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Is there a geographical pattern in the breeding system of a complex of hermaphroditic slugs (Mollusca: Gastropoda: *Carinarion*)?

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Allozyme analyses of the hermaphroditic slugs *Arion* (*Carinarion*) *fasciatus*, *A*. (*C*.) *circumscriptus* and *A*. (*C*.) *silvaticus* have suggested that the three species in North America and north-west Europe predominantly reproduce uniparentally, most probably by selfing. We used allozyme electrophoresis to investigate the population genetic structure of these species throughout a larger part of their native European distribution. Our results show that the previously reported 'species' specific allozyme markers are no longer valid if populations from central Europe are investigated, and *A*. *fasciatus* and *A*. *silvaticus* appear to be 'paraphyletic' taxa. In contrast to the general belief that selfing organisms show low gene diversities, the high selfing rates in N-NE European *Carinarion* do not necessarily result in low gene diversities. Moreover, our data suggest a geographical pattern in the prevalence of outcrossing, at least in *A*. *fasciatus*, with selfing in N-NE Europe and a mixed breeding system (i.e. selfing and outcrossing) in central Europe. Possible scenarios for the disjunct distribution of breeding systems in *Carinarion* are discussed.

Keywords: allozymes, breeding systems, hermaphroditic slugs, homozygous multilocus genotypes, population genetics.

Introduction

One of the main concerns of population genetics is to understand how genetic variation is distributed within and among populations. One important factor is the breeding system, which mediates the way genes are transmitted across generations (e.g. Jarne & Städler, 1995). Hermaphroditic animals provide opportunities to study the evolution of breeding systems, in particular selfing vs. outcrossing. Pulmonate gastropods are all hermaphrodites (Heller, 1993) and show a wide variety of breeding systems, involving obligate outcrossing, obligate selfing, parthenogenesis and mixed breeding systems [for a review see Selander & Ochman (1983)]. Jarne (1995) showed that the genetic variability, as estimated by the mean number of alleles per locus, and gene diversity are reduced in selfing pulmonates when compared to outcrossing species. Experimental studies also showed that many gastropods are either almost complete selfers or complete outcrossers [e.g. Jarne (1995)]. A few basommatophoran freshwater snails exhibit a mixed mating system with much interindividual variation in the selfing rate (Jarne & Städler, 1995 and references therein), but in these species selfing rates are high, while little is known about the geographical distribution of selfing and outcrossing (Viard *et al*., 1997).

Previous allozyme studies on North American and Irish populations of the slug subgenus *Carinarion* Hesse 1926 have suggested that its three species, viz. *Arion* (*Carinarion*) *fasciatus* (Nilsson 1823), *A*. (*C*.) *circumscriptus* Johnston 1828 and *A*. (*C*.) *silvaticus* Lohmander 1937 each consist of one or two homozygous multilocus genotypes (MLGs) as a result of a uniparental breeding system (McCracken & Selander, 1980; Foltz *et al*., 1982). This led Selander & Ochman (1983) to claim that the species strictly reproduce by selfing. Recently, Backeljau *et al*. (1997) and Jordaens *et al*. (1998) reported that in NW Europe *A*. *fasciatus*, *A*. *silvaticus* and *A*. *circumscriptus* are not single MLGs but

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complexes of many co-occurring MLGs, thus confirming a uniparental reproductive mode.

However, the fact that (1) at least the copulation of *A. fasciatus* has been described once (Gerhardt, 1935), (2) the occurrence of spermatophores is reported regularly in *Carinarion* (e.g. Jordaens *et al.*, 1996) and (3) nine heterozygote individuals have been found in two Belgian (one *A. silvaticus* and two *A. circumscriptus*), one Polish (one *A. fasciatus*) and one German population (five *A. silvaticus*) (in 884 specimens screened for seven polymorphic loci), may imply that outcrossing between MLGs, though very rare, is not impossible (Jordaens *et al.*, 1996; Backeljau *et al.*, 1997; Jordaens *et al.*, 1998). Based on a limited number of additional observations in an Austrian population, Jordaens *et al.* (1996) and Backeljau *et al.* (1997) even tentatively suggested a geographical pattern in the prevalence of outcrossing. Interestingly, several heterozygotes possibly represented interspecific hybrids (Jordaens *et al.*, 1996; Backeljau *et al.*, 1997), indicating that the species may not be reliably separated by their MLGs alone.

Against this background, the present contribution aims at: (1) estimating the amount of species-specific differentiation in outcrossing *Carinarion* populations; (2) investigating geographical patterns in the prevalence of outcrossing in *Carinarion*; and (3) comparing the genetic variability (i.e. mean number of alleles per locus and gene diversities) of selfing and outcrossing populations. To this end, we surveyed allozyme variation in a large number of native *Carinarion* populations from N-NW and central Europe.

Materials and methods

Sampling and electrophoresis

Vertical polyacrylamide gel electrophoresis (PAGE) was used to assess allozyme variation in 45 *Carinarion* populations (Table 1). The total sampling area ranged from south Sweden in the north to the Czech Republic, Austria and Switzerland in the south. All three species are common in this area except for *A. fasciatus* which has not been recorded from Belgium and The Netherlands. Each sample consisted of slugs collected from a few m^2 only, to avoid Wahlund effects. Species were identified following Lohmander (1937) and Waldén (1955). Eight polymorphic enzyme loci were surveyed: esterase Q (*EsQ*, EC 3.1.1.1), fumarate hydratase (*Fumh*, EC 4.2.1.2), alanine aminotransferase (*Alat*, EC 2.6.1.2), phosphoglucomutase (*Pgm*, EC 2.6.1.1), lactate dehydrogenase (*Ldh*, EC 1.1.1.47) and three leucylalanine aminopeptidase loci (*Pep*, EC 3.4.11). Sample preparation and experimental procedures followed Backeljau (1987) and Backeljau *et al.* (1997).

Table 1 List of populations, numbers of specimens and numbers of MLGs (in parentheses) of *A. fasciatus*, *A. silvaticus* and *A. circumscriptus*, Nei's (1978) gene diversity (H_{exp}) and observed heterozygosity (H_{obs}). †These populations were analysed by Jordaens *et al.* (1998)

Country	*A. fasciatus*	H_{obs}	H_{exp}	*A. silvaticus*	H_{obs}	H_{exp}	*A. circumscriptus*	H_{obs}	H_{exp}	Total	H_{obs}	H_{exp}
Sweden												
S1: Göteborg	20(1)	0	0	1(1)	0	0	1(1)	0	0	22(3)	0	0.055
S2: Genarp	24(1)	0	0	8(2)	0	0.029	4(1)	0	0	36(4)	0	0.156
S3: Baskemölla	—			5(1)	0	0	—			5(1)	0	0
S4: Lund	1(1)	0	0	2(1)	0	0	2(1)	0	0	5(3)	0	0.289
S5: Lund	2(1)	0	0	3(1)	0	0	5(1)	0	0	10(3)	0	0.268
S6: Lund	39(1)	0	0	—			1(1)	0	0	40(2)	0	0.019
Poland												
P1: Muszkowice	—			16(2)	0	0.064	—			16(2)	0	0.064
P2: Augustow	4(1)	0	0	—			—			4(1)	0	0
P3: Bialystok	2(1)	0	0	—			—			2(1)	0	0
P4: Bialowieza	17(1)	0	0	—			2(2)	0	0.167	19(3)	0	0.074
P5: Zgorzelec	26(4)	0.063	0.095	—			—			26(4)	0.063	0.095
P6: Zgorzelec	23(2)	0.071	0.063	—			—			23(2)	0.071	0.063

Germany												
G1: Görlitz	12(2)	0.052	0.061	—			—			12(2)	0.052	0.061
G2: Görlitz	—			—			16(2)	0	0.043	16(2)	0	0.043
G3: Schmilka	4(2)	0	0.071	—			—			4(2)	0	0.071
G4: Görlitz	—			—			14(2)	0.080	0.064	14(2)	0.080	0.064
G5: Treseburg	—			3(2)	0	0.067	5(2)	0	0.044	8(4)	0	0.250
G6: Javernick	4(1)	0	0	3(1)	0	0	—			7(2)	0	0.198
Belgium												
B1: Mortsel	—			5(3)	0	0.111	1(1)	0	0	6(4)	0	0.167
B2: Genk	—			14(1)	0	0	—			14(1)	0	0
B3: Houx	—			—			24(1)	0	0	24(1)	0	0
B4: Nîsmes	—			—			8(1)	0	0	8(1)	0	0
B5: Yvoir	—			—			24(1)	0	0	24(1)	0	0
B6: St.-Antonius†	—			26(3)	0.005	0.137	—			26(3)	0.005	0.137
B7: St.-Antonius†	—			21(3)	0	0.055	1(1)	0	0	22(4)	0	0.084
B8: Halle†	—			24(2)	0	0.048	—			24(2)	0	0.048
B9: Halle†	—			24(4)	0	0.078	—			24(4)	0	0.078
B10: Pittem†	—			—			24(2)	0	0.056	24(2)	0	0.056
B11: Gent†	—			1(1)	0	0	19(2)	0	0.039	20(3)	0	0.094
Czech Republic												
C1: Námest	41(2)	0	0.022	—			—			41(2)	0	0.022
C2: Naloucany	9(2)	0	0.026	—			—			9(2)	0	0.026
Switzerland												
SW1: Langnau	—			24(2)	0	0.022	—			24(2)	0	0.022
Liechtenstein												
L1: Schaanwald	—			13(2)	0	0.034	—			13(2)	0	0.034
Austria												
A1: Graz	135(4)	0.052	0.075	—			—			135(4)	0.052	0.075
A2: Graz	50(2)	0.025	0.038	—			—			50(2)	0.025	0.038
A3: Graz	18(1)	0.118	0.125	—			—			18(1)	0.118	0.125
A4: Graz	33(2)	0	0.021	—			—			33(2)	0	0.021
A5: Graz	49(3)	0.038	0.099	—			—			49(3)	0.038	0.099
A6: Bischofshofen	30(3)	0.058	0.070	—			—			30(3)	0.058	0.070
A7: Horn	28(2)	0.036	0.078	—			—			28(2)	0.036	0.078
A8: Vienna	40(1)	0	0	—			—			40(1)	0	0
A9: Altenburg	—			46(2)	0.003	0.013	—			46(2)	0.003	0.013
A10: Altenburg	12(3)	0.042	0.111	—			—			12(3)	0.042	0.111
A11: Steinegg	—			13(1)	0	0	—			13(1)	0	0
A12: Inner Laterns	—			17(3)	0	0.053	—			17(3)	0	0.053

Staining recipes were adapted from Harris & Hopkinson (1976).

Data analysis

The program BIOSYS-2 [updated version of BIOSYS-1 of Swofford & Selander (1981)] was used to calculate allele frequencies, Nei's (1978) unbiased expected heterozygosities (i.e. gene diversity H_{exp}) and observed heterozygosities (H_{obs}). Tests for genotypic (i.e. linkage) disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) were performed with the program GENEPOP v. 3 [updated version of GENEPOP v. 1.2 of Raymond & Rousset (1995)]. LD was tested for each population separately. HWE was evaluated with a probability test, which corresponds to the 'exact HW test' of Weir (1996). For all tests a sequential Bonferroni correction was applied to correct for multiple testing (Rice, 1989). For each population, GENEPOP v. 3 was used to estimate F_{IS} values according to Weir & Cockerham (1984) as a measure of heterozygote deviation. We estimated the selfing rate S using the classical relationship $F_{IS} = S/(2 - S)$ (e.g. Viard *et al.*, 1997). Tests for HWE and LD and the calculation of S were restricted to populations with more than 20 individuals to avoid problems of small sample sizes.

Nei's (1978) genetic distance (D) was calculated between MLGs and used for UPGMA clustering and nonmetric multidimensional scaling (NMDS) with the NTSYS program v. 1.80 (Rohlf, 1993).

Results

Species differentiation

The species-specific alleles of *A. fasciatus* (i.e. *Pgm-1*^{85}), *A. silvaticus* (i.e. *Alat*^{100}) and *A. circumscriptus* (i.e. *EsQ*^{100} and *Fumh*^{100}) reported by Backeljau *et al.* (1997) in material from NW Europe, were now also found in other species, so that none of the species is characterized by species-specific alleles. This also makes *A. fasciatus* and *A. circumscriptus* more polymorphic than hitherto reported, so that at least *A. silvaticus* and *A. fasciatus* show comparable gene diversities (Table 1).

Combined with the studies of Backeljau *et al.* (1997) and Jordaens *et al.* (1998), a total of 46 different MLGs are now recorded in *Carinarion*: 13 in *A. fasciatus*, 24 in *A. silvaticus* and nine in *A. circumscriptus*. Two MLGs were shared by *A. fasciatus* and *A. silvaticus*. Due to ties (e.g. Backeljau *et al.*, 1996), 11 alternative UPGMA topologies were found. In all trees, *A. fasciatus* and *A. silvaticus* appeared 'paraphyletic' (Fig. 1). In three topologies also *A. circumscriptus* appeared 'paraphyletic'.

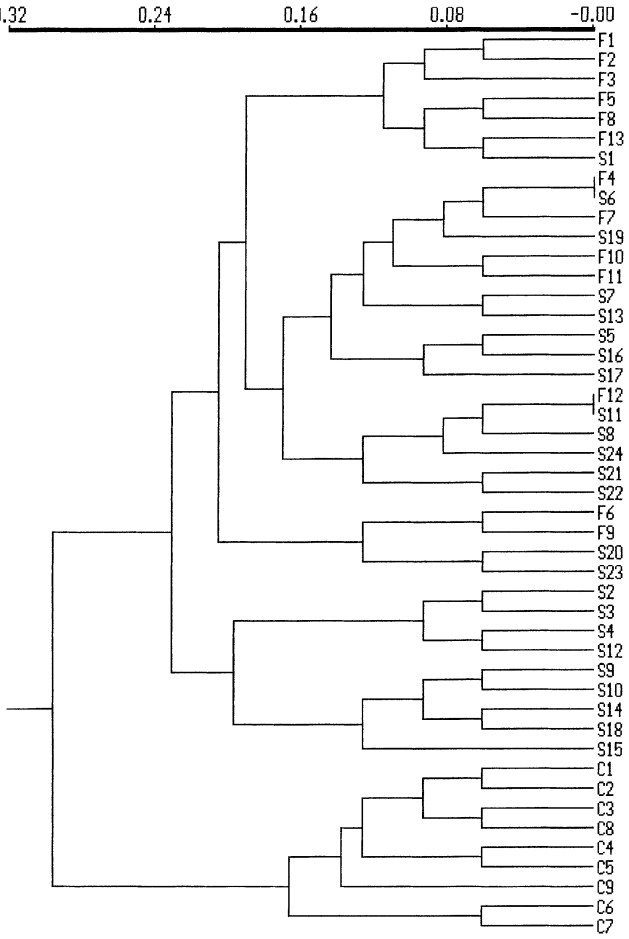

Fig. 1 UPGMA dendrogram of Nei's (1978) genetic distances between 46 *Carinarion* MLGs: *A. fasciatus* (F1–F13), *A. silvaticus* (S1–S24) and *A. circumscriptus* (C1–C9).

The average interstrain genetic distance and the NMDS plot (stress:0.37) (Fig. 2) suggested a close relationship between *A. fasciatus* and *A. silvaticus* genotypes (average $D = 0.21 \pm 0.10$, range: 0.00–0.53) and a more distinct position of *A. circumscriptus* genotypes (*A. fasciatus–A.circumscriptus*: average $D = 0.28 \pm 0.08$, range: 0.06–0.53; *A. silvaticus–A. circumscriptus*: average $D = 0.31 \pm 0.10$, range: 0.13–0.64). Intraspecific genetic distances between strains were comparable for the three species (*A. fasciatus*: average $D = 0.15 \pm 0.07$, range: 0.06–0.35; *A. silvaticus*: average $D = 0.18 \pm 0.08$, range: 0.04–0.44; *A. circumscriptus*: average $D = 0.13 \pm 0.06$).

Variation in breeding system

Significant LD was found in 24 tests (out of 65 possible tests) at a 5% significance level. Twenty significant values were recorded in populations with high selfing

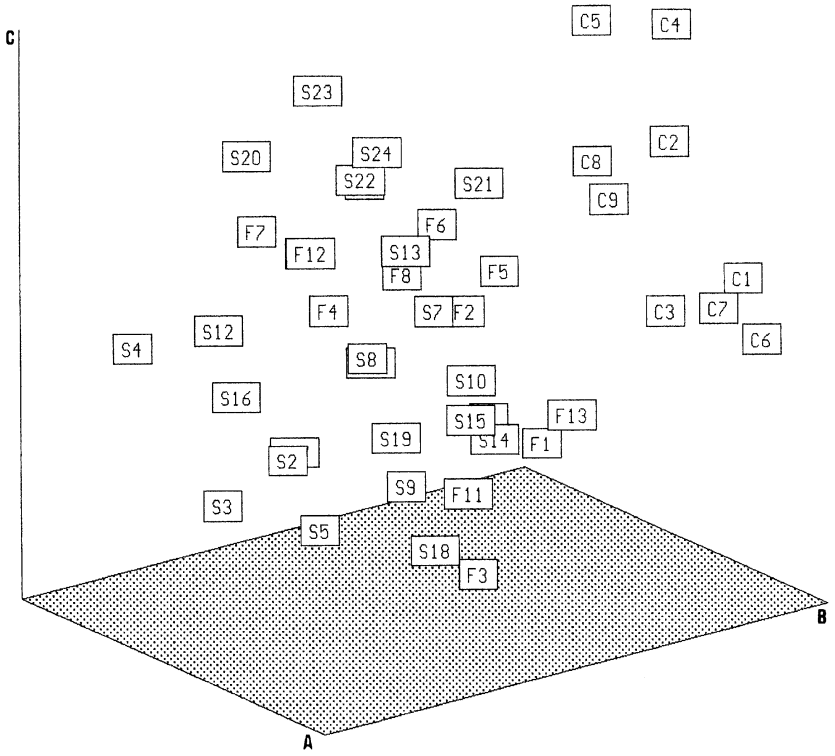

Fig. 2 Two-dimensional projection of Nei's (1978) distances between 46 *Carinarion* MLGs as obtained by NMDS: *A. fasciatus* (F1–F13), *A. silvaticus* (S1–S24) and *A. circumscriptus* (C1–C9).

rates. Yet, after applying the sequential Bonferroni procedure, only two tests (between *Pgm*-1 and *Alat* and between *Est* and *Fumh* in S2) remained significant.

In N-NW European *Carinarion* populations (Belgium and Sweden) no heterozygotes were observed (i.e. $F_{IS} = 1$ in all cases), except for a single *Ldh* heterozygote in St.-Antonius (B6) ($F_{IS} = 0.960$, Table 2; Jordaens *et al.*, 1998) resulting in a mean F_{IS} value of 0.996 and high selfing rates (Table 2; Fig. 3). There were no indications for the presence of null alleles. In many situations genotype frequencies deviated significantly from HWE (Table 2). In central Europe (other countries), however, heterozygous individuals were found in 11 out of 23 multi-MLG populations and in one single-MLG population (i.e. A3) (Table 1; Fig. 3), yielding a wide range of F_{IS} values < 1 (0.553 ± 0.377) and lower selfing rates (Table 2; Fig. 3). At many loci genotype proportions did not deviate significantly from HWE (Table 2). Moreover, genotype proportions at the *Pgm*-1 locus never deviated from HWE expectations in populations with heterozygotes. In contrast, *Alat* and *Pep* genotype proportions sometimes deviated significantly from HWE expectations in populations with heterozygotes (*Alat* in A1, A2, A5, *Pep*-1 in A1 and A5 and *Pep*-2 in P5 and A7).

Genetic variability

H_{exp}, H_{obs} and the number of MLGs in each population are given in Table 1. Neither the mean number of alleles per locus (Mann–Whitney *U*-test: all populations $P = 0.89$; populations with $n \geq 20$ $P = 0.59$), nor gene diversity (Mann–Whitney *U*-test: all populations $P = 0.06$; populations with $n \geq 20$ $P = 0.13$) were higher in outcrossing populations (i.e. populations with heterozygotes) compared to selfing populations (i.e. populations without heterozygotes). Compared to Backeljau *et al.* (1997) and Jordaens *et al.* (1998), we found two new alleles in central Europe, i.e. *Pep-1*^{115} and *Pgm-1*^{110} (Table 2).

Discussion

Taxonomic implications

Our study shows that the species specificity of the allozyme markers reported by Backeljau *et al.* (1997) is no longer valid if material from central Europe is included. The occurrence of outcrossing in some populations, the lack of species-specific allozyme markers, the 'paraphyly' of *A. fasciatus* and *A. silvaticus* and the observation that two MLGs are shared by *A. fasciatus* and *A. silvaticus* provide new support to Backeljau *et al.*'s (1997) questioning of the species status of at least *A. fasciatus* and *A. silvaticus*. Therefore, we suggest that *Carinarion* would be considered as a single species which in N-NW Europe may be divided into a number of MLGs which in some areas hybridize.

Table 2 Allele frequencies, F_{IS} estimates, selfing rates (S) and exact p-values for HWE (*$P < 10^{-6}$) of populations with more than 20 individuals. Pep-3 was monomorphic in all populations $: F_{IS} not calculated because one of the two alleles is represented only once; see Raymond & Rousset, 1995)

	Population																						
Locus	S1	S2	S6	P5	P6	B3	B5	B6	B7	B8	B9	B10	B11	C1	SW1	A1	A2	A4	A5	A6	A7	A8	A9
Ldh																							
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.750	1.000	1.000	0.038	1.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
83	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.962	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F_{IS}								0.901			1.000		1.000										
HWE								*			0.02		0.03										
Pgm																							
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.010	0.000	0.000	0.000	0.000
100	0.045	0.083	0.025	0.000	0.000	0.000	0.000	0.000	0.045	1.000	0.192	0.000	0.050	0.000	1.000	0.985	0.970	0.091	0.949	0.000	0.000	0.000	1.000
93	0.045	0.250	0.000	0.404	0.543	1.000	1.000	1.000	0.955	0.000	0.808	1.000	0.950	0.000	0.000	0.000	0.030	0.000	0.020	0.304	0.393	0.000	0.000
85	0.910	0.667	0.975	0.596	0.457	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.011	0.000	0.909	0.020	0.696	0.607	1.000	0.000
F_{IS}	1.000	1.000	1.000	−0.019	−0.117				1.000		1.000		1.000			−0.009	−0.021	1.000	−0.026	−0.080	0.416		
HWE	0.0006	*	0.01	1	0.68				0.02		*		$			1	1	0.0001	1	1	0.05		
Alat																							
100	0.045	0.222	0.000	0.000	0.000	0.000	0.000	1.000	0.955	1.000	1.000	0.000	0.050	0.098	0.042	0.207	0.090	0.000	0.112	0.033	0.000	0.000	0.022
88	0.955	0.788	1.000	1.000	1.000	1.000	1.000	0.000	0.045	0.000	0.000	1.000	0.950	0.902	0.958	0.793	0.910	1.000	0.888	0.967	1.000	1.000	0.978
F_{IS}	1.000	1.000							1.000				1.000	1.000	1.000	0.327	0.640		0.900	1.000			1.000
HWE	0.02	*							0.02				0.03	*	0.02	0.0004	0.0013		*	0.02			0.01
EsQ																							
100	0.045	0.111	0.025	0.000	0.000	1.000	1.000	0.000	0.045	0.000	0.000	1.000	0.950	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
88	0.955	0.899	0.975	1.000	1.000	0.000	0.000	1.000	0.955	1.000	1.000	0.000	0.050	1.000	0.958	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
F_{IS}	1.000	1.000	1.000						1.000				1.000		1.000								
HWE	0.02	*	0.01						0.02				0.03		0.02								
Fumh																							
100	0.045	0.111	0.025	0.000	0.000	1.000	1.000	0.000	0.045	0.000	0.000	1.000	0.950	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
93	0.955	0.899	0.975	1.000	1.000	0.000	0.000	0.423	0.818	0.000	0.962	0.000	0.050	1.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
86	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.577	0.136	1.000	0.038	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F_{IS}	1.000	1.000	1.000					1.000	1.000		1.000		1.000										
HWE	0.02	*	0.01					*	*		0.02		0.03										
Pep-1																							
115	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.136	0.040	1.000	0.418	0.033	0.000	0.000	0.033
100	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	0.952	1.000	1.000	1.000	1.000	1.000	1.000	0.864	0.960	0.000	0.582	0.967	1.000	1.000	0.967
F_{IS}									1.000							0.296	−0.032		0.629	1.000			0.662
HWE									0.02							0.003	1		*	0.02			0.03
Pep-2																							
100	0.000	0.000	0.000	0.846	0.000	0.000	1.000	0.962	0.955	0.208	0.038	0.261	0.150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000
91	1.000	1.000	1.000	0.154	1.000	1.000	0.000	0.038	0.045	0.792	0.962	0.739	0.850	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929	1.000	1.000
F_{IS}				1.000				1.000	1.000	1.000	1.000	1.000	1.000								1.000		
HWE				*				0.02	0.02	*	0.02	*	0.0003								0.001		
F_{IS}	1.000	1.000	1.000	0.339	−0.11	—	—	0.960	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.298	0.337	1.000	0.706	0.187	0.540	—	0.796
S	1.000	1.000	1.000	0.506	0.000	—	—	0.980	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.459	0.504	1.000	0.828	0.315	0.701	—	0.886

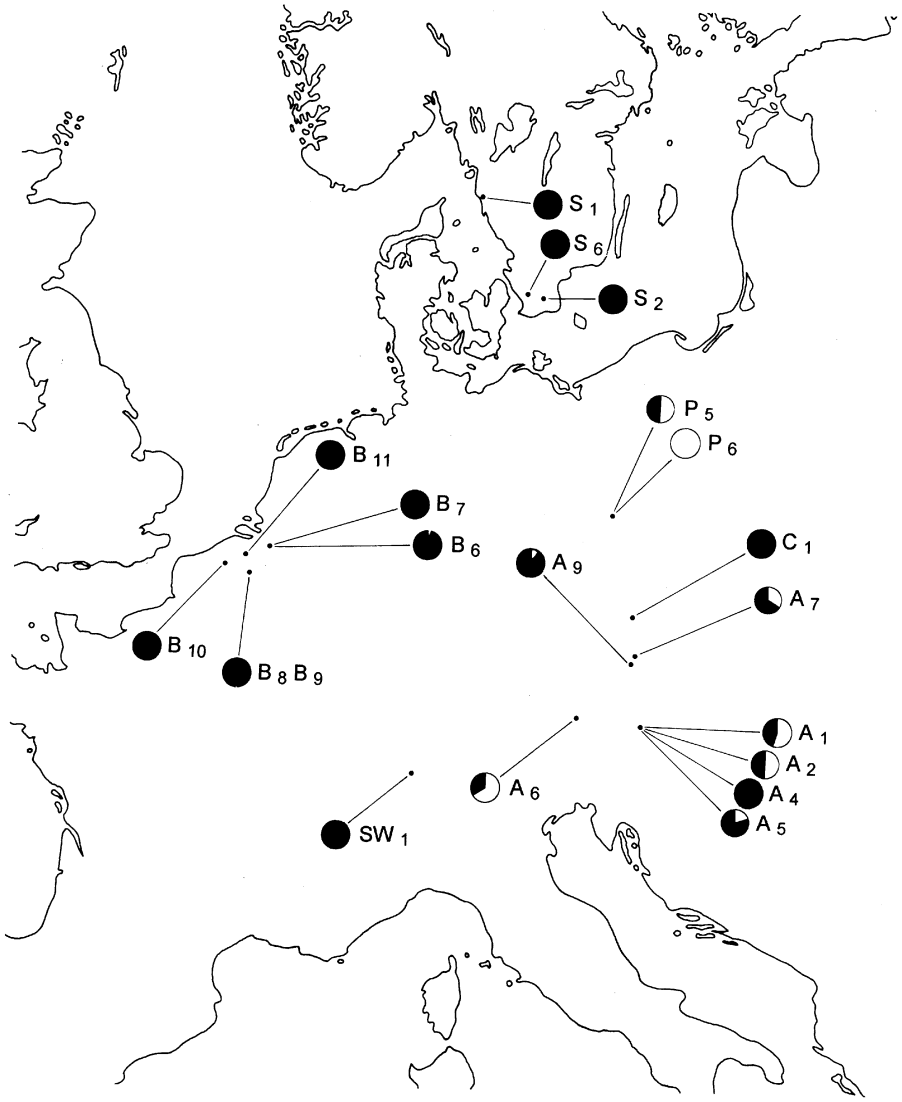

Fig. 3 Inferred selfing rates (solid sections) and outcrossing rates (open sections) in *Carinarion* populations with more than 20 individuals.

Distribution of genetic variance and breeding system

The high F_{IS} values in N-NW European *Carinarion* populations result from the near absence of heterozygotes and thus confirm earlier reports of high selfing rates in these populations (Foltz *et al*., 1982; Backeljau *et al*., 1997; Jordaens *et al*., 1998). Population densities in many N-NW European *Carinarion* populations are low (personal observation; Jordaens *et al*., 1998) and thus we may expect a strong influence of genetic drift on the genetic structuring of these populations. The genetic consequences of genetic drift may be amplified by selfing, which may explain the low variability in some populations (e.g. Njiokou *et al*., 1993; Jarne, 1995; Viard *et al*., 1997).

Nevertheless, our results contradict the claim of Selander & Ochman (1983) that *Carinarion* strictly reproduces by self-fertilization, since the occurrence of heterozygous individuals in central Europe may indicate some degree of outcrossing. Outcrossing may even be the more common reproductive mode in this area since several loci do not deviate significantly form HWE expectations, at least in *A. fasciatus*, although significant departures from HWE may suggest some degree of selfing in these populations too.

S-value estimates from population genetic data assume that the observed F_{IS} values are only influenced by the selfing rate. Of course, this may not be true, because other factors such as spatial and temporal variance in allele frequencies may also contribute to F_{IS} values. Yet, population genetic data have the advantage

over single-generation estimates, because they reflect selfing rates averaged over several generations (Jarne & Städler, 1995). It is evident that this mating-system heterogeneity across the range of the *Carinarion* complex would have been more convincingly demonstrated if we had performed experiments to directly estimate the outcrossing rate, via crossing experiments involving two or more MLGs. However, all our attempts to outcross *Carinarion* (even with animals from populations with low selfing rates) have failed, even when individuals were previously isolated for long periods, which in other gastropod species stimulates copulation (Heike Reise & Janet Leonard, personal communications).

The high selfing levels in N-NW European populations and the lower selfing levels in central European populations support Jordaens *et al.*'s (1996) and Backeljau *et al.*'s (1997) suggestion of a geographical pattern in the prevalence of outcrossing, at least in *A. fasciatus*. This is to our knowledge the first indication of such patterns of mating systems in hermaphroditic animals. The possible differential distribution of selfing and outcrossing *Carinarion* populations could be ascribed to 'geographical self-fertilization', somewhat similar to 'geographical parthenogenesis', i.e. the disjunct distribution of sexual and asexual populations (e.g. Fussey, 1984; Hughes, 1989; Enghoff, 1994). The biological meaning of this disjunct distribution of breeding systems currently remains unresolved.

The Quaternary cold periods in Europe are thought to have influenced the amount and distribution of intraspecific genetic variation in many animals and plants (Taberlet *et al.*, 1998). The more northerly distribution of parthenogenetic populations of several species may be attributed to the high colonization ability of clones after the last glaciations (Vogel *et al.*, 1999). Similarly, we presume that selfing *Carinarion* MLGs were able to (re)colonize Europe much faster than outcrossing *Carinarion*. This seems likely since Baur & Bengtsson (1987) and Bengtsson & Baur (1993) showed that selfing gastropods are better colonizers than outcrossers. There is a general perception that central and northern Europe were colonized by range expansion from Mediterranean refugia (viz. the Iberian peninsula, Italy and the Balkans) at the end of the last glaciation and that the northern part of Europe has been colonized primarily from the Balkan and Iberian refugia (e.g. Taberlet *et al.*, 1998). Since *Carinarion* is absent from the Iberian peninsula (Castillejo, 1997), it is probable that *Carinarion* has (re)colonized N-NW Europe from Balkan refugia. For several small mammals Bilton *et al.* (1998) provided strong support for recolonization that may have occurred from glacial refugia in central Europe–western Asia. If one of these scenarios is true, then we expect also high outcrossing levels in the Balkan area. Although these models of postglacial range expansion from south or central European refugia is well supported for some temperate animals and plants, there is an alternative scenario. Some of the north European *Carinarion* populations may have survived the last glaciation (or several glaciations) in local, ice-free refugia and (re)colonized the rest of N-NW Europe from these refugia. However, this concept of glacial refugia in northern Europe has today lost much of its credibility (Bilton, 1994). Moreover, it will be almost impossible to distinguish between this and the former models (Tollefsrud *et al.*, 1998). Obviously, alternative explanations, such as possible ecological and altitudinal differences between NW-European and central European populations may explain such a pattern in breeding systems as well, but the data are too preliminary to allow adequate testing of these alternatives.

Contrary to current models (e.g. Selander & Ochman, 1983; Jarne, 1995), gene diversities and mean numbers of alleles per locus in selfing and outcrossing *Carinarion* populations were of the same magnitudes. Indeed, although many selfing populations only contain one MLG, about 50% of the populations contain more than one MLG (Backeljau *et al.*, 1997; Jordaens *et al.*, 1998). Possibly, *Carinarion* does not fit into current models.

It is evident from our study that our knowledge is incomplete and before firm conclusion can be made, a clearer understanding of the phylogeography of *Carinarion* is required. Nevertheless, our data indicate that population genetic data of hermaphroditic animals may provide supplementary information for postglacial phylogeographic scenarios.

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