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Regional structuring of genetic variation in short-lived rock pool populations of *Branchipodopsis wolfi* (Crustacea: Anostraca)

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Abstract The genetic structure of three metapopulations of the southern African anostracan *Branchipodopsis wolfi* was compared by analysing allozyme variation at four loci (PGM, GPI, APK, AAT). In total, 17 local populations from three sites (metapopulations) were analysed from rock pools in south-eastern Botswana ranging from 0.2 to 21 m^2 in surface area. In three populations we found significant deviations from Hardy-Weinberg (H-W) equilibrium at one or more loci due to heterozygote deficiencies. Genetic variability at one site was significantly lower than at the other sites, which may be linked to a greater incidence of extinction and recolonisation, as the basins at this site are shallower and have shorter hydrocycles. Across all local populations, a significant level of population differentiation was revealed. More than 90% of this variation was explained by differentiation among sites (metapopulations), although this differentiation did not correlate with geographic distance, or with environmental variables. Genetic differentiation among populations within metapopulations was low, but significant at all sites. At only one of the sites was a significantly positive association measured between genetic and geographic distance among local populations. Our data suggest that persistent stochastic events and limited effective long-range dispersal appear to dominate genetic differentiation among populations of *B. wolfi* inhabiting desert rock pools. The lack of association between geographic distance and genetic or ecological differences between rock pool sites is indicative of historical stochastic events. Low heterozygosity, the significant deviations from H-W equilibrium, and the large inter- but low intra-site differentiation are suggestive of the importance of short-range dispersal. Gene flow between metapopulations of *B. wolfi* appears to be seriously constrained by distances of 2 km or even less.

Key words Divergence · Spatial genetic structure · Gene flow · Dispersal · Egg banks

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Introduction

Despite an assumed high dispersal capacity of resistant resting stages (Hutchinson 1967), significant genetic variation is generally observed among local populations of freshwater invertebrates with complex life cycles. This has been explained by persistent founder effects (Hebert 1974a, 1987a; Boileau and Hebert 1991; Boileau et al. 1992; Berg and Garton 1994; Boileau and Taylor 1994), temporally fluctuating selection (Lynch 1987), and local adaptation (Hebert 1974b; Mort and Wolf 1986; De Meester 1996). Despite significant differentiation in allele frequencies, the association between genetic and geographic distance in zooplankton populations is weak at best (Innes 1991; Lynch and Spitze 1994; Davies et al. 1997; Vanoverbeke and De Meester 1997; but see Giessler 1997). Gene flow estimates derived from gene frequency divergence in invertebrate pond taxa are often low, even though the presence of resistant resting eggs and the rapid colonisation of new habitats suggest high dispersal capacities (De Meester 1996). Boileau et al. (1992) suggested that such poor fit between estimates of dispersal and gene flow is caused by the non-equilibrium nature of these populations and reflect persistent founder events, and Vanoverbeke and De Meester (1997) argue that the genetic differentiation among *Daphnia* populations can be inflated due to genetic drift in cyclic parthenogenetic populations characterised by a low clonal diversity. In populations of the obligately sexual anostracan *Branchinecta coloradensis*, estimates of gene flow corresponded well with ecological estimates of dispersal of propagules via salamanders (Bohonak 1998). Bohonak (1999) argues that dispersal makes a measurable contribution to population genetic differentiation across a ma-

jority of animal species. More information is obviously needed on the effect of geographic distance in shaping the genetic structure of freshwater zooplankton and the effectiveness of resting egg dispersal.

Freshwater anostracans (fairy shrimps) produce banks of resistant eggs that may promote dispersal and gene flow, and their habitats are often small and of an insular nature, characteristics that promote divergence. Studies of population genetic structure in obligately sexual zooplankton are meagre compared to the vast information from cyclically and obligately parthenogenetic species. Studies on species from very short-lived desert pools may be particularly informative, as the impacts of chance processes such as dispersal, colonisation and genetic drift are expected to be higher in such habitats than in the lakes and ponds which are traditionally studied. The limited available information on the genetic structure of freshwater anostracans generally reveals significant levels of local differentiation (Boileau et al. 1992; Riddoch et al. 1994; Davies et al. 1997; Bohonak 1998). This pattern was interpreted by Bohonak (1998) as a balance between drift and gene flow, while Boileau et al. (1992) states that it is all historical artifact.

The southern African fairy shrimp *Branchipodopsis wolfi* is particularly abundant in desert rock pools. As these habitats are very old, their fauna is likely to have reached a drift-gene flow equilibrium (Riddoch et al. 1994). The particular topography of the rocky escarpments in south-eastern Botswana creates a range of geographic distances between pools and sites, generating a suitable design to test for a relation between geographic and genetic distance. Riddoch et al. (1994) found genetic evidence for isolation by distance (maximium: 80 m) between seven populations of *B. wolfi* on one of the rock bluffs also included in the present study and argued that this was probably caused by wind dispersal.

With our study we extend the earlier observations by Riddoch et al. (1994) to a wider geographic scale by the incorporation of two additional rock pool sites. Genetic variation at four polymorphic enzyme loci is studied within and among these three sites. The analysis of the regional structuring of genetic variation will generate more reliable conclusions on the roles of geographic distance for resting egg dispersal and gene flow than was hitherto possible on the basis of a limited number of populations from one site only. The selection of ten more pools (17 in total), furthermore, results in a wider range of pool types permitting a more thorough interpretation of genetic diversity patterns in relation with habitat characteristics such as pool size, pool hydroperiod and water quality.

Materials and methods

Study animal

Branchipodopsis species are distributed widely in southern and eastern Africa (Hamer and Appleton 1996) and are the only anostracans in the region to persist in short-lived rock pools. *B. wolfi* is the most widely distributed and morphologically variable species of the genus (Hamer and Appleton 1996). It matures rapidly (3–4 days) and is very fecund (daily broods of 30–150 resting eggs) under laboratory conditions (Brendonck et al. 1998). Broods are shed at once and eggs sink as loose particles to the bottom. Hatching is light- and temperature-dependent, and not all eggs hatch during a single inundation (Brendonck et al. 1998). This generates egg banks that vary among pools and seasons between 1,000 and 220,000 eggs m^{-2}, corresponding to population sizes of 200–500,000 viable diapausing eggs per pool (Brendonck and Riddoch, in press a).

Study sites

All three sites are situated in south-eastern Botswana. Each location consists of a compact granite outcrop with morphometrically variable pool basins, which are a maximum of 80 m apart. The surface area of the pools ranges from 0.2 to 21 m^2. Two of the rocky outcrops are located in the vicinity of Gaborone (Kgale Hill, KH, and Kgale Siding, KS), and the third one is located 50 km to the west (Thamaga, Th) (Fig. 1). During the wet season (October–April), pools fill intermittently. The average hydroperiod of individual pools ranges from 5 days to more than 1 month. As the permanent dam near KS (Fig. 1) is about 70 m lower than the level of the KS rock pools, it has no influence on the hydrology of these pools with granite basement. Th basins are generally more shallow (8.3±3.0 cm, n=13) and have less vegetation (33% of pools) than pools at KS (9.0±2.6 cm deep, 53% of pools vegetated, n=17) and KH (12.6±3.5 cm deep, 50% of pools vegetated,

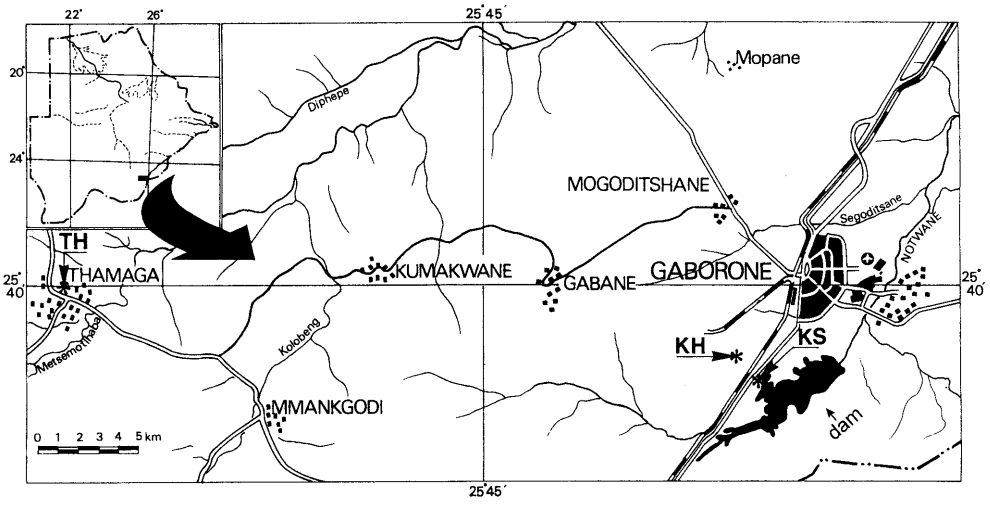

Fig. 1 Geographic location of the studied metapopulations (sites) of *Branchipodopsis wolfi* in south-eastern Botswana (*KS* Kgale Siding, *KH* Kgale Hill, *TH* Thamaga). *Inset*: Botswana

n=4). Th pools also have a shorter mean observed hydroperiod (12±3 days) than pools at the other sites (KS: 18±8 days; KH: 23±12 days) (L. Brendonck, unpublished work). Pools with aquatic vegetation (*Limosella* sp.) have a sediment bed (containing the resting eggs) of about 1 cm covering the entire surface of the basin, while unvegetated pools have a loose sediment layer barely covering the underlaying rock.

A total of 17 pools varying in morphometry and representing a range of geographic distances were selected for study. Seven pools each were selected at KS and Th, and three at KH. Three KS populations (A, J, K) were previously analysed by Riddoch et al. (1994, Fig. 2.2.1: pools 7, 3 and 4, respectively).

Environmental variables

The selected pools were monitored daily for conductivity (μS cm^{-1}), pH, temperature (°C) and depth from day 4 to day 11 after filling. Surface area, maximum depth, surface area/maximum depth ratio, maximum volume and the percentage vegetation cover were also measured for each pool. These variables were selected because they may affect *B. wolfi* growth and population size, and are associated with pool hydroperiod. On the basis of yearly observations in December/January (1993–1998) we also estimated the average duration of a hydrocycle and the percentages of hydroperiods that exceeded 15 days. A period of 15 days is enough for reproduction even when low temperatures or high population densities delay maturation. An additional three pools were sampled (KS-K, Th-2, Th-8), but not monitored for conductivity, pH, temperature and depth. Differences among sites for these variables were assessed by ANOVA and post hoc contrasts among sites were conducted using Bonferroni tests (Systat 8, SPSS Inc.).

Ecological similarities between all 17 pools were analysed by means of hierarchical clustering on the basis of standardised values of habitat characteristics using Ward's amalgamation method and Euclidean distances (Systat 8, SPSS Inc.).

Genetic analysis

Approximately 60 specimens per pool were collected between 27 and 29 November 1995, at which time the animals were about 10

Table 1 Estimates of genetic variability at four loci in 17 populations of *Branchipodopsis wolfi* from three sites (*KH, KS, Th*), SEs in parentheses

Population	Mean heterozygosity				
	Mean sample size per locus	Mean number of alleles per locus	Number of polymorphic loci (0.99 criterion)	H_o direct-count	H_e unbiased estimate
Kgale Hill					
KH-3	39.8 (3.9)	2.8 (0.8)	3	0.359 (0.120)	0.435 (=0.154)
KH-4	39.5 (0.5)	2.8 (0.8)	3	0.336 (0.118)	0.417 (0.142)
KH-6	46.3 (2.7)	3.5 (1.0)	3	0.321 (0.108)	0.474 (0.164)
Mean		3.0		0.348	0.442
Kgale Siding					
KS-A	49.5 (0.3)	2.5 (0.3)	4	0.334 (0.049)	0.374 (0.055)
KS-C	34.8 (2.8)	2.8 (0.3)	4	0.446 (0.042)	0.460 (0.038)
KS-F	39.5 (0.3)	2.3 (0.3)	4	0.412 (0.054)	0.420 (0.051)
KS-J	27.5 (1.2)	3 (0.4)	4	0.339 (0.074)	0.440 (0.046)
KS-K	38.8 (0.5)	2.5 (0.3)	4	0.355 (0.029)	0.396 (0.057)
KS-L	53.5 (3.3)	2.8 (0.5)	4	0.442 (0.049)	0.425 (0.059)
KS-M	39.8 (0.3)	2.8 (0.3)	4	0.389 (0.028)	0.466 (0.019)
Mean		2.7		0.396	0.426
Thamaga					
Th-1	31.0 (2.4)	2 (0.4)	3	0.219 (0.093)	0.247 (0.106)
Th-2	48.5 (0.5)	1.8 (0.3)	3	0.195 (0.136)	0.248 (0.127)
Th-3	42.8 (0.3)	2.5 (0.5)	3	0.154 (0.093)	0.218 (0.113)
Th-8	36.0 (0.0)	2.5 (0.6)	3	0.208 (0.099)	0.238 (0.115)
Th-9	34.8 (3.0)	2 (0.4)	3	0.259 (0.118)	0.221 (0.098)
Th-10	24.0 (5.0)	2 (0.7)	2	0.145 (0.084)	0.230 (0.134)
Th-12	34.8 (2.8)	1.8 (0.3)	3	0.219 (0.095)	0.235 (0.103)
Mean		2.1		0.210	0.234

days old. Specimens were transported alive to the laboratory where they were snap-frozen in liquid nitrogen. Whole organism homogenates were screened for protein variation using Titan III cellulose acetate gels (Helena Laboratories) following the methodology of Hebert and Beaton (1993). After screening for polymorphy and quality of staining, four enzyme loci were retained: PGM (EC 2.7.5.1), GPI (EC 5.3.1.9), APK (EC 2.7.3.3), and AAT (EC 2.6.1.1). These loci were also used in a previous study on some KS populations using the starch gel method (Riddoch et al. 1994). To align banding patterns from different runs and populations, we used a *Daphnia magna* clone as reference. In addition, half of one individual from each run was used in the subsequent run. Runs that gave bad staining resolution were dropped from the analysis resulting in variable sample sizes per locus (Table 1).

Descriptive statistics, *F*-statistics and hierarchical analyses were carried out using Biosys-1 release 1.7 (Swofford and Selander 1989). Exact tests for Hardy-Weinberg equilibrium, exact tests for population differentiation, unweighted pair-group method with arithmetic averaging (UPGMA) clustering, and Mantel tests were performed with TFPGA (Miller 1997a). These applications were selected from the various programs according to performance and quality of the resulting graphs and tables. Where concurrent applications were available, results generated by one selected program were always compared with those of another program. All such comparisons revealed almost identical results confirming the reliability of the selected applications.

The percentage of polymorphic loci was calculated using the 0.99 criterion. Average heterozygosity was estimated based on the proportion of heterozygous individuals (H_o, "direct count" method) and according to the unbiased estimate (H_e) of Nei (1978). The calculation of the exact probability of rejecting Hardy-Weinberg (H-W) equilibrium at each variable locus was performed using the conventional Monte Carlo method (Guo and Thompson 1992) using ten batches of 2000 permutations each. As the standard error of all estimated *P*-values was smaller than 0.01, there was no need to increase the number of batches. A sequential Bonferroni test was applied to correct for multiple testing (Rice 1989). Statistical genotypic independence between two loci at all populations was analysed with a Markov chain method to estimate (without bias) exact probabilities (genepop-1.2.; Raymond and Rousset 1995a). Differences between sites in levels of genetic diversity (numbers of alleles per locus, heterozygosity) were analysed by ANOVA with post hoc Bonferroni multi-comparison tests (Systat 8, SPSS Inc.).

To determine significant differences in allele frequencies among all populations and among populations at each site, the exact test of Raymond and Rousset (1995b) was applied using a Markov chain method with 1000 dememorisation steps and 2000 permutations per batch. The numbers of batches were adjusted between 20 and 40 to obtain standard errors of less than 0.01. Additionally, the Fisher (1954) combined probability test was employed as a global test over loci to determine the overall significance in allele frequencies. *F*-statistics were used to analyse genetic differentiation among populations. For each variable locus, F_{is}, F_{it}, and F_{st} values were computed for the complete set of populations as well as for each site separately, F_{st} being calculated according to Nei (1977; G_{st}). A weighted average of *F*-values across alleles was calculated for each polymorphic locus, and a mean value was then determined for all polymorphic loci. Finally, a hierarchical analysis of population differentiation combined across loci was performed using the formulation of Wright (1978), with populations, sites and the total set of populations as the different levels of population organisation. To further illustrate the pattern of observed genetic differentiation, a hierarchical cluster analysis using UPGMA (Sneath and Sokal 1973) was constructed on the basis of the original distance metric of Rogers (1972). To generate increased confidence in the constructed tree, a bootstrapping sampling procedure was applied with 1000 permutations. The association of Rogers' genetic distance with log-transformed geographic distance for each pair of populations within and among sites was analysed using the test of matrix correspondence with 10,000 permutations of Mantel (1967).

As greater habitat size usually goes together with greater population size and greater number of micro-habitats may affect the genetic diversity of the considered populations, we assessed the association between measures of habitat size (surface area, maximum depth, surface area/maximum depth ratio, volume) and inundation period of the studied rock pools with genetic diversity (numbers of alleles, heterozygosity) of the respective *B. wolfi* populations. As in some cases (e.g. Riddoch 1993; Johannesson et al. 1995) allozyme frequencies correlated to environmental variables, we have tested for an association between allele frequencies and all environmental variables measured. Associations between environmental variables and genetic characteristics (genetic diversity measures, allele frequencies) were assessed with principal component analysis run for a correlation matrix in a varimax rotation mode and with the Pearson correlation coefficient with Bonferroni adjusted probabilities (Systat 8, SPSS Inc.).

The genetic structure of the three populations (KSA, J, K) previously described by Riddoch et al. (1994) (sampling date: February 1992) were compared with the present results (sampling date: November 1995). For each population, allele frequencies at each locus were compared between years with an exact test of contingency (Miller 1997b).

Results

Environmental variables

Clustering of all 17 pools by environmental similarity (both hydrological and physical/chemical characteristics) revealed more frequent clustering of KH pools with KS pools than with Th pools, five of which formed a separate group (Fig. 2).

There were significant differences among study sites with respect to conductivity (ANOVA, F=4.405, 2 *df*, P=0.039), temperature (F=88.313, 2 *df*, P<0.001), and average depth (F=7.436, 2 *df*, P=0.015) of the study pools. The selected pools at Th had a significantly

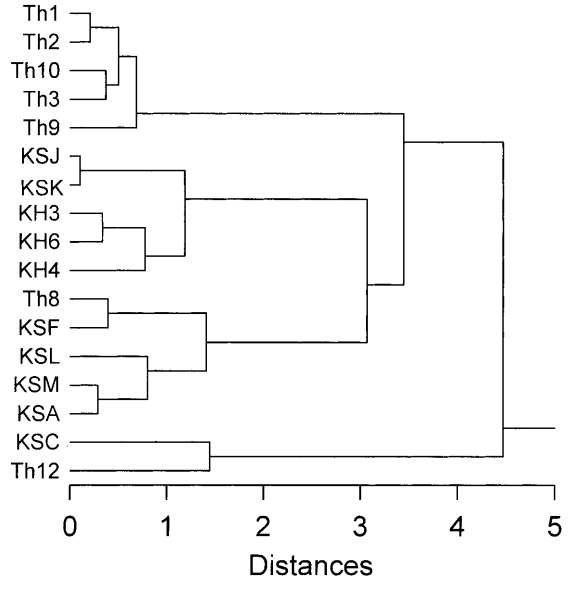

Fig. 2 Hierarchical clustering of 17 study pools from three metapopulations (*KH, KS, Th*) on the basis of standardised values of ecological variables using Ward's amalgamation method and Euclidean distances (Systat)

(P<0.05) lower conductivity (16.4±6.7 µS cm^{-1}) than pools at KS (26.8±5.6 µS cm^{-1}) and at KH (25.5±5.9 µS cm^{-1}). The average temperature of the studied pools was different among all sites (Th: 29.3±0.5°C; KS: 26.3±0.6°C; KH: 24.5±0.6°C). The average depth of studied Th pools (6.5±2.9 cm) was significantly less than that of pools at KH (10.1±3.5 cm) but not significantly different from the average depth of KS pools (8.3± 4.1 cm). The mean values of all other monitored variables of water quality and habitat size and inundation period were not significantly different among sites.

Genetic analysis

Table 1 shows that the populations were polymorphic at four (all KS populations), three (all others except Th-10), or two (Th-10) of the four loci. At KH and Th, the APK locus was monomorphic, whereas there were two alleles in the KS populations. There was an overall significant difference among sites for numbers of alleles (ANOVA, F=13.079, 2 df, P=0.001) as well as for heterozygosity (H_e) (F=118.820, 2 df, P<0.001). Thamaga populations had a significantly (P<0.01) lower number of alleles, and lower heterozygosity levels than populations at KH and KS. Using exact p-values allele frequencies differed significantly (P<0.05) from H-W expectations due to heterozygote deficiencies at at least one locus in 8 of the 17 populations (all KH populations, two KS (KS-J and K-SM) and three Th (Th-2, Th-3 and Th-8) populations). After sequential Bonferroni correction, deviations from H-W equilibrium remained significant in only three populations: KH-3: GPI (P=0.0003); KH-6: PGM (P<0.0001), GPI (P<0.0001) and Th-2: PGM (P<0.0001). There was no significant (P>0.01) linkage disequilibrium between locus pairs in any population.

Table 2 F-statistics based on allele frequencies within *B. wolfi* populations from three metapopulations (KH, KS, Th). Probabilities of the observed differences in allele frequencies according to exact tests for population differentiation (Raymond and Rousset 1995a, 1995b) are also indicated. Overall significance over loci was determined using Fisher's combined probability test

Locus	F_{is}	F_{it}	F_{st}
A All 17 populations			
PGM	0.164	0.458	0.351***
GPI	0.139	0.334	0.227***
APK	0.056	0.451	0.418***
AAT	0.104	0.261	0.175***
All	0.124	0.379	0.291***
B All 3 KH populations			
PGM	0.212	0.225	0.016***
GPI	0.310	0.335	0.037**
AAT	0.119	0.149	0.034**
All	0.224	0.247	0.029***
C All 7 KS populations			
PGM	0.098	0.109	0.012
GPI	0.019	0.070	0.052***
APK	0.056	0.106	0.053***
AAT	0.116	0.126	0.011**
All	0.076	0.105	0.031***
D All 7 Th populations			
PGM	0.219	0.228	0.012
GPI	0.013	0.028	0.016
AAT	0.086	0.113	0.029**
All	0.132	0.151	0.021*

*P<0.05, **P<0.01, ***P<0.001

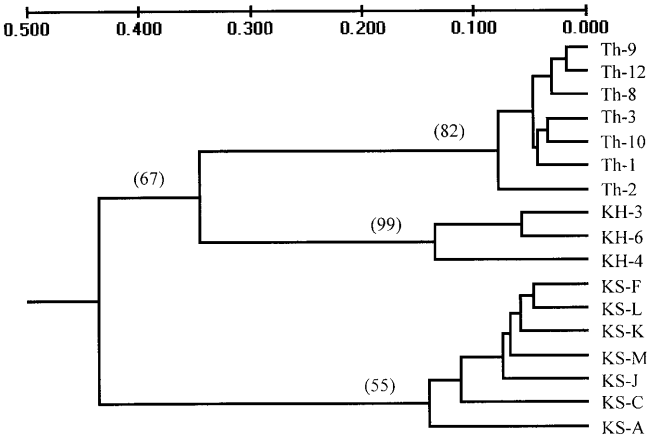

Fig. 3 UPGMA dendrogram based on the original genetic distance of Rogers (1972) (corresponding distance values are given on the *scale*) and pairwise comparisons of 17 populations of *B. wolfi* from three metapopulations (*KH, KS, Th*). Bootstrap values over loci presented in *parentheses*

Allele frequencies were significantly (P<0.001) heterogeneous for all loci across all populations (Table 2A). Within sites, allelic heterogeneity was significant averaged over all loci (Th: P=0.0246; KH and KS: P<0.001) (Table 2B–D). When all 17 populations were pooled, a large proportion of total inbreeding (F_{it}) was due to genetic differentiation among populations (F_{st}) (Table 2A). The relatively high F_{is} values (inbreeding within pools, about one-third of total) are indicative of some substructuring within local populations. Within sites local genetic differentiation was low averaged over all loci (F_{st}: 0.021 for Th, –0.031 for KS) but significant (Table 2B–D). Hierarchical analysis of F-statistics across all loci for all populations (Wright 1978) indicated that most (>90%) of the variance in allele frequencies among *B. wolfi* populations was due to differences among the three sites. Clustering of populations also shows clearly differentiated sites (Fig. 3). Populations at the two neighbouring sites (KH and KS) were genetically not more related to each other than to the Th site, which is 50 km apart (Figs. 1, 4A). The average genetic distances between KH and KS populations and between KS and Th populations were significantly (Tukey pairwise comparison, P<0.05) higher than the genetic distance between KH and Th pools (Fig. 4A). This lack of a clear geographical pattern in the genetic structure of the sites resulted in low bootstrap values (<70%) (Fig. 3). Mantel's test revealed a strong association between genetic and log-transformed geographic distance over all populations (r=0.8807; P=0.001) which mainly resulted from the significant

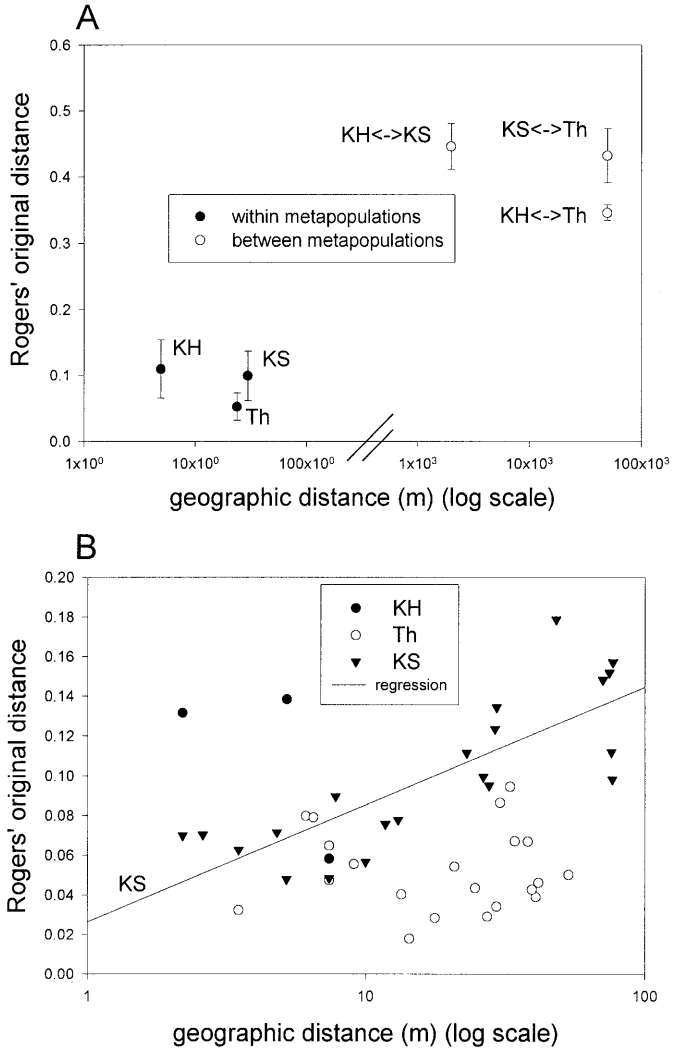

Fig. 4 Relationship between **A** the average geographic distance (*logarithmic scale*) and the average value (±SD) of Rogers' original distance metric within and among three metapopulations (*KH, KS, Th*) of *Branchipodopsis wolfi*, and **B** the geographic distance (*logarithmic scale*) and Rogers' original distance metric for all pairwise comparisons of populations within each metapopulation. The *regression line* shows the positive relation for the KS data

Table 3 Loadings of habitat variables on the components (PC) of two principal component analyses (PCAs) including **A** measures of genetic diversity and **B** allele frequencies. Each PCA was run for a correlation matrix in a varimax rotation mode. In both analyses the association of interest lay in the first factor

Variables	PC1	PC2	PC3	PC4
A Including measures of genetic diversity				
Temperature	–0.836	0.276	–0.397	–0.147
H_e	0.830	–0.248	0.169	0.439
Number of alleles	0.776	–0.413	0.184	0.113
Conductivity	0.738	0.410	0.041	0.398
H_o	0.630	–0.119	0.096	0.695
pH	0.509	0.610	0.085	–0.315
Maximum volume	–0.063	0.974	0.101	0.037
Surface	–0.173	0.972	–0.007	0.035
Surface/depth	–0.315	0.924	–0.146	0.042
%Successful floodings	0.056	–0.115	0.955	–0.126
Observed duration	0.191	–0.051	0.901	0.202
Maximum depth	0.310	0.293	0.842	–0.152
%Polymorphic loci	0.240	–0.062	0.153	0.910
%Vegetation cover	0.111	0.163	–0.328	0.779
%Variance explained	25.212	26.721	20.246	17.769
B Including allele frequencies				
GPI_D	0.940	–0.213	–0.138	0.189
GPI_E	–0.889	–0.077	0.037	–0.153
PGM_A	0.865	–0.181	–0.110	0.169
AAT_G	0.707	0.328	–0.274	0.019
Temperature	–0.681	–0.498	0.191	–0.256
GPI_B	0.591	0.348	0.107	0.199
AAT_C	–0.529	0.463	0.313	0.085
pH	0.526	–0.101	0.650	0.067
PGM_B	0.522	0.765	–0.124	0.063
AAT_B	–0.508	–0.778	0.104	–0.096
PGM_E	–0.013	–0.984	0.070	–0.002
PGM_I	–0.192	0.971	–0.078	0.041
APK_G	–0.210	0.955	–0.008	0.050
APK_B	0.210	–0.955	0.009	–0.050
PGM_H	–0.449	–0.782	0.299	–0.148
%Vegetation cover	0.076	0.677	0.171	–0.500
Conductivity	0.253	0.645	0.494	–0.079
Maximum volume	–0.131	–0.050	0.965	0.106
Surface	–0.192	–0.109	0.943	–0.001
Surface/depth	–0.277	–0.176	0.875	–0.130
AAT_E	–0.117	0.341	0.569	0.457
%Successful floodings	0.135	0.114	–0.110	0.942
Observed duration	0.339	0.413	–0.022	0.771
Maximum depth	0.385	0.056	0.301	0.753
PGM_D	0.243	–0.105	–0.179	–0.222
GPI_G	–0.005	0.232	–0.042	–0.478
%Variance explained	21.930	29.264	15.859	11.798

association within the KS site (r=0.8097; P=0.006) (Fig. 4B). Mantel's correlations for the Th and KH sites were not significant (r=–0.0145, r=–0.6853; P=0.5, P=0.8) (Fig. 4B).

For the purpose of this study, the PCA results of interest lie in the individual principal components (PC) which include measures of genetic diversity (Table 3A) and allele frequencies (Table 3B) as major variables to separate out the habitat characteristics that were best correlated with these genetic variables. The first PCA revealed an association on the first factor between measures of genetic diversity (H_e, numbers of alleles) and temperature (negatively correlated). The second analysis indicated an association on the first factor between temperature and allele frequencies at the GPI, PGM and AAT loci. There was no significant correlation between any of the measures of genetic diversity and any measure of pool size or duration. There was a significant (P<0.05) correlation between average pool temperature and genetic diversity measures, and between temperature and allele frequencies at the PGM, GPI and AAT loci of the respective populations.

For the three populations (KSA, J, K) that were studied in both 1992 and 1995, allele frequencies at the four studied loci were not significantly different (P>0.05) between the two sampling dates, suggesting that the among-year variation in allele frequencies is low.

Discussion

Genetic variation within populations

In three out of the 17 populations of *B. wolfi* studied, a significant deviation from H-W equilibrium with heterozygote deficiency was observed at one or several loci. Significant deviations from H-W equilibrium due to heterozygote deficiencies were also found in populations of *Branchinecta sandiegonensis*, another anostracan occurring in short-lived pools (Davies et al. 1997). These observations on freshwater anostracans from ephemeral pools are in contrast to patterns in temporary cladoceran populations that generally are in H-W equilibrium (Hebert 1987b). Heterozygote deficiencies can be explained by selection, non-random mating, inbreeding, or population subdivision (Wahlund effect). Genotype-dependent larval mortality in short-lived pools could be an important selective factor, but so far there is no obvious reason why, in our study, this should affect the KH populations with a high incidence of significant deviations from H-W more than the hydrologically similar KS populations (Fig. 2) where deviations from H-W were less pronounced. Selective differences between sites related to predation or competition were not studied and can, therefore, not be excluded. Assortative mating has so far not been found in any anostracan and is therefore considered as an unlikely explanation for the observed heterozygote deficiencies in *B. wolfi*. It could, however, be cryptic and hard to detect in the field. Although active population densities in *B. wolfi* can be low (L. Brendonck and B. Riddoch, personal observations) and may cause temporary bottlenecks during one hydroperiod (generation), strong inbreeding is not likely in zooplankton species with persistent egg banks, especially in the case of *B. wolfi* where the shallow egg banks are not structured and where all eggs are exposed to hatching stimuli. The most likely cause of the observed heterozygote deficiency is a Wahlund effect resulting from the simultaneous hatching of eggs from different (often bottlenecked) generations, and/or from mixing of eggs from several pools by overflows after heavy rains. Some dried *B. wolfi* eggs float when rehydrated, and these hatch significantly better than equally viable sinking eggs (Brendonck et al. 1998; Brendonck and Riddoch, in press b). This phenotype could promote short-range dispersal and mixing of populations. The short average distance between pools at the KH site in comparison with the average distance between pools at the other sites (Fig. 4A) supports this hypothesis. Relatively high levels of sub-structuring of local populations (F_{is}) in *B. wolfi* (Table 2) may also result from such mixing of populations due to overflows.

The local populations studied at Th had a significantly lower number of alleles and heterozygosity than the studied populations at the other sites. We found no significant correlation between pool size/duration and genetic diversity in our limited set of pools. However, when all 13 Th pools are considered, they had significantly shorter average hydroperiods than the entire set of pools at the other sites (see Study sites). The low genetic variability at the Th site may therefore be caused by the loss of genetic variation due to severe bottlenecks in the egg banks or to repeated extinctions and recolonisations of at least some populations depleting the total genetic variation of populations at one site. Loss of genetic variation due to bottlenecking is experimentally demonstrated in other organisms (e.g. McCommas and Bryant 1990; Leberg 1992), and Gilpin (1991) showed that the effective size of a metapopulation with turnover may be only a fraction of its census size. Even though the production of resting eggs by *B. wolfi* reduces the likelihood of true extinctions, the threats may be different in Th pools because some are very shallow and it is conceivable that the resting egg bank of a given pool may be flushed away during heavy rains. Future studies will also evaluate the importance of egg bank sizes in determining genetic diversity at these sites.

Genetic differentiation among populations

UPGMA clustering of Rogers genetic distance grouped populations into three main clusters corresponding to the three sites. There was a moderate level of genetic differentiation within (Rogers distance <0.15) and a high level of differentiation among these entities (Rogers distance 0.35–0.45). The structure of each rock pool site does not conform to the original metapopulation concept of Levins (1970), as in our study system the habitat patches (pools) are of unequal quality and local populations have different chances of extinction (Brendonck and Riddoch, in press a). However, as each site consists of discrete local populations among which there is gene flow, by migration of wind-blown (Brendonck and Riddoch 1999) or overflowing resting eggs or even adults (authors, personal observations), the setting of populations at each site meets "the broader view of a metapopulation that no longer has population turnover as a necessary characteristic" (Hanski and Gilpin 1997). The term "metapopulation" can, therefore, be used interchangeably with "site" in our study.

Across all populations studied, the level of among-population genetic differentiation (F_{st}=0.291) is higher than local differentiation in the freshwater anostracans *Artemiopsis stefanssoni* (F_{st}=0.075) (Boileau et al. 1992) and *Branchinecta coloradensis* (F_{st}=0.124) (Bohonak 1998), but comparable to local differentiation in *B. paludosa* (F_{st}=0.360) (Boileau et al. 1992) and much lower than in a set of *B. sandiegonensis* populations (F_{st}=0.657) sampled on a spatial scale of up to 50 km (Davies et al. 1997). In obligately sexual brine shrimps, the reported levels of population differentiation varied between F_{st}=0.24 in *Artemia franciscana* and F_{st}=0.12 in *A. salina* (Abreu-Grobois and Beardmore 1982; Abreu-Grobois 1987), whereas only a weak overall genetic differentiation was observed among nine *A. sinica* populations (F_{st}=0.02–0.25) separated by as much as 1000 km

(Naihong et al., in press). The level of among-population genetic differentiation observed in anostracans is relatively high in comparison with other obligately sexual zooplankton such as calanoid copepods (Boileau and Hebert 1988; Boileau et al. 1992), but moderate compared to cyclically parthenogenetic cladocerans (Innes 1991; Carvalho 1994; Hebert and Wilson 1994; Lynch and Spitze 1994; Thier 1994). Obviously, these comparisons should be interpreted carefully because of the evident confounding effects of number and types of allozyme loci, different geographic scales and of different habitat types that are considered in these studies. By incorporating two more metapopulations of *Branchipodopsis wolfi* in our analysis, the F_{st} value observed by us has increased fourfold in comparison with the estimate in Riddoch et al. (1994) (F_{st}:≈0.07). In addition, it has been indicated that the high F_{st} value observed in cladocerans is due to the parthenogenetic mode of reproduction rather than to differences in gene flow per se. Data presented by Vanoverbeke and De Meester (1997) strongly suggest that erosion of genetic diversity in small *Daphnia magna* populations, due to clonal selection during the parthenogenetic phase, may increase differentiation among populations because of genetic drift.

At the within metapopulation level, differentiation is significantly (Tukey pairwise comparison, $P<0.05$) lower at Th than at KH and KS (Table 2, Fig. 4A). This inferred high level of gene flow at Th corresponds with direct observations of dispersal by wind of resting eggs at that site (Brendonck and Riddoch 1999). Th pools have a larger surface, are more shallow and are less vegetated than pools at the other sites, making them more prone to droughts and wind-borne dispersal.

It seems most likely that genetic divergence among local populations and metapopulations of *B. wolfi* results from low dispersal capabilities and long lasting founder effects. Stochastic events linked to the founding of anostracan populations by a small number of individuals are considered to be very important in short-lived and fragmented pools (Davies et al. 1997). These initial gene frequency differences between populations may persist for thousands of generations as a consequence of a founder-flush event (Boileau et al. 1992; Boileau and Taylor 1994; Nichols and Hewitt 1994). Rapid population growth in *B. wolfi*, implied by the short maturation time and high fecundity, is in agreement with the assumptions of a founder-flush process.

On the basis of the limited set of environmental variables screened, there is no evidence that high genetic divergence among sites resulted from differences in selective environments experienced by the studied specimens during the short inundation period. Conductivity, temperature and average depth of the study pools varied significantly among sites but the hydrologically and phenotypically most similar sites (KS and KH) were genetically most divergent (see Figs. 2, 3, 4A). This is consistent with expectations that allozymes are selectively neutral (e.g. Kimura 1983; Futuyama 1986; but see e.g. Riddoch 1993; Johannesson et al. 1995). It is very unlikely that the measured correlation between average pool temperature and allele frequency at three loci (GPI, PGM, AAT) at the same time is the result of selection, as this association is usually only manifested at the GPI locus (Riddoch 1993). It is, therefore, more likely that this association is caused by chance due to the low genetic diversity at the Th site that also held pools with the highest average water temperature. Of course, our assessment of the environmental characters was limited, and more information is required on differences in biotic variables (predation, parasitism, competition).

Genetic divergence between populations may also result from temporally fluctuating selection mimicking genetic drift (Lynch 1987). The similarity in allele frequencies among years of the three KS populations studied by us and Riddoch et al. (1994), suggests, however, genetic stability over the short term. Frequent extinction/recolonisation events in a natural metapopulation structure (Wade and Mc Cauley 1988; Piertny and Carvalho 1995; but see Slatkin 1985; Dybdahl 1994; Harrison and Hastings 1996) and bottleneck-flushes (Taggart et al. 1990) may under some conditions also promote among-population genetic divergence, but are also implausible explanations in the present case, because diapausing egg banks are extensive at the genetically most structured KS site (Brendonck and Riddoch, in press a), making true extinction unlikely. Adult *B. wolfi* population densities vary in space and time, and because pools often hold less than a few hundred animals, it may seem likely that drift could be rapid (Boileau and Taylor 1994). However, delayed hatching from the mixed egg bank may reintroduce lost alleles from previous years and stabilise allele frequencies (Hairston and DeStasio 1988; Boileau and Hebert 1991; Davies et al. 1997), hence reduce the likelihood that genetic drift may occur rapidly.

The high level of genetic differentiation among metapopulations and the weak genetic structuring within individual metapopulations results in a strong overall effect of geographic distance. Inter-metapopulation differentiation was, however, not markedly associated with distance. KH and KS metapopulations, separated by less than 2 km, were genetically more different than the KH and Th sites, which are about 50 km apart (Fig. 4A). This suggests that a distance of less than 2 km may already be an effective barrier to gene flow in this species. This estimate corresponds with a distance of about 3 km that was considered to be an effective barrier to gene flow among *Daphnia pulex* populations (Innes 1991). In our study, the average geographic distances among all pools at each of the three sites did not correlate with the corresponding average levels of population differentiation (Fig. 4A). In individual metapopulations, a positive relationship between geographic and genetic distance was only clear at KS (Fig. 4B). Pools KSA and KSC, separated by more than 20 m from each other and from the other pools, were clearly differentiated, as illustrated in the UPGMA dendrogram (Fig. 3) and as revealed by the significant Mantel's correlation between genetic

and geographic distance at this site. A similar pattern was revealed by Riddoch et al. (1994) based on samples obtained in 1992. The spatial genetic structuring of *B. wolfi* metapopulations (low intra-, high inter-metapopulation differentiation) suggests limited effective dispersal. Wind was suggested to be a short-range vector at KS (Riddoch et al. 1994), but its effectiveness in dispersal of resting stages could only be shown at the Th site (Brendonck and Riddoch 1999). At KS, water birds may be an important vector due to the presence of a large dam in the neighbourhood (Fig. 1), while at the Th site cattle are abundant. In addition, part of the egg bank of *B. wolfi* floats after inundation and may represent an efficient means of short-range dispersal by overflow between pools (Brendonck and Riddoch, in press b). The limited long-range dispersal in *B. wolfi*, causing a strong regional patterning of gene pools, may also be an important factor in explaining the high morphological variability between regions observed in this species (Hamer and Appleton 1996) which may eventually lead to allopatric speciation. The potential for divergence is usually enhanced in organisms with a subdivided population structure (Templeton 1982). In addition, the rapid loss of genetic variation due to chance events, as here suggested for the Th populations, may be a precursor to speciation (Mayr 1954; Carson and Templeton 1984). Our results suggest that the metapopulations of *B. wolfi* are important units of evolution.

To estimate the relative importance of stochastic events versus natural selection in shaping the spatial genetic structure of this species, future research will focus on the genetic aspects of colonisation and dispersal, and on patterns of genetic differentiation in ecologically relevant traits such as life history traits, under common garden conditions. The integration of information on these ecologically relevant traits and (quasi)neutral traits may shed light on the mechanisms causing genetic differentiation at the within or among metapopulation level in these anostracans that are characteristic of very short-lived desert pools.

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