilarial prevalence map. As expected, there was a close overlap between the parasite resistance surface map and the microfilarial prevalence map. The concurrence between the parasite resistance surface maps and the microfilarial prevalence map also validates landscape genetics model output. However, the correlation between the vector resistance surface and the microfilarial prevalence map was not as strong as for the parasite resistance surface. The correlation breaks down mainly in the western parts of the transition ecological region, which had high conductance for the parasites but low microfilarial prevalence. This could be due to the combination of reasons mentioned above for contrasting high parasite resistance and low vector resistance in low soil moisture areas viz. lack of human population and the greater proportion of *O. ochengi* limiting the vectorial capacity for *O. volvulus*. Nevertheless, anticipating land-use changes that leads to the availability of human host in these regions might pose a risk of onchocerciasis transmission being established in currently low human population density areas in the western parts of the transition ecological region.

### Implications

For the first time in the context of onchocerciasis, we utilise the landscape genetics framework to incorporate the parasite and the vector genetic data with the environmental data. This approach takes us a step ahead in not necessarily "delineating" but inferring about onchocerciasis transmission zones. Here, we have transformed the metrics of genetic connectivity into a resistance surface and the geneflow map giving insights into transmission zones and the source-sink dynamics. Further, the bivariate fusion map can be used to visualise the areas with low resistance and high prevalence, which might act as residual infection pockets even after continuous interventions. Inferences like these might be vital in making spatially explicit onchocerciasis control decisions. For example, in the current study, we can hypothesize that communities in the Pru river basin are one of the critical connecting areas with low resistance for the parasite and the vector geneflow and high onchocerciasis prevalence. Since this region has a confluence of perfection for parasites and flies, MDAi alone might not be sufficient to eliminate onchocerciasis transmission in these areas. We might have to complement it with vector control interventions (like slash and clear strategy (Smith et al., 2019)).

Eliminating onchocerciasis transmission in the connecting areas might facilitate onchocerciasis elimination in other surrounding areas. However, it is not to say that the other areas might not act as the source of infection, particularly if the infection is well controlled in the Pru basin. When there is a high transmission level in other peripheral communities, there is a high chance of infection being recruited to communities in Pru. Recent modelling work suggests that low endemic areas can act as a source to re-ignite transmission in MDAi-controlled onchocerciasis endemic areas (McCulloch et al., *pers comm,* Vos et al., 2021). Resistance surfaces could be used to prioritise interventions at a larger spatial scale with spatial heterogeneity in interventions. Specifically, areas with low parasite resistance and high prevalence should be prioritised for MDAis, areas with low vector resistance should be prioritised for vector interventions, and the areas with a low resistance to both the parasites and the vector should be prioritised for MDAi complemented with vector interventions. However, for the spatial scale of the current study, where all the communities are well-connected via areas of low resistance, a widespread MDAi needs to be maintained.

The absence of isolation by distance among the vector and parasite populations suggests that the connectivity between the river basins was maintained via ecological features elucidating the possibility of transmission across river basins. With the landscape genetics approach, we show that vectors are far more mobile through the landscape than would be suggested by just looking at breeding sites alone. Therefore, it is fair to say that the river basins, particularly in the context of the transition ecological region of Ghana, might not form the biological basis of the intervention unit. It is not unfair to propose a single and larger Great Volta river basin (Sam Armoo *pers. comm.*). Further, transmission zones or intervention units might not be isolated and static but rather dynamic. This further strengthens the fact that we need to have good MDAi coverage over a large geographical scale for it to be effective.

The first clinical trials of MDAi began in Asubende, a community in the Pru river basin, and unsurprisingly, SOR was reported first here (Awadzi, Attah, et al., 2004; Awadzi, Boakye, et al., 2004; Osei-Atweneboana et al., 2011). As shown by this study and other studies, Asubende is the ecologically favourable area for onchocerciasis, characterised particularly by high biting rates, vector density and vector mobility (Frempong et al., 2016; Lamberton et al., 2015). Therefore, with the reports of SOR in these regions and evidence of transmission from these areas, the possibility of spreading the SOR strains cannot be ignored. One can expect the consequences of SOR to be spread over an extensive geographical range than just the focus within which the MDAi is no longer effective. We have a prevalence source that is not controlled by MDAi, which will result in contamination of the gene pool outside of that focus by the SOR genotype. There is a double penalty, a short-term penalty where some areas act as a source of infection irrespective of SOR and a long-term penalty where the SOR genotype might disseminate more widely.

### Limitations and future directions

Despite the potential of landscape genetic approaches in understanding onchocerciasis transmission, some associated limitations exist. The vector or the parasite mobility inferred from the geneflow might not represent the current processes. However, these are the result of the vector and parasite migration that occurred in the recent past. Therefore, even if this is not definite proof of what is happening right now, this could happen in the future. Similarly, high vector mobility might not necessarily mean high vector density or high vector biting rates. High biting rates are crucial for the high endemicity of the disease, whereas the vector mobility might help maintain or even amplify onchocerciasis endemicity. Here we assume that if the vector has high mobility in the areas of high prevalence, there is a likely possibility of high transmission events. However, incorporating vector abundance data and annual biting rates might further enrich the insights from the approach.

There are some caveats specific to the current study that could be improved in future studies. First, the sampling density and the spatial coverage of the samples could increase the accuracy of the estimated resistance surfaces. The samples in this study were initially collected for the population genetic study. Therefore, future landscape genetic studies should consider dense and stratified uniform sampling across space and environmental gradients (Balkenhol, 2016; Leempoel et al., 2017). Due to the unavailability of the nuclear sequence data, the analysis was done using the mitochondrial sequence data, which lacks recombination and thus might provide a low signal of gene flow (Hedtke et al., 2020). We recommend using nuclear data in future landscape genetics studies. Further, the analysis was done at a single spatial scale. Therefore, different environmental factors might prove to be significant at different spatial scales, either coarser or finer. Thus, the relationship pattern between the environmental variables and their resistance to the gene flow may differ in other regions.

There are seasonal shifts in the species distributions of black flies, which could be challenging to capture with samples from a single time frame. We cannot be sure if the spatial trends hold with respect to time. Therefore, temporal sampling would be more relevant in observing the changes in the resistance surface due to seasonal fluctuation. Temporal data would help us gain insights on changes in resistance surface and thus the transmission zone with respect to time. Further, blackflies could exist as a metapopulation with local extinction and re-colonisation dynamics (Hedtke et al., 2020). Therefore, temporal sampling should occur at a similar period of the year when we can select the same species for analysis. Finally, it is essential to note that high resistance does not necessarily mean habitat unsuitability for the blackflies but rather observed unsuitability for the movement of the blackflies based on the genetic data. Nevertheless, this could be a powerful approach to spatially transforming population genetic connectivity estimates, accounting for ecological variables and gaining insights into transmission zones.

## Conclusion

We have demonstrated that the lack of isolation by distance, i.e., geographic distance failing to explain the genetic distance, in the transition ecological region of Ghana was well elucidated by considering the environmental variables. Both the parasite and vector populations from communities across the river basins in the transition ecological region of Ghana were connected through specific ecological features. We transformed population genetic estimates of the vector and the parasites into a spatial map which gives us insight into transmission zones and source-sink dynamics of onchocerciasis transmission. Environmental variables such as elevation and soil moisture were significantly associated with the parasite gene flow; similarly, the soil moisture and precipitation were significantly associated with the vector gene flow. In addition, the pre-MDAi microfilarial prevalence analysis found that environmental variables such as slope, soil moisture and temperature seasonality were significantly associated with the microfilarial prevalence. The fusion maps of the resistance surfaces and the prevalence map indicated the central Pru basin as the area with low resistance values for both the parasite and the vector populations and high microfilarial prevalence. Therefore, in areas like Pru, which are also characterised by low vector resistance, MDAi alone might not be successful in eliminating transmission and are recommended to be complemented with vector control. Finally, we have used a novel landscape genetics framework for the first time in the context of onchocerciasis to add a spatial dimension to the population genetic estimates and gain insights into onchocerciasis transmission in the transition ecological region of Ghana. This approach could be translatable to any other vector-borne disease and other endemic regions around the world.

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