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Population genetics of the *Schistosoma* snail host *Bulinus truncatus* in Egypt

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ABSTRACT

The tropical freshwater snail *Bulinus truncatus* serves as an important intermediate host of several human and cattle *Schistosoma* species in many African regions. Despite some ecological and malacological studies, there is no information on the genetic diversity of *B. truncatus* in Egypt. Here, we sampled 70–100 snails in ten localities in Upper Egypt and the Nile Delta. Per locality, we sequenced 10 snails at a partial fragment of the cytochrome c oxidase subunit 1 gene (*cox*1) and we genotyped 25–30 snails at six microsatellite markers. A total of nine mitochondrial haplotypes were detected, of which five were unique to the Nile Delta and three were unique to Upper Egypt, indicating that snail populations may have evolved independently in both regions. Bayesian clustering and hierarchical *F*-statistics using microsatellite markers further revealed strong population genetic structure at the level of locality. Observed heterozygosity was much lower compared to what is expected under random mating, which could be explained by high selfing rates, population size reductions and to a lesser extent by the Wahlund effect. Despite these observations, we found signatures of gene flow and cross-fertilization, even between snails from the Nile Delta and Upper Egypt, indicating that *B. truncatus* can travel across large distances in Egypt. These observations could have serious consequences for disease epidemiology, as it means that infected snails from one region could rapidly and unexpectedly spark a new epidemic in another distant region. This could be one of the factors explaining the rebound of human *Schistosoma* infections in the Nile Delta, despite decades of sustained schistosomiasis control.

1. Introduction

The human parasite *Schistosoma haematobium* is a blood fluke that is responsible for about 112 million infections in Africa and the Arabian Peninsula, causing a chronic and debilitating disease known as urogenital schistosomiasis (Gryseels et al., 2006). While the adult worms reproduce sexually within the human blood vessels, their offspring cycle obligatorily through an intermediate snail host each generation by means of clonal reproduction (Rollinson and Simpson, 1987). The geographic distribution and transmission of these parasites are therefore strongly linked to the geographic distribution and population dynamics of their intermediate snail host. Ecological changes following the construction of water barrages have led to more suitable biotic and abiotic snail environments, resulting in the increase or invasion of urinary schistosomiasis in Cameroon, Cote d'Ivoire, Ghana, Mali, Namibia, Senegal and Sudan (Chitsulo et al., 2000).

Schistosoma haematobium is transmitted by several snails of the genus *Bulinus*, which are freshwater hermaphrodite planorbids. The genus comprises 37 recognized *Bulinus* species that have an extensive distribution throughout Africa, the East African islands and some areas in the Middle East and the Mediterranean (Brown, 1994). Several of these *Bulinus* species transmit schistosomes, of which *B. truncatus* is one of the most important intermediate host of various *Schistosoma* species of human and cattle (Rollinson and Simpson, 1987; Brown 1994). Tropical freshwater snails such as *B. truncatus* inhabit various kinds of transient habitats such as irrigation systems, rivers and ponds where water availability (volume and surface) varies greatly across seasons (Brown, 1994; Jarne, 1995). Temporal and/or geographical variations in ecological conditions cause large fluctuations in snail population sizes, possibly resulting in population extinctions and recolonization. In addition, *B. truncatus* are true hermaphrodites that preferentially self-fertilize (selfing), but with possibility for cross-fertilization (outcross-

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ing) when a male counterpart is present (Jarne and Charlesworth, 1993). A remarkable feature of *Bulinus* is the capacity for strong and rapid population increase, which is related to the ability of self- and cross-fertilization, in response to environmental stimuli (e.g. temperature, rainfall, etc.). Population bottlenecks and selfing have a strong impact on the population demography and will therefore change the genetic composition of snail populations (Viard et al., 1996). Both are expected to reduce the amount of genetic diversity within a population, while increasing genetic differentiation among them. *Bulinus truncatus* is therefore an interesting species to study the consequences of mating and bottlenecks on population genetic structure.

In the present study we studied the (distribution of) genetic diversity of *B. truncatus* populations in Egypt. Six microsatellites markers and one mitochondrial DNA marker (a 490 bp fragment of the cytochrome c oxidase subunit 1 gene – *cox*1) were used to genetically characterize *B. truncatus* snails from ten localities in the Nile Delta and Upper Egypt. This is the first genetic study on *B. truncatus* in Egypt, and the first study that investigates the population genetics of *B. truncatus* using both mitochondrial and nuclear markers.

2. Material and methods

2.1. Sample collection and DNA extraction

Bulinus truncatus snails were collected in ten sites along the Nile River (Egypt) during the beginning of the dry season (June-July) in 2012. Sampling sites were selected based on previous evidence of extensive human water contact, high human infection rates and snail abundance. Eight sites were situated in Lower Egypt that contains the Delta of the Nile River, while two sites were located in Upper Egypt (Fig. 1; Table 1). The direct distance between the sites ranged from 3.9 km between Al Kanisa and Al Masaken to 568 km between El Beheira and Qena.

Bulinus truncatus snails were initially identified based on shell morphology using the field identification key of Kristensen (Kristensen and Christensen, 1989), but see also the recent review of Lofty and Lofty (Lotfy and Lotfy, 2015). The samples were collected without chronometry from their habitats by two researchers and subsequently conserved in 70% ethanol. Therefore, we do not have specific data on the infection status of the snails, as they were not subjected to shedding. Per site 70–100 *B. truncatus* snails were successfully sampled. Of these, 25–30 snails were randomly selected per site for microsatellite genotyping (see below). Of these, 10 snails were randomly selected per site for mitochondrial DNA sequencing (see below). Note that we did not keep track which 10 snails exactly, out of the 25–30 genotyped snails, were sequenced. Hence, we cannot link a single *cox1* sequence with a single microsatellite multilocus genotype. Genomic DNA was extracted from snails using the QiAamp DNA Mini Kit (Qiagen®, France) following the manufacturer's instructions. A Nanodrop ND-1000 spectrophotometer was used to quantify DNA and check DNA purity.

2.2. Mitochondrial DNA sequencing

A fragment of the cytochrome c oxidase subunit 1 gene (*cox*1) was amplified using primers HCO2198 and LCO490 (Folmer et al., 1994). A 25 µL PCR mixture was set up with 0.5 U Taq polymerase (Qiagen), 5 µL 10 x buffer, 1–2 µL $MgCl_2$ (depending on template and primers), 0.8 µL dNTPs, and 0.5 µL from each primer (20 ng/µL). A standard DNA concentration of 500 ng/µL was used as template for all PCR reactions. Cycling conditions started with a preheat step at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 10 s, annealing at 45 °C for 30 s and extension at 72 °C for 1 min, and finished with a final extension step at 72 °C for 10 min. PCR products were purified using QIAquick PCR Purification Kit (Qiagen®, France), sequenced in both directions (forward and reverse) using the BigDye v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems) and read using the automated DNA fragment analyzer ABI-377 (Applied Biosystems).

2.3. Microsatellite genotyping

Bulinus truncatus samples were furthermore genotyped using six previously published microsatellite primers (Bt1, Bt4, Bt6, Bt12, Bt13 and Bt23) (Jarne et al., 1994). The forward primers were labeled with 6-FAM and NED to allow multiplexing (Table 2). PCR reactions were carried out using the Qiagen Multiplex PCR Kit (Qiagen®, France) in 25 µL volumes including 0.5 µL (20 ng/µL) of each primer, 12.5 µL of Multiplex PCR Master Mix (providing a final concentration of 3 mM MgCl2, 3 × 0.85 mL). A standard concentration of 500 ng/µL was used as template for all the PCR reactions. Cycling conditions were the following: a preheat step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing that decreased from 59 °C to 49 °C (2 °C/5 cycles) for 30 s and extension at 72 °C for 45 s, and a final extension step at 72 °C for 10 min. PCR products were analyzed using an ABI Prism 310 Genetic Analyser and allele sizes were manually verified using GENEMAPPER v4.0 (Applied Biosystems).

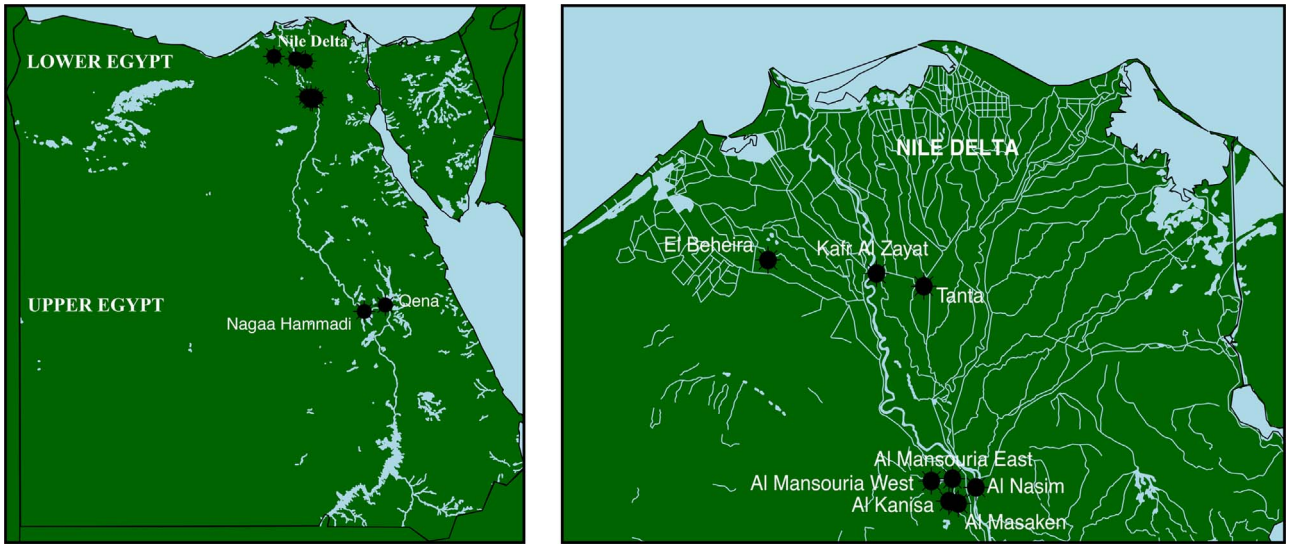

Fig. 1. Map of Egypt showing the ten sampling localities of *Bulinus truncatus*. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)