

## Direct and indirect measures of dispersal in the fairy shrimp *Branchipodopsis wolffi* indicate a small-scale isolation-by-distance pattern

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### Abstract

We compared dispersal rates and patterns using both spatial genetic structure as well as direct measures of dispersal in *Branchipodopsis wolffi*, a freshwater anostracan inhabiting clusters of spatially fragmented and temporally unpredictable ephemeral rock pools in southeastern Botswana. For a total of 29 populations from three rock pool sites, the active population component was subjected to allozyme analysis for four loci and gene flow between populations was estimated. For a subset of the pools, we quantified the number of viable floating dormant eggs and larvae dispersing into overflow traps during flood events. Genetic and geographic distances were significantly correlated within each site. Gene flow estimates indicated from 0.6 to 227 migrants per generation. This relatively high effective dispersal rate corresponds with our direct observation of peak dispersal between pools during floods. Up to 784 viable dormant eggs and 301 larvae were trapped at one overflow during one single rainfall event. We determined that a distance of 50 m is already an effective barrier to gene flow for this species. There is ample effective genetic communication between different populations within each rock pool site, but this communication is limited by distance.

Dispersal is a key process influencing the dynamics and evolution of populations and species (Mayr 1963). At regional scales, dispersal ranges set the spatial limits for colonization of new sites (e.g., Jenkins and Buikema 1998; Cáceres and Soluk 2002; Louette and De Meester 2005) and influence the probability of extinction of local populations (e.g., Ciofi and Bruford 1999; Vos et al. 2001). Among-population dispersal is also an important determinant of metapopulation structure, defining the units within which dynamics and evolution are considered (e.g., Harrison and Hastings 1996). Understanding patterns and consequences of dispersal is also important for several applied topics, including viability analysis of fragmented populations (e.g., Mossman and Waser 2001; Jenkins et al. 2003), risk evaluation of escape of genetically modified organisms into natural populations (e.g., Ellstrand and Hoffman 1990), control of epidemic diseases, and invasion of exotic species (e.g., Havel et al. 2002; Colautti et al. 2005).

Many lentic freshwater habitats are island-like in topographic structure and display strong temporal heterogeneity, particularly in unpredictable ephemeral habitats. Long-term persistence of zooplankton species in geographically variable and temporally unstable habitats can be accomplished through dispersal and diapause strategies (Bilton et al. 2001). The importance of dispersal in the ecology and evolution of zooplankton is currently subject to much debate. Because of the possession of dormant stages, many freshwater invertebrates have, since Darwin (1859), been characterized as frequent, widespread dispersers, an idea consistent with their supposedly cosmopolitan distribution. In recent decades, however, detailed morphological (e.g., Frey 1982) and several molecular (e.g., Hebert and Wilson 1994) studies have shown a strong provincialism and the existence of cryptic species, indicating that the notion of cosmopolitanism in zooplankton is incorrect. A number of recent studies on the role of dispersal in structuring zooplankton communities have yielded contrasting views. Shurin (2000), in an experimental field study, found little evidence for an effect of dispersal limitation in shaping local zooplankton community structure in ponds. This is consistent with the observations of rapid colonization of new ponds in natural settings by Louette and De Meester (2005) and of artificial pools by Cohen and Shurin (2003). Together with the increasing amount of studies showing the effect of birds as important vectors (e.g., Figuerola et al. 2005), these studies suggest that dispersal rates generally are high (for review see De Meester et al. 2002; Havel and Shurin 2004). This

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interpretation of limited dispersal limitation contrasts, however, with findings of Jenkins (1995) and Jenkins and Buikema (1998), in a study monitoring the colonization of environmentally similar new ponds, of a strong effect of stochasticity in the pattern of community assembly, leading them to reject the notion that dispersal in most freshwater taxa is frequent and widespread. This is supported by the observations of Jenkins and Underwood (1998). Cáceres and Soluk (2002) also stressed that increased attention should be focused on the potential role of dispersal limitation for understanding the structure and functioning of aquatic communities (for review see Bohonak and Jenkins 2003).

Clusters of pools with variable levels of geographic isolation offer a good system for studying genetic patterns of isolation by distance as an indirect measure of effective dispersal. *Branchipodopsis wolfi* is a specialist anostracan, inhabiting mainly highly unpredictable and very ephemeral rock pools organized in relatively dense clusters throughout semiarid southern Africa. In such small rock pools with variable phenology and topographic organization, stochastic processes such as dispersal, colonization, and genetic drift are expected to have greater effects on the population genetic structure than in larger permanent and more stable habitats (e.g., Boileau and Taylor 1994).

Riddoch et al. (1994) revealed significant genetic differentiation among seven *B. wolfi* populations at one site in southeastern Botswana. Although genetic distances were more similar among neighboring pools, there was no significant pattern of isolation by distance. Averaged over all pools, the estimated number of migrants per generation ( $N_m$ ) was between two and four. Brendonck et al. (2000) extended this study to a total of 17 pools distributed over three sites. Most of the genetic differentiation could be attributed to the subdivision in geographically separated clusters, whereas genetic differentiation between populations was low and only significant at one site. There was no correlation between genetic and geographic distances between the rock pool sites that were separated by 2 to 50 km, respectively. Within the individual sites, a significant isolation-by-distance pattern could only be confirmed at one of the three sites. Both the studies by Riddoch et al. (1994) and Brendonck et al. (2000) thus indicate that isolation by distance is only weakly important at a local scale, whereas the pattern becomes random from distances of 2 km or even less. The strength of this conclusion was weakened by the limited resolution in the spatial analyses in these studies (Zeller et al. 2006). Also, the exact constraining distance for efficient dispersal for this species could not be determined because of a gap in the distance covered between 75 m and 2 km. By using egg traps at different sites around and between pools, Brendonck and Riddoch (1999) empirically demonstrated limited short-range wind-borne dispersal of viable dormant eggs. These authors speculated that dispersal of floating eggs and larvae by means of overflows is probably a more important mechanism of dispersal and gene flow. If this is the case, then we would expect a strong pattern of isolation by distance, as dispersal by overflows is a very local process.

Given the controversy over the importance of dispersal in freshwater zooplankton and the lack of high-resolution studies on patterns of genetic variation in a large number of regional populations as a measure of dispersal efficiency, we here build further on previous work by increasing the spatial resolution and combining our genetic analysis with direct measures of dispersal through overflows in the field. Our study aims to quantify whether and from what distance dispersal limitation has a detectable effect on the population genetic structure of local populations of *B. wolfi*. We report on spatial patterns of genetic differentiation among 29 *B. wolfi* populations from three geographically separated rock pool sites. Two of these sites were also examined in Brendonck et al. (2000). Another site, separated by less than 2 km from the nearest one of these, was included to increase the resolution of the spatial analysis. To have a direct measure of dispersal by means of overflows, we also quantified numbers of viable floating dormant eggs and hatched larvae flowing among rock pools during heavy rains.

## Methods

*Study animal*—The anostracan genus *Branchipodopsis* is distributed throughout southern and eastern Africa. It is the only anostracan genus in this region that can persist in temporary rock pools in which the inhabitants are exposed to unpredictable hydrological conditions combined with a harsh physical environment. *B. wolfi* is obligately sexual and oviparous, with dormant eggs produced throughout the adult life span. When pools fill with water, part of the dormant eggs produced in earlier growing seasons hatch (Brendonck et al. 1998). As a result of this partial hatching, dormant eggs produced by different generations build up dormant egg banks. These mixed and long-lived egg banks reach densities of up to 220,000 eggs  $m^{-2}$  (Brendonck and Riddoch 2000) and serve as a means to bridge dry conditions and buffer for demographic catastrophes due to early drying.

*Study site and sampling*—Populations in the current study are situated in the hardveld region of southeastern Botswana, characterized by granite outcrops with clusters of ephemeral rock pools. The populations are clustered in three geographically separated sites, two at Thamaga and one at Kgale Siding (further referred to as Th-I, Th-II, and KS). The two Th sites are about 200 m apart, whereas Th and KS are separated by 50 km (Fig. 1). The maximum distance between two pools at the same site was about 200 m (Th-II). In all three sites, spatial positions were measured with a 100-m tape measure. Generally, Th pools are shallower and have shorter hydroperiods than KS pools (average hydroperiod: Th-I =  $10 \pm 1.7$  d, Th-II =  $11 \pm 2.0$  d, KS =  $16 \pm 3.0$  d [Hulsmans et al. unpubl. data]). The hydroperiod of the individual pools ranges from 1 d to more than 1 month. At Th-I, Th-II, and KS 9, 15 and 5 pools, respectively, had sufficient population sizes for analysis.

From the 29 populations, 60 specimens per pool were collected using 200- $\mu$ m nets during December 1998 and

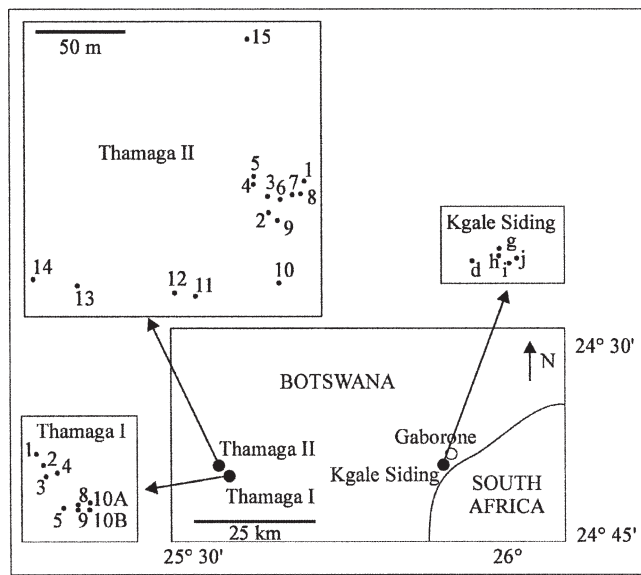


Fig. 1. Geographic location of the three rock pool sites in Botswana (capital Gaborone) with indication of the populations of *Branchipodopsis wolffi* studied within each of the sites. The scale within the Thamaga II site indicates the distance within each of the sites.

January 1999. Specimens were immediately stored at  $-80^{\circ}\text{C}$  before being transported to Belgium in liquid nitrogen. In the laboratory, they were stored again at  $-80^{\circ}\text{C}$  until genetic analysis.

**Allozyme electrophoresis**—On the basis of Riddoch et al. (1994) and Brendonck et al. (2000), four allozyme loci that stained well and could be interpreted accurately were used to assess genetic variation: phosphoglucosmutase (*PGM*, EC 5.4.2.2), glucose-6-phosphate isomerase (*GPI*, EC 5.3.1.9), arginine kinase (*ARK*, EC 2.7.3.3), and amino aspartate transferase (*AAT-I*, EC 2.6.1.1). Whole-organism homogenates were screened for protein variation using Titan III cellulose acetate gels (Helena Laboratories). Staining recipes and procedures followed Hebert and Beaton (1993) with minor modifications. To align banding patterns from different runs and populations, standards (i.e., half of an individual that was run previously and a specimen of a genetically characterized *Daphnia magna* clone) were included in each electrophoretic run. All heterozygotes stained consistently with known quaternary structure (Hebert and Beaton 1993). Allelic variants were designated by numeric values relative to the most common allele, which was arbitrarily assigned mobility of 100. Individuals that could not be scored accurately were not analyzed.

**Data analysis**—Deviations from Hardy–Weinberg equilibrium and genotypic linkage disequilibrium across loci and populations were assessed using the exact test available in GENEPOP 3.3 (Raymond and Rousset 1995). Genetic variability measures, including mean number of alleles per locus (*A*), percentages of polymorphic loci based on the 0.99-criterion (*P*), and observed and expected heterozygos-

ity ( $H_O$  and  $H_E$ ) were calculated for each population using GENETIX 4.04 (Belkhir et al. 2002). To determine the degree of genetic differentiation among populations, pairwise  $F_{ST}$  estimates (following Weir and Cockerham 1984) were calculated using GENEPOP 3.3 (Raymond and Rousset 1995). The significance levels were adjusted for multiple tests using the sequential Bonferroni correction (Rice 1989). Differences between sites in levels of genetic diversity measures were analyzed by analysis of variance using the software package STATISTICA version 6 (StatSoft Inc. 2004).

We characterized the spatial genetic structure by partitioning the genetic variance among rock pool sites and populations using hierarchical *F*-statistics (Weir and Cockerham 1984) in the context of an island model, computed with the software package TFPGA 1.3 (Miller 1997). The average inbreeding coefficient within populations ( $F_{IP}$ ) and within the total of all populations ( $F_{IT}$ ) was computed, and the proportion of genetic variance was determined for both among populations within sites ( $F_{PS}$ ) as well as among sites ( $F_{ST}$ ). To determine whether the observed genetic structure can be explained by genetic isolation through geographic distance, we plotted pairwise  $F_{ST}/(1 - F_{ST})$  ratios against the interpopulation geographic distances following Rousset (1997). The significance of the relation between genetic differentiation and geographic distance was assessed by means of a Mantel test, performing 10,000 randomizations, using the software package IBD 1.5 (Bohonak 2002).

The number of effective migrants between each pair of populations was estimated as  $Nm = (1/F_{ST} - 1)/4$  (Wright 1931). By plotting the values of  $Nm$  against the geographic distances, we estimated the constraining distance for efficient dispersal.

**Short-range dispersal by means of overflows**—To quantify outflow of dormant propagules and hatched larvae of *B. wolffi* during abundant rain, overflow traps were mounted between individual rock pools at Th-I and KS. Naturally eroded outflows of these pools were, at approximately 0.5 to 1 m downstream of the source pool, canalized by means of plasticine to force all overflowing water to pass through a 200-mL vial with 100- $\mu\text{m}$  bottom net. In total, 11 different overflows between different pools were studied during three rainfall events. One pair of these connected pools was analyzed genetically. After abundant rainfall events, vials were removed and rinsed thoroughly to collect all trapped organisms. New traps were put in position to await new rains. In the laboratory, *B. wolffi* eggs and larvae were counted under the dissection microscope. Eggs were tested for their viability by squeezing the propagules and testing for the presence of a yolky embryo. At one instance of abundant rain, pools were overflowing while we were at the site, and the rate of outflowing propagules per hour could be quantified.

## Results

**Within-population genetic diversity**—Over a total of 1,611 individuals, we found four alleles at locus *PGM* and locus

Table 1. Genetic variability estimates at four loci in 29 populations of *Branchipodopsis wolfi* at three sites (Kgalé Siding [KS], Thamaga I [Th-I], Thamaga II [Th-II]): mean number of alleles per locus (A), mean number of polymorphic loci based on the 0.99-criterion (P), and observed and expected heterozygosity averaged over all loci ( $H_O$  and  $H_E$ ) with indication of significant deviations from Hardy–Weinberg equilibrium for one or more loci in the concerned population.

Population	A	P	$H_O$	$H_E$
<b>Kgalé Siding</b>				
KSD	3	4	0.5490	0.5517
KSG	2.5	4	0.3923	0.3747
KSH	2.5	4	0.4060*	0.3644
KSI	2.5	4	0.4353	0.4483
KSJ	2.5	4	0.4148**	0.3950
Mean	2.6	4	0.4395	0.4268
<b>Thamaga I</b>				
Th-I1	1.7	2	0.0805	0.0951
Th-I2	1.7	2	0.0524	0.0616
Th-I3	1.5	2	0.0763	0.0687
Th-I4	1.75	2	0.0688	0.0760
Th-I5	2	2	0.1287	0.1163
Th-I8	1.5	2	0.0745	0.0674
Th-I9	1.5	2	0.1223	0.1089
Th-I10A	1.5	2	0.1250	0.1249
Th-I10B	1.5	2	0.0858*	0.1229
Mean	1.6	2	0.0905	0.0935
<b>Thamaga II</b>				
Th-II1	2	3	0.0890**	0.1374
Th-II2	2	3	0.1590	0.1631
Th-II3	1.5	2	0.0833	0.0763
Th-II4	2	3	0.0751	0.0854
Th-II5	1.75	2	0.0641*	0.0747
Th-II6	1.75	2	0.0550	0.0683
Th-II7	2	3	0.1006	0.0925
Th-II8	2	2	0.0551	0.0562
Th-II9	2	3	0.0711	0.0748
Th-II10	1.75	2	0.1776	0.1846
Th-II11	1.75	2	0.1691	0.1495
Th-II12	2.25	2	0.1701*	0.1843
Th-II13	2	3	0.1103*	0.1660
Th-II14	2	3	0.1892*	0.1906
Th-II15	1.75	3	0.2730	0.2879
Mean	1.9	2.5	0.1228	0.1328
Mean overall populations	1.9	2.6	0.1652	0.1735

\*  $p < 0.05$ .

\*\* significant after sequential Bonferroni correction.

*AAT-1*, three alleles at locus *GPI*, and two alleles at locus *ARK*. Genetic variability measures for the 29 *B. wolfi* populations are shown in Table 1. There were significant differences among sites in number of alleles ( $F_{2, 26} = 43.48$ ,  $p < 0.000001$ ), heterozygosity ( $H_E$ :  $F_{2, 26} = 70.80$ ,  $p < 0.000001$ ;  $H_O$ :  $F_{2, 26} = 63.09$ ,  $p < 0.000001$ ), and number of polymorphic loci based on the 0.99-criterion ( $F_{2, 26} = 19.47$ ,  $p < 0.000007$ ). The KS populations revealed a significantly higher (Tukey post-hoc,  $p < 0.006$ ) genetic diversity than the Th-I and Th-II populations. For the five populations at the KS rock pool site the mean number of alleles per locus ranged from 2.5 to 3.0. At the Th-II site (15 populations), between 1.5 and 2.25 alleles were found per

locus. For the nine Th-I populations these numbers ranged from 1.5 to 2. At the KS site all four selected loci had multiple alleles. Based on the 0.99-criterion three loci (*PGM*, *ARK*, *AAT-1*) were polymorphic in the Th-II populations, whereas at the Th-I site only two were variable (*PGM* and *GPI*). Heterozygosity averaged across loci ranged from 0.0524 (Th-I2) to 0.5490 (KSD) for observed heterozygosity ( $H_O$ ) and from 0.0616 (Th-I2) to 0.5517 (KSD) for expected heterozygosity ( $H_E$ ). Observed heterozygosities  $H_O$  were significantly higher (Tukey post-hoc,  $p < 0.0002$ ) in KS populations ( $H_O$ , mean = 0.4395) compared to those in Th-II ( $H_O$ , mean = 0.1228) and Th-I ( $H_O$ , mean = 0.0905). The same pattern (Tukey post-hoc,  $p < 0.0002$ ) was found for expected heterozygosities  $H_E$  (KS:  $H_E$ , mean = 0.4268; Th-II:  $H_E$ , mean = 0.1328; Th-I:  $H_E$ , mean = 0.0935). Only 9 of 76 tests for Hardy–Weinberg equilibrium revealed significant departures at the 5% significance level, and only two of them remained significant after sequential Bonferroni correction: KSJ (*PGM*) and Th-II1 (*AAT-1*). All disequilibria were attributed to a deficit of heterozygotes. Tests of linkage disequilibrium for each locus pair within each population were all nonsignificant after sequential Bonferroni correction. The different loci can therefore be considered as providing independent information on population genetic structure.

*Between-population genetic differentiation*—The hierarchical partitioning of spatial genetic variance is presented in Table 2. Genetic differentiation among sites was highly significant ( $F_{ST} = 0.4698$ ). Analysis across all loci and all populations indicated that about 47% of the total variance in allele frequencies among *B. wolfi* populations was attributable to differences between the three sites. The neighboring Th sites were genetically more similar ( $F_{ST} = 0.4456$ ) than they were to the KS site, situated about 50 km to the east (Th-I – KS:  $F_{ST} = 0.5242$ ; Th-II – KS:  $F_{ST} = 0.5051$ ), but genetic differentiation was highly significant between all three sites. Within the KS and Th-II sites, genetic differentiation between populations averaged over all loci was significant (Th-II:  $F_{PS} = 0.1272$ ; KS:  $F_{PS} = 0.0470$ ). Within Th-I the among-population genetic differentiation ( $F_{PS} = 0.0220$ ) did not differ significantly from zero. The mean value of  $F_{IT}$  was significantly greater than zero. As both  $F_{PS}$  and  $F_{ST}$  are also significant, homozygote excess in the total sample was caused both by subdivision within as well as among sites.

*Correlation between genetic and geographic distance*— $F_{ST}/(1 - F_{ST})$  ratios for all pairs of populations increased with geographical distance in all three rock pool sites (Fig. 2), revealing an isolation-by-distance pattern (Rousset 1997). A Mantel test indicated a highly significant relation ( $p = 0.0002$ ) between  $F_{ST}/(1 - F_{ST})$  ratios and geographic distance at the large Th-II site (max. distance between pools: 199 m), with the distance separating the populations accounting for 54% of the variation in genetic differentiation ( $r = 0.7322$ ). Within the KS site (max. distance between pools: 24 m), a significant relation ( $p = 0.0478$ ) between geographic and genetic distance existed,

Table 2.  $F$ -statistics at different hierarchical levels (within the total of all populations [ $F_{IT}$ ], among populations within sites [ $F_{PS}$ ], among sites [ $F_{ST}$ ], and within populations [ $F_{IP}$ ]) for 29 populations of *Branchipodopsis wolffi* at three sites (Kgale Siding [KS], Thamaga I [Th-I], Thamaga II [Th-II]).

Populations	Locus	$F_{IT}$	$F_{PS}$	$F_{IP}$	
Kgale Siding	<i>PGM</i>	0.2169	0.0075	0.2110	
	<i>GPI</i>	0.1009	0.1045	-0.0040	
	<i>ARK</i>	-0.0262	0.0336	-0.0619	
	<i>AAT-1</i>	0.0317	0.0695	-0.0406	
	Over all loci	0.0783	0.0470*	0.0327	
Thamaga I	<i>PGM</i>	0.0684	0.0289	0.0406	
	<i>GPI</i>	0.0366	0.0068	0.0300	
	<i>ARK</i>				
	<i>AAT-1</i>	-0.0003	-0.0021	0.0018	
	Over all loci	0.0583	0.0220	0.0371*	
Thamaga II	<i>PGM</i>	0.2395	0.1417	0.1139	
	<i>GPI</i>	0.3808	0.3428	0.0578	
	<i>ARK</i>	0.0416	0.0463	-0.0049	
	<i>AAT-1</i>	0.0886	0.0271	0.0632	
	Over all loci	0.2018*	0.1272*	0.0855*	
Overall populations		$F_{IT}$	$F_{PS}$	$F_{ST}$	$F_{IP}$
	<i>PGM</i>	0.6474	0.5968	0.5578	0.1255
	<i>GPI</i>	0.1901	0.1729	0.0397	0.0209
	<i>ARK</i>	0.4737	0.4969	0.4790	-0.0461
	<i>AAT-1</i>	0.3839	0.3829	0.3516	0.0017
	Over all loci	0.5388*	0.5114*	0.4698*	0.0561

Tests of significance through bootstrapping over loci.  
\*  $p < 0.05$ .

with 35% of the variation in genetic differentiation explained by geographic distance ( $r = 0.5949$ ). Also at the Th-I site (max. distance between pools: 42 m) we did observe a significant relation ( $p = 0.0223$ ) between geographic distance and genetic differentiation, with 19% of the variation in genetic differentiation explained by geographic distance ( $r = 0.4295$ ).

Estimates of  $Nm$  within and between sites indicate high gene flow between pools at short distances (within sites: up to 227 effective migrants per generation) (Fig. 3). For most pairs of populations separated by 50 m or more, the

number of migrants per generation became very small (populations separated by less than 50 m: mean  $Nm = 32 \pm 36.5$ ; populations separated by 50 m or more: mean  $Nm = 3 \pm 7.4$ ).

*Direct estimate of short-range dispersal by means of overflows*—A considerable number of viable dormant eggs and larvae of *B. wolffi* were captured in overflow traps, with up to 784 viable eggs and 301 larvae trapped during

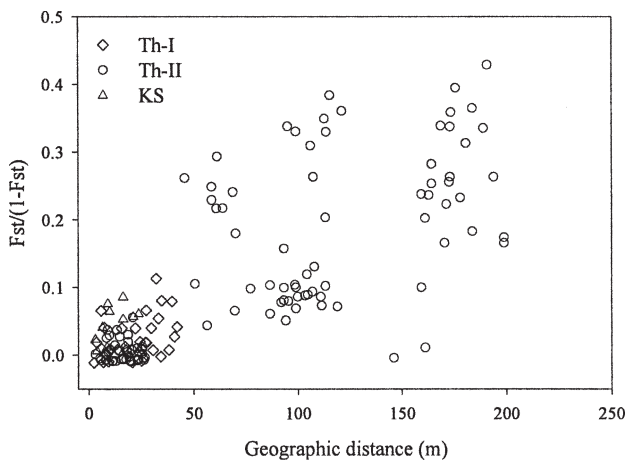


Fig. 2. Isolation-by-distance plot of  $F_{ST}/(1 - F_{ST})$  versus geographic distance (m) of all pairwise combinations of *Branchipodopsis wolffi* populations within each of the three rock pool sites. Kgale Siding (KS), Thamaga I (Th-I), Thamaga II (Th-II).

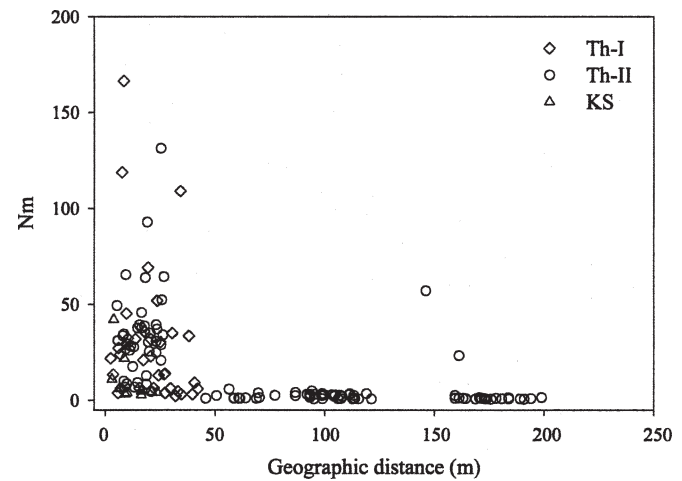


Fig. 3. Plot of estimated number of effective migrants per generation ( $Nm$ ) versus the geographic distance (m) of all pairwise combinations of *Branchipodopsis wolffi* populations within each of the three rock pool sites. Kgale Siding (KS), Thamaga I (Th-I), Thamaga II (Th-II).

Table 3. Number of viable dormant eggs and larvae of *Branchipodopsis wolfi* captured in overflow traps at Thamaga I and Kgale Siding during three rain events. The roman numerals indicate different overflow traps. At one occasion (Thamaga I, 08 Feb 00) dispersal rates could be calculated.

Rains	Overflow	Eggs	Larvae	Total
Thamaga I				
25 Jan 00	I	5	0	5
	II	70	0	70
	III	120	0	120
	IV	42	0	42
	V	0	0	0
	VI	25	0	25
07 Feb 00	I	784	301	1,085
	II	451	157	608
	III	54	2	56
	IV	21	8	29
	V	7	13	20
	VI	0	0	0
08 Feb 00	I	85 (57/h)	70 (46/h)	155 (103/h)
	II	192 (128/h)	71 (46/h)	263 (175/h)
	III	2 (1/h)	0	2 (1/h)
	IV	0	0	0
	V	18 (12/h)	19 (12/h)	37 (25/h)
	VI	0	0	0
Kgale Siding				
10 Jan 04	I	0	0	0
	II	0	0	0
	III	0	0	0
	IV	51	1	52
	V	48	1	49
11 Jan 04	I	0	0	0
	II	0	0	0
	III	11	0	11
	IV	15	0	15
	V	12	0	12
12 Jan 04	I	1	0	1
	II	0	0	0
	III	12	0	12
	IV	9	3	12
	V	10	3	13

a single overflow event associated with a shower (Table 3). Trapping of dispersing propagules while pools were overflowing revealed a dispersal rate of between 0 and 128 eggs and 0 and 46 larvae per hour.

There were marked temporal differences in ranking of pools with regard to numbers of dispersants, particularly at Th-I. For instance, Th-I overflow I only delivered five dispersants on 25 January 2000 but 1,085 dispersants on 07 February 2000.

## Discussion

Our study has revealed a significant relation between geographic and genetic distance for four allozyme loci in the anostracan *B. wolfi* inhabiting ephemeral pools on three geographically separated rock pool sites in southeastern Botswana. This consistent pattern indicates that distance is an important constraint on the effective dispersal (i.e.,

dispersal resulting in gene flow) of active organisms or their dormant eggs of this species between pools within each site. Geographic distance explained 19–54% of the variation in genetic differentiation among populations at the three sites. The smaller maximum geographic distances between any two pools at both the KS (24 m) and Th-I site (42 m) in comparison with Th-II (199 m) probably explains the observed variation in the effect of geographic isolation among these sites. The stronger isolation-by-distance pattern at KS in comparison with Th-I, in turn, may be related to the fact that Th basins are more shallow, have a lower incidence of aquatic vegetation, and a shorter mean observed hydroperiod than those at KS. These characteristics of the Th-I pools make them more susceptible to droughts and overflows and thus may facilitate dispersal of dormant eggs through wind action or by flushing events under heavy rainfall.

Gene flow estimated on the basis of pairwise  $F_{ST}$  measures within sites ranged from 0.6 to 227 effective migrants per generation ( $N_m$ ). Between sites, this number was limited to a maximum of 0.31 migrants per generation, resulting in a significant and large genetic differentiation between all three sites. Averaged over all populations within each site,  $N_m$  (Th-I:  $N_m = 11.1$ ; Th-II:  $N_m = 1.7$ ; KS:  $N_m = 5.1$ ) was comparable to that reported by earlier studies on these sites in Riddoch et al. (1994) (KS:  $N_m = 3.1$ ) and Brendonck et al. (2000) (Th-I:  $N_m = 11.7$ ; KS:  $N_m = 7.8$ ). Our plot of  $N_m$  versus geographic distance (Fig. 3) indicates a distance of about 50 m as an effective barrier to intense gene flow in *B. wolfi*. Our results therefore indicate that there is ample effective genetic communication between different populations within each rock pool site, but that this communication is limited by distance.

The indirect estimates of gene flow based on  $F$ -statistics are based on a number of assumptions: genes move with equal likelihood among all populations, gene flow and genetic drift have equilibrated, the rate of migration greatly exceeds that of mutation, and the genetic markers used are selectively neutral (Whitlock and McCauley 1999). Slatkin and Barton (1989), however, demonstrated that this indirect method gives reasonably accurate gene flow estimates for both the island and the stepping stone model. Mutation rates are also usually lower than migration rates for allozymes (Ouborg et al. 1999). Natural selection is unlikely to have shaped the pattern, as most allozyme markers are considered quasineutral and we obtained largely concordant results when considering the loci separately. As the studied rock pool populations are at least several thousand years old (Carney et al. 1994), the permanently inhabiting fauna of these pools is likely to have reached genetic equilibrium. Slatkin (1993) has argued that a clear pattern of isolation by distance, such as observed in our study, is only detectable at equilibrium. On the basis of these arguments, most assumptions to extrapolate levels of gene flow in our study system hold and our gene flow estimates obtained from the indirect approach may be considered reliable. Yet, we want to make two cautionary remarks. First, the extrapolation of gene flow estimates to dispersal pattern should be made

carefully. Gene flow can, to some extent, be uncoupled from dispersal rate by low hatching of immigrant eggs, interpool heterogeneity in abiotic stresses, or competition with locally adapted resident populations (De Meester et al. 2002). Consequently, patterns of gene flow (effective dispersal) may underestimate dispersal rates (movement of individuals or eggs), underlining the need for direct observations. Our observations on dispersal rates indeed indicate that the number of individuals dispersing through overflows may be very high. A second cautionary remark interferes more strongly with our estimate of gene flow itself. If reduced establishment success in habitats that are already occupied by resident populations uncouples dispersal rates to some extent from gene flow (De Meester et al. 2002), then it follows that colonization (i.e., dispersal) rates are higher than rates of genetic exchange. It is then conceivable that, contrary to the argument made in Slatkin (1993), part of the pattern of isolation by distance is caused by colonization dynamics, and effective gene flow may be lower than estimated. In our study system, it is conceivable that the pattern of isolation by distance for pools separated by more than 50 m is largely reflecting colonization dynamics and persistent founder effects (Boileau et al. 1992; De Meester et al. 2002), whereas the pattern of neighboring pools (<50 m) is strongly influenced by ongoing gene flow associated with overflows. This is consistent with results of a recent study by Zeller et al. (2006) on the genetic structure of two freshwater calanoid copepod species with a different dispersal potential. Genetic and geographic distance were positively correlated on a small spatial scale (<100 km) in the species producing dormant eggs, whereas no pattern of isolation by distance could be detected in the species lacking dormant eggs. On a large spatial scale (1,340 km), no pattern of isolation by distance was found in either species. These authors argued that the observed species-specific differences at short distances were probably caused by differences in short-distance dispersal and current gene flow. Our results indicate that these processes might even be important on a much smaller spatial scale: at distances of 50 m or more, gene flow might not be strong enough to erode founder effects. The genetic structure at larger scales (from which the extent depends upon habitat and species) might, therefore, reflect colonization dynamics, whereas at a smaller spatial scale, contemporary gene flow might be more important.

Studies using neutral genetic markers have often reported pronounced genetic differentiation among even nearby populations of passively dispersing freshwater invertebrates, suggesting low levels of gene flow. This apparent paradox of high dispersal and low gene flow may reflect the resilient effects of founder events (Boileau et al. 1992; Brendonck et al. 2000; De Meester et al. 2002). Early colonizers develop such large populations that genetic contributions from later colonists are mathematically minor. The potential to colonize whole habitats from a few propagules with the capacity for rapid population growth may insure that allele frequencies established during initial founder events are resistant to decay for several thousands of generations, even with substantial rates of gene flow. In addition, the development of

extensive dormant egg banks creates a powerful buffer against the impact of new invaders by maintaining population size at high levels and by increasing generation time. The impact of new immigrants may be further reduced by selection due to rapid adaptation of the resident population to local environmental conditions. Gene flow therefore most probably is less effective in organisms showing high population growth rates, large population sizes, dormant egg banks, and a high capacity for local adaptation. Much of the structure in genetic variation among populations of aquatic organisms that is seen at larger scale may therefore be shaped by colonization events rather than contemporary gene flow.

Zooplankton, either as dormant propagules or as active individuals, are potentially dispersed via wind, water, and animal vectors (Bilton et al. 2001). Our results on dispersal of eggs and larvae through overflows suggest that these play an important role in the communication between neighboring populations. Up to 784 viable dormant eggs and 301 larvae were trapped during one single rainfall event in one overflow. At one occasion during abundant rains, a dispersal rate of up to 128 eggs and 46 larvae per hour was recorded. As such overflows are common between the pools, dispersal of eggs and larvae may be considered an effective means of short-range dispersal. Dispersal between pools by means of overflows is a unidirectional process. Donor populations therefore may experience outflow of eggs and larvae but no inflow, and are therefore at greater risk of bottlenecks in the dormant egg bank and even extinction. For the one pair of populations connected through overflow (from KSJ to KSI) that was also genetically analyzed, we observed an  $F_{ST}$  value of approximately zero and a slightly lower observed and expected heterozygosity in the source pool than in the target pool. Our direct measures also suggest that temporal heterogeneity in levels of dispersal is important. Possibly, overflow is most effective when pools flood from being completely dry, in a single rainfall event, because the sediment is less likely to be bound up by vegetation and moisture, and such rains are likely to be more intense, mixing up the sediment more effectively.

Our observations on dispersal rates through overflows only reflect one way of dispersal and are limited in time and space. Thus they do not allow a quantitative comparison between direct and indirect measures of gene flow. Studies that directly quantify dispersal among a set of zooplankton populations are rare. Bohonak and Whiteman (1999) estimated that between one and 30 viable *Branchinecta coloradensis* dormant eggs were moved annually between pools via salamanders. They also observed that the observed dispersal estimates were very similar to the estimated gene flow. Michels et al. (2001) estimated dispersal rates as high as 40,000 zooplankton individuals per hour in some overflows connecting ponds.

A significant isolation-by-distance relation, as in *B. wolffi*, was found in other freshwater anostracans as well. Davies et al. (1997) reported a moderate positive relation between genetic and geographic distance in the anostracan *Branchinecta sandiegonensis* sampled on a spatial scale of up to 50 km (distance between closest pools: 280 m). High genetic divergence ( $F_{ST} = 0.51$ ) was observed on this scale. Between

0.051 and 1.61 effective migrants per generation were estimated between each pair of populations. Bohonak (1998) reported an isolation-by-distance pattern in the anostracan *B. coloradensis*. Between valleys separated by 5–10 km, a high degree of differentiation ( $F_{ST} = 0.77$ ), which corresponds to 0.07 effective migrants per generation, was observed. On a local scale ( $\leq 110$  m), populations were genetically similar ( $F_{ST} = 0.13$ ) and gene flow was estimated to be 1.7 individuals exchanged within clusters of pools each generation. Boileau et al. (1992) reported  $F_{ST}$  values of 0.075 and 0.360 for Canadian populations of *Artemiopsis stefansoni* and *Branchinecta paludosa*, corresponding to estimates of 3.1 and 0.4 individuals, respectively, exchanged per generation between populations separated by distances of less than 1 km. Weeks and Duff (2002) also report significant genetic divergence ( $F_{ST} = 0.16$ ) among populations of another large branchiopod, the clam shrimp *Eulimnadia texana*, that were separated by only hundreds of meters. Estimates of effective migration rate in this study were less than two migrants per generation. In general, all these results seem to be in agreement with the results obtained in our study: high genetic differentiation on a scale of more than 100 m, low genetic differentiation at a very local scale.

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