Papyrus swamps, hypoxia, and faunal diversification: variation among populations of *Barbus neumayeri*

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To test whether patches of papyrus swamp contribute to diversification of populations of non-air-breathing fishes, the gill morphology of *Barbus neumayeri* was compared between a papyrus swamp and several tributaries which differed in oxygen regime. Total gill filament length differed among sites and was negatively related to dissolved oxygen availability, supporting strong selection pressure for low-oxygen tolerance in the swamp interior. Among recaptures of marked *B. neumayeri* over a 4·5-year period among the focal swamp and connected stream and river sites, 93% of fish were recovered at the site of capture. Some of the individuals that moved crossed physicochemical gradients and traversed long distances within the swamp/stream system. This movement rate would theoretically be sufficient to homogenize gene frequencies among populations. However, randomly amplified polymorphic DNA (RAPD) markers indicated significant genetic differentiation among sites and no relationship between genetic differences and geographical distances among sites suggesting habitat-specific selection pressures on dispersers, rather than insufficient dispersal.

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INTRODUCTION

Understanding patterns of dispersal in populations and their evolutionary consequences is a key issue in ecology. For fishes, the availability of dissolved oxygen is one abiotic factor that can limit habitat quality and dispersal pathways (Roberts, 1975; Kramer, 1983a, 1987; Chapman & Liem, 1995). Fishes show a diversity of morphological, physiological, and behavioural adaptations to oxygen scarcity including: the development of air-breathing organs, large gill surface area, change in oxygen carrying capacity of the blood, anaerobic metabolism, low metabolic rate, morphological specializations for exploitation of the oxygen-rich surface layer, changes in activity, and habitat selection (Lewis, 1970; Galis & Barel, 1980; Hochachka, 1982; Kramer, 1983a, 1987; Perry & McDonald, 1993; Graham, 1997). These adaptations have associated costs and benefits which vary with ecological circumstance and affect the ability of fishes to use or disperse through hypoxic waters (Kramer, 1983a,b, 1987). Although much effort has been directed towards describing the physiological and morphological

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adaptations to deoxygenation in fishes, we understand very little about the role of oxygen-scarce waters as barriers or biological filters, and their demographic and zoogeographical consequences.

Oxygen scarcity is widespread in tropical fresh waters, particularly in floodplain pools, inundated forests, and permanent swamps (Carter & Beadle, 1930; Carter, 1955; Kramer et al., 1978; Welcomme, 1979; Junk et al., 1983; Chapman et al., 1998). In East Africa, hypoxia is prevalent in extensive dense wetlands dominated by papyrus (Cyperus papyrus) and Miscanthidium violaceum (Carter, 1955; Beadle, 1981; Chapman et al., 1998) and may pose an effective barrier to fish dispersal (Roberts, 1975). The few accounts of papyrus and Miscanthidium swamp fish faunas include many air breathers (Protopterus aethiopicus Heckel, Clarias spp., Ctenopoma muriei (Boulenger); Polypterus senegalus Cuvier; Carter, 1955; Beadle, 1981; Chapman, 1995; Chapman & Liem, 1995; Chapman et al., 1996a,b), though also some water-breathing fishes. The latter tend to be species with extremely efficient oxygen uptake mechanisms. For example, Chapman et al. (1996b) discovered remnant populations of a small mormyrid, Petrocephalus catostoma (Günther), in wetland lagoons surrounding Lake Nabugabo, Uganda after its population had disappeared from the main lake following the introduction of Nile perch Lates niloticus (L.). This tiny electric fish survives by virtue of a low metabolism, a low critical oxygen tension, large gill surface area, and inverted swimming during aquatic surface respiration (ASR) to expose its subterminal mouth to the surface of the water (Chapman & Chapman, 1998). Water breathers are also common in ecotonal areas between wetlands and the open waters of a lake or river where oxygen levels are higher (Hickley & Bailey, 1986; Chapman et al., 1996a,b). However, many of these species do not penetrate the hypoxic swamp interior producing a sharp transition in species composition that corresponds to a sharp transition in physicochemical conditions in wetland ecotones (Rosenberger, 1997).

Despite the limited diversity of fishes within papyrus swamps and other heavily vegetated wetlands, these habitats may contribute to the maintenance of faunal structure and diversity. For air-breathing fishes like many clariid catfishes and lungfishes, large swampy divides characterized by chronically low oxygen conditions may not limit dispersal. However, for non-air breathers that cannot tolerate low oxygen conditions, dense swamps may limit movement and serve as a barrier to dispersal leading to diversification and speciation. For water breathers that can survive in dense wetlands, the use of and dispersal through these habitats is still likely to be limited by the spatial and temporal patterns of variation in oxygen content within the wetland and the efficiency of oxygen uptake in the species. This may lead to geographical variation between wetland populations and populations from open water sections of the drainage, particularly in traits related to oxygen uptake. To examine this idea we compared the gill size of a small African cyprinid Barbus neumayeri Fischer 1884 from the Rwembaita Swamp, a dense papyrus swamp in western Uganda, to a population of B. neumayeri from a well-oxygenated, everflowing section of the Mpanga River into which the swamp eventually drains (Chapman & Liem, 1995). These sites are separated by c. 7 km but occur in the same drainage area. For a fish of a given body weight, total gill filament length was much larger in the Rwembaita Swamp population, suggesting greater capacity for oxygen uptake (Chapman

& Liem, 1995). Additional studies revealed significant differences in the respiratory behaviour between *B. neumayeri* from the Rwembaita Swamp and those from open water populations in the same river system (Olowo & Chapman, 1996). These data support the significance of papyrus swamps in promoting variation among populations; however, it remains unclear whether papyrus swamps actually reduce mixing of populations leading to genetic differentiation.

In this study we ask whether patches of papyrus swamp create a coarse grained environment and contribute to diversification of populations of non-air breathers. To address this objective we first expand on our earlier study of variation in gill morphology by documenting gill size at sites progressively closer to the Rwembaita Swamp with different oxygen levels, to examine whether gill size varies over very small geographical scales and the degree to which oxygen can explain variation in gill size. Second, we document rates of dispersal of *B. neumayeri* over a 4·5-year period among the Rwembaita Swamp and connected stream and river sites. Finally, we quantify genetic variation among populations of *B. neumayeri* in the same swamp/river system using randomly amplified polymorphic DNA (RAPD) assay. Our findings indicate the potential of papyrus swamps in contributing to diversification of *B. neumayeri* populations, and more generally to the natural process of faunal isolation and reunification, a process that may be responsible for much of the character and evolutionary lability of tropical freshwater fish faunas.

MATERIALS AND METHODS

STUDY SITE AND SPECIES

The study was conducted in Kibale National Park, western Uganda (0°13′–0°41′N and 30°19′–30°32′E). Approximately 60% of the 766 km^2 park is moist evergreen forest, and the remainder is a mosaic of wetland, grassland, plantations of pine, thicket, and colonizing forest (Butynski, 1990). Kibale Forest is drained by two major everflowing rivers, the Dura and Mpanga Rivers; both are tributaries of Lake George (Fig. 1). These rivers are fed by numerous small forest streams, many of which are intermittent.

Mean annual rainfall in the Kibale Forest (1977–1996) has averaged 1678 mm (range 1205–2139 mm). There are distinct wet and dry seasons which are bimodal in distribution. May–August and December–February tend to be drier than other months.

The primary study site, Rwembaita Swamp, is one of the larger papyrus (*Cyperus papyrus*) swamps in the park (*c*. 6·5 km in length) and feeds the Njuguta River, a tributary of the Mpanga River (Fig. 1). Several small intermittent streams also feed into the Rwembaita Swamp, and much of the system runs through an area that was logged selectively 30 years ago. In the dense papyrus stands, which reach up to 5 m in height, the terminal brush-like umbels form a closed canopy, so that the interior of the papyrus forest is dark and cool. The shaded swamp limits mixing and minimizes incident light, which, in combination with high rates of organic decomposition of huge amounts of water-logged vegetation, produces extremely hypoxic waters (Carter, 1955; Thompson, 1976; Beadle, 1981; Chapman & Liem, 1995; Chapman *et al.*, 1998). In the valley swamps of the Kibale Forest, the papyrus mats are generally not floating, but lie on the mud. During the drier periods, open water in the swamp is restricted to small pools and deeper channels. During the rainy periods, the papyrus swamp is transformed into a maze of interconnected channels, large pools, and inundated grassland areas.

One of the two fish species inhabiting the Rwembaita Swamp is *B. neumayeri*, which reaches a maximum length of 12·5 cm in the swamps and rivers of Kibale Forest. *Barbus*
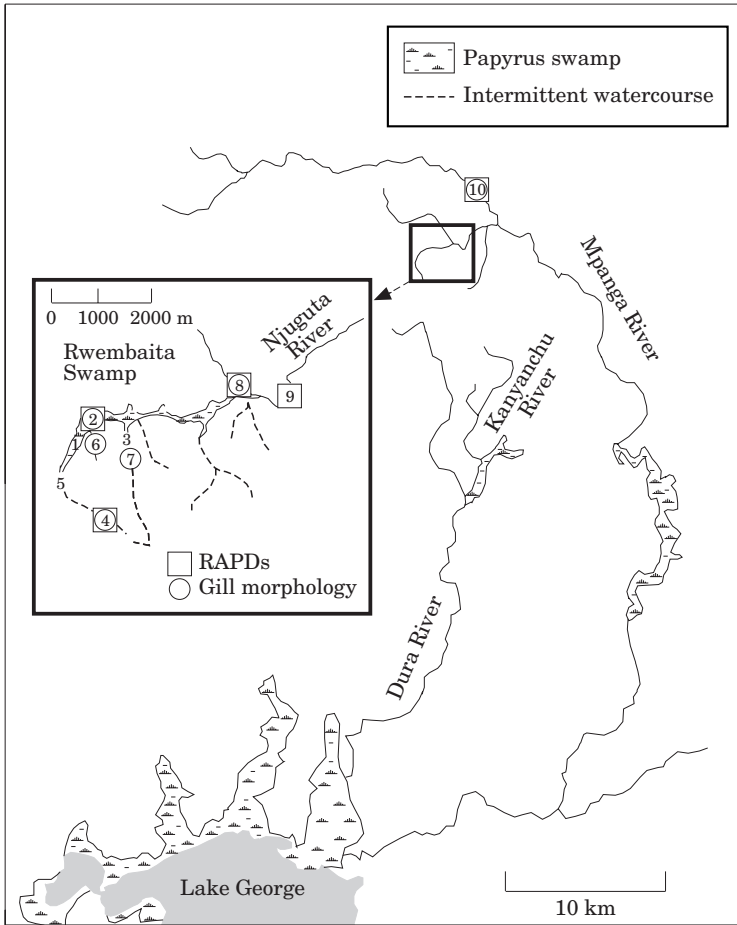
Fɪɢ. 1. Map of the major river systems that drain Kibale National Park, Uganda (Dura River and Mpanga River), and the sampling sites used for environmental sampling (sites 1–10), dispersal studies (sites 1–8), gill morphological measurements (sites 2, 4, 6, 7, 8, and 10), and genetic analyses (sites 2, 4, 8, 9, and 10). Sites 1–3 are located in dense papyrus swamp; sites 4–7 are intermittent habitats; and sites 8–10 are everflowing river sites.

neumayeri feeds principally on small insect larvae, aquatic plants, and detritus (Corbet, 1961; Chapman, unpubl. data). The species is widely distributed in East Africa (Greenwood, 1962, 1966), and is found in a variety of habitats within Kibale National Park including seasonal streams, papyrus swamps, and everflowing streams and rivers (Olowo & Chapman, 1996). *Clarias liocephalus* Boulenger, an air-breathing clariid catfish (Clariidae), is the other species (Chapman, 1995).

ENVIRONMENTAL CONDITIONS

Mean dissolved oxygen concentration values were recorded monthly at three sites in the Rwembaita Swamp (sites 1–3, Fig. 1) and at four intermittent stream sites (sites 4–7, Fig. 1) from July 1993–July 1996; and at three everflowing river sites, site 8 from July 1993–July 1996, site 9 from July 1993 to July 1994, and site 10 from July 1996 to July 1997. At each site duplicate readings of dissolved oxygen and water temperature were taken at three to six stations, with the exception of site 4 where there were 13 stations.

Data were collected using a YSI meter (Model 51B or Model 95). The large number of stations at site 4 was necessary to ensure that some stations remained with water during the driest months. In the closed cover of the forest and papyrus swamp, diel variation in dissolved oxygen and water temperature tended to be very low (Chapman & Liem, 1995). Therefore, measurements were taken once per sampling period between 1100 and 1500 hours. Rainfall data were collected at the Makerere University Biological Field Station, located *c*. 3 km from the Rwembaita Swamp (site 2).

GILL MORPHOLOGY

We compared the gill morphology of *B. neumayeri* from Rwembaita Swamp with that from five other sites, selected to produce a range of variation in mean oxygen levels and distance from the Rwembaita Swamp. These included three intermittent stream sites (sites 4, 6, and 7) and two everflowing river sites (sites 8 and 10). Sites 6, 7, and 8 were all within 150 m of dense papyrus swamp. Fish were live-captured with minnow traps and preserved in paraformaldehyde. The morphological parameters most easily and accurately measured for large numbers of small fish are those related to gill filament length. Total gill filament length was meured for 10 fish from each population using standard methods (Muir & Hughes, 1969; Hughes, 1984). For each fish, the branchial basket was removed, and the four gill arches from the left side of the basket were separated. For each hemibranch of the gill arches, the length of every fifth gill filament was measured. Two successive measurements along a hemibranch were averaged and multiplied by the number of filaments in the section between the two filaments. Filament lengths were summed for the four hemibranchs and multiplied by two to produce an estimate of total gill filament length (TFL). Although total gill surface area is a better indicator of oxygen uptake capacity, total gill filament length is generally correlated with the area of respiratory surface (Palzenberger & Phola, 1992), and it was assumed that a longer total gill filament length in one population as compared to another of the same species reflected a greater capacity to extract oxygen from the water.

Analysis of covariance (ANCOVA) was used to compare total gill filament length among populations with body weight (total weight of preserved specimens) as the covariate. Both variables were log transformed. Adjusted means (sample means adjusted for a common mean body weight and a common regression line) and their standard errors were calculated from the ANCOVA analysis, and the a posteriori Sidak test was used to test for significant differences between pairs of sites. A polynomial regression was used to examine the relationship between weight-adjusted mean total gill filament length and mean dissolved oxygen levels of the six sites.

DISPERSAL

Field studies on the movement patterns of *B. neumayeri* among swamp and connected stream and river sites were carried out over a 4·5-year period which began in July of 1993. Dispersal of *B. neumayeri* was examined on a monthly basis among sites 1–8 (three sites within the Rwembaita Swamp, four intermittent stream sites which feed into the swamp, and the Njuguta River, into which the swamp drains, Fig. 1). All sites were monitored over the 4·5-year period, except for site 8 which was included in the first 3 years only. Each month, fish were trapped using metal minnow traps set overnight and baited with bread. Traps were generally set in sequence between 0900 and 1400 hours and pulled in the same sequence the following day. One to five traps were set at each station for which environmental data were collected. The number of traps varied depending on the amount of open water available, and all traps were set to allow captured fish access to the water surface. Each captured *B. neumayeri* was measured (total length, L_T), and fish were marked according to site (1–8) using different colours of tattoo ink injected into the muscle with a fine gauge hypodermic needle (Chapman & Kramer, 1991; Chapman & Liem, 1995; Rosenberger, 1997). Fish were held in plastic buckets during processing and then returned to the site of capture. Marks tended to last for 1–2 months, so a new set of fish was marked each month at all sites. Approximately 20 000 fish were captured marked, and released during the study. Occasionally, forest elephants visit the sampling

sites in the swamp and stream systems, and their activities drastically change the morphometry of certain stations, particularly in the papyrus swamp. We attempted to sample the same stations at each site throughout the study, but occasional changes in location were necessary subsequent to elephant visits.

Differences in the mean size of recaptured fish that had moved between sites (dispersers) and those that were recaptured at the same site (residents) were evaluated with a *t*-test. Since very few fish were recaptured at a site different from their original capture site, all dispersal events were summarized relative to the distance and oxygen gradient covered in the move.

RAPD ANALYSIS

The randomly amplified polymorphic DNA (RAPDs) assay was used to examine genetic differentiation among *B. neumayeri* from the Rwembaita Swamp (site 2, Fig. 1), one intermittent stream (site 4), two sites on the Njuguta River (sites 8 and 9), and an everflowing site on the Mpanga River (site 10). Papyrus swamps separate sites 4, 2, 8, and 9 (Fig. 1). It is unknown whether papyrus swamps choke the river valleys between sites 9 and 10, because this area has not been explored in detail.

Tissue samples were collected from fish and macerated in high salt buffer (saturated NaCl, 250 mM EDTA pH 7·5; 20% DMSO) to prevent DNA degradation. DNA was extracted successfully from tissue samples kept in this buffer at room temperature for >1 year. High molecular weight DNA was isolated from small portions of tissue using methods described in Coffroth & Mulawka (1995). Briefly, tissue samples ($\sim$0·5 cm^2) were macerated with CTAB buffer (1·4 M NaCl, 100 mM Tris-HCl pH 8·0, 20 mM EDTA, 2·0% hexadecyl trimethyl-ammonium bromide (CTAB) and 0·2% 2-mercaptoethanol) and proteinase K (0·1 mg ml^{-1}). Samples were incubated for 1–4 h at 65° C followed by phenol-chloroform-isoamyl alcohol extractions and EtOH precipitation overnight at –20° C. The DNA was resuspended in 200 µl of sterile water with RNAse A (0·5 µl per sample of 10 mg ml^{-1} stock solution). Five to 10 ng of DNA were added to 10·5 µl of PCR mix [100 µM each dNTPs, 0·5 µM primer and 0·5 U Primezyme® (Biometra Corp., Tampa, FL, U.S.A.)]. To minimize variation among reactions, all of the reaction components were combined in a master mix with a volume necessary for the number of samples to be amplified. The RAPD amplification programme consisted of an initial cycle of 94° C for 2·5 min, 35° C for 1 min, 72° C for 2 min followed by 45 cycles of 94° C for 1 min, 35° C for 1 min, and 72° C for 2 min, with 72° C for 10 min final extension. PCR products were analysed on a 1·6% agarose-synergel (1 : 1; Diversified Biotech, Boston, MA, U.S.A.) gel and stained with ethidium bromide. DNA of known size and intensity were provided by pGEM® DNA markers (Promega Corp., Madison, WI, U.S.A.). Polaroid photographs of each gel were digitized using a Kodak® digital camera. The intensity and molecular weight of each band was determined using Kodak Digital Science Image analysis software (version 2.02; Eastman Kodak Co. Scientific Imaging Systems, New Haven, CT, U.S.A.). The 126-bp fragment from the size marker was selected to provide the minimum intensity threshold.

The RAPD procedure was adopted because it is a simple and readily available molecular tool that permits evaluation of intraspecific genetic structure across a range of spatial scales, and does not require any prior DNA sequence information for primer development (Grosberg *et al*., 1996). To minimize weaknesses associated with this procedure, individuals from each of the five sites were run together on each gel to minimize gel run differences. For each gel, potential bands that equalled or exceeded the minimum intensity were scored as present. All individuals were run for each primer two or more times and only those bands that amplified consistently were used in the analysis.

Presence/absence data were used to calculate genetic similarity indices for each pair of individuals using the RAPDistance program (version 1.04, Armstrong *et al*., 1994). Genetic variation among and within sites was assessed using analysis of molecular variance (AMOVA, Excoffier *et al*., 1992; Stewart & Excoffier, 1996). Significance of variance components and genetic distances (Φ-statistics) were tested against randomly constructed null distributions.
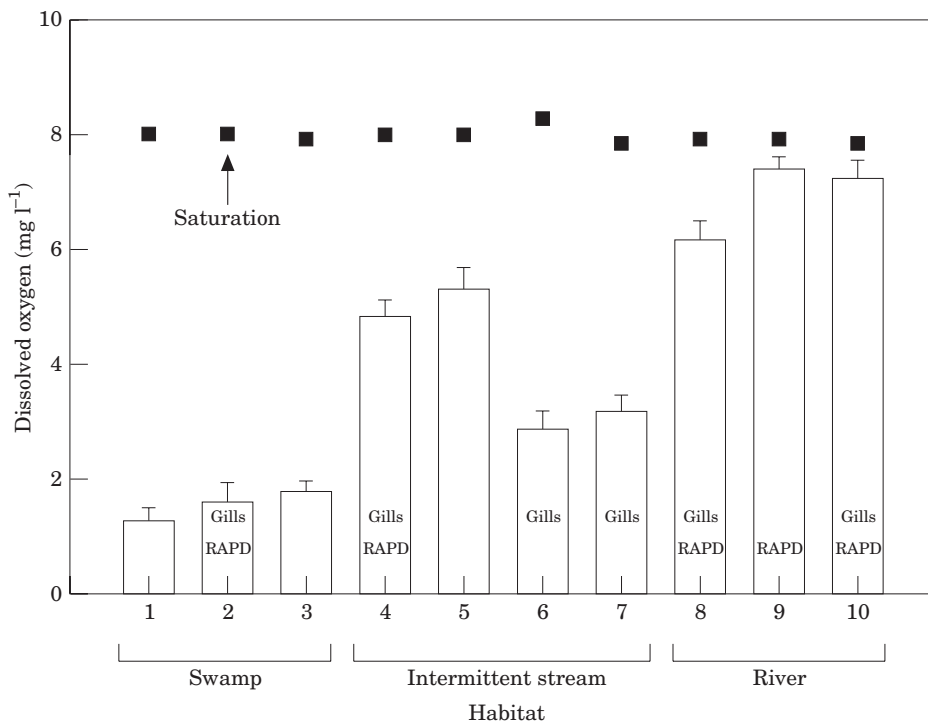
F~IG~. 2. Mean dissolved oxygen levels (mg l^{-1} ± s.e.) for 10 sites in the Mpanga River drainage of western Uganda; three sites within the dense papyrus of the Rwembaita Swamp (sites 1–3); four intermittent stream sites (sites 4–7); and three everflowing river sites (sites 8–10). Each value represents the mean of duplicate samples at a series of microsites taken monthly within each system over a 3-year period (sites 1–8) or a 1-year period (sites 9 and 10). Saturation values for each site are indicated based on the average temperature of the site over the years of study. Sites for genetic (RAPD) analyses and measurements of total gill filament length for *Barbus neumayeri* are indicated on the bars.

RESULTS

ENVIRONMENTAL CONDITIONS

Average monthly oxygen levels were extremely low at all three sites in the Rwembaita Swamp over the 3 years of study (site 1: 1.22 ± 0.18 mg l^{-1}, s.e.; site 2: 1.61 ± 0.27 mg l^{-1}; site 3: 1.79 ± 0.19 mg l^{-1}, Fig. 2). Peak values were observed during seasonal flooding when levels were often >3 mg l^{-1} (Fig. 3). At the four intermittent stream sites, dissolved oxygen levels were significantly higher than in the swamp but varied markedly among sites ranging from an average of 2.92 ± 0.27 mg l^{-1} at site 6 to 3.13 ± 0.17 mg l^{-1} at site 7, 4.83 ± 1.36 mg l^{-1} at site 4, and 5.35 ± 1.73 mg l^{-1} at site 5 (Fig. 2). Seasonal trends were evident with higher values during seasonal flooding (Fig. 3). Dissolved oxygen concentration in both the swamp and intermittent stream sites fell well below saturation values (Fig. 2). Dissolved oxygen values were high throughout most of the sampling periods at both sites in the Njuguta River (site 8: 6.21 ± 0.17 mg l^{-1}; site 9: 7.37 ± 0.23 mg l^{-1}) and at the Mpanga River (site 10: 7.23 ± 0.29 mg l^{-1}, Figs 2 and 3).
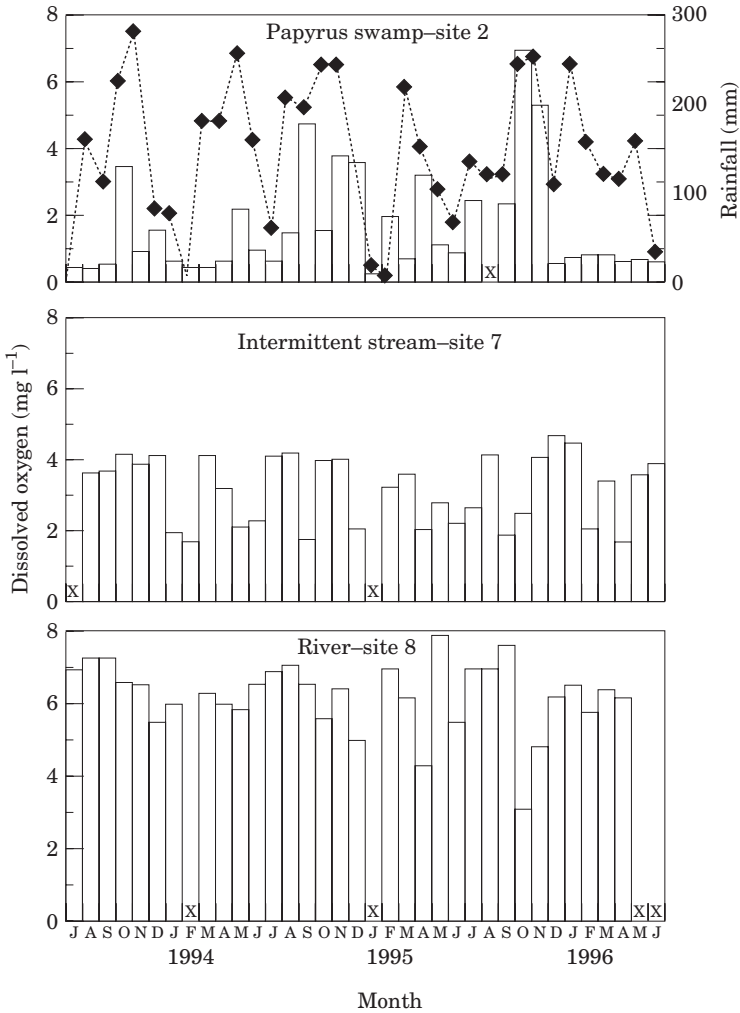
FIG. 3. Mean dissolved oxygen levels (mg l^{-1}) for three sites in Kibale National Park, Uganda: a papyrus swamp (Rwembaita Swamp, site 1, Fig. 1), a small intermittent stream (site 7, Fig. 1), and an everflowing river (Njuguta River, site 8, Fig. 1). Each value represents the mean of duplicate samples at a series of microsites within each system. Seasonal variation in oxygen levels in the Rwembaita Swamp is shown relative to the seasonal pattern of rainfall (mm). No data are available for the sampling periods indicated by (X) due to the activity of forest elephants.

Water temperature averaged 17·3° C across all sites and ranged from 16·8° C at site 6 (an intermittent stream situated in dense forest) to 17·9° C in the everflowing Mpanga River (site 10, Fig. 4). Water temperature showed little variation over the year, with an average range at 4·2° C over the 3 years of study.

GILL MORPHOLOGY

In all populations, total gill filament length increased with body mass (bilogarithmic plots, $r^2 = 0.78$ to 0·95, $P < 0.01$ to $P < 0.001$). An ANCOVA indicated no difference in the slopes of the relationships of total gill filament length and body weight among the six populations ($F = 0.92$, $P = 0.48$); however,
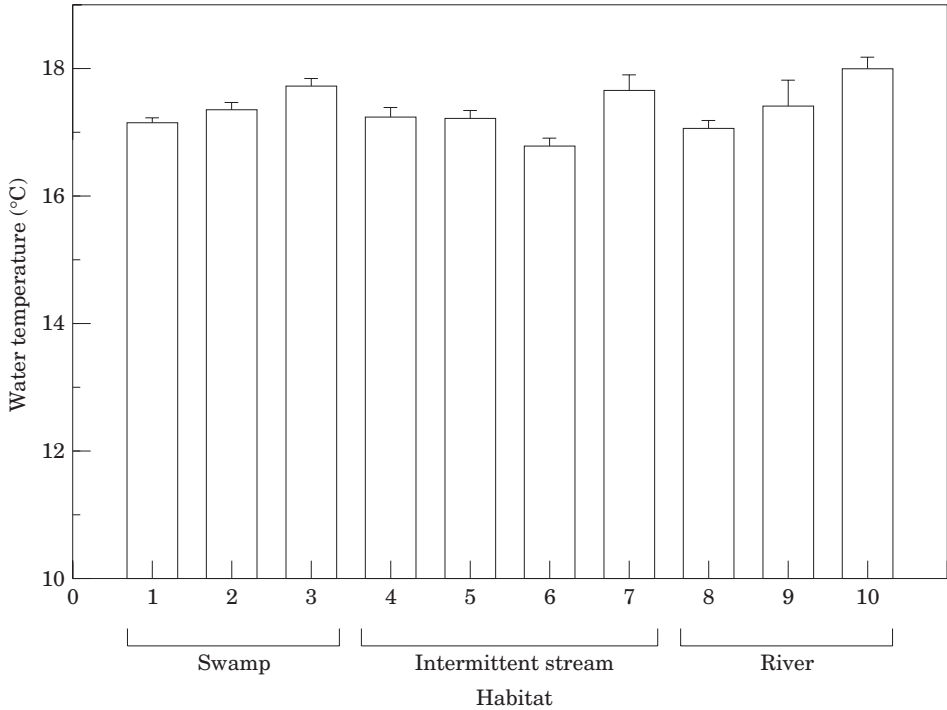
FIG. 4. Mean water temperature (°C ± S.E.) for 10 sites in the Mpanga River drainage of western Uganda: three sites within the dense papyrus of the Rwembaita Swamp (sites 1–3); four intermittent stream sites (sites 4–7); and three everflowing river sites (sites 8–10). Each value represents the mean of duplicate samples at a series of microsites taken monthly within each system over a 3-year period (sites 1–8) or a 1-year period (sites 9 and 10).

the intercepts were significantly different ($F=21·96$, $P<0·001$). For a fish of a given body weight, total gill filament length was larger in the swamp populations (adjusted mean gill size=174·6 cm) than any of the other five populations (adjusted mean: site 4=120·5 cm, site 6=134·9 cm, site 7=127·4 cm, site 8=110·4 cm, site 10=113·8 cm, Fig. 5). Among the small-gilled populations, total gill filament length was smaller at sites 8 and 10 than at site 6. Among the six sites, adjusted mean total gill filament length was negatively related to mean oxygen levels (Fig. 6); and a polynomial regression was used to produce a best-fit line ($y=0·53x^2–0·89x+2·43$, $r^2=0·98$, $P=0·002$). The shape of the relationship suggests that gill size decreases quickly with increasing oxygen availability down to an asymptote (Fig. 6).

Palzenberger & Pohla (1992) reviewed the literature on gill morphology of fishes. From their data set for 28 water-breathing freshwater species, they extracted the mean slope of significant regressions for gill morphological parameters including total gill filament length and body weight. They set the lowest and highest mean values within each parameter range to 0 and 100%, respectively, to create a range of values for each gill parameter. This permitted them to express the values of a species as a percentage within the range of values for freshwater fishes. For total gill filament length, *B. neumayeri* ranged from an
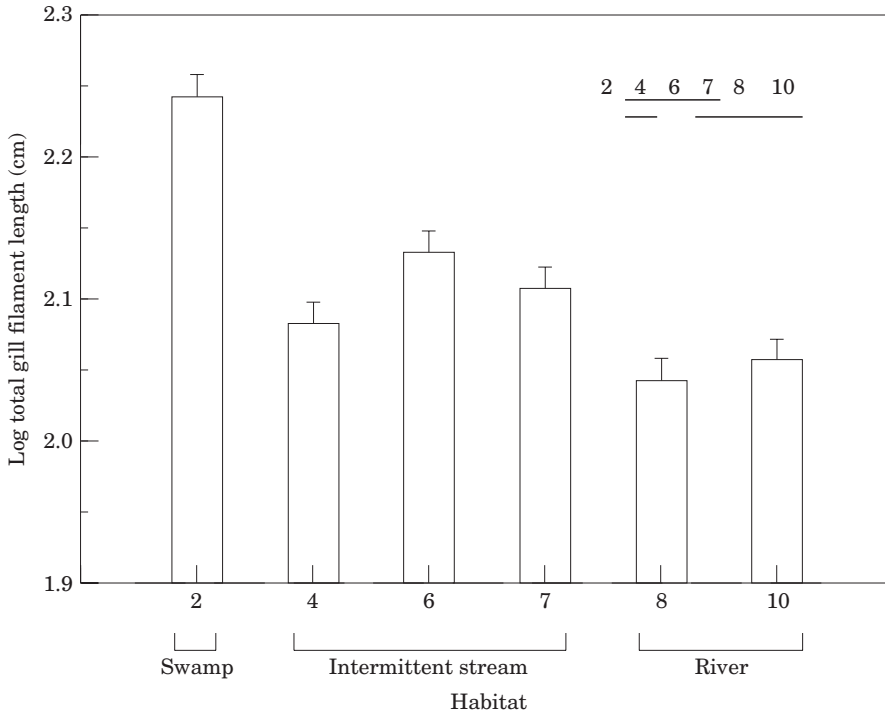
FIG. 5. Weight-adjusted mean total gill filament length (log transformed, ± S.E.) for six populations of *Barbus neumayeri* from sites in the Mpanga River drainage of western Uganda. Sites include a dense papyrus swamp (Rwembaita Swamp, site 2, Fig. 1), three intermittent stream sites (sites 4, 6, and 7, Fig. 1), and two everflowing river sites (sites 8 and 10, Fig. 1). Underlined sites are not significantly different at *P*<0·05 (ANCOVA, Sidak test).

average of 45·6% of the range of freshwater fishes at site 10, an everflowing river to an average of 60·4% of the range in the Rwembaita Swamp.

DISPERSAL

Of the 256 *B. neumayeri* that were recaptured over the study, 93·4% were recaptured in the same site in which they had been marked (residents). Of the 6·6% of the fish that had dispersed (*n*=17), eight had dispersed to another site within the same system (e.g. within Mikana stream or within the Rwembaita Swamp, Fig. 7). Nine fish dispersed from the stream habitat to swamp habitat or *vice versa*, and therefore from sites which produced small-gilled fish to and from sites which produced large-gilled fish (Fig. 7). Dispersers were significantly larger (mean total length=8·1 ± 0·2 cm, S.E.) than residents (mean total length=7·2 ± 0·1 cm, *t*=2·55, *P*=0·011). Eighty-one per cent of the dispersal events occurred during wet seasons when high waters may produce both higher oxygen levels and pathways for movement. It should be noted that our estimate of dispersal is conservative, since it is possible that some of the fish that we failed to recapture had moved to a site that we did not sample.

RAPD ANALYSIS

Of the dozen primers evaluated, two were chosen for this analysis for their consistency in scoring across sites and among individuals (Table I). Individuals
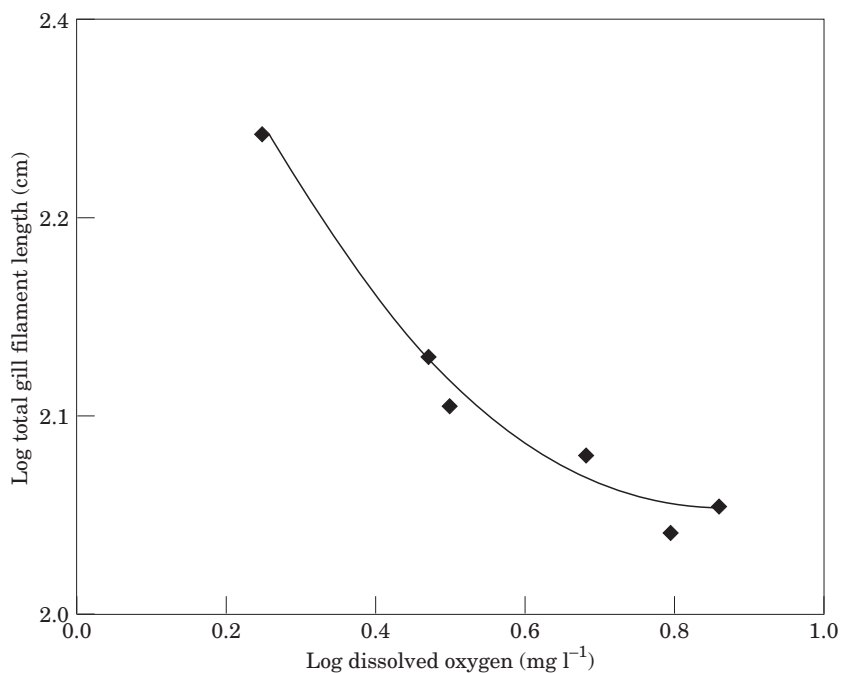
Fig. 6. Relationship between mean weight-adjusted total gill filament length (log transformed) and mean dissolved oxygen concentration of the environment for six populations of *Barbus neumayeri*. All sites are located in the Mpanga River drainage of western Uganda and range from dense papyrus swamp to well-oxygenated everflowing river habitats.

produced from nine to 30 bands for the two primers with an average of nearly 19 bands per individual. Bands used in this analysis varied in size from 200 to 1200 bp. In general any one band was rare; on average a given band occurred in only 20% of the individuals sampled (Table I). Only a small number of the bands were common among sites and individuals. Of the 93 bands only 13 (14%) were found in 30% or more of the individuals. No bands were exclusive to any one site. None the less there was significant genetic differentiation among sites (Table II). Total genetic variance attributable to between-population differences was significant, indicating genetic differentiation among the sampled populations even given the small sample sizes. Examination of genetic differentiation between pairs of sites indicates that seven of the 10 pair-wise comparisons show significant genetic differences. There was a marginally significant difference between sites 2 and 4 ($P=0.082$). Sites 4 and 8, and 2 and 10 were not significantly different from one another. There was no relationship between genetic differences and geographical distances between sites.

DISCUSSION

PAPYRUS SWAMPS AND STRUCTURAL ADAPTATIONS TO HYPOXIA

Papyrus swamps are extensively distributed in East and Central Africa and dominate much of the 85 000 km^2 of permanent swamp on the African continent (Beadle & Lind, 1960; Beadle, 1981; Thompson & Hamilton, 1983). Their extent
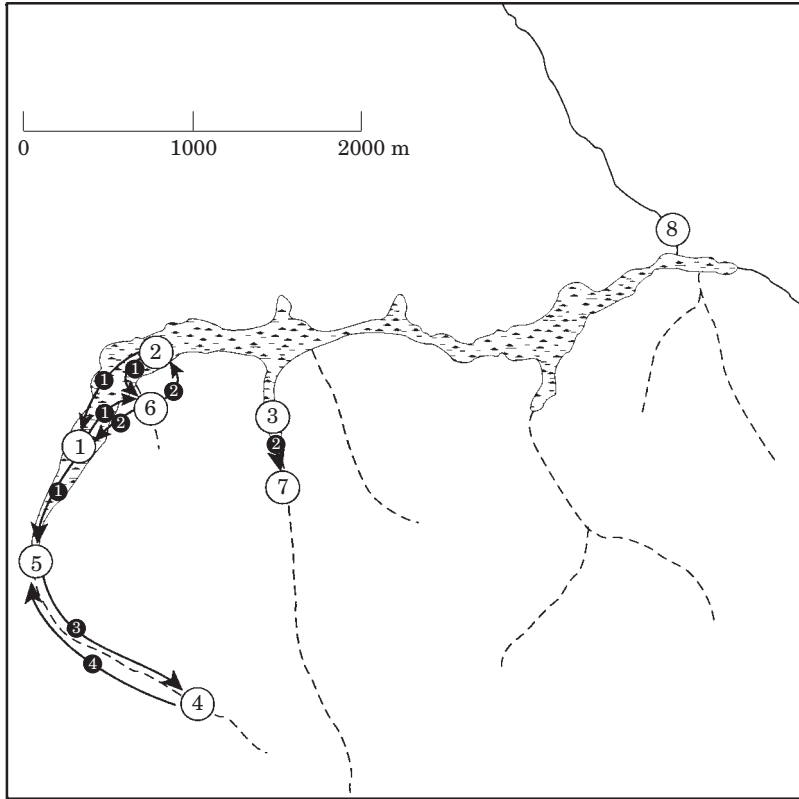
FIG. 7. Patterns of movement for individual *Barbus neumayeri* marked and recaptured between July 1993 and January 1998 in the Rwembaita Swamp system of Kibale National Park, Uganda. The number of dispersers is indicated in a black circle on each movement trajectory. Movement was examined among sites 1–8.

TABLE I. Sequence of RAPD PCR primers used to assess genetic differentiation among *Barbus neumayeri* populations

Primer no.	Sequence	Total no. of bands scored	Band frequencies mean (range)	Mean no. of bands scored/individual
233	5′-CTG AAG CGG A-3′	49	20·3% (4–53)	9·9
376	5′-CAG GAC ATC G-3′	44	19·7% (9–37)	8·7
Total		93	20·0%	18·6

makes them a habitat of great ecological importance, and our results provide several lines of evidence to suggest that these swamps may pose an ecological and genetic barrier for *Barbus neumayeri*. These include: variation in gill morphology among populations that relates to oxygen concentration, limited movement of *B. neumayeri* among swamp and feeder stream sites, and genetic

TABLE II. Analysis of molecular variance (AMOVA) for 68 individuals of *B. neumayeri* using 93 RAPD bands and genetic distances (Φ-statistics) among sites are shown on upper diagonal; lower diagonal shows significance levels based upon probability that distances obtained from random null distribution are greater than observed genetic distances (Excoffier *et al.*, 1992). (Null distribution based upon 5000 iterations)

AMOVA

Source of variation	d.f.	SSD	MSD	Variance component	% of total	*P* value
Among sites	4	0·91	0·227	0·006	3·83	0·038
Within sites	63	9·30	0·148	0·147	96·17	

Genetic distances

Sites	Sample size	2	4	8	9	10
2	15		0·025	0·069***	0·052***	0·014
4	15	0·082		0·010	0·024*	0·037*
8	15	0·000	0·271		0·049***	0·050**
9	21	0·000	0·025	0·000		0·046***
10	16	0·157	0·020	0·013	0·000	

P=*<0·05; **<0·01; ***<0·001.

differences among populations from different neighbouring sites in the drainage. If similar patterns are observed in other non-air-breathing species, these habitats may have important broadscale impacts on the zoogeographical distribution of non-air breathers in the region.

The low dissolved oxygen levels found in the Rwembaita Swamp are not unusually low for papyrus swamps or other heavily vegetated swamps in the region (Beadle, 1932; Carter, 1955; Rosenberger, 1997; Chapman *et al.*, 1998), and would represent stressful conditions for any temperate species (Doudoroff & Shumway, 1970; Davis, 1975). *Barbus neumayeri* survives these extreme conditions by virtue of a low metabolism, high haemoglobin, very efficient use of aquatic surface respiration, habitat selection behaviour, and large gills (Chapman & Liem, 1995; Olowo & Chapman, 1996; Chapman & Chapman, unpubl. data). Given the severe oxygen scarcity characteristic of these systems, one would anticipate strong selection pressure for low-oxygen tolerance that would be relaxed in connected open water systems. For non-air breathers, such as *B. neumayeri*, this may lead to variation among swamp and open water forms in characteristics related to oxygen uptake.

Variation in gill morphology among swamp and open water sites was examined as an indicator of response to low oxygen conditions. Gill filament length was much greater in the swamp population than in the open water sites falling in the upper range for freshwater fishes (60·4%), and varied among the six sites in relation to oxygen availability. In response to severe hypoxia, *B. neumayeri* uses aquatic surface respiration (ASR, Kramer & Mehegan, 1981)

at the air–water interface, ventilating its gills with water from the surface (Chapman & Liem, 1995; Olowo & Chapman, 1996). This is a widespread adaptation to hypoxia among non-air-breathing fishes living in periodically hypoxic habitats (Gee *et al.*, 1978; Kramer & McClure, 1982; Winemiller, 1989; Chapman *et al.*, 1995). However, ASR thresholds (PO_2 level at which ASR is initiated) for swamp-dwelling *B. neumayeri* are very low relative to other tropical freshwater fishes and open water populations of *B. neumayeri* (Kramer & McClure, 1982; Chapman & Liem, 1995; Olowo & Chapman, 1996). Large gill size may contribute to efficient oxygen uptake capacity and facilitate a lower ASR threshold.

The observed interdemic variation in the gill morphology of *B. neumayeri* may be due to underlying genetic differences. However, environmentally induced phenotypic variation (phenotypic plasticity) and/or the interaction of genetic and environmental influences may also contribute to the observed differences in gill size. There are a growing number of studies which have documented phenotypic plasticity in the jaw, skull, and body shape of fishes (Meyer, 1987; Wimberger, 1991, 1992, 1993; Day *et al.*, 1994), and phenotypic plasticity in the gill morphology of the African cichlid *Pseudocrenilabrus multicolor* Scholer, has been documented in response to oxygen availability. Recently, there have been several suggestions that such environmentally induced phenotypic variation can be selectively advantageous and can contribute to the origin of novel traits (Stearns, 1989; West-Eberhard, 1989; Thompson, 1991). Future studies examining the extent to which variation in gill morphology among populations of *B. neumayeri* has an environmental basis, a genetic basis, or represents significant genotype by phenotype interactions (genetic variation that must be present for selection to influence plasticity) will indicate the potential of papyrus swamps in contributing to the morphological diversification of *B. neumayeri* populations.

DISPERSAL AND GENETIC DIFFERENTIATION

Rates of dispersal of *B. neumayeri* from the Rwembaita Swamp system and its tributaries were low. Only 3·5% of the recaptured fish had moved from stream habitat to swamp habitat or *vice versa*, and therefore from sites which produced small-gilled fish to and from sites which produced large-gilled fish. In addition, the only fish which moved from an intermittent feeder stream into the swamp were those from site 6, the stream with the lowest oxygen concentration and the largest gills of the stream/river sites. The maximum distance moved was 1520 m between sites 4 and 5. Most of the dispersal events occurred during wet season conditions when high waters may produce both higher oxygen levels and pathways for movement, and most moves were by fish large enough to be sexually mature. More *B. neumayeri* seem to reach a mature reproductive stage before the seasonal rains than at other times of the year, and reproductive activities peak with seasonal peaks of precipitation (Frankl & Chapman, unpubl. data). Therefore, it is quite possible that reproductively active individuals were among the dispersing individuals.

Although our recovery of marked individuals was low, clearly individuals crossed physicochemical gradients, traversed long distances, within the swamp/ stream system, and included potentially reproductively mature fish. Theoretically the movement rate observed would be sufficient to homogenize gene

frequencies among the populations (Wright, 1978). In addition, our dispersal estimate is conservative, since some individuals not recaptured may have moved to sites not sampled. The observed genetic differences among populations may therefore not reflect insufficient dispersal (genetic isolation), and may represent selection within populations. For example, the low oxygen conditions in the swamp may limit the growth, survival, or reproduction of small-gilled fishes. The large difference in gill size among the swamp and open water sites suggests a morphological cost to swamp-dwelling which may affect competitive abilities of swamp-dwelling *B. neumayeri* that move into an open water system. We have found significant differences in the streamline and trophic morphology of the African cichlid *Pseudocrenilabrus multicolor victorize*, that correspond to intraspecific differences in gill size (Chapman *et al.*, unpubl. data). Such functional–morphological tradeoffs may limit the success of phenotypes crossing oxygen gradients. Alternatively, assortative mating whereby individuals choose mates that are phenotypically like themselves, may greatly limit genetic exchange among populations even if dispersers survive in the new system. Differences in colour between swamp and some river populations is notable and may provide a mechanism for stock differentiation.

In conclusion, this study suggests that *B. neumayeri* populations differ significantly in gill morphology in response to intersite variation in oxygen availability. Whether, the observed variation in gill morphology represents genetic or phenotype variation or their interaction is unknown. However, movement among populations is theoretically sufficient to homogenize gene frequencies. It is proposed that the observed genetic differences between populations may reflect habitat-specific selection pressures.

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