Overground vs underground, a genetic insight into dispersal and abundance of the Cape dune mole-rat

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Abstract
Molecular methods are commonly used to investigate cryptic populations that are difficult to locate or observe directly. The population dynamics of many subterranean organisms have been overlooked, at least in part, due to the absence of appropriate molecular markers. Recent studies in African mole-rats have raised questions about the modes of dispersal and mate acquisition. Here we apply a suite of 25 microsatellite markers to test the overground/underground dispersal hypotheses. Using these data we also apply an approach to estimate population size and look for signal of demographic expansion or contraction. The genetic data suggest that the same breeding population extends between locations (~50km), with elevated inbreeding coefficients suggestive of some degree of isolation of the urban location. Low genetic differentiation between study sites supports the proposed high levels of vagility of dispersing individuals overground. We find a signal of long-term population decline of *B. suillus* in this region. Their adherence to mesic conditions potentially recommends *B. suillus* of utility in monitoring the proposed climate-induced desiccation of the Western Cape. Of potential interest is the discovery of a second divergent population at the rural location, with microsatellite data suggesting contemporary reproductive isolation and a mitochondrial divergence putatively dated at around 0.3 million years.

Keywords: African mole-rats, Bathyergidae, *Bathyergus suillus*, population genetics, population size

Introduction

The high energetic cost of burrow extension and strict adherence to particular soil and moisture conditions has long been considered to limit the potential for movement and range expansion in fossorial organisms (e.g. Bennett & Faulkes, 2000). Contrary to this, an increasing body of evidence suggests much higher vagility of subterranean mammals such as the enigmatic mole-rats (Bray et al. 2012; Patzenhauerova et al. 2010). These examples describe above-ground movement for both mate acquisition and presumed dispersal despite poor morphological adaptations to such actions (Nemec et al. 2007). While low vagility systems are characterised by highly localised population structure between occupied patches of habitat (Gaines, 1980), rapid long-distance (i.e. above-ground) dispersal is expected to result in low genetic differentiation within a single Hardy-Weinberg population spread over a wide area. Species that naturally occupy patchy landscapes are often characterised by high vagility, and examples exist of those that have successfully prospered or colonised even heavily urbanised areas (Angold et al. 2006). Those species with lower dispersal potential may be more restricted by interruptions of suitable habitat, more dependent on linkage corridors, and sensitive to barriers such as roads (Marsh et al. 2008; Wang et al. 2008). Urbanisation is expected to present many barriers to subterranean mammal dispersal,

resulting in fragmented and isolated populations in comparison with an open rural landscape (Dickman & Doncaster, 1989).

The Cape dune mole-rat *Bathyergus suillus* in particular has a distribution limited to lowland areas (<300m) (De Graaf, 1981), overlapping some of the highest density human population and farming areas across the South African southern coastline. Being the largest subterranean rodent (Jarvis and Bennett 1991; Kotze et al. 2006), *B. suillus* is considered a pest species due to the damage caused to vehicles and buildings by extensive burrow systems undermining foundations, roads, and railway lines (Skinner & Chimimba, 2005). Despite being commonly found in both rural (Thomas et al. 2009) and urban locations in the Western Cape (Hart et al. 2007) little is known about the abundance or dispersal dynamics of the Cape dune mole-rat. Burrow systems can extend up to 100m, similar to the average spacing distance between systems (Thomas et al. 2009). Understanding the dynamics of one subterranean organism may also provide insights into the other more vulnerable species sharing similar ecological requirements. Although still considered abundant, historical information implies far greater population sizes; one account suggests the need to travel by rhino trails as overland movement was so treacherous due to the profusion of burrows (Bennett & Faulkes, 2000).

Molecular markers circumvent the obvious difficulties encountered with monitoring subterranean populations. Here we assess the dispersal ability of a fossorial mammal by determining the genetic population structure across 1km and 50km. This sampling structure allows for the assessment of long-distance dispersal ability, as well as the effect of urbanisation as a possible barrier to dispersal. Patterns of genetic diversity and differentiation between two locations as well as demographic modelling are employed for the first time in this species. Specifically we ask: (i) Is there evidence to suggest that *B. suillus* is capable of dispersing long-distances (overground hypothesis), or do we see highly structured spatial patterns of genetic variation consistent with subterranean dispersal (underground hypothesis)? (ii) What does demographic modelling tell us about abundance and can we discern any signal of population size trajectory in this species?

Methods

Sampling

Tissue samples were collected from 20 individuals at each of two sites in an urban location (Cape town Airport; 33˚58'S, 18˚37'E) separated by ~1km, and 18 individuals were sampled at a rural location ~50km away (Darling; 33° 22'S, 18° 23'E: Figure 1). Sampling involved the use of multiple traps placed in tunnels in areas of mole-rat activity. Although the rural location was sampled from within 1km in an area with no known discontinuities, two distinct genetic groups emerged comprising 9 individuals. Two additional samples available on GenBank were included from the range extremes of Rondawel and Stilbaai (cytochrome *b* data only; Faulkes et al. 2004). The collection and processing of all biological material used in this study was approved by the University of Cape Town Animal Ethics Committee (AEC#:2003/V&/JOR) and University of Pretoria ethics committee (AUCC#:040702/015). Muscle tissue samples were taken and stored in ethanol (See Hart et al. 2006 for details).

Genotyping/sequencing

Genotypes were generated from 25 microsatellite markers developed for *B. suillus* and related species; BS01-BS08 (Bray et al. 2011) CH1, CH3, DM1, DM5, DM7 (Burland et al. 2001) Gcap02, Bsuil06, Bsuil04, Chott03, Bsuil02, Gcap07, Bsuil01, Chott05, Cmech (03, 04, 09), Gcap10, (Ingram, unpublished thesis). These markers were applied in four multiplexes using the Qiagen Multiplex kit with standard conditions (45 cycles, 60˚C annealing temperature). A proportion of individuals were retyped (~15%) to confirm scoring. Partial cytochrome b (cyt*b*) fragments were generated for 28 individuals (15 urban, 13 rural) using universal vertebrate primers (T. Hoareau, unpublished; 40 cycles, 60˚C annealing temperature).

Genetic variation and population structuring

Microsatellite heterozygosity scores and linkage disequilibrium for all locus pairs were calculated in GENETIX4.05 (Belkhir et al. 2004), as well as population-level summary statistics. All loci were tested for conformation to Hardy Weinberg Equilibrium and for the

presence of null alleles using GENEPOPv4.0.10 (Rousset, 2008). To uncover cryptic population structure, the Bayesian model-based clustering algorithm STRUCTURE (Pritcherd et al. 2000) was applied. This approach groups individuals into K homogenous clusters (populations). The appropriate K value was chosen through the Evanno et al. (2005) selection approach, using the mode of the ΔK distribution. Values of K were set to vary from 1 to 5, running 20 simulations from different starting points. Burn-ins were set at 10^5 followed by 10^5 steps for data collection. Average and standard deviation in log estimated likelihood [L(K)] was calculated for each K (admixture model, correlated allele frequencies). To test population isolation between the locations, and sub-regions thereof, gene identity was estimated using ESTIM 1.0 (Vitalis & Couvet, 2001). The rate of loss of variation in finite populations is dependent on population size, with coalescence times scaling inversely with effective population size. The parameter F is an average value for per-locus gene identities and was used as a proxy for within-population genetic drift. In this way this approach provides a simultaneous estimate of effective population size and migration rate. Cytochrome *b* sequence data was cleaned and aligned in BIOEDIT (Hall 2005) and diversity measures calculated using MEGA5 (Tamura et al. 2005).

Demographic modelling

A Bayesian approach for detecting and quantifying demographic expansion or contraction was applied to the microsatellite data in MSVAR 1.3 (Storz & Beaumont 2002). Contemporary (N_1) and ancestral (N_0) population sizes and the time since the start of the demographic event are quantified under an exponential model of population size change. Run priors were log-normal and set to vary in all combinations of 4 and 5 for the parameters N_1 and N_0 with standard deviations of 2. Each of the eight runs was run for $1x10^7$ iterations with a thinning interval of $1x10^4$. The first 10% of each run was removed to avoid any bias of initial conditions before being analysed using in R (Ihaka & Gentleman, 1996). Initially each chain was checked by eye for convergence before comparison between chains using the gelman statistic (<1.2). Median estimates were calculated from a concatenated dataset containing the outputs of all runs. The 95% confidence in posterior distributions were calculated between the 2.5% and 97.5% intervals. Generation time for the analysis was calculated using T=(*a*+*b)*/2 (Pianka, 1978), *a* being age at maturation, *b* being longevity. Maturation and longevity were estimated at 1 and 5 years (based on age class data (Hart et al. 2007), and an estimate of longevity of 3.2 years for a smaller solitary mole-rat species (Puzachenko, 1996)). A second approach, this time for the detection of recent population size changes (either bottleneck or growth; Cornuet & Luikart 1999) was also applied to the microsatellite data. This analysis compared 10^4simulated H_E values against the observed dataset using the Wilcoxon's signed rank test. Under the recommendation of Peery *et al*. (2012) that multistep mutations are generally underestimated, we applied only the Two-Phase mutational model under the following proportions of multisteps; 0.25, 0.35, and 0.45.

Phylogenetics and Phylogeography

Time since separation between the different mitochondrial lineages (excluding Rondawel and Stilbaai) was calculated from the cyt*b* data through the BEAST application (Drummond & Rambaut, 2007). The nucleotide substitution model was determined through JMODELTEST (Posada, 2008) according to the AIC. The nucleotide substitution model with the lowest AIC for the cytB sequences was TIM2 + G, therefore we used this model in our phylogenetic analyses. Both species models (Yule; Birth-death) were tested in addition to all options for the clock models, and the tree with the highest likelihood was chosen (run length $1x10^9$ iterations, sampling every $1x10^5$). The final model run was the relaxed exponential clock under the birth-death speciation model with the silvery mole-rat (*Heliophobius argenteocinereas*) and a cane-rat (*Thryonomys swinderianus*) as outgroups. Convergence was confirmed through comparison of four chains ($1x10^7$ iterations, sampling every $1x10^3$, minus 10% burnin) in Tracer (Rambaut & Drummond, 2007) using uniform priors. The molecular clock was calibrated using the fossil-dated common ancestor of the two older nodes; that of *Heliophobius* and *Bathyergus* (19.5 million years ago (mya); SD 0.5; Lavocat, 1973), and that of *Thryonomys* and *Bathyergus* (34 million years ago (mya); SD 0.5; Lavocat, 1973). A minimum spanning network of the cytb haplotypes was generated using TCS (Clement et al. 2000).

Results

Genetic variation and population structuring

No significant signal of linkage disequilibrium was detected between the 25 microsatellite loci, estimates of null allele frequencies were below the recommended limit (0.2; Dakin & Avise 2004: Table S1), removal of loci with higher estimates was not seen to affect the analysis so all loci were included. Genotyping error was calculated to be ~0.01 incorrect alleles/genotype, missing data information is available in Table S1. Two distinct genetic clusters were determined within the data according to STRUCTURE (Figure 1), half of the rural individuals

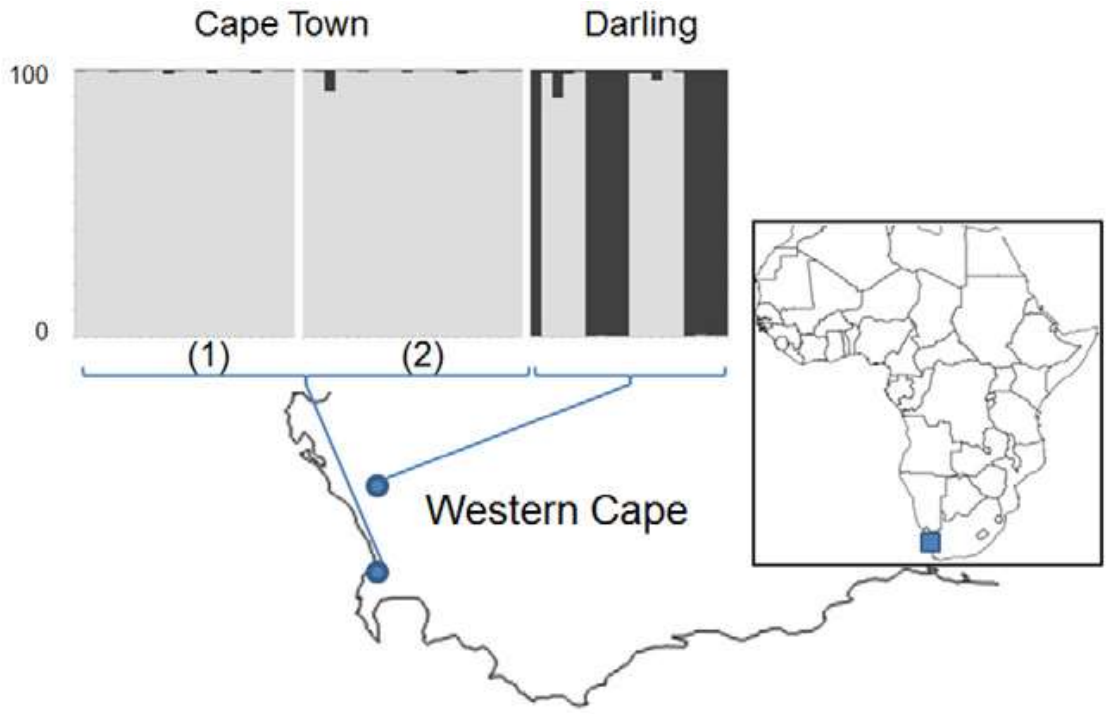

Figure 1 Bayesian assignment of individuals into genetic clusters based on microsatellite markers for Cape Town sites 1 and 2, and the rural location in Darling.

(n=9) formed a distinct genetic cluster. A low but significant F_{ST} was found between the two sub-regions within the urban location (F_{ST}=0.02; p=0.002). Both urban and rural groups showed high levels of genetic variation, the combined urban group displayed higher F_{IS} and gene identity measures (F) than the combined rural (Table 1). A total of 705 base pairs of

Table 1 Expected (H_E) and non-biased (H_{NB}) heterozygosity, mean number of alleles per locus (A), F_{IS}, average one-locus identity probabilities (F), and F_{ST}.

Locations	**n**	**H_E**	**H_{NB}**	**A**	**F_{IS}**	**F**	**F_{ST}**
Urban 1	20	0.54	0.56	5.2	0.11	0.11	
Urban 2	20	0.51	0.53	5.3	0.06	0.16	U1-U2=0.02
Urban combined	40	0.54	0.55	6.4	0.1	0.18	
Rural 1	9	0.51	0.54	3.9	-0.07	0.15	
Rural 2	9	0.64	0.68	4.9	-0.007	-0.07	R1-R2=0.17
Rural combined	18	0.65	0.67	6.4	0.06	-0.07	U-R=0.09

sequence data were generated for cytb and added to those already available (GenBank accession numbers; KC153980-KC153987 and AY425911, AY425912 respectively). Nucleotide diversity was 0.005 with 11 parsimony informative sites within *B. suillus*.

Demographic modelling

The Storz and Beaumont (2002) method was used to identify any signal of historical population expansion or decline and to give an estimate of the magnitude and timing of this event. Due to the strong population structuring at the rural location, analysis concentrated on the combined urban individuals (see Chikhi et al. 2010). Current effective population size was estimated to have a median of 1600 (95% HPD: 300-7300). This approach identified a population decline (Figure S1), with an ancestral population size of 12000 (HPD: 3700-45000). The start of this decline was estimated to be a median of 2500 years ago (HPD: 250-58000). For comparative purposes, an approach for specifically investigating more recent population expansion or decline was also applied (Cornuet & Luikart, 1999); No significant signal of either a recent population bottleneck or expansion was detected for any of the three mutation models (data not shown).

Phylogeography

A single weakly supported divergence was seen from the consensus Bayesian Inference phylogeny of *B. suillus* cyt*b*, corresponding with those individuals separated by the microsatellite analyses (Figure 2a). According to the molecular dating approach the estimate of the age of this split was at least 0.56 million years old (median = 3.23; 95% CI: 0.56-7.84). The cyt*b* haplotypes corresponding with the Darling-specific genetic cluster for the microsatellite data are grouped with the northern haplotype in Rondawel (Figure 2b).

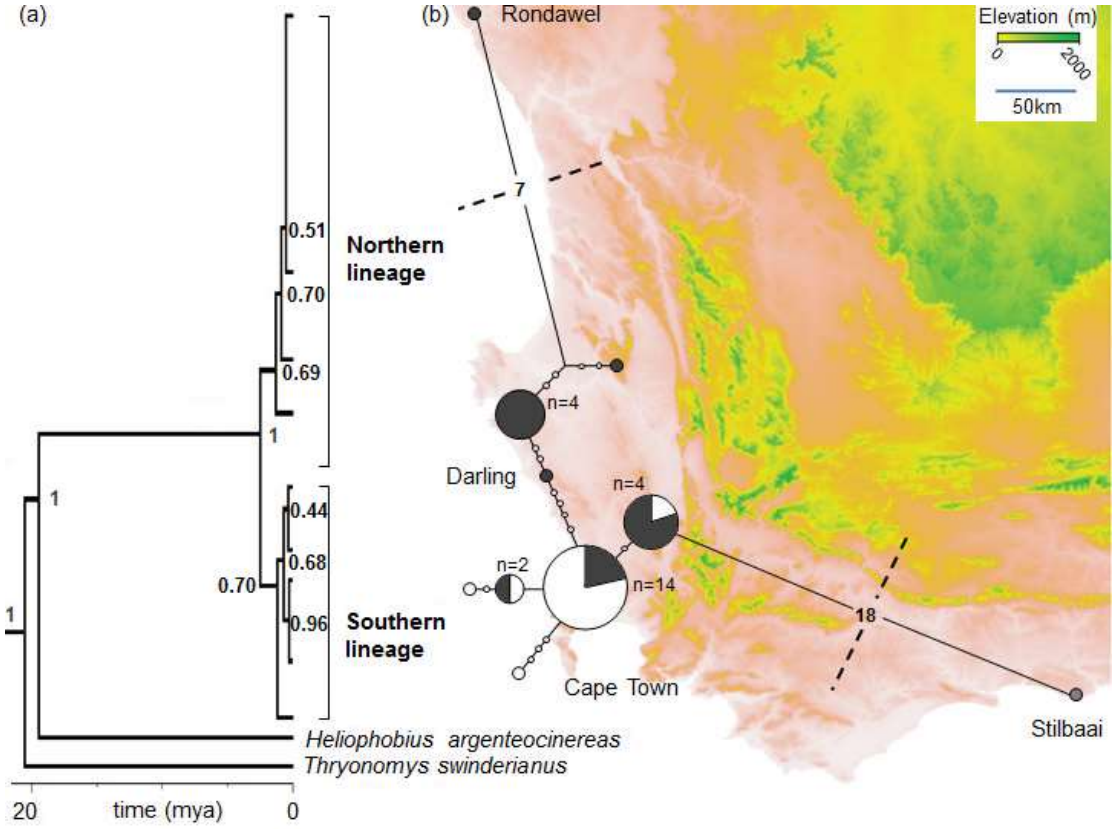

Figure 2 (a) Consensus Bayesian Inference cytb phylogeny for *B. suillus* using the silvery mole-rat (*Heliophobius argenteocinereas*) and a cane-rat (*Thryonomys swinderianus*) as outgroups. Support values given at each node. (b) Maximum parsimony haplotype network for cytb in *B. suillus (n=28)*. Cape Town and Darling haplotypes shown in white and grey respectively.

Discussion

Partitioning of genetic diversity; urban isolation?

Mole-rats have been shown to exhibit poor adaptation to above-ground movement (Nemec et al. 2007) and exhibit low dispersal ability (e.g. Rado et al. 1992). The classical description of subterranean mammals suggests that most disperse up to a few hundred metres, followed by

a sedentary lifestyle with only minor boundary changes to their territories (Nevo 1979). Even species where initial dispersal is overground, like the European mole, have only been noted to progress 2-3km/year (Haeck 1968). The consequence of short dispersal distances (corresponding with belowground dispersal; Rado et al. 1992) are seen in spalacid populations where distinct genetic structure and diversity of chromosome numbers can be seen spatially (Wahrman et al. 1969). Consistent with the expectations of the overground hypothesis, the *B. suillus* sampled here showed the same genetic cluster at both locations 50km apart, demonstrated by microsatellite and mitochondrial data. with microsatellite variation being comparably high in both. Despite this obvious landscape connectivity and high variation at both locations (microsatellites; >6 alleles/locus; H_E = >0.5; Table 1), dispersal into the urban location shows evidence of being restricted as seen in the identity measures, and arguably also in the F_{IS} values (Table 1). Whilst isolation is influenced by dispersal barriers, population density is also important to gene-flow across the landscape, which is also likely to be lower in urban locations.

Population size and trends

Figures for abundance are scarce in mole-rat literature, those that exist are often not a true representation of wild conditions (e.g. 1000/km^2, *Cryptomys hottentotus*; Genelly 1965). An averaged estimate for spalacids in Israel suggest densities of ~100/km^2 (Nevo et al. 1982). Estimates based on the Storz and Beaumont (2002) method suggest an effective population in the Cape Town region to be not more than 7,300 breeding females. A rough figure for the extent of this area is in the region of ~2000km^2 (approximate area of lowland extending between Cape Town and Darling). Crucially, the total area actually inhabited by this species is unknown, and to even approach the density seen in *Spalax* would need to be as low as ~70km^2. What is clear is that this is markedly less than the ancestral population size suggesting that *B. suillus* has undergone a historical long-term decline in this area. Mole-rats adhere to specific soil types, and have been shown to be sensitive to levels of precipitation (Davies & Jarvis, 1986). If the decline estimated here is linked to historical aridification (there have been four major events in the last 12,000 years; Stokes et al. 1997), we might expect to see an increase in rate of decline in view of accelerated aridification in near-future climate model predictions (Thomas 2004). Although it is interesting to note that until now there is no evidence of strong recent decline, as might be expected as a reaction to either increased human settlement, or recent climatic perturbations. As a common species *B. suillus* might be appropriate to monitor these effects, as competition for space increases and climate change accelerates.

Regional population break

Some confusion in comparing the two locations is apparent due to the presence of an additional genetic cluster at the rural location. Although likely to be artificially elevated through low sample sizes, the magnitude of the sub-structuring in the rural location was unexpectedly high (F_{ST}= 0.17). The same nine rural animals clearly represent a diverse population in both the nuclear and mitochondrial markers (Fig 1&2), although it must be reiterated that the mitochondrial divergence was only weakly supported. Gene identity measures also single out this divergent rural cluster as being far more outbred with an F value of -0.07 relative to those of 0.11-0.16 for the two urban and remaining rural clusters. These latter F values are high and suggestive of population isolation, comparable to that seen in breeds of domestic cattle kept at low numbers (Bray et al. 2009). That the divergent rural genetic cluster shows low gene identity implies a large and outbred source population. The high membership (>99%) to this rural genetic cluster, with little gene flow into other individuals suggests that there is some kind of barrier to genetic mixing. No clear physical barrier is evident and similar ecological conditions exist throughout the rural sampling area (*Lolium perennae* and *Trifolium repens* ground cover with a largely sandy loam). The genetic variation within the rural site is striking given the high genetic similarity between the remaining rural individuals and those from 50km away in the urban location. The estimated intra-lineage separation time (based on cyt*b*) is comparable to the time for speciation in other rodent taxa; per lineage diversification rates in *Rattus* have been estimated at up to 3.0 per million years (Rowe et al. 2007). *Bathyergus suillus* has been regularly reported in the fossil record in the Western Cape for the last ~0.3 million years (Mathews et al. 2005). Morphological descriptions can belie molecular evolution, with isolation and/or adaptation being responsible for the divergence of these lineages

throughout this period. It remains an intriguing question as to which was the driving influence in this case.

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Supplementary information;

Figure S1 Evidence of population size change in *B. suillus* from microsatellite-based estimates of contemporary (NO) and ancestral (N1) population sizes (prior distributions shown as dashed lines; as determined through MSVAR 1.3.

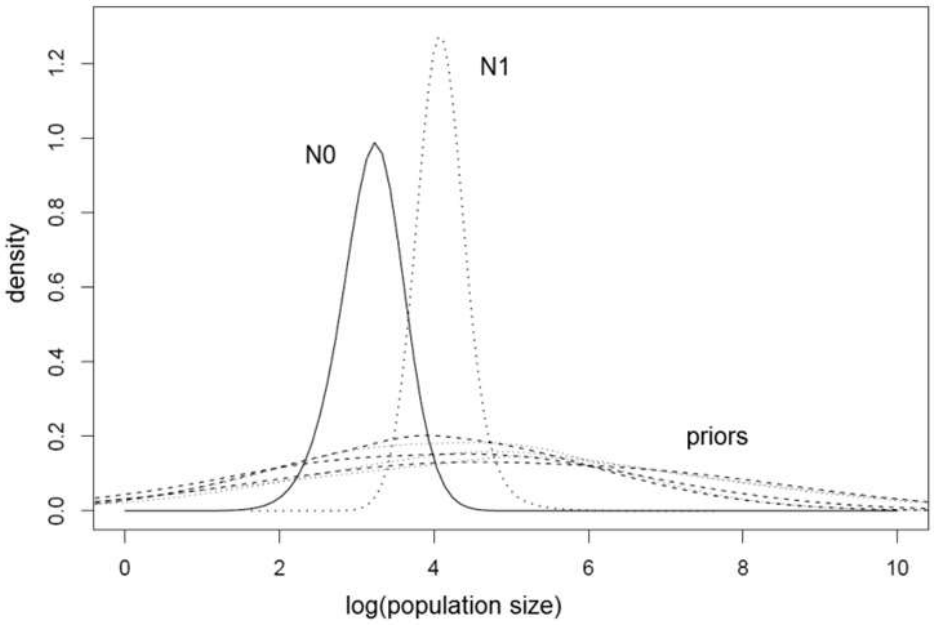

Table S1 Locus summary information; Number of alleles (A_{max}), estimated proportion of null alleles (Null), F_{IS} (Probability of significant deviation from Hardy-Weinberg equilibrium; P<0.05=*), and percentage missing of the total 116 alleles/locus (% miss).

Locus	A_{max}	Null	F_{IS}	% miss
BS02	3	0	0.14	4
BS03	3	0	-0.04	0
BS04	2	0	-0.21	0
BS05	5	0	0.44*	0
BS06	5	0.19	-0.14	5
BS08	3	0	0.47	2
A39	8	0	0.25	0
Gcap02	7	0	0.00	8
Bsuil06	18	0.11	0.03	0
Bsuil04	14	0.04	-0.05	2
Chott03	9	0.05	-0.11	0
CH1	11	0.04	0.00	28
Bsuil02	6	0	-0.21	0
A5	8	0.04	0.12*	0
Bsuil01	10	0	0.17	0
Gcap07	10	0	0.19	2
D7	7	0.05	0.06	1
D5	11	0.19	0.23	0
dChott05	8	0	0.03*	13
Ch3	7	0.04	-0.21	3
Cmech03	9	0	0.31*	2
Gcap10	10	0.04	-0.06	15