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Bulinus globosus (Planorbidae; Gastropoda) populations in the Lake Victoria basin and coastal Kenya show extreme nuclear genetic differentiation

Silvester Nyakaana[a,*], J. Russell Stothard[b], Allen Nalugwa[a], Bonnie L. Webster[c], Charles N. Lange[d], Aslak Jørgensen[e], David Rollinson[b], Thomas K. Kristensen[f]

[a] *Makerere University, Institute of Environment and Natural Resources, Molecular Genetics Laboratory, P.O. Box 7298, Kampala, Uganda*
[b] *CITD, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK*
[c] *Wolfson Wellcome Biomedical Laboratories, Biomedical Parasitology Division, Department of Zoology, Natural History Museum, Cromwell Road, London SW7 5BD, UK*
[d] *National Museums of Kenya, P O. Box 40658 – 00100, Nairobi, Kenya*
[e] *Laboratory of Molecular Systematics, The Natural History Museum of Denmark, University of Copenhagen, Sølvgade 83, 1307 Copenhagen K, Denmark*
[f] *The Mandahl-Barth Research Centre for Biodiversity and Health, DBL-Parasitology, Health and Development, Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 57, DK-1871 Frederiksberg C, Denmark*

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ABSTRACT

Bulinus globosus, a key intermediate host for *Schistosoma haematobium* that causes urinary schistosomiasis, is a hermaphroditic freshwater Planorbid snail species that inhabits patchy and transient water bodies prone to large seasonal variations in water availability. Although capable of self-fertilizing, this species has been reported to be preferentially out crossing. In this study, we characterized the population genetic structure of 19 *B. globosus* populations sampled across the Lake Victoria basin and coastal Kenya using four polymorphic microsatellite loci. Population genetic structure was characterized and quantified using F_{ST} statistics and Bayesian clustering algorithms. The four loci used in this study contained sufficient statistical power to detect low levels of population genetic differentiation and were highly polymorphic with the number of alleles per locus across populations ranging from 16 to 22. Average observed and expected heterozygosities across loci in each population ranged from 0.13 to 0.69 and from 0.39 to 0.79, respectively. Twenty-five of the seventy-six possible population-locus comparisons significantly deviated from Hardy–Weinberg equilibrium proportions after Bonferroni corrections, mostly due to the deficiency of heterozygotes. Significant genetic differentiation was observed between populations and Bayesian inferences identified 15 genetic clusters. The excess homozygosity, significant inbreeding and population genetic differentiation observed in *B. globosus* populations are likely to be due to the habitat patchiness, mating system and the proneness to cyclic extinction and recolonization in transient habitats.

1. Introduction

The freshwater self-fertile hermaphroditic snail species, *Bulinus globosus* (Jarne et al., 1991) is an intermediate host of the human parasite *Schistosoma haematobium* that is responsible for urinary schistosomiasis in sub-Saharan Africa (Webbe, 1981; Brown, 1994). It is estimated that more than 207 million people are infected with schistosomiasis worldwide and that 85% of these cases are in Sub-Saharan Africa (WHO, 2010).

B. globosus occupies a wide variety of often temporary and patchily distributed freshwater habitats (Brown, 1994). Patchiness of the habitat distribution and the availability of freshwater in time and space (Brown, 1980) have been identified to greatly influence the patterns of genetic variation exhibited by this host snail species (Jarne and Delay, 1991). Recurrent cycles of desiccation of the temporary water pools during the dry season followed by flooding during the wet season expose these snail populations to repeated extinction and recolonization events which accentuate genetic drift and inbreeding leading to reduced within-population variation and increased between-population differentiation (Slatkin, 1985a; Wade and McCauley, 1988). Therefore, a detailed understanding of the spatial distribution of genetic variation of *B. globosus* in relation to the habitat requirements and reproductive biology is crucial in understanding the ecology and epidemiology of urinary schistosomiasis thus leading to its enhanced and targeted control.

In accordance with the reproductive assurance hypothesis which postulates that selection favors self-fertilization in circumstances where potential mates are scarce or absent (Darwin, 1877,

* Corresponding author at: Makerere University, Institute of Environment and Natural Resources, Molecular Biology Laboratory, P.O. Box 7298, Kampala, Uganda. Tel.: +256 392 947428/702 848789; fax: +256 414530134.
E-mail addresses: snyakaana@muienr.mak.ac.ug, snyakaana@botany.mak.ac.ug, snyakaana@gmail.com (S. Nyakaana).

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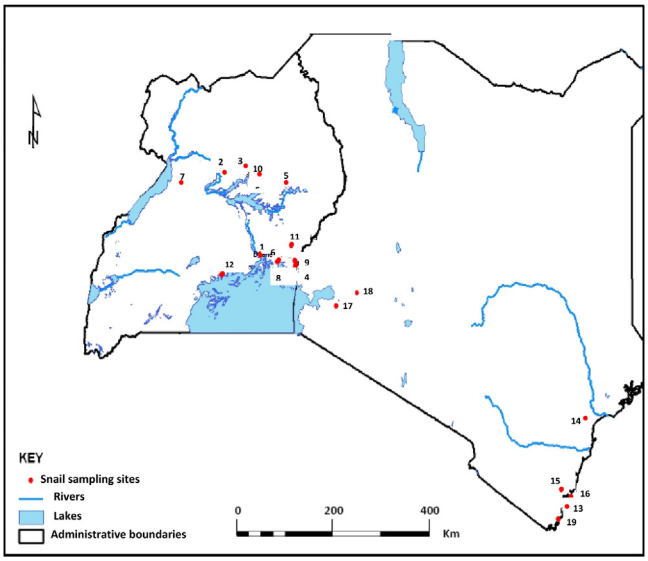

Fig. 1. Map showing *Bulinus globosus* sampling localities in Uganda and Kenya for the period 2006–2009. The numbering of the sampled localities is as given in Table 1.

1878), the selfing mode of reproduction is of major ecological significance in hermaphroditic self-fertilizing snail species in spite of the gene transmission advantage associated with out-crossing. The recurrent temporal desiccation and flooding of *B. globosus* habitats inevitably exposes this species to recurrent bottlenecks (Brown, 1994) and consequently evolution of self-fertilization in this species has enabled it to become a superior colonizer of mate-scarce environments (Brown, 1980).

With the advent of the polymerase chain reaction (PCR) in the mid-1980s (Saiki et al., 1985, 1988) and the recent successful isolation and characterization of new neutral and highly polymorphic genetic markers such as microsatellites in most species, these new genetic tools have increasingly proved to be extremely robust in detecting within and between-population genetic variation in a number of species. In this study, four polymorphic microsatellite loci of *B. globosus* previously isolated and characterized by Emery et al. (2003) have been used to investigate population structure of *B. globosus* populations sampled from Kenya and Uganda fresh water bodies.

2. Materials and methods

2.1. Study sites

One hundred and eighty samples were collected across 19 localities in spatially distributed in Uganda and Kenya as indicated in Fig. 1. The habitats ranged from freshwater lake shores, ponds, rivers, irrigation schemes, temporary water bodies, ditches and roadside water pools (Table 1) and were not connected in any way by any intervening waterways. However, this does not preclude potential gene flow between populations as birds and other animals have been reported to transport snails and/or their eggs over land between water bodies that are not hydrologically connected (Boag, 1986; Green and Figuerola, 2005). Taxonomic identification of the snails to species level based on shell morphological characteristics was achieved using standard field identification keys (Brown, 1994; DBL-WHO, 1998; Kristensen, 1987). Snails were then preserved and stored in 70% alcohol for prospective DNA extraction and genetic characterization using molecular techniques.

The small number of samples collected from the different localities is attributable to patchiness and relatively desiccated state of the habitats as much of the sampling was conducted during the dry season.

2.2. DNA extraction, amplification and genotyping

Genomic DNA was extracted from the foot tissue of each sample using the DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, USA) in accordance with the manufacturer's protocol. Four microsatellite loci (BgZ2, BgZ3, BgZ4 and BgZ5) out of the six loci previously isolated from *B. globosus* (Emery et al., 2003) were optimized and used to amplify samples from the 19 populations in order to quantify the partitioning of genetic variation within and between the different populations. Loci BgZ1 and BgZ6 were not used as they could not be optimized for most samples collected from localities in Uganda.

2.3. Genetic diversity analysis

Genetic diversity indices such as number of alleles per locus per population (A), observed heterozygosity (H_O) and expected heterozygosity (H_E) corrected for small sample size (Nei, 1987) (computed using GENEPOP version 4) (Rousset, 2008) were used to quantify intra-population diversity at each locus. Fisher's exact tests (Raymond and Rousset, 1995) as implemented in GENEPOP version 4.0 (Rousset, 2008) were used to test for conformity to Hardy–Weinberg equilibrium genotypic proportions and pair-wise linkage disequilibria between loci and the significance of the associated P-values was adjusted by applying the sequential Bonferroni correction (Rice, 1989) across all populations and all loci to correct for type 1 error due to multiple comparisons. Wright's inbreeding coefficient F_{IS}, was calculated following the method of Weir and Cockerham (1984) as implemented in Arlequin version 3.1 (Excoffier et al., 2005).

2.4. Population structure analysis

ARLEQUIN software version 3.1 (Excoffier et al., 2005) was used to quantify the extent of inter-population genetic differentiation and extent of inbreeding using Wright's F_{ST} statistics based on the infinite alleles model, IAM (Weir and Cockerham, 1984).

Simulations were conducted on the dataset using the software POWSIM (Ryman and Palm, 2006) employing various combinations of N_e and t (where N_e is the effective population size and t is the time since divergence, respectively) to determine whether the microsatellite data of the four loci used contained sufficient statistical power to detect low levels of population genetic differentiation. We assessed both the type I error (α) (the probability of rejecting H_O when it is true) and the type II error (β), which is the probability of rejecting (H_O: genetic homogeneity) when it is false. POWSIM estimates the power of the genetic loci using information on sample size, number of samples, number of loci, and allele frequencies for any hypothetical degree of true differentiation quantified as F_{ST} (Ryman and Palm, 2006). The significance of the tests was assessed with Fisher's exact tests.

The freeNA software was used to calculate the null allele frequency at each locus in each population (Chapuis and Estoup, 2007). The same software was also used to calculate the global F_{ST} values at each locus with and without the excluding-null-allele (ENA) correction method. The significance of the difference between the two categories of F_{ST} values was assessed using a t-test.

STRUCTURE 2.3.4 (Pritchard et al., 2000) was used to enumerate the potential number of populations within our samples. STRUCTURE uses a Bayesian clustering approach to assign individuals to K populations, where the posterior probabilities of observing the data given alternative values of K can be estimated with or without prior assumptions based on geographic sampling or species membership (Pritchard et al., 2000). Structure divides sampled individuals into