MALAKOLOGISCHE ABHANDLUNGEN

Staatliches Museum für Tierkunde Dresden

Band 20 | Ausgegeben: 5. Dezember 2000 | Nr. 3

Mating system in Valvatidae – a preliminary study (Gastropoda: Heterostropha)

With 1 figure and 7 tables

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Abstract. The genetic structure of two European valvatid species (Gastropoda: Heterostropha): *Valvata piscinalis* (O. F. MÜLLER, 1774) and *V. pulchella* STUDER, 1820 was studied to infer the amount of selfing in those hermaphrodite gastropods. Three populations of *V. piscinalis* and one of *V. pulchella*, all from Poland, were examined for seven enzyme systems, coded by ten loci. In *V. pulchella*, and one population of *V. piscinalis*, all the ten loci were monomorphic, in the other two populations of *V. piscinalis*, three polymorphic loci (GPI, HBDH, IDH-1) were detected. High interspecific and low intraspecific variation was observed. Within the polymorphic populations, the percentage of polymorphic loci (30 %) was rather low, and mean heterozygosity (0.049-0.051 observed, and 0.067-0.095 expected) was low. Exact multilocus and multipopulation tests, as well as multilocus tests for the two polymorphic populations showed a statistically significant homozygote excess. However, no significant departure from HWE was found as concerns GPI in all populations and IDH-1 in one population. f values were not correlated between loci, and θ confidence interval for HBDH did not overlap the intervals for the other two polymorphic loci. D-statistics indicated that the main source of disequilibrium was interpopulation differentiation, despite the relatively high (1.013-3.594) Nm values calculated from θ. The results point to stochastic processes and perhaps also selection, but certainly neither selfing nor inbreeding, to be responsible for the observed heterozygote deficits.

Key words. *Valvata*, Heterostropha, hermaphrodite gastropod, selfing, mating system, population genetic structure, allozyme electrophoresis, F-statistics, D-statistics, homozygote excess

Introduction

Simultaneous hermaphrodites are potentially selfing animals. It, however, is generally believed that cross-fertilization gives an advantage to the animal so is preferred and predominates, notwithstanding that in some cases it is self-fertilization that natural selection favours (see JARNE, VIANEY-LIAUD & DELAY 1993 for review). There are numerous studies on the mating systems in hermaphrodites, many of them dealing with pulmonate gastropods: freshwater basommatophorans (CAIN 1956, JARNE, VIANEY-LIAUD & DELAY 1993, WETHINGTON & DILLON 1997) as well as terrestrial stylommatophorans (FOLTZ et al. 1982, HILLIS 1989, JORDAENS et al. 1998). The results suggest that cross-fertilization predominates except for several cases of more or less obligatory selfers, and that even those species that usually avoid self-fertilization still retain the ability to self-fertilize if there is no partner for copulation. Nothing, however, is known about the mating systems in the Heterostropha FISCHER, 1885 where the Valvatidae belong.

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The species belonging to the family Valvatidae, especially *Valvata piscinalis* (O. F. MÜLLER, 1774), are widely known as having extremely variable shells. Numerous forms have been described, the systematic status of many of which remaining enigmatic (FALNIOWSKI 1989). Along with this variability, *V. piscinalis* is usually very little varied when looked at within a given population. It has been suggested (e.g. FALNIOWSKI 1989) that this pattern of variation can be explained by the relatively common occurrence of selfing in this species.

The aim of the present paper was to study the genetic structure of three populations of *V. piscinalis* and one of *V. pulchella* STUDER, 1820 by means of allozyme electrophoresis, to obtain some data on the mating system in valvatids. Such one-generation study is not adequate to address all questions that have arisen concerning this, yet it should suffice to answer the main question, whether valvatids reproduce predominantly by random mating and cross-fertilization, or not and thus some other mechanisms are involved.

Material and methods

The material was collected with a sieve, at the following localities:

1. Pińczów, South Poland, an old channel of the Nida River at Skrzypiów village by the town of Pińczów; a shallow, muddy, stagnant basin, distant from the river channel by about 200 m, much eutrophized, with aquatic vegetation on the bed.
2. Nieciecz, about 300 km to the NE of locality 1, about 10 km to the SE of the town of Łomża; an old channel of the River Narew seasonally connected with the river; muddy bottom with aquatic plants, at a depth of about 2 m.
3. Czarnocin, North-East Poland, about 315 km to the NE of locality 1, about 12 km to the N of locality 2; an old channel of the Narew River connected with the river, situated at about 500 m to the west of Czarnocin village; a muddy bottom covered with aquatic vegetation, at a depth of about 2 m.
4. Drozdowo, about 300 km to the NE of 1, 1.2 km to the NE of 2, 11 km to the SE of 3; shallow, seasonal pools in the valley of the Narew, situated about 500 m to the west of Drozdowo village; on grass.

The snails were kept in aerated and cooled aquaria, and electrophoresized within a few days. Individuals for electrophoresis were freezed at –18 °C for 10 minutes and then dissected on ice. The hepato-pancreas was homogenized in a glass homogenizer, in 50-70 µl (depending on snail size) of homogenizing solution (100 ml distilled water, 10 mg NADP, 10 mg NAD, 100 µl β-mercaptoethanol). The homogenates were centrifuged at 11 000 RPM for 10 min. and used immediately for cellulose acetate electrophoresis following the protocol of RICHARDSON, BAVERSTOCK & ADAMS (1986). The cellogel strips were from MALTA, Italy; the other chemicals from SIGMA, USA. The enzyme names and E.C. codes are after MURPHY et al. (1996).

All data were analyzed with GENEPOP (RAYMOND & ROUSSET 1995), to compute exact tests for HWE (GUO & THOMPSON 1992, ROUSSET & RAYMOND 1995) and f (= F_{IS} in WRIGHT 1978) for each polymorphic population and for each polymorphic locus, with exact probabilities. Relatedness (HAMILTON 1971, QUELLER & GOODNIGHT 1988), and F-statistics over all populations of *V. piscinalis*, following the notation and computational procedure introduced by WEIR & COCKERHAM (1984) and WEIR (1990, 1996), were computed with WEIR's FSTAT (WEIR 1990, GOUDET 1995), the confidence intervals estimated with jackknife and bootstrapping techniques (WEIR 1990). FSTAT was also applied to calculate θ and Nm values for all possible pairs of populations. The Nm values between population 4 and the others, as between the two species, are probably of no biological meaning, but are given for comparison, to scale observed intraspecific values (in fact, any real gene flow between the population 1 and the other two of *V. piscinalis* is also hardly probable). Composite linkage disequilibrium coefficient Δ_{ij} (BLACK & KRAFSUR 1985), corrected correlation coefficient R_{ij} (BLACK & KRAFSUR 1985), and D-statistics of OHTA (1982) were computed with LINKDOS (GARNIER-GERE & DILLMANN 1992, based on BLACK & KRAFSUR 1985). The proportion of progeny produced by self-fertilization was estimated for each polymorphic locus in each population, according to the formula given by HEDRICK (1983): $S = (4pq-2h)/(4pq-H)$, where p and q are allele frequencies, and H is the proportion of heterozygous individuals, for a given locus. Mean heterozygosity and genetic distances were computed with BIOSYS-1 (SWOFFORD & SELANDER 1981).

Table 1: Allele frequencies in populations 1–4: 1 – Pińczów, 2 – Nieciecz, 3 – Czarnocin, 4 – Drozdowo. In parentheses numbers of specimens assayed. Alleles in order of decreasing mobility. AAT – Aspartate Aminotransferase (EC 2.6.1.1), ALP – Alkaline Phosphatase (EC 3.1.3.1), GPI – Glucose-6-phosphate Isomerase (EC 5.3.1.9), HBDH – 3-Hydroxybutyrate Dehydrogenase (EC 1.1.1.30), IDH – Isocitrate Dehydrogenase (EC 1.1.1.42), MDH – Malate Dehydrogenase (EC 1.1.1.37), PGDH – Phosphogluconate Dehydrogenase (EC 1.1.1.44). Enzyme names and EC numbers after MURPHY et al. (1996).

	Population					Population			
Locus	1	2	3	4	Locus	1	2	3	4
AAT-1	(18)	(33)	(42)	(40)	IDH-1	(18)	(22)	(29)	(40)
A	1.000	1.000	1.000	0.000	A	1.000	0.909	0.879	0.000
B	0.000	0.000	0.000	1.000	B	0.000	0.091	0.121	0.000
AAT-2	(18)	(33)	(42)	(40)	C	0.000	0.000	0.000	1.000
A	1.000	1.000	1.000	1.000	IDH-2	(18)	(31)	(41)	(40)
ALP	(18)	(33)	(42)	(40)	A	1.000	1.000	1.000	1.000
A	1.000	1.000	1.000	1.000	MDH-1	(18)	(33)	(41)	(40)
GPI	(18)	(33)	(42)	(40)	A	1.000	1.000	1.000	0.000
A	1.000	0.803	0.810	0.000	B	0.000	0.000	0.000	1.000
B	0.000	0.197	0.190	0.000	MDH-2	(18)	(33)	(41)	(40)
C	0.000	0.000	0.000	1.000	A	1.000	1.000	1.000	1.000
HBDH	(18)	(29)	(39)	(40)	PGDH	(18)	(33)	(40)	(40)
A	0.000	0.000	0.000	1.000	A	0.000	0.000	0.000	1.000
B	1.000	0.655	0.923	0.000	B	1.000	1.000	1.000	0.000
C	0.000	0.345	0.077	0.000					

Results

Seven enzyme systems, coded by ten presumed loci (Table 1) gave well resolved and always interpretable zymograms. In one population of *V. piscinalis* (population 1), and in *V. pulchella* (population 4) no polymorphic locus was detected. In the other two populations of *V. piscinalis* (populations 2 and 3) three polymorphic loci were found: GPI, HBDH, and IDH-1. There was no private allele in the studied populations of *V. piscinalis*, the only difference between population 2 and 3 being in the frequencies of the common alleles, while population 1 was fixed at one of the alleles found in populations 2 and 3 in each polymorphic locus (Table 1).

In populations 2 and 3 the mean number of alleles per locus was 1.30, and 30 % of loci were polymorphic (Table 2). In these polymorphic populations, the mean observed heterozygosity was 0.049-0.051, and the mean expected heterozygosity was 0.067-0.095, depending on

Table 2: Genetic variability at ten loci in all populations (Standard errors in parentheses). *: a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95; **: unbiased estimate (NEI 1978).

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity Direct count	Hardy-Weinberg expected**
Valvata piscinalis					
1. Pińczów	18.0 (±0.0)	1.00(±0.00)	0.0	0.000 (±0.000)	0.000 (±0.000)
2. Nieciecz	31.3 (±1.1)	1.30(±0.15)	30.0	0.051 (±0.028)	0.095 (±0.053)
3. Czarnocin	39.9 (±1.3)	1.30(±0.15)	30.0	0.049 (±0.033)	0.067 (±0.036)
Valvata pulchella					
4. Drozdowo	40.0 (±0.0)	1.0 (±0.00)	0.0	0.000 (±0.000)	0.000 (±0.000)

	1.	2.	3.	4.
1.	*.***	0.015	0.005	0.916
2.	0.168	*.***	0.006	0.866
3.	0.129	0.070	*.***	0.882
4.	0.775	0.775	0.775	*.***

Table 3: Above diagonal: NEI unbiased genetic distance; Below diagonal: CAVALLI-SFORZA & EDWARDS arc distance.

population (Table 2). Within *V. piscinalis*, NEI's unbiased genetic distances were very small (0.005-0.015), despite that the geographic distance between population 1 and the other two was quite long, but the said genetic distance between *V. piscinalis* and *V. pulchella* (Table 3) was considerable (0.866-0.916). The same concerns CAVALLI-SFORZA & EDWARDS arc genetic distance (Table 3).

Comparisons between the expected and observed mean heterozygosity mentioned above suggest a heterozygote deficit. The levels of probability yielded by the GENEPOP exact tests assuming a heterozygote excess were not significant. The GENEPOP exact tests assuming a heterozygote deficit for all the populations and loci indicated a highly significant multipopulation and multilocus deficit (Table 4). A highly significant homozygote excess was detected for both polymorphic populations by the multilocus tests. The multipopulation tests, however, showed no significant deficit at GPI (Table 4). In each of the two polymorphic populations, a highly significant heterozygote deficit was detected at HBDH, whereas at IDH-1 a similar deficit was observed only in population 3.

Theoretically, from the proportion of heterozygous individuals (Table 4) and allele frequencies (Table 1) a proportion of progeny produced by self fertilization can be estimated, according to the formula given by HEDRICK (1983), for each population and each locus (Table 4). The calculated values of those estimates were often high, ranging from 0 to about 78%, but in each population they were inconsistent between loci (Table 4). Similarly

Table 4: f values for each locus and each polymorphic population, with exact probabilities (calculated with GENEPOP by complete enumeration); H_0 = HWE (Hardy and Weinberg equilibrium), P-val – probability of H_0 (computed with an assumption of H_1 = homozygote deficit); W&C – f computed after WEIR & COCKERHAM (1984), R&H – f computed after ROBERTSON & HILL (1984); Matr. – number of matrices analyzed. Multipopulation (by locus) and multilocus (by population) unbiased estimates of Hardy and Weinberg exact values, calculated by complete enumeration with Genepop are also given, standard errors in parentheses; H_1 = heterozygote deficit; for H_1 = heterozygote excess both multilocus and multipopulation tests resulted in not significant levels of probability. f over all loci computed with FSTAT; H_{OB} – observed number of heterozygotic specimens, H% – proportion of heterozygotic specimens (in per cent), S% – proportion of progeny produced by self fertilization (in per cent), according to the formula of HEDRICK (1983).

population	2. Niecziec			3. Czarnocin				Multipopulation
locus	P-val	W&C	R&H	Matr.	P-val	W&C	R&H	Matr.	test (P-val)
GPI	0.0774	+0.343	+0.350	7	0.8292	-0.069	-0.070	9	0.2235 (±0.0008)
H_{OB}/H%/S%	7/21.21/49.58				14/33.33/0.00				
HBDH	0.0043	+0.554	+0.570	11	0.0077	+0.647	+0.660	4	0.0000 (±0.0000)
H_{OB}/H%/S%	6/20.69/70.32				2/5.13/77.98				
IDH-1	0.1378	+0.468	+0.485	3	0.0322	+0.525	+0.540	4	0.0048 (±0.0001)
H_{OB}/H%/S%	2/9.09/62.13				3/10.34/67.89				
multilocus test (P-val)	0.0001 (±0.0000)				0.0009 (±0.0000)				0.0000 (±0.0000)
f over all loci	0.462				0.241				

Table 5: F statistics and Relatedness (Relat.) over all populations of *Valvata piscinalis*, for all polymorphic loci, computed with FSTAT; probability (that f or F or θ is not > 0) < 0.00007, except fo the values given in italics, being not significant, values marked with ' (p within the range: 0.00000-0.00014), marked with " (p within the range: 0.00021-0.00650), marked with ^ (p equals 0.00057), values marked with ~ (p equals 0.01679) and values marked with * (p equals 0.04043); Mean o-pop: mean and standard deviation (in brackets) computed with jacknifing over populations; Mean o-loci: mean and s.d. (in brackets) computed over loci; confidence intervals: Boot o-loci - bootstrapping over loci (95% and 99% respectively), Pas – permutting alleles within samples (95% and 99%), Pat – permutting alleles within total (95% and 99%), Pgt – permutting genotypes within total (95% and 99%).

	Allele	F	θ	f	Relat.
GPI	1	0.163*	0.050~	*0.118*	0.087
	2	0.163*	0.050~	*0.118*	0.087
	All	0.163*	0.050~	*0.118*	0.087
Mean o-pop		0.082* (±0.233)	-0.014 (±0.098)	*0.089* (±0.238)	-0.006 (±0.163)
HBDH	2	0.667	0.201	0.583'	0.241
	3	0.667	0.201	0.583'	0.241
	All	0.667	0.201	0.583'	0.241
Mean o-pop		0.669 (±0.013)	0.279 (±0.146)	0.556' (±0.055)	0.335 (±0.174)
IDH-1	1	0.514^	*0.016*	0.506"	0.022
	2	0.514^	*0.016*	0.506"	0.022
	All	0.514^	*0.016*	0.506"	0.022
Mean o-pop		0.511^ (±0.045)	*-0.011* (±0.057)	0.516" (±0.034)	-0.012 (±0.075)
Over all loci		0.439	0.103	0.375	0.144
Mean o-loci		0.437 (±0.220)	0.111 (±0.073)	0.355 (±0.204)	0.164 (±0.090)
Confidence intervals					
Boot o-loci	95%	[0.163 0.667	[0.016 0.201]	[0.118 0.583]	[0.022 0.241]
	99%	[0.163 0.667]	[0.016 0.201]	[0.118 0.583]	[0.022 0.241]
Pas	95%	[-0.011 0.249]	[0.107 0.112]	[-0.138 0.158]	[-.--- -.---]
	99%	[-0.044 0.293]	[0.106 0.113]	[-0.177 0.209]	[-.--- -.---]
Pat	95%	[-0.120 0.131]	[-0.015 0.026]	[-0.118 0.132]	[-.--- -.---]
	99%	[-0.128 0.182]	[-0.017 0.040]	[-0.143 0.183]	[-.--- -.---]
Pgt	95%	[0.413 0.426]	[-0.022 0.038]	[0.403 0.426]	[-.--- -.---]
	99%	[0.413 0.430]	[-0.025 0.059]	[0.395 0.427]	[-.--- -.---]

inconsistent were the values of the intrapopulation inbreeding coefficient f (WEIR 1980, 1986, F_{IS} in notation of WRIGHT: Table 4). For all loci f was much higher in population 2 (0.462) than in 3 (0.241: Table 4). f quantifies departures from Hardy and Weinberg equilibrium (HWE) and may reflect the degree of inbreeding within populations.

For the polymorphic loci, F-statistics and relatedness were computed over the three studied populations of *V. piscinalis* (Table 5). The random model of WEIR (1990, 1996) was applied to numerical resampling. The confidence intervals, computed by bootstrapping over loci, as well as permuting alleles within total and permuting genotypes between total, pointed to f as the main source of variation, because the confidence intervals for the coancestry coefficient θ had always been much narrower and contained much lower values

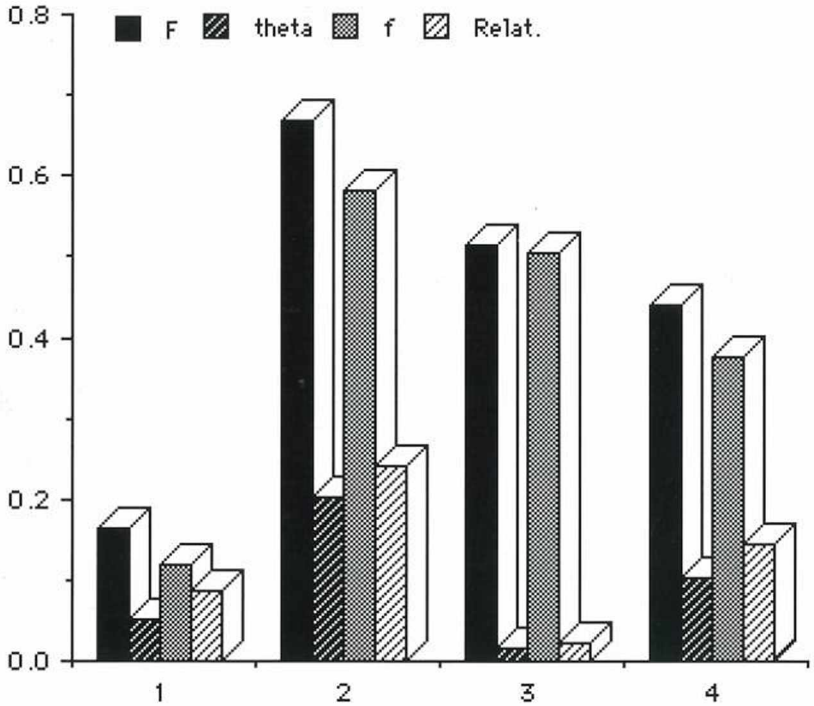

Fig. 1: F-statistics (F, f, θ and relatedness) computed with FSTAT for each locus over all populations of *Valvata piscinalis* (1 – GPI, 2 – HBDH, 3 – IDH-1, 4 – average for all the polymorphic loci).

than those for either the intrapopulation inbreeding coefficient f or overall inbreeding coefficient F (Table 5). The same concerns the mean values and standard deviations computed by jackknifing over loci. The means over populations (Table 5) computed for particular loci indicate that the intervals of the coancestry coefficient θ do not overlap between loci. For GPI, the confidence intervals of F and θ overlap, and f is not significantly higher than 0, thus HWE can be postulated. For HBDH and IDH-1, the confidence intervals of F and θ do not overlap and f is high. It is noteworthy that for IDH-1, the θ value is not significant. The values of relatedness are rather high only for HBDH and negligible for the other two polymorphic loci (Table 5 and Fig. 1).
The value of θ over all loci is 0.103, and the estimate of migrant number computed from it equals 2.177. Coancestry pairwise coefficient and the corresponding Nm values for each pair of populations were also computed (Table 6). Apart from the interspecific values, which are extremely high and low respectively, within *V. piscinalis* θ is low (0.0650-0.1979) and Nm moderately high (1.013-3.594).

Linkage disequilibrium computed with FISHER's technique indicated a statistically significant disequilibrium between HBDH and GPI. In population 3, for HBDH-GPI, composite disequilibrium Δ_{ij} (the best disequilibrium coefficient for analysis of field data, assuming neither random mating nor gametes' frequencies: BLACK & KRAFSUR 1985) equalled ±0.04656, the corrected correlation coefficient R_{ij} was ±0.35644, $\chi^2 = 4.96$, $p \leq 0.0260$. For all three *V. piscinalis* populations the values were: $\Delta_{ij} = \pm 0.03611$, $R_{ij} = \pm 0.21031$, $\chi^2 = 3.80$, $p \leq 0.0511$. OHTA's D-statistics (Table 7) was computed for each pair of loci. D'^2_{IS} was always

	1.	2.	3.	4.
1.	*.****	1.013	2.544	-.---
2.	0.1979	*.****	3.594	0.018
3.	0.0895	0.0650	*.****	0.014
4.	1.0000	0.9325	0.9461	*.***

Tab. 6: Below diagonal: pair-wise θ values; above diagonal: pair-wise Nm values, computed with FSTAT.

higher than $D'_{ST}{}^2$, and $D_{ST}{}^2$ was always higher than $D_{IS}{}^2$. In each case, $D'_{IS}{}^2$ and $D_{IT}{}^2$ concerning the HBDH locus were relatively high, the highest values assumed for IDH-1, GPI and IDH-2.

Discussion

The high values of the unbiased NEI genetic distance between *V. pulchella* and each of the three populations of *V. piscinalis* are increased by the lack of polymorphic loci in *V. pulchella,* even so they are reliable enough for the (sub)genus level rather to be postulated (cf. FALNIOWSKI et al. 1996 for references). This is supported with morphological data. FALNIOWSKI (1989, 1990) observed well marked differences in the radulae and reproductive organs of these valvatids. The total lack of genotypic polymorphism in the studied *V. pulchella* may be due to founder events or may suggest that this rather small and isolated population went through a bottleneck. The same concerns *V. piscinalis* in population 1.

The interpopulation variation within *V. piscinalis*, reflected in both unbiased NEI genetic distance and CAVALLI-SFORZA & EDWARDS arc genetic distance, is very small for even intraspecific comparisons (FALNIOWSKI et al. 1996 for references). All the interpopulation differences detected consist in various frequencies of the same pairs of alleles at each of the

Tab. 7: Ohta's D-statistics: variance components of linkage disequilibrium for *Valvata piscinalis*. Given only pairs of loci with at least one nonzero value.

components:	within subpopulation		between subpopulation		total
loci compared	$D_{IS}{}^2$	$D'_{IS}{}^2$	$D_{ST}{}^2$	$D'_{ST}{}^2$	$D_{IT}{}^2$
HBDH - IDH-1	0.00100	0.19832	0.04840	0.00018	0.19850
HBDH - IDH-2	0.00000	0.17073	0.04391	0.00000	0.17073
HBDH - 6PGDH	0.00000	0.15971	0.04391	0.00000	0.15971
HBDH - GPI	0.00422	0.17989	0.06081	0.00510	0.18499
HBDH - MDH-1	0.00000	0.15944	0.04391	0.00000	0.15944
HBDH - MDH-2	0.00000	0.15944	0.04391	0.00000	0.15944
HBDH - AAT-1	0.00000	0.15944	0.04391	0.00000	0.15944
HBDH - AAT-2	0.00000	0.15944	0.04391	0.00000	0.15944
HBDH - ALP	0.00000	0.15944	0.04391	0.00000	0.15944
IDH-1 - IDH-2	0.00000	0.01923	0.00544	0.00000	0.01923
IDH-1 - 6PGDH	0.00000	0.01923	0.00544	0.00000	0.01923
IDH-1 - GPI	0.00244	0.09378	0.02815	0.00073	0.09451
IDH-1 - MDH-1	0.00000	0.01923	0.00544	0.00000	0.01923
IDH-1 - MDH-2	0.00000	0.01923	0.00544	0.00000	0.01923
IDH-1 - AAT-1	0.00000	0.01923	0.00544	0.00000	0.01923
IDH-1 - AAT-2	0.00000	0.01923	0.00544	0.00000	0.01923
IDH-1 - ALP	0.00000	0.01923	0.00544	0.00000	0.01923
IDH-2 - GPI	0.00000	0.05225	0.01813	0.00000	0.05225
6PGDH - GPI	0.00000	0.05010	0.01813	0.00000	0.05010
GPI 1 - MDH-1	0.00000	0.04834	0.01813	0.00000	0.04834
GPI 1 - MDH-2	0.00000	0.04834	0.01813	0.00000	0.04834
GPI 1 - AAT-1	0.00000	0.04674	0.01813	0.00000	0.04674
GPI 1 - AAT-2	0.00000	0.04674	0.01813	0.00000	0.04674
GPI 1 - ALP	0.00000	0.04674	0.01813	0.00000	0.04674

three polymorphic loci, no private allele having been found. The values of genetic distances are small, notwithstanding that population 1 is distant from the other two (2 and 3) by about 300 km. Compared with the literature, the proportion of polymorphic loci, as well as the mean number of alleles per locus, are quite low. This values, however, are not exceptionally low for prosobranchs (FALNIOWSKI et al. 1996).

The mean expected heterozygosity in both polymorphic populations is below the average for prosobranchs (see FALNIOWSKI et al. 1996 for references). The mean observed values are in both cases lower than the expected ones, thus, in general, a heterozygote deficit is shown. This has also been confirmed by the results of the exact multipopulation and multilocus test, as well as by the multilocus tests for each of the two polymorphic populations. Such a deficit might suggest selfing or inbreeding to be common in the considered populations. This, however, is not the case in *V. piscinalis*.

As concerns the GPI locus, the populations were at HWE. HEDRICK's formula quantifies departures from HWE, thus possible levels of selfing, but gives those estimates in spite of the source of disequilibrium. Nothing better than such estimates is available with data on one locus, but if one had information about more loci and populations, more profound analysis would be possible. In this study, HEDRICK's estimates as well as the values of the inbreeding coefficient f, are inconsistent between loci and populations. In fact, out of the three polymorphic loci, only HBDH showed a statistically significant homozygote excess in both polymorphic populations. To postulate selfing, f values should be correlated between loci, and in *V. piscinalis* this has not been noted.

The low level of interpopulation differentiation is reflected also in the low values of the coancestry coefficient θ the main source of variation is the intrapopulation inbreeding coefficient f. The low values of θ do not confirm selfing either; if the latter had occurred, the coancestry coefficient would have assumed high values even between the two very close populations (HILLIS 1989), thus between 3 and 4. The low values of θ together with the relatively high values of Nm, which mean a high level of gene flow, suggest that it is not much probable that Wahlund's effect is the cause of the observed homozygote excess. Wahlund's effect, however, cannot be excluded, since the values of θ and Nm may depend on scale. Low θ and high Nm between either close or distant populations do not imply that there could not be otherwise if parapatric subpopulations within one population were considered, such comprising breeding communities that may inhabit very small area each (GRANT & UTTER 1988, DAY 1990, JOHNSON, HOLBORN & BLACK 1993).

θ confidence intervals at particular loci do not overlap, thus the occurrence of some disturbing forces, neither selfing nor inbreeding, must be postulated (WEIR 1990). FALNIOWSKI, MAZAN & SZAROWSKA (1999) discuss the possible reasons, other than inbreeding, for homozygote excess. Some of them may concern *V. piscinalis*. One of the sources of the homozygote excess observed in this species, most of all in HBDH, may be selection. On the other hand, D-statistics (see below) has not indicated a strong epistatic selection. Thus, we postulate stochastic factors like genetic drift and some bottlenecks, and/or a significant admixture of migrants in the history of the populations. The water level at localities 2 and 3 is very unstable and at droughts it falls, which would result in partial desiccation of the habitats. This, in turn, would considerably reduce the population size. On the other hand, during floods, the snails may be transported by water from one locality to another. Strong heterozygote deficiency in HBDH locus may also be an artifact, caused by masking heterozygotes by a null allele. Although probable, this cannot be proved with our data and needs further research.

Δ_{ij} and R_{ij} were low but statistically significant for HBDH-GPI in population 3, and almost significant for all populations (probability that $R_{ij} = 0$: $p \leq \approx 0.05$). OHTA's D-statistics indicated relatively high values of $D'_{IS}{}^2$ and $D_{IT}{}^2$ for HBDH, thus some linkage component in

the observed homozygote excess at HBDH can be postulated. $D'_{IS}{}^2$ is the expected variance of the correlation of two alleles at two loci of one gamete in a subpopulation relative to that of the total population; it is the variance of the correlation of the two alleles at two loci on one chromosome in a deme relative to that in the total population (OHTA 1982). The general pattern, in which the values of $D'_{IS}{}^2$ are always higher than those of $D'_{ST}{}^2$, and the values of $D_{ST}{}^2$ are always higher than those of $D_{IS}{}^2$, points to this being a nonsystematic disequilibrium (OHTA 1982, BLACK & KRAFSUR 1985): the main source of the disequilibrium is limited migration, certainly not the epistatic natural selection that favours gametes with the same combinations in every deme (OHTA 1982). The strong epistatic selection in only part of the demes must also be excluded. It is noteworthy all the more because F-statistics has given other results: Nm's between populations were between 1.013 and 3.594. According to the classic one-locus and two-allele model of WRIGHT (1969) to prevent genetic differentiation between populations due to genetic drift, it suffices if $4Nm > 1$. This, however, is not so where there are more alleles and more loci (OHTA 1982), and Nm as high as 10 is necessary to postulate a panmixia (COCKERHAM & WEIR 1993, SLATKIN 1993). In fact, there was obviously no gene flow between population 1 and the others that were about 300 km distant from the former. It seems that D-statistics reflects the biological reality better than θ and the resulting Nm values do.

The main conclusion is that, notwithstanding the homozygote excess observed concerning some loci, in *V. piscinalis* selfing is rare or does not occur. At present, it is impossible to decide what the main source of this excess is. The most probable are genetic drift, linkage disequilibrium and, possibly, selection.

Acknowledgments

We wish to thank M. Grużewski, M.Sc., for his help in collecting snails. This study was supported by a Committee of Scientific Research (KBN) grant BW/IZ/23/97.

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(Received on May 18, 2000)