Ecology, 88(12), 2007, pp. 3032–3043

EXTINCTION, RECOLONIZATION, AND DISPERSAL THROUGH TIME IN A PLANKTONIC CRUSTACEAN

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Abstract. Dormant propagule banks are important reservoirs of biological and genetic diversity of local communities and populations and provide buffering mechanisms against extinction. Although dormant stages of various plant and animal species are known to remain viable for decades and even centuries, little is known about the effective influence of recolonization from such old sources on the genetic continuity of intermittent populations under natural conditions. Using recent and old dormant eggs recovered from a dated lake sediment core in Kenya, we traced the genetic composition of a local population of the planktonic crustacean *Daphnia barbata* through a sequence of extinction and recolonization events. This was combined with a phylogeographic and population-genetic survey of regional populations. Four successive populations, fully separated in time, inhabited Lake Naivasha from ca. 1330 to 1570 AD, from ca. 1610 to 1720 AD, from ca. 1840 to 1940 AD, and from 1995 to the present (2001 AD). Our results strongly indicate genetic continuity between the 1840–1940 and 1995–2001 populations, which are separated in time by at least 50 years, and close genetic relatedness of them both to the 1330–1580 population. A software tool (Colonize) was developed to find the most likely source population of the refounded 1995–2001 population and to test the number of colonists involved in the recolonization event. The results confirmed that the 1995–2001 population most probably developed out of a limited number of surviving local dormant eggs from the previous population, rather than out of individuals from regional (central and southern Kenya) or more distant (Ethiopia, Zimbabwe) populations that may have immigrated to Lake Naivasha through passive dispersal. These results emphasize the importance of prolonged dormancy for the natural long-term dynamics of crustacean zooplankton in fluctuating environments and suggest an important role of old local dormant egg banks in aquatic habitat restoration.

Key words: ancient DNA; Colonize software; Daphnia; *dispersal; egg bank; extinction; habitat restoration; paleogenetics; recolonization; seed bank; storage effect.*

INTRODUCTION

Dormancy, or the occurrence of hypometabolism at certain stages of the life cycle, is a ubiquitous strategy used by organisms to bridge unfavorable periods (Brendonck and De Meester 2003). The formation of so-called resistant stages or propagules is found in a wide variety of life forms, including animals such as crustaceans, rotifers, insects, nematodes, flatworms, sponges, mollusks (reviewed in Williams 1998), flowering plants (reviewed in Thompson et al. 1996), algae (Worm et al. 2001), bacteria (Madigan et al. 2005), and fungi (Aimé and Miller 2002). Because these dormant propagules (seeds, spores, or eggs) can remain viable for several decades or even centuries (McGraw et al. 1991, Hairston et al. 1995, Thompson et al. 1996, Cáceres 1998), they can theoretically hatch and recolonize a local habitat long after the active population has become extinct due to temporary unsuitability of ecological conditions. As a result, such reservoirs or banks of dormant propagules can have important ecological and evolutionary consequences for contemporary communities. First, dormant egg banks provide a buffer against local extinction and can increase local diversity through the storage effect (Chesson 1983, 1994, Kalisz and McPeek 1993). They can increase the rate of evolutionary change by providing a wide genetic spectrum, from multiple overlapping generations, on which selection can act (Hedrick 1995). Alternatively they can also slow down the rate of evolution, e.g., when dormant eggs from populations adapted to a different selective environment continue to hatch and dilute the genetic composition of the modern population (Hairston and De Stasio 1988). More generally, egg banks offer unique opportunities for ecological and evolutionary research, as they allow reconstruction of past selection pressures and population genetic changes through time (reviewed in De Meester et al. 2007).

The ecological and evolutionary impact of a dormant egg or seed bank critically depends upon the extent to which they actually contribute to contemporary populations and communities. Although laboratory experi-

Manuscript received 11 September 2006; revised 23 January 2007; accepted 21 March 2007; final version received 13 April 2007. Corresponding Editor: K. L. Cottingham.

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ments have shown that propagules of many species can remain viable for up to several centuries (Hairston et al. 1995, Thompson et al. 1996), little is known about the relative genetic contribution of such very old local propagules vs. that of propagules immigrating from contemporary populations (i.e., on the relative importance of dispersal in time and dispersal in space). To some extent, a few case studies have addressed the importance of local recruitment for population reestablishment after extinction (e.g., Pake and Venable 1996, Faustova et al. 2004, Binks et al. 2005) or that of dispersal from nearby lakes (e.g., Pollard et al. 2003). However, considering that efforts to restore disturbed habitats and ecosystems to their natural condition often follow upon several decades of unfavorable habitat conditions for indigenous species, we need to find out over which time scale local recruitment is effectively possible. This is especially true for aquatic ecosystems in lakes and ponds, where old dormant propagule banks become buried deeper and deeper under accumulating sediments that hamper hatching cues. More fundamentally, knowing the time scale over which local propagule banks are relevant to long-term population dynamics is important for insight in the genetic continuity and effective generation time of populations, in metapopulation dynamics, and in the maintenance of local and regional genetic variation over time.

Members of the planktonic crustacean genus *Daphnia* (Crustacea: Anomopoda) are large (1–5 mm) invertebrate grazers, mainly of phytoplankton. They have the capacity to produce dormant eggs encapsulated by tough chitinous egg cases (ephippia), which eventually sink to the lake bottom and are resistant to physical and chemical deterioration. Accumulating year after year in successive sediment layers, these banks of dormant eggs provide an historical archive of past population dynamics, also long after they are no longer viable. In Lake Naivasha, a characteristically fluctuating tropical freshwater ecosystem (Verschuren et al. 2000*b*) and one of Kenya's largest lakes, four species of *Daphnia* occur today (Mergeay et al. 2006*a*). The record of fossil ephippia in dated sediment cores, however, indicates that populations of at least eight *Daphnia* species have occurred in Lake Naivasha within the past century (Mergeay et al. 2004). One species, *Daphnia barbata* Weltner, suddenly reappeared around 1995 after an absence of more than 50 years. In the present study we use paleogenetic methods on recent and old dormant eggs to investigate whether this modern active population was founded from old but still viable dormant eggs in the local egg bank or, alternatively, followed immigration from other, regional or more distant populations. Taking advantage of the detailed late-Holocene paleolimnological record of Lake Naivasha (Verschuren 2001), we used mitochondrial DNA sequencing and variation at nuclear DNA microsatellites markers to reconstruct the population-genetic dynamics of *D. barbata* in Lake Naivasha through time. We then compared the genetic composition of the modern *D. barbata* population of Lake Naivasha with that of successively older local populations (i.e., extracted from increasingly older time horizons in the sediment archive) and with that of modern regional (Kenya) and more distant populations (Ethiopia, Zimbabwe).

Methods

Lake Naivasha is a large ($\sim$135 km^2) and currently shallow (maximum depth [z_{max}] $\approx$ 5 m; excluding Crescent Island Crater) freshwater lake located at 1885 m above sea level just south of the Equator in Kenya's Eastern Rift Valley (Fig. 1). Crescent Island Crater (CIC) is a relatively small (1.9 km^2) and deep (15.2 m in 2001) paraboloid depression of volcanic origin, submerged in the eastern portion of Lake Naivasha and works as a sediment trap for the much shallower northeastern sector of the lake (Fig. 1c; Verschuren 1999). In contrast to the main basin of Lake Naivasha, CIC never stood dry during the past 1800 years (Verschuren 2001). As a result of the very high sediment input (on average 1 cm/yr since 1900 AD; Fig. 2) and absence of wind-driven resuspension on its deep lake floor, this small and steep-walled crater basin has accumulated a continuous and undisturbed high-resolution sediment archive of climate and environmental change (Verschuren et al. 2000*a*).

Sediment coring and processing

A short core (NC01-1S: 0–152 cm depth, covering the period ca. 1812–2001 AD) and a long core (NC01-D: 88–764 cm depth, ca. 240–1920 AD), both retrieved from near the deepest point of CIC in July–August 2001 (Fig. 1c), were used to construct a continuous record of the local population history of *D. barbata* over the last 700 years (Fig. 2). The 6.6-cm diameter (NC01-1S) or 5-cm diameter (NC01-D) cores were sectioned in 2-cm increments, after removing the outermost 2 mm to avoid contamination of adjacent increments by mud smeared along the core tube wall. The 240 sediment slices were sieved through 150-μm mesh, and the retained organic residue was scanned at 30× magnification for the presence of *Daphnia* ephippia. Ephippia of *D. barbata* were identified using diagnostic characters first documented through study of ephippial females in modern Kenyan populations (Mergeay et al. 2005), then counted and stored in 100% ethanol for genetic analysis. To confirm that lack of fossil *D. barbata* ephippia in certain core intervals reflected true absence of this species from the contemporary local zooplankton community (i.e., to check against the possibility that a substantial population of *D. barbata* may have existed in Lake Naivasha that did not produce ephippia), we searched subsamples from six selected sediment depths lacking *D. barbata* ephippia (28–30 cm, ca. 1990 AD; 46–48 cm, ca. 1977 AD; 136–138 cm, ca. 1827 AD; 160–162 cm, ca. 1785 AD; 190–192 cm, ca. 1746 AD; 280–282 cm, ca. 1624 AD) for the presence of *Daphnia* postabdominal claws. For this analysis, 1 mL

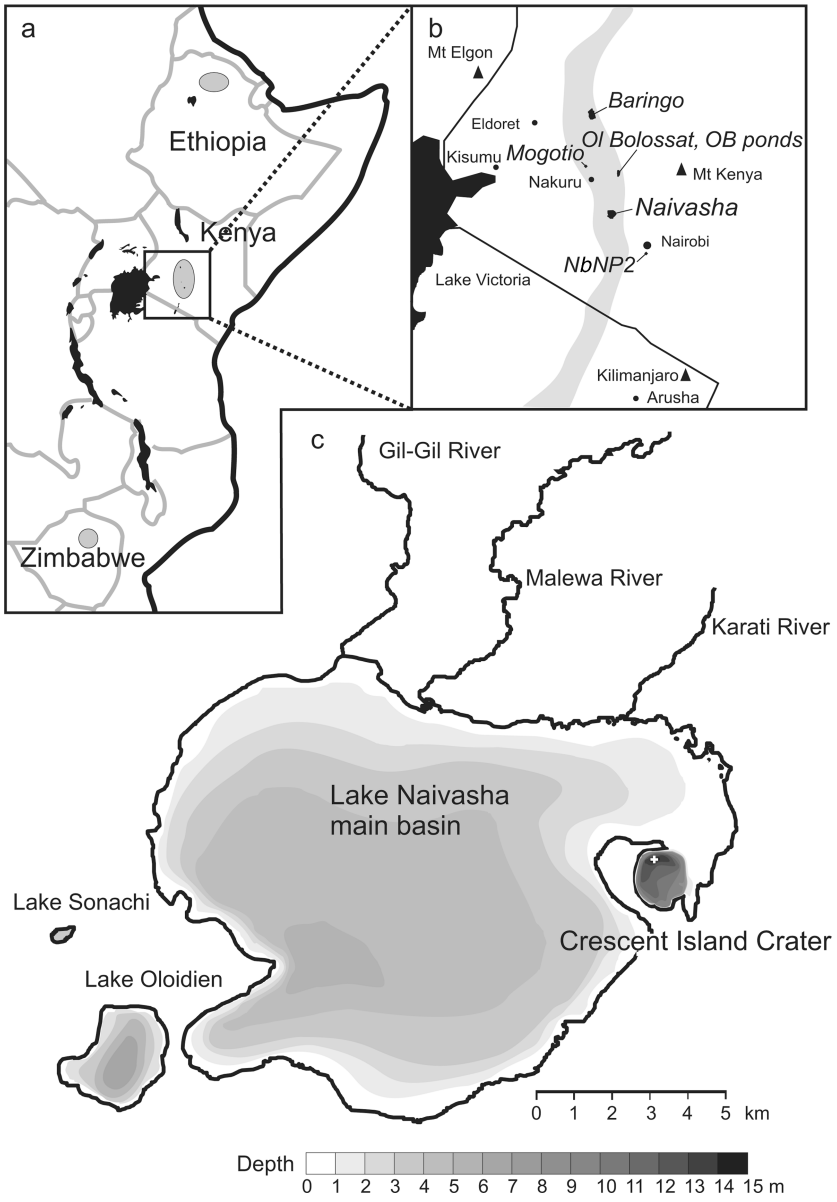

FIG. 1. Sample sites. (a) Map of East Africa showing the sampling areas (gray ovals) in Ethiopia, Kenya, and Zimbabwe. (b) Location of regional Kenyan samples. The elongated light gray zone represents the eastern Rift Valley bottom. (c) Bathymetric map of Lake Naivasha and Crescent Island Crater (modified from Åse et al. [1986] to reflect the 2003 AD water level and lake contour), showing the coring location (white cross).

subsamples were stirred in warm 10% potassium hydroxide solution for 45 min, sieved through 50-μm mesh, and scanned at 50×. *Daphnia barbata* postabdominal claws are distinguished from those of other regional *Daphnia* species (see Mergeay et al. 2005 for an overview) by the size and shape of the first and second pecten of spines at the base of the claws.

Sediment chronology was established by detailed lithostratigraphic correlation to cores from the same location in CIC that had been dated directly by ^{210}Pb and ^{14}C (Verschuren 2001). The chronology of core NC01-1S is based on a series of stratigraphic marker horizons corresponding to known historical events and stratigraphic correlation with the Pb^{210}-dated core NC93-1S (Verschuren 2001, Mergeay et al. 2004). Core NC01-D was dated using lithostratigraphic correlation with the ^{14}C-dated core NC93-2L (Mergeay 2005). Age at depth for each 2-cm core slice was estimated by linear interpolation between the 10 time-marker horizons so established over the 700-year period.

Regional sampling

A field survey in central and southern Kenya during January 2003 yielded zooplankton collections and

TABLE 1. General characteristics of the sampled sites and genetic characteristics of populations.

Site	Country	Code	Geographical coordinates	Maximum depth (m)	Area (ha)	Distance (km)	H (n)	AR	F_{IS}
Lake Naivasha 1998 AD	Kenya	N1998	00°46′20″ S, 36°21′40″ E	14.5	15 000	0	1 (7)	3.3	0.123
Lake Naivasha 1880 AD	Kenya	N1880	00°46′20″ S, 36°21′40″ E	15.5†	15 000	0	1 (4)	6.6	0.532*
Lake Naivasha 1550 AD	Kenya	N1550	00°46′20″ S, 36°21′40″ E	28†	>30 000	0	1 (3)	3.8	0.034
Nairobi National Park Hyena Dam	Kenya	NbNP2	01°20′18″ S, 36°48′38″ E	1.5	1	75	1 (4)	3.8	−0.227
Lake Ol Bolossat	Kenya	OlBol	00°09′55″ S, 36°25′59″ E	2	2000	60	1 (5)	4	0.103
Ol Bolossat ponds	Kenya	OB23	00°10′06″ S, 36°26′01″ E	1.5	0.03	59	1 (4)	3.3	−0.002
Lake Baringo	Kenya	Barin	00°39′07″ N, 36°03′38″ E	4	10 800	141	1 (5)	4.9	0.088
Mogotio Kapchelukung Dam	Kenya	Mogot	00°05′00″ N, 36°05′00″ E	1.5	0.9	95	1 (5)	2.8	0.321*
Lake Chivero	Zimbabwe	Chive	17°49′02″ S, 30°34′04″ E	27	2630	2165	2 (4)	NA	NA
Bokoro Dam	Ethiopia	Bokor	14°11′48″ N, 39°34′26″ E	3.5	3.8	1689	3 (5)	NA	NA
Dibdibo Dam	Ethiopia	Dibdi	14°15′38″ N, 39°05′03″ E	8.8	14.8	1690	4 (4)	NA	NA
Adi Gela Dam	Ethiopia	AdiGe	13°07′45″ N, 39°00′38″ E	4.3	17	1578	3 (5)	NA	NA
Mai Sessella Dam	Ethiopia	MaiSe	14°04′27″ N, 39°01′40″ E	9	21.8	1644	3 (5)	NA	NA
Korir Dam	Ethiopia	Korir	13°44′58″ N, 39°36′46″ E	4	13.7	1646	5 (5)	NA	NA

Notes: Distance is in reference to Lake Naivasha. Abbreviations: H, haplotype number (the number of sequenced individuals is given in parentheses); AR, allelic richness; F_{IS}, fixation index within subpopulations; NA, not applicable.

* P value significant after Bonferroni sequential correction.

† Maximum depth is based on a lake level reconstruction (Verschuren 2001).

surface sediment samples from 40 standing waters, including most of the few natural freshwater lakes occurring in this region (Mergeay et al. 2006*a*). *Daphnia barbata* was found at 11 of these sites (excluding the main and satellite basins of Lake Naivasha), but only five samples contained sufficient ($n > 14$) individuals or intact dormant eggs to allow population-genetic comparison with the Lake Naivasha populations (Table 1, Fig. 1a, b). The distance of these five regional waters to Lake Naivasha ranges from 60 to 140 km and from 60 to 220 km when including the other six sites where *D. barbata* was found at low abundance. Apart from the large East African lakes Victoria and Turkana (located 170 and 370 km away, respectively), no other regional waters are known to contain *D. barbata* (Uku and Mavuti 1994, Green 1995, Mergeay et al. 2005). As other studies have documented long-distance dispersal events in *Daphnia* (Hairston et al. 1999, Duffy et al. 2000, Mergeay et al. 2006*b*), we also included five *D. barbata* populations from Ethiopia and one from Zimbabwe, located approximately 1500–2000 km from Lake Naivasha (Table 1, Fig. 1a).

DNA extraction and amplification

For analysis of the ancient DNA in old dormant eggs, single eggs (or remnants) were picked out of the ephippium and transferred to UV-sterilized, 200-μL microfuge tubes in 10 μL proteinase K-buffer (16 mmol/L $(NH_4)_2SO_4$, 67 mmol/L Tris-HCl pH 8.8, 0.01% Tween-20, 7% dithiothreitol, and 0.5 mmol/L proteinase K). As the two eggs contained in a single ephippium are not genetically independent of one another, only one egg per ephippium was used for genetic analysis. Samples were incubated at 56°C for 1 h followed by 10 min at 95°C and 2-min centrifugation. Pre-polymerase chain reaction (PCR) steps of ancient DNA samples were always carried out in a separate, fully equipped laboratory in which no PCR is allowed, using UV-sterilized material and presterilized filter tips to avoid contamination from exogenous DNA. Every PCR plate included three blank extraction controls and three controls without template to ascertain no contamination had taken place.

We sequenced DNA of the mitochondrial 12S rRNA gene (12S) from three to seven individuals of each modern and ancient *D. barbata* population to produce a conservative initial estimate of the genetic relatedness between them. DNA sequencing followed the laboratory protocol described in Mergeay et al. (2005). The quality of DNA in eggs dating from the period 1545–1555 AD (termed the N1550 sample) was inadequate to sequence large DNA fragments. Instead a fragment of 170 nucleotides was first amplified and sequenced using the internal primers 12S_int1F (5′ GAGAGTGACGGGCGATATG 3′) and 12S_intR (5′ ACTTCAGGTCAAGGTGCAG 3′), then further processed following the normal protocol for 12S amplification. The PCR products were excised from agarose gels and purified using the GFX PCR DNA and gel band purification kit (Amersham Biosciences, Buckinghamshire, UK). Approximately 10–50 ng of purified fragment was sequenced using 3.2 pmol of the forward primer and the ABI Big Dye Terminator Kit (Applied Biosystems, Foster City, California, USA). The sequencing reaction products were analyzed using an ABI 3130 capillary DNA sequencer.

Until now, no microsatellite DNA markers have been developed specifically for *D. barbata,* but since previous research has shown that nuclear microsatellite loci are regularly conserved between *Daphnia* species (Colbourne et al. 2004, Brede et al. 2006), we screened more than 30 candidate microsatellite loci developed for *D.*

magna and *D. pulex* (J. K. Colbourne, *unpublished data*; Colbourne et al. 2004) for amplification, variation, and presence of tandem repeats in *D. barbata*. Three such microsatellite marker loci were eventually selected for population genetic analyses of *D. barbata* populations: Dp339 (primers 5′ cgcctccctccgtctattct 3′ and 5′ cccagcgtgtgacatctcaat 3′) and Dp291 (primers 5′ gaagaattcggtcggtgtgg 3′ and 5′ tcgaaaccgtctcgtctcgt 3′) amplify trinucleotide repeats, while Dma35R (primers 5′ cagccatcagtaaccagac 3′ and 5′ gaacgaggtctggaaaacg 3′) amplifies a dinucleotide repeat. The PCR cycles consisted of 20 s at 95°C, 20 s at 52°–55°C and 20 s at 72°C, followed by a final elongation at 72°C for 5 min. The PCR mixes contained 3 mmol/L $MgCl_2$ for loci Dma35R and Dp291 and 4 mmol/L $MgCl_2$ for locus Dp339. The PCR conditions were set to 40 cycles for old and degraded eggs and to 30–35 cycles for recent eggs and adult individuals from the modern regional populations. The PCR products were separated and visualized on a LI-COR 4200 system (LI-COR Biosciences, Lincoln, Nebraska, USA) using a 6% polyacrylamide gel (Sequagel XR, National Diagnostics, Charlotte, North Carolina, USA) or on an ABI 3130 capillary sequencer (Applied Biosystems), which were always run in parallel with a sizing standard supplied by the manufacturers. Allele sizes and genotypes were assessed with GeneImagIR 4.03 software (Scanalytics, Billerica, Massachusetts, USA) for polyacrylamide gels and Genemapper 3.7 (Applied Biosystems) for capillary sequencing.

Phylogeographic and population genetic analysis

We aligned the 12S mitochondrial DNA sequences (GenBank accession numbers AM412571–AM412584) in Mega3 (Kumar et al. 2004) and from it calculated the pairwise number of differences between haplotypes. The TCS software package (Clement et al. 2000) was used to construct a 95% maximum parsimony network of haplotypes, treating gaps in the sequence as the fifth possible nucleotide state.

Following this exploratory genetic analysis of all available samples, we subjected all populations (both old and modern regional) that contained the same 12S haplotype as the modern Lake Naivasha population to more in-depth population-genetic analyses. We calculated measures of allelic richness (AR) of microsatellite loci within populations and *F* statistics in the Fstat software package (version 2.9.3; Goudet 2001), while genetic distances (Nei's minimum distance) were calculated in the Genetix software package (version 4.05.2; Belkhir et al. 2004). These genetic distances were used to build an unweighted pair group method with arithmetic mean (UPGMA) tree using Statistica 6.0 (Statsoft, Tulsa, Oklahoma, USA).

Due to founder events the allelic composition of a newly founded population may deviate strongly from that of the source population and inflate the genetic distance between them (Boileau et al. 1992). To test for the strength of founder events affecting newly established *D. barbata* populations in Lake Naivasha, we used the software program Bottleneck (Cornuet and Luikart 1996), which exploits the fact that in populations that have recently experienced a genetic bottleneck (due to a significant reduction in effective population size), the allele numbers are reduced faster than the allele diversity (Cornuet and Luikart 1996). We used the two-phased model of mutation with default settings, using 1000 iterations. The Wilcoxon signed ranks test was used to determine the statistical significance of excess gene diversity. The qualitative mode shift method implemented in Bottleneck was used as an additional estimator.

Determining the propagule size and source

Although pairwise genetic distances provide an indication of genetic relationships between populations and hence on possible source populations, they do not provide information on the number of founding individuals through which one population originated from another. We addressed this problem by designing a randomization test that estimates the number of founders needed to explain the differences in observed allele frequencies between source and target population. This test calculates probabilities for the minimum and maximum number of colonizers, estimates the most likely number of colonizers for a specific pair of target and source populations, and allows comparison of alternative source populations. Hence, it produces estimates of which populations are likely source populations and of the number of colonizers needed from each of these populations. The program implementing the randomization procedure Colonize (compatible with Linux and Windows operating systems; see Supplement for details and software) yields for a given source–target combination separate probabilities for the minimum number and the maximum number of colonizers. By combining both probabilities, one can subsequently calculate a joint probability for how "likely" each number of colonizers is in explaining the observed pattern. The test was repeated for each possible source population (except N1550) using the data for loci Dp291 and Dp339. For populations for which data were available for locus Dma35R, we also repeated the test using all three loci. In addition, all possible source populations were pooled into one "regional" super-population (all modern Kenyan populations excluding N1998) by averaging absolute frequency counts and rounding up to the nearest integer (the randomization procedure uses absolute frequency data as input). For each source population and number of colonizers, 10^4 randomizations were performed for 1–50 colonizers (20 batches of 500 permutations, giving an average probability and standard error), using the rare alleles correction provided by the program (see Supplement for details of this procedure).

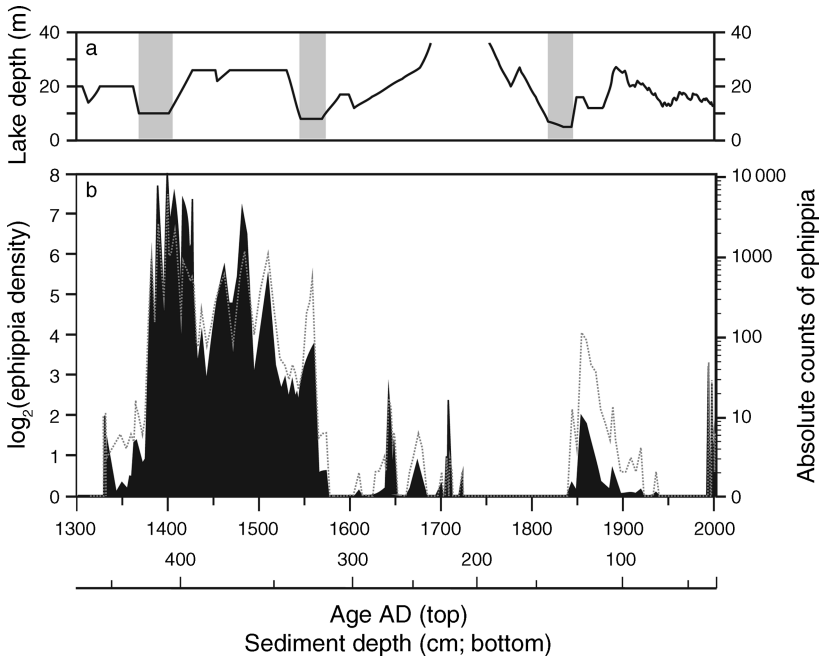

FIG. 2. (a) Reconstructed lake depth in Crescent Island Crater (CIC; Verschuren 2001). Gray bars indicate periods during which the main basin of Naivasha stood dry and only CIC still held water. (b) Changes in the abundance of *Daphnia barbata* ephippia in Lake Naivasha since 1300 AD, with indication of sediment depth. Densities are expressed as numbers of ephippia per gram dry sediment mass ($\log_2$ scale, left axis, black graph) and as absolute counts ($\log_{10}$ scale, right axis, stippled line).

RESULTS

Demographic change of Lake Naivasha D. barbata *through time*

The distribution of fossil ephippia in the CIC sediment record (Fig. 2) reveals that *D. barbata* in Lake Naivasha has experienced three cycles of population extinction and recolonization over the past 700 years. Following a long period of high population abundance that started in the 14th century, the population crashed coincidently with a lake level decline in the late 16th century. This happened at the onset of a prolonged episode of dry climatic conditions known regionally as the Nyarubanga drought (Webster 1979). Recolonization took place ca. 1610 AD during the subsequent lake level rise, but this 17th-century *D. barbata* population apparently failed to establish a strong foothold. After about a century of erratic presence it disappeared by 1730 AD, when the lake level was at a maximum (Fig. 2). Lack of post-abdominal claws of this species in mid and late 18th century sediments (two depth horizons checked, dated to ca. 1746 AD and 1785 AD) argues against persistence of the population without production of dormant eggs. According to the ephippial record (Fig. 2), *D. barbata* only recolonized Lake Naivasha after renewed lake filling ended the so-called Laparanat-Mahlatule drought of the early 19th century. This population reached its peak abundance in the mid to late 19th century, followed by a long phase of gradual decline until disappearing in the first half of the 20th century. Its last occurrences in core NC01-1S are dated to ca. 1920 (four ephippia) and ca. 1936 (one ephippium), but the combined evidence from this and three other cores covering 20th-century lake history (Mergeay et al. 2004) indicates that this population persisted until ca. 1940, however at very low densities. *Daphnia barbata* then remained absent for at least 50 years, until a new population appeared suddenly around 1995 and persisted at least until fieldwork for this study (January 2003), when *D. barbata* was the dominant large zooplankton species in Lake Naivasha.

Phylogeographic analyses

DNA sequencing of the mitochondrial 12S rRNA gene yielded one unique haplotype (H1) for all Kenyan *D. barbata* samples (Table 1), including all seven samples of old dormant eggs from which intact DNA could be extracted. These old samples represent two of the three historical Lake Naivasha populations: 14th to 16th century (N1550) and mid 19th to early 20th century (N1880). The five Ethiopian populations sampled yielded three different haplotypes (H3–H5), and the single population from Zimbabwe also has a distinct haplotype (H2). Further, the short, 170-base pair (bp) DNA sequence obtained from the N1550 population is sufficient to characterize its haplotype as distinct from the Ethiopian and Zimbabwean haplotypes.

The Kenyan haplotype H1 differs from the other haplotypes at between three and eight nucleotide positions, including inserts and deletions. In the

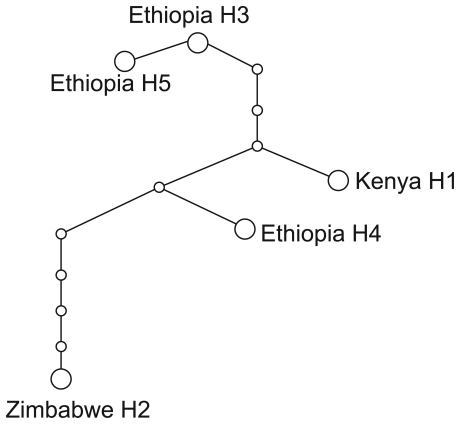

FIG. 3. Unrooted haplotype network representing the relationship between the five 12S haplotypes of *Daphnia barbata* (H1–H5). Each circle represents one nucleotide substitution. All Kenyan populations solely consisted of H1.

haplotype network (Fig. 3) it is positioned within the cluster of Ethiopian haplotypes, but clearly distinct from each of them. Although chances are small that the sampled Ethiopian lakes were the original source of a recolonization event in Lake Naivasha attributable to long-distance dispersal, their phylogeographic relationships do provide information on the regional origin of such a hypothetical source population, because the phylogeography of zooplankton taxa typically display a strongly regional structure (e.g., Taylor et al. 1998, Gomez et al. 2000, Penton et al. 2004, Degelas and De Meester 2005). Our results thus suggest that all sampled Kenyan populations of *D. barbata* represent a single, geologically recent range expansion from Ethiopia. More important in the context of this study, they imply that only Kenyan populations, either from Lake Naivasha itself or from lakes within the wider region, delivered the likely founders of the N1998 and N1880 Lake Naivasha populations, as they all share haplotype H1.

Population genetics of Lake Naivasha and regional D. barbata *populations*

In total, 1080 recent and old ephippia of *D. barbata*, distributed over the four discrete core sections in which remains of the four successive Lake Naivasha populations are preserved, were opened and checked for dormant eggs. Only 209 of these contained dormant eggs, or remains thereof, suitable for genetic analysis. No suitable eggs were found in the core section representing the population of 1610–1750 AD, as virtually all ephippia recovered were empty or contained strongly degenerated egg remains. Similarly, ephippia deposited during 1905–1940 AD were empty or contained only degenerated eggs. Attempts to amplify DNA reliably from such samples failed. We therefore focused on the 1998 population (N1998: ca. 1995–2001 AD), the late 19th-century population (N1880: ca. 1860–1900 AD) and the 16th-century population (N1550: ca. 1545–1555 AD), which all yielded fair amounts of well-preserved dormant eggs (Fig. 2).

Samples of the three Lake Naivasha populations consistently amplified DNA fragments of the expected size for at least one locus. Locus Dma35R could only be amplified using the most recent dormant eggs, and we failed to amplify DNA from the oldest population (N1550) using locus Dp291 as well. In the five regional populations with adequate sample sizes (total $n = 114$ individuals or dormant eggs), all loci were amplified successfully (Appendix A). Overall, allelic richness was highest in N1880 (Table 1; AR = 6.6). All other populations showed values of AR very similar to one another (between 2.8 and 4.9), indicating that the genetic diversity in N1998 (N1998: AR = 3.3) is comparable to that found in the regional pool. The fixation index (F_{IS}; Table 1) shows that N1880 had a large heterozygote deficit ($F_{IS} = 0.532$, $P = 0.002$), in contrast to the other Lake Naivasha populations. This may be an artifact of our mixing of (temporal) subpopulations that differ from each other genetically ("Wahlund effect"), because we sampled the N1880 population over a longer time period (~40 years) than the other studied populations (<20 years). If correct, this may also explain the relatively high allelic richness in N1880.

The Wilcoxon signed ranks test in Bottleneck did not identify any of the sampled populations as having experienced a recent bottleneck (P value between 0.13 and 0.50, depending on the population). Mode-shift analysis in Bottleneck did suggest a shifted mode (and hence a recent population size reduction or founder event) in populations NbNP2 and OB23, but actually the sizes of these two samples were too low for this specific test to be statistically valid (minimum 30 individuals; Cornuet and Luikart 1996). Nevertheless, the results on the ancient and recent Naivasha populations indicated an allelic composition that is in good agreement with large population sizes and low genetic drift. In the N1550 sample this is not surprising, as this had been a large and stable population for over 200 years (Fig. 2). Hence we do not expect founder effects to have inflated the genetic distance between modern or old Lake Naivasha populations and their putative source population (Boileau et al. 1992).

Pairwise genetic distances between Lake Naivasha and other Kenyan populations were pronounced, but there were substantially smaller distances between recent (N1998) and old (N1880, N1550) Lake Naivasha populations (Fig. 4). Populations N1998 and N1880 cluster closely together (Nei's minimum distance $\approx$ 0.13), with N1550 as a sister to both of these (Nei's minimum distance $\approx$ 0.16), indicating that the three temporally separated Lake Naivasha populations are more closely related to one another than to any of the four main regional populations sampled (Nei's minimum distance 0.21–0.23). In the genetic loci analyzed, the relatedness between modern and old Lake Naivasha

populations is comparable to that between the modern populations of Lake Ol Bolossat (OlBol) and Ol Bolossat ponds (OB23) ~350 m away.

Estimating the propagule source and size

Using the sampled regional Kenyan populations as potential source population, we used the Colonize randomization test to estimate the likelihood that each of those particular populations founded the modern Lake Naivasha population (N1998) and the associated likely number of colonizing individuals (Appendix B). For all populations except N1880, the joint probability for minimum and maximum number of colonizers as determined in Colonize was zero. Only population N1880 was a likely founder, with $P > 0.05$ for a range of 5–12 colonizing individuals and a maximum probability (0.08) for eight colonizers (Fig. 5, Appendix B). In addition, although the pooled regional sample gave a very low joint probability (but above zero) at four to seven colonists, the 0.05 probability limits for minimum and maximum number of colonizers did not allow an acceptable range of colonizer numbers (Appendix B; the minimum number of colonizers [five] is higher than the maximum number of colonizers [three]). Together this shows that the local dormant egg bank of N1880 (which lasted marginally until ca. 1940) was the most likely source of the N1998 population when compared to the other regional populations and that the number of colonizers was on the order of 10 individuals.

Discussion

Validation of the ephippial record of demographic change

Given that the ephippial record of *D. barbata* in CIC suggests complete absence of this species in Lake Naivasha zooplankton for three periods of time lasting between 30 and 110 years (ca. 1580–1610 AD, ca. 1730–1840 AD, and ca. 1940–1995 AD), the question arises whether lack of ephippia in a single sediment core implies true absence in the living community. Three lines of evidence indicate that (1) the observed abundance patterns of fossil ephippia through time are indeed a reliable indicator of past population abundance at the order-of-magnitude scale and (2) that a 2-cm slice of the studied cores (which represents on average three years of deposition on 20 or 38 cm^2 of the lake bottom and is thus equivalent to annual deposition on 60 or 115 cm^2) is an adequate sample size to monitor species presence or absence in a zooplankton community.

First we note that postabdominal claws are skeletal remains that (1) are diagnostic at the species level, (2) are present on both sexually and asexually reproducing individuals, and (3) are hence produced in much higher quantities than ephippia. Absence of *D. barbata* claws in the three main core sections that also lack *D. barbata* ephippia strongly argues against the hypothetical persistence during those periods of a Lake Naivasha *D. barbata* population that did not produce dormant eggs. Second, the abundance patterns of *D. barbata* ephippia through time in core NC01-1S (representing the more recent portion of the CIC record, ca. 1812–2001 AD) are highly consistent with those found in three other cores (NC91-1S, NC91-2S, and NC93-1S) that cover the same time period but were taken in different parts of the crater basin (Mergeay et al. 2004). They all show a gradual decline of *D. barbata* ephippia from the end of the 19th century to the 1920s and probable disappearance by ca. 1940. The early part of this decline can be attributed to a gradual change towards less favorable habitat conditions associated with natural lake-level fluctuation, but the final blow was probably given by exotic planktivorous fishes introduced to Lake Naivasha in the 1920s and 1930s (Mergeay et al. 2004). In the four cores combined (replicate samples together representing annual deposition on 460 cm^2 of lake bottom) only two strongly damaged partial ephippia were recovered from sediments dated to between 1940 and 1995 (Mergeay et al. 2004: Fig. 2b). Considering their bad preservation state compared to the vast majority of our study material, these specimens most probably represent secondary redeposition of old (pre-1940) ephippia due to sediment disturbance in the periphery of the basin.

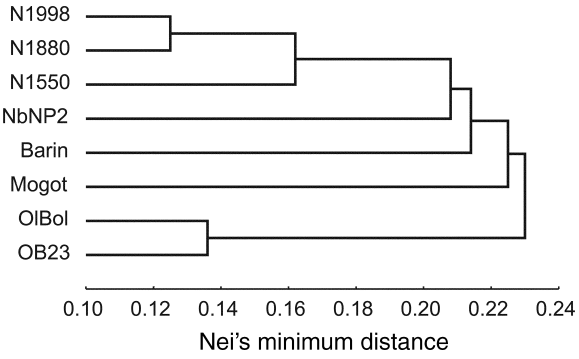

FIG. 4. Unweighted pair group method with arithmetic mean (UPGMA) tree calculated from Nei's minimum genetic distance among the different *Daphnia barbata* populations with 12S haplotype H1.

Third, our results are fully consistent with the accumulated data from extensive live zooplankton surveying in Lake Naivasha between the 1930s and early 1990s. Mavuti and Litterick (1981) failed to detect *D. barbata* during 24 consecutive monthly surveys between July 1978 and July 1980 at four sampling stations distributed over the lake, on each occasion filtering 700 L of water per locality. D. M. Harper (*unpublished manuscript*) did not find *D. barbata* during occasional sampling between 1982 and 1984, nor in regular monthly surveys from July 1984 to April 1986. Uku and Mavuti (1994) also did not find *D. barbata* in 1990 and 1991. In agreement with these data, sediments in our cores deposited during each of these periods are devoid of *D. barbata* ephippia. Furthermore, although neither Worthington and Ricardo (1936), Jenkin (1934),

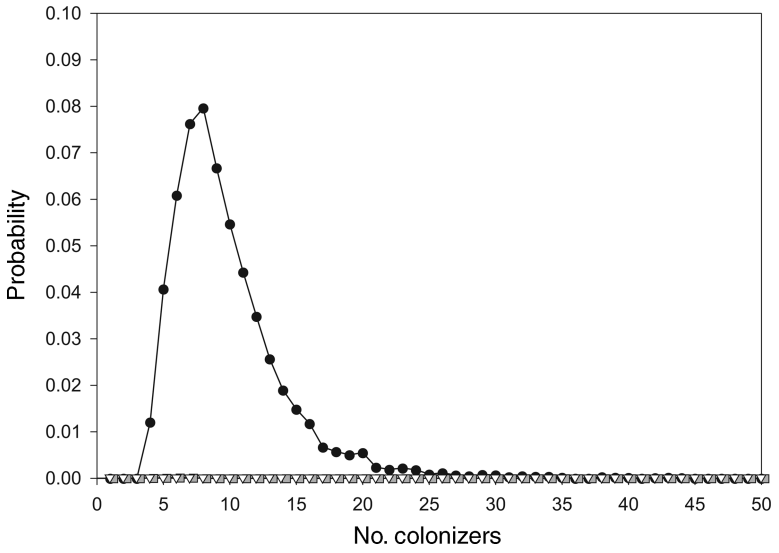

FIG. 5. Graphical representation of the joint probability score calculated by Colonize, using N1998 as sink and all other populations as source. Data for two loci were used (Dp291 and Dp339). Key to symbols: solid circles, probability score for population N1880; open triangles, probability score for pooled regional samples; gray squares, probability scores for Barin, Mogot, OlBol, OB23, NbNP2 (all zero scores) populations. Only population N1880 showed high probability scores.

or Lowndes (1936) detected *D. barbata* in Lake Naivasha during early collecting efforts in 1929 and 1930, we did record this species occasionally in sediments deposited around that period. This supports the conclusion of previous studies (Frey 1960, Vandekerkhove et al. 2005) that species detection thresholds are lower in sediment samples than in average live zooplankton samples. Combined, these three lines of evidence demonstrate that the stratigraphic changes of ephippium abundance in Lake Naivasha sediments reflect genuine order-of-magnitude demographic change in the historical *Daphnia* populations (cf. Verschuren and Marnell 1997).

Prior to the 20th century, demographic change in the Lake Naivasha *D. barbata* population often occurred during episodes of dramatic lake-level change. Its disappearance shortly after 1550 AD coincided with drastic lake-level decline during the Nyarubanga drought, and its reappearance around 1840 AD coincided with rapid lake transgression following the Laparanat-Mahlatule drought. In contrast, both the apparent extinction around 1940 AD and recolonization in the mid-1990s can be linked to anthropogenic influences. Fish introductions since the 1920s have involved at least nine exotic fish species, most of them specifically intended to boost fisheries. The resulting increase in fish predation pressure is what probably led to the demise of *D. barbata* in Lake Naivasha a decade later (Mergeay et al. 2004). Recolonization around 1995 despite the continued presence of zooplanktivorous fish must have been facilitated by the marked increase in water column turbidity that has developed in recent decades, due to eutrophication of the lake and massive soil erosion from surrounding farmland, thus reducing the efficiency of visual predators (Mergeay et al. 2004).

Local and regional genetic differences

DNA sequencing of the 12S mitochondrial gene showed that all Kenyan populations, including the old and modern Lake Naivasha populations, shared the same mitochondrial haplotype. In contrast, Ethiopian and Zimbabwean populations were clearly distinct from the Kenyan populations at this level. As a result, in our data set only Kenyan *D. barbata* populations are eligible as sources for the modern (post-1995) Lake Naivasha population. Comparison of allelic distributions and pairwise genetic distances among the local and regional Kenyan populations further suggested that this modern population was founded from the local dormant egg bank, by then more than 50 years old. The Colonize test clearly showed that only the previous Lake Naivasha population (ca.1860–1940 AD) was a likely source of propagules for its modern population and that probably aproximately 10 individuals were involved in this recolonization event. Given the large size of Lake Naivasha (ca. 150 km^2), this is a very low number. Admittedly, Colonize assumes that initial allele frequencies remain stable, whereas genetic drift in a small, newly established population may artificially scale down the estimated number of colonizers. In fact, we found no evidence of genetic drift in the modern population that would have pointed to a recent genetic bottleneck. Also the genetic diversity of the modern population and the fixation index F_{IS} do not suggest a strong reduction in effective population size. Combined, our results indicate that the modern Lake Naivasha population was indeed established by a limited number of individuals of the

previous Lake Naivasha population and that, however limited in number, these initial colonizers provided sufficient allelic diversity and rapidly expanded to an abundance large enough to prevent substantial genetic drift.

In similar fashion the 16th-century Lake Naivasha population was more related to the later Naivasha populations than to other regional populations. Although the evidence was scored on only one locus, it indicates a certain degree of genetic continuity between the 16th-century and 19th-century populations and suggests that the 16th-century dormant egg bank (probably indirectly through the 17th-century population) was at least partly involved in *D. barbata*'s 19th-century recolonization of Lake Naivasha.

Likelihood of local vs. regional colonization

Controlled hatching experiments have shown that dormant eggs of *Daphnia* can remain viable for at least 125 years (Cáceres 1998). To what extent such old eggs are involved in natural recolonization in lakes, however, was not known. Our data suggest that *D. barbata* was able to recolonize Lake Naivasha from dormant eggs that were at least 50 years old. *Daphnia* and other planktonic crustaceans have high effective dispersal rates, colonizing newly established habitats from the regional pool within months (Louette and De Meester 2005). Even very large distances are easily overcome, as witnessed by the speed at which *D. lumholtzi* has spread over North America since 1993 (Havel et al. 2000) or at which a single invading clone of *Daphnia pulex* spread across Africa in at most a few decades (Mergeay et al. 2006*b*).

Once a fluctuating aquatic habitat becomes suitable again for a given species, the origin of an establishing population will be strongly determined by the relative speed at which regional propagules arrive in the lake, hatch, grow, and reproduce, compared to surviving local propagules. The probability that old eggs, often buried under tens of centimeters of sediment, reach the surface evidently declines with age. Yet this disadvantage may be compensated by their huge numerical advantage compared to immigrants even after several decades to a century. Indeed, if only 1% of the eggs buried in Lake Naivasha sediments during the period 1860–1940 survived until the 1990s, which is $\sim$50 times smaller than experimental data on two *Daphnia* species (Cáceres 1998), then still an astounding 30×10^9 viable dormant eggs would have been waiting to surface, hatch, and recolonize the lake as the active modern population. Clearly, the genetic continuity after recolonization that we observe in the present study suggests that local hatching from old dormant eggs must be a common phenomenon.

There are several plausible ways in which deeply buried dormant eggs can surface again and recolonize the local habitat by dispersal through time. In large shallow lakes such as Lake Naivasha, probably the most important process is wind-induced sediment resuspension. In contrast with the deep-crater basin where an undisturbed sediment record can accumulate, much of the shallow wind-stressed main basin of Lake Naivasha is shallower than the critical depth for fine-grained sediment deposition, and therefore a large area of lake bottom is frequently subject to sediment resuspension (Verschuren 1999). Especially during occasional heavy storms, wind action likely causes substantial local resuspension of old sediments. Alternatively, and perhaps of greater importance in small wind-sheltered lakes, deep sediment reworking also occurs when hippopotami walk over the lake floor or when large fish or other animals wallow in the mud.

The importance of long-term dormancy: beyond Daphnia

Several striking parallels can be drawn between the inhabitants of fluctuating lakes in arid and semiarid regions and those of pioneer habitats in terrestrial ecosystems. Pioneer plant species have to cope with irregular and unpredictable bouts of habitat disturbance and short habitat availability, since succession and facilitation rapidly favor stronger competitors. They often have small and easily dispersed seeds, with high longevity (Thompson et al. 1996, Bekker et al. 1998). Likewise in fluctuating lakes, suitable and hostile habitat conditions alternate repeatedly as lake level drops or rises unpredictably. In Lake Naivasha, for example, there have been at least eight important cycles of lake-level rise and decline over the past 1800 years, and about as many extinction and recolonization cycles of *D. barbata* (Mergeay 2005). Thus, whereas the role of dormant eggs for population persistence in intermittent and ephemeral habitats (with seasonal habitat unsuitability) is evident, we here show that long-lived dormant stages may also promote genetic continuity in systems that show shifts in habitat quality over much longer time scales. Using a zooplankton model, we have shown that dormant propagules can truly act as time travelers under natural conditions, bridging extended periods of unsuitable conditions and contributing actively to modern populations. Consequently, species with seemingly short (active) generation times paradoxically appear capable to extend their generation time to several decades, maybe even centuries. As short-lived plant and zooplankton species are also excellent dispersers, they thus seem to maximize regional persistence by bet-hedging in both space and time. In addition to emphasizing the ecological and evolutionary importance of long-lived dormant egg banks, the results of this study confirm that dormant egg banks can significantly contribute to biodiversity conservation, in allowing the reestablishment of indigenous populations following restoration of natural ecosystems after multiple decades of absence due to anthropogenic disturbance. Dormant egg banks not only reduce the problem of dispersal limitation in the restoration of isolated ecosystems, they may also favorably promote persistence of local genotypes against exotic genotypes.

Acknowledgments

This study was carried out with permission of the Permanent Secretary of the Ministry of Education, Science and Technology of Kenya under research permit 13/001/11C to D. Verschuren and funded by the K.U.–Leuven Research Fund (project OT/04/23) and the Research Foundation-Flanders (project G0086.00). The authors thank K. Mavuti (University of Nairobi, Kenya), W. Shivoga (Egerton University, Kenya), S. Higgins (Kijabe Ltd., Naivasha), the Lake Naivasha Riparian Association, and the Lake Naivasha Yacht Club for logistic support, and H. Eggermont and K. De Maeyer for assistance in the field. T. Dejenie provided samples from Ethiopia; M. Barson from Zimbabwe. Two anonymous referees provided constructive suggestions on a previous version of the manuscript. J. Mergeay was supported by a Ph.D. fellowship from the Institute for the Promotion and Innovation of Science and Technology in Flanders (IWT, Belgium) and is currently supported by a postdoctoral grant of the K.U.–Leuven Research Fund.

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APPENDIX A

A table showing sample sizes and allele frequencies of the eight studied Kenyan populations of *Daphnia barbata* at microsatellite loci Dp291, Dp339, and Dma35R (*Ecological Archives* E088-188-A1).

APPENDIX B

Output summary of the Colonize randomization procedure using population N1998 as sink, repeated for LN1880, Barin, Mogot, NbNP2, O1Bol, OB23, and the pooled regional samples (excluding the ancient N1880 and N1550 samples) (*Ecological Archives* E088-188-A2).

SUPPLEMENT

Colonize: a tool to estimate the number of individuals colonizing a new habitat (*Ecological Archives* E088-188-S1).