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Spatio-temporal genetic structure and gene flow between two distinct shell morphs of the planktonic developing periwinkle *Littorina striata* (Mollusca: Prosobranchia)

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ABSTRACT: The planktonic developing periwinkle *Littorina striata* produces both nodulose and smooth shells, which were originally regarded as 2 separate species. Although both morphs occur microsympatrically, their distribution is not random. Nodulose shells predominate at wave-sheltered sites, whereas smooth shells are more common at wave-exposed sites. The degree of genetic similarity between the 2 shell types and their microgeographic spatio-temporal genetic structuring were investigated using allozyme electrophoresis. This indicated that: (1) both morphs share a common gene pool, (2) gene flow between populations is high and of comparable magnitude to gene flow between both morphs, (3) the population genetic structure of *L. striata* remains stable over a sampling period of 3 yr, and (4) genetic and morphological distances between populations are not correlated. These results thus confirm the conspecific status of the 2 shell types and suggest that shell variability and spatial patterning in *L. striata* persist in the presence of intense gene flow.

KEY WORDS: *Littorina striata* · Planktonic development · Gene flow · Spatio-temporal variation · Shell morphology · Allozymes

INTRODUCTION

Many intertidal prosobranchs have shells that are highly polymorphic with respect to colour, size, shape and sculpture (e.g. Struhsaker 1968, Crothers 1981, 1992, Boulding & Van Alstyne 1993, Chapman 1994, Johannesson 1996, Hull et al. 1996, McQuaid 1996, Reid 1996). Although most of this variability can be explained in functional terms (e.g. Crothers 1981, Seeley 1986, McQuaid 1996, Reid 1996), it often remains unclear whether shell polymorphisms are maintained and patterned by natural selection or ecophenotypic plasticity. Current theories usually invoke natural selection to explain shell variation and its patterning in non-planktonic developers (e.g. Seeley 1986, Chapman 1994, Johannesson & Johannesson 1996), as non-planktonic developing prosobranchs have limited dispersal abilities and are therefore subjected to greater genetic population differentiation (e.g. Scheltema 1971, Crisp 1978), as seen for instance in the non-planktonic developing littorinids *Littorina saxatilis* (Johannesson et al. 1993, Rolán-Alvarez et al. 1996, Johannesson & Tatarenkov 1997) and *L. mariae* (Tatarenkov & Johannesson 1994). There is, however, growing evidence that prosobranchs with high dispersal abilities (i.e. with planktonic development) may also be genetically more subdivided than is currently assumed (e.g. Palumbi 1994 and references therein). Moreover, some planktonic developers display a considerable amount of shell variability too (e.g. Stiven 1992, Karakousis et al. 1993, Frid & Fordham 1994, Reid 1996 and references therein, Reimer & Tendengren 1996).

The periwinkle *Littorina striata* King & Broderip, 1832, is one such polymorphic planktonic developing

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prosobranch (Rosewater 1981, Reid 1996, De Wolf et al. 1997). The species produces 2 major shell morphotypes: one with a spirally sculptured, nodulose shell, the other having a nearly smooth shell without nodules (e.g. Rosewater 1981, Reid 1996, De Wolf et al. 1997). Recent attempts to describe and interpret the microscale patterning and functionality of this polymorphism (Britton 1995, Vedel & Depledge 1995, De Wolf et al. 1997) suggested that at least part of the shell variability shows ecological correlates (Britton 1995, De Wolf et al. 1997). Earlier authors even went so far as to suggest that both morphotypes represent different species (e.g. Dunker 1853, Weinkauff 1882). Although it is now generally accepted that only 1 species is involved (e.g. Reid 1996), it is still unclear whether and to what extent the 2 morphotypes are genetically similar. This is equivalent to asking whether both morphotypes share a common gene pool. If so, it remains to be investigated how they maintain their morphological integrity, differentiation and ecological patterning (De Wolf et al. 1997).

As a first step in the resolution of these issues we applied allozyme electrophoresis to estimate the degree of gene flow and spatio-temporal genetic differentiation among populations and morphotypes of *Littorina striata* at a microgeographic scale. To this end we used the locations and material that were analyzed for morphometric variation by De Wolf et al. (1997).

MATERIAL AND METHODS

Littorina striata. *L. striata* is an endemic periwinkle in the Macaronesian archipelagos (Azores, Madeira, Canary Islands and Cape Verde Islands), where it is common in the upper littoral and the littoral fringe of rocky shores (Rosewater 1981, Reid 1996). At Ilheu de Vila Franca do Campo (hereafter referred to as Ilheu), a drowned volcanic crater situated 1 km off the south coast of the island of São Miguel (Azores), the 2 morphotypes of *L. striata* co-occur microsympatrically, even though they are not randomly distributed (Britton 1995, De Wolf et al. 1997). The nodulose morph, which is on average smaller and lighter than the smooth morph, is mainly found at the sheltered lagoon inside of the crater, whereas the larger and heavier smooth morph dominates the crater's wave-exposed outside (De Wolf et al. 1997). Regardless of the morphotype, shells from the lagoon are on average smaller, less globose and have a smaller aperture compared to shells from the wave-exposed outside. In addition, both morphs enlarge their aperture size within a few months when transplanted from the lagoon to the outside (De Wolf et al. 1997). Yet, within a wave exposure regime, the smooth morph has on average a larger aperture (De Wolf et al. 1997).

Allozyme electrophoresis. *Littorina striata* (n = 1078) was collected annually (August 1992, July 1993 and July 1994) at 8 sites in Ilheu, yielding a total of 24 samples (i.e. 3 consecutive samples per site). Four sites were situated in the sheltered lagoon inside the crater (1 to 4), while the 4 other sites were located at the wave-exposed outside of the crater (5 to 8). Two outside locations were low-shore populations (5 and 7), the 2 others were high-shore populations (6 and 8). For a description of the area and sampling sites, see Morton (1990) and De Wolf et al. (1997).

Specimens were starved for 4 d and subsequently frozen and stored at −80°C. Each individual was morphologically characterized by De Wolf et al. (1997). Soft body parts were prepared for vertical polyacrylamide gel electrophoresis (PAGE) as described by Backeljau & Warmoes (1992). A preliminary screening of 38 enzymes yielded 4 well-resolved, polymorphic loci that could be used for population genetic analysis: glucose phosphate isomerase (GPI, E.C. 5.3.1.9), 6-phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44) and malate dehydrogenase (MDH, E.C. 1.1.1.37) were resolved in a Tris/citric acid buffer at pH 8.0, while mannose phosphate isomerase (MPI, E.C. 5.3.1.8) was analyzed using a Tris/glycine electrode buffer and a Tris/HCl gel buffer, both at pH 9.0. Enzyme stainings were adapted from Harris & Hopkinson (1976).

Statistical analysis. Genotype frequency departures from Hardy-Weinberg (HW) equilibrium conditions were tested with chi-squared and exact probability tests. Chi-squared tests were performed using the CHIHW program (v. 3.1) of Zaykin & Pudovkin (1993), which applies a pseudo-probability test based on the Monte Carlo procedure (1000 permutations) proposed by Roff & Bentzen (1989). Exact probabilities were estimated with the GENEPOP software package v. 1.1 (Raymond & Rousset 1995), which applies the Markov chain method proposed by Guo & Thompson (1992).

For each population, linkage disequilibria were tested for all pairwise loci comparisons, using the GENEPOP software package v. 1.1 (Raymond & Rousset 1995).

As several mollusc species show a relationship between individual heterozygosity and size (e.g. Koehn & Gaffney 1984, Gaffney et al. 1990, Zouros & Pogson 1994), we tested this possibility in *L. striata* too, because it could affect our population genetic analysis, given the differential size and morphotype distribution of *Littorina striata* at Ilheu. Therefore, for each sampling year we calculated and tested Spearman Rank correlations between individual heterozygosity (the number of allozyme loci at which a specimen is heterozygous, i.e. 0 to 4) and individual values for shell height, shell width, aperture height, aperture width, height of the shell top, total weight and body weight.

These calculations were performed using the STATISTICA v. 5.0 software (Statsoft, Tulsa, OK, USA).

Allele frequency heterogeneities between populations and morphotypes were tested per sampling year with R×C contingency tables. The heterogeneity between morphotypes was assessed for the lagoon and outside specimens separately, in order to reduce possible spatial/ecological differentiation between the sampling areas. In addition, temporal allele frequency heterogeneities were tested within each population, among all 3 sampling years. All these analyses were performed with the GENEPOP v. 1.1 (Raymond & Rousset 1995) package, which applies a Markov chain method (Guo & Thompson 1992) to obtain unbiased estimates of the Fisher exact test statistics.

The degree of genetic differentiation between populations was estimated per sampling year by means of Wright's (1965) F and Nei's (1973, 1977) G statistic as implemented by the programs BIOSYS (Swofford & Selander 1989) and GENESTAT v. 3.31 (Lewis 1992). Because F_{ST}/G_{ST} values were not significantly different from 0, the amount of gene flow (Nm) between populations was only inferred from private allele frequencies, i.e. the frequencies of alleles that are unique to 1 population only (Slatkin 1985a, Slatkin & Barton 1989). These latter were calculated with the program GENESTAT v. 3.31 (Lewis 1992).

Finally, per sampling year, Prevosti distance (Wright 1978) was calculated between populations using the program BIOSYS (Swofford & Selander 1989). The relationship between these distances and the squared Mahalanobis distances among the same populations, calculated from the morphological data of De Wolf et al. (1997), was evaluated with a Mantel test, using 1000 permutations, implemented by the NTSYS v. 1.80 program (Rohlf 1993). The distance matrices are available upon request.

A significance level ,of 5% was used throughout. The sequential Bonferroni technique was employed to correct for false assignments of significance by chance alone (multiple testing problem) (Rice 1989).

RESULTS

Allele frequencies and the results of the HW tests are provided in Appendix 1, which shows that over the 3 yr, and irrespective of the test statistic used, only 3 cases of HW deviations were observed. After sequential Bonferroni correction for multiple testing, none of the 3 deviations remained significant. No linkage disequilibria were detected (available upon request).

With the exception of 1 case (body weight in 1993), no significant Spearman Rank correlations were found between individual heterozygosities and size/weight variables (Table 1). However, this particular correlation was, here too, no longer significant after Bonferroni correction. Hence, mere size related heterozygosity differences were not expected to affect our analyses.

Table 2 shows the results of the analysis of allele frequency heterogeneities between both morphotypes, within the lagoon and outside of the crater, in order to correct for possible genetic heterogeneity due to wave exposure differences. After sequential Bonferroni correction, no significant genetic heterogeneity was detected between the morphotypes (Table 2). Table 3 shows the results of the analysis of allele frequency heterogeneities between populations (i.e. mixture of both morphotypes) per sampling year. Out of 300 tests, only 21 were significant. However, after sequential Bonferroni correction, none of these tests remained significant. Likewise, no significant genetic heterogeneity was detected between the sampling years, after sequential Bonferroni correction (Table 4).

Table 1. *Littorina striata*. Spearman Rank correlations between individual heterozygosity and morphometric characteristics (HS= shell height; WS = shell width; HA = aperture height, WA = aperture width; HT = shell top height; TW = total weight; BW = body weight)

Variable	1992		1993		1994	
	R	p	R	p	R	p
HS	−0.1135	0.1835	0.1547	0.0973	0.0022	0.9802
WS	−0.1291	0.1298	0.1644	0.0778	−0.0747	0.3984
HA	−0.0918	0.2822	0.1650	0.0775	−0.0465	0.5997
WA	−0.0067	0.4353	0.1722	0.0645	−0.0351	0.6916
HT	−0.0964	0.2588	0.1400	0.1350	−0.0048	0.9571
TW	−0.0761	0.3735	0.1547	0.0972	−0.0365	0.6806
BW	−0.0950	0.2661	0.2003	0.0311	−0.0223	0.8013

Table 2. *Littorina striata*. Genetic heterogeneity analysis. Probabilities of exact tests for comparison of smooth and nodulose morphotypes, within the lagoon and outside

Locus	1992		1993		1994	
	Lagoon	Outside	Lagoon	Outside	Lagoon	Outside
GPI	0.8311	0.8056	0.4513	0.8899	0.9384	0.4888
MPI	0.1168	0.0772	0.6755	0.3956	0.8974	0.3297
PGD	0.3262	0.6979	0.0421	0.0051	0.3679	0.0758
MDH	1.0000	1.0000	0.3552	0.2576	0.3988	1.0000

Table 3. *Littorina striata*. Analysis of allele frequency heterogeneity tests between populations, using exact probabilities for pairwise sampling site comparisons. Out of a possible 300 tests, only those are shown where at least 1 significant heterogeneity was scored. na = test statistic non-applicable, due to monomorphicity of the considered locus

Population	1992			1993			1994		
Locus:	GPI	MPI	PGD	GPI	MPI	PGD	GPI	MPI	PGD
1-4	0.7681	0.7052	0.5719	0.2410	0.1547	na	0.0239	0.2345	0.2552
1-5	0.9185	0.0346	0.2761	0.4265	0.7351	0.4936	0.1826	1.0000	1.0000
1-8	0.7567	0.0602	1.0000	0.1614	0.1204	0.0255	0.1510	1.0000	1.0000
2-4	0.2210	0.6367	0.3309	0.0205	0.7970	0.2600	0.3037	0.0948	0.4537
2-5	0.3682	0.0019	0.4558	0.6044	0.1343	0.3645	0.8926	0.5667	0.1607
2-6	0.1928	0.0052	0.1957	0.9882	1.0000	0.3561	0.7124	0.6567	1.0000
2-8	0.8900	0.0023	0.9138	1.0000	0.8112	0.4881	0.6590	0.6645	0.0786
3-4	0.7679	0.0415	0.5902	0.1074	0.1386	na	0.2504	0.5366	0.2449
3-7	0.9557	0.5934	1.0000	0.1171	0.3937	0.0493	0.6517	0.3966	0.6359
3-8	0.7074	0.6997	0.8314	0.2086	0.1135	0.0107	0.7132	0.8003	1.0000
4-5	0.4500	0.0010	0.0341	0.0213	0.1111	0.5087	0.3347	0.2476	0.2513
4-6	0.1047	0.0110	0.0177	0.0740	1.0000	1.0000	0.0231	0.1106	0.4868
4-7	0.5600	0.3059	1.0000	0.0367	0.7956	0.1360	0.1380	1.0000	0.2320
4-8	0.4903	0.0102	0.4737	0.0344	1.0000	0.0434	0.5217	0.1654	0.0929
5-6	0.0333	0.8256	1.0000	0.4869	0.1389	0.4907	0.1581	0.8078	0.1095

Table 4. *Littorina striata*. Analysis of allele frequency heterogeneity tests within each population, among all 3 sampling years. na = test statistic non-applicable, due to monomorphicity of the considered locus

Site	GPI	MPI	PGD	MDH
1	0.7093	0.3006	0.0956	na
2	0.6562	0.0049	0.9078	0.2880
3	0.5723	0.5239	0.0576	na
4	0.8312	0.0546	0.4433	0.58968
5	0.0540	0.1053	0.0395	0.30456
6	0.8165	1.0000	0.1477	na
7	0.3142	0.5205	0.0393	na
8	0.3812	0.9340	0.0295	0.8140

The overall mean F_{ST} (G_{ST}) values were small and not significantly different from 0, suggesting little or no population differentiation (Table 5). Hence, private allele based Nm estimates were high (i.e. Nm $\gg$ 1) (Table 6), though fluctuated depending on the gene flow calculation method used (i.e. Slatkin 1985a or Slatkin & Barton 1989), or the sampling year that was considered (Table 6).

The Mantel tests of the matrix correlation between the Prevosti and squared Mahalanobis distances per sampling year revealed no significant relationships (Table 7). Hence, genetic and morphometric data were apparently not correlated.

DISCUSSION

The low mean F_{ST} (G_{ST}) values reported here for *Littorina striata* are comparable to corresponding values observed in other prosobranchs with planktonic development (e.g. Maestro et al. 1982, Kartavtsev & Zaslavskaya 1983, Janson 1985a, b, Brown 1991, Johannesson 1992, Macaranas et al. 1992, Ford & Mitton 1993, Karakousis et al. 1993, Parsons & Ward 1994, Dayan & Dillon 1995, Benzie & Williams 1996), whereas they are considerably lower than F_{ST} (G_{ST}) values of non-planktonic developing species (e.g. Knight et al. 1987, Grant & Utter 1988, Johannesson & Johannesson 1990, Ward 1990, Rolan-Alvarez et al. 1995, Johnson & Black 1996). Moreover, the genetic population structure of *L. striata* appears temporally stable, as no temporal effects could be detected in the genetic heterogeneity

Table 5. *Littorina striata*. Summary of F_{ST} statistic (Wright 1965) and G_{ST} statistic (Nei 1973, 1974) at all loci [significance of F_{ST} was tested by means of the Pearson chi-squared statistic for an M × N contingency table with (M – 1)(N – 1) degrees of freedom where M = number of populations and N = number of alleles]

Locus	1992		1993		1994	
	F_{ST}	G_{ST}	F_{ST}	G_{ST}	F_{ST}	G_{ST}
GPI	0.010	0.000	0.023	0.011	0.015	0.003
MPI	0.032	0.022	0.015	0.002	0.010	0.000
PGD	0.016	0.006	0.031	0.017	0.009	0.000
MDH	0.011	0.002	0.010	0.000	0.016	0.005
Mean	0.015	0.005	0.022	0.009	0.013	0.001

Table 6. *Littorina striata*. Gene flow estimates based on private alleles, for 1992, 1993, 1994 and for all 3 sampling years. P(1): frequency of private alleles; N_{sam}: sample size

	1992	1993	1994	Overall
P(1)	0.0085	0.0290	0.0135	0.014
N_{sam}	50.1875	35.4338	38.5625	41.3646
Nm (Slatkin 1985a)	50.0186	6.2359	26.0442	22.5928
Nm (Slatkin & Barton 1989)	26.6414	5.0466	16.2412	14.2647

Table 7. *Littorina striata*. Mantel test for correlation between Prevosti genetic and squared Mahalanobis distances

Sampling year	Matrix correlation	p
1992	0.1161	0.6750
1993	0.1172	0.7167
1994	0.1516	0.7587

analysis and as annual F_{ST} (G_{ST}) values were highly similar. Depending on which gene flow calculation method is used (i.e. Slatkin 1985a vs Slatkin & Barton 1989), different Nm estimates can be obtained (De Wolf et al. 1995), though in this study, with all Nm values well over 1, we conclude that they are of comparably high magnitude. Hence, gene flow estimates are high and comparable among the 3 sampling years. With Nm values well over 1 it is expected that population differentiation due to random genetic drift should not occur (e.g. Slatkin 1985b). Similarly, there is no genetic differentiation between the 2 morphotypes, which supports their conspecific status. Hence, the shell polymorphism in *L. striata* and its microscale spatial/ecological patterning seem to persist in the presence of a substantial amount of gene flow, which counteracts genetic population differentiation at the 4 loci investigated.

The ecological patterning of the 2 morphotypes in *Littorina striata* (i.e. nodulose shells are more abundant at wave-sheltered sites, while smooth shells dominate at wave-exposed shores) seems at first sight comparable to the distribution of 2 shell morphotypes in the non-planktonic developing periwinkle *L. saxatilis* along the Galician coast (Spain) (Johannesson et al. 1995, Rolán-Alvarez et al. 1996). There, it appears that upper, less wave-exposed shore levels are occupied by ridged shells, while the lower, heavily wave-exposed shore levels are dominated by smooth shells (Rolán-Alvarez et al. 1996). In mid-shore zones where both morphs occur together and may produce 'hybrid' shells, there is behavioural evidence for non-random mating (Johannesson et al. 1995, Rolán-Alvarez et al. 1995). In addition, allozyme analyses have shown that for a given shore, gene flow between shell forms is lower compared to gene flow within each morphotype, even though there seems to be no significant genetic differentiation between the 2 morphs (Johannesson et al. 1993, Rolán-Alvarez et al. 1996). Furthermore it has been shown in Swedish populations of *L. saxatilis* that genetic differentiation is not only caused by limited gene flow but can also result from specific habitat related effects (Johannesson & Tatarenkov 1997). The situation of the Galician *L. saxatilis* morphotypes has been interpreted as indicating partial reproductive isolation, which may represent a possible case of incipient sympatric speciation (Johannesson et al. 1995).

Despite the similarity between the morphological patterning in *Littorina striata* and Galician *L. saxatilis*, our data suggest that the 2 morphotypes in *L. striata* are not reproductively isolated and therefore it is unlikely that they would represent another case of incipient sympatric speciation. Yet, we have no behavioural data on the possibility of non-random mating. Given the fundamentally different developmental modes between *L. striata* (planktonic larvae) and Galician *L. saxatilis* (brooder releasing crawling juveniles), one might wonder whether the mechanisms that are responsible for the comparable microscale morphological patterning in both species are similar too.

Rolán-Alvarez et al. (1996) suggested that selection maintains the differential microenvironmental distribution of the smooth and ridged shells in *Littorina saxatilis*. In *L. striata*, in contrast, it is much less clear whether, and to what extent, selection determines morphological patterning. This issue is more complicated in *L. striata*, as selection and/or plasticity may act before, during or after settlement of the larvae, a problem not encountered in *L. saxatilis*, whose offspring immediately crawl around in the neighbourhood of the adults.

A transplantation experiment executed at the same sites where the present material was sampled suggested that some aspects of shell morphology (i.e. aperture size) of *Littorina striata* may be plastic (i.e. ecophenotypic). There is, however, no clear evidence of nodulose shells becoming smooth or vice versa during lifetime (Reid 1996), so that shell sculpture in *L. striata* seems to be fixed. De Wolf et al. (1997) discussed the possible functional significance of several shell features in *L. striata* at Ilheu, but such considerations are not sufficient to decide about underlying mechanisms.

In *Littorina picta* (now *Nodilittorina hawaiiensis*), another planktonic developing littorinid with sculptural variation, nodulation is maintained by selection (Struhsaker 1968). In contrast, our findings reveal a

spatio-temporal homogeneity of allelic frequencies and thus an abscence of genetic differentiation between both morphs of *L. striata*. If selection, rather than plasticity is responsible for the observed morphological heterogeneity and patterning in in this species, as is supposed to be the case in *L. saxatilis* and in *L. picta*, then it should be very intense in order to overcome the homogenizing effects of high gene flow which seem to exist between both morphs.

Obviously, further genetic studies, using more variable genetic markers are needed to support the current data, as molecular markers could reveal genetic differences that remained undetected in the present work.

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Appendix 1. *Littorina striata*. Allele frequencies, observed (H_{obs}) and expected (H_{exp}) heterozygosity levels, chi-squared (p-χ^2) and exact probabilities (p-ext) for deviation from Hardy-Weinberg equilibria. na: test statistic non-applicable, due to (1) monomorphic loci or (2) quasi monomorphic loci (i.e. 2 alleles are present, but one is represented only once)

Locus		1	2	3	4	5	6	7	8
1992									
GPI	(N)	40	49	44	61	37	39	49	92
	A	0.013	0.000	0.011	0.016	0.014	0.013	0.020	0.005
	B	0.325	0.276	0.307	0.369	0.365	0.256	0.276	0.277
	C	0.013	0.020	0.045	0.033	0.000	0.000	0.051	0.033
	D	0.600	0.643	0.614	0.533	0.554	0.718	0.612	0.620
	E	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.016
	F	0.050	0.061	0.023	0.033	0.068	0.013	0.041	0.049
	H_{obs}	0.475	0.571	0.432	0.492	0.568	0.359	0.531	0.500
	H_{exp}	0.532	0.507	0.527	0.577	0.555	0.418	0.545	0.536
	p-χ^2	0.148	0.665	0.179	0.027	0.405	0.681	0.645	0.424
	p-ext	0.755	0.795	0.178	0.013	0.412	0.679	0.657	0.252
MPI	(N)	41	36	44	47	37	40	38	90
	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.049	0.014	0.114	0.032	0.162	0.138	0.079	0.133
	C	0.951	0.986	0.886	0.968	0.838	0.863	0.912	0.867
	D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	H_{obs}	0.098	0.028	0.136	0.064	0.216	0.225	0.158	0.244
	H_{exp}	0.093	0.027	0.201	0.062	0.272	0.237	0.145	0.231
	p-χ^2	1.000	1.000	0.079	1.000	0.237	1.000	1.000	0.696
	p-ext	1.000	na	0.075	1.000	0.215	0.548	1.000	1.000
PGD	(N)	41	49	51	58	39	39	41	90
	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
	B	0.976	0.969	0.980	0.991	0.936	0.923	0.988	0.965
	C	0.024	0.031	0.020	0.009	0.064	0.077	0.012	0.028
	D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
	H_{obs}	0.049	0.061	0.039	0.017	0.128	0.103	0.024	0.089
	H_{exp}	0.048	0.059	0.038	0.017	0.120	0.142	0.024	0.086
	p-χ^2	1.000	1.000	1.000	1.000	1.000	0.194	1.000	1.000
	p-ext	1.000	1.000	1.000	na	1.000	0.187	na	1.000
MDH	(N)	41	50	52	57	39	37	48	90
	A	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.017
	B	1.000	1.000	1.000	0.991	1.000	1.000	1.000	0.983
	H_{obs}	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.033
	H_{exp}	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.033
	p-χ^2	na	na	na	1.000	na	na	na	1.000
	p-ext	na	na	na	na	na	na	na	1.000
1993									
GPI	(N)	38	36	39	26	38	38	40	39
	A	0.026	0.000	0.051	0.000	0.039	0.000	0.000	0.000
	B	0.316	0.222	0.282	0.442	0.197	0.237	0.237	0.218
	C	0.053	0.014	0.013	0.000	0.026	0.013	0.000	0.013
	D	0.579	0.736	0.641	0.538	0.684	0.724	0.724	0.712
	E	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000
	F	0.026	0.028	0.013	0.019	0.039	0.026	0.026	0.050
	H_{obs}	0.526	0.417	0.462	0.346	0.447	0.447	0.525	0.436
	H_{exp}	0.561	0.408	0.507	0.514	0.489	0.419	0.433	0.417
	p-χ^2	0.148	1.000	0.519	0.082	0.326	0.903	0.270	1.000
	p-ext	0.105	1.000	0.582	0.070	0.438	1.000	0.369	1.000

Appendix 1 (continued)

Locus		1	2	3	4	5	6	7	8
1993 (continued)									
MPI	(N)	38	36	39	28	31	37	39	37
	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.066	0.125	0.064	0.143	0.048	0.135	0.115	0.149
	C	0.934	0.875	0.936	0.857	0.952	0.865	0.885	0.851
	D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	H_{obs}	0.079	0.194	0.128	0.214	0.097	0.270	0.231	0.243
	H_{exp}	0.123	0.219	0.120	0.245	0.092	0.234	0.204	0.253
	p-χ^2	0.109	1.000	1.000	1.000	1.000	0.610	0.642	1.000
	p-ext	0.130	0.434	1.000	0.440	1.000	1.000	1.000	1.000
PGD	(N)	34	35	40	25	34	37	37	36
	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	1.000	0.957	1.000	1.000	0.956	0.986	0.946	0.917
	C	0.000	0.043	0.000	0.000	0.015	0.014	0.054	0.083
	D	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000
	H_{obs}	0.000	0.086	0.000	0.000	0.029	0.027	0.108	0.111
	H_{exp}	0.000	0.082	0.000	0.000	0.085	0.027	0.102	0.153
	p-χ^2	na	1.000	na	na	0.013	1.000	1.000	0.199
	p-ext	na	1.000	na	na	0.014	na	na	0.202
MDH	(N)	34	35	30	25	37	38	37	38
	A	0.000	0.014	0.000	0.000	0.014	0.000	0.000	0.000
	B	1.000	0.986	1.000	1.000	0.986	1.000	1.000	1.000
	H_{obs}	0.000	0.029	0.000	0.000	0.027	0.000	0.000	0.000
	H_{exp}	0.000	0.028	0.000	0.000	0.027	0.000	0.000	0.000
	p-χ^2	na	1.000	na	na	1.000	na	na	na
	p-ext	na	na	na	na	na	na	na	na
1994									
GPI	(N)	40	35	33	38	38	40	39	40
	A	0.013	0.000	0.000	0.000	0.000	0.013	0.038	0.000
	B	0.250	0.343	0.303	0.408	0.408	0.275	0.256	0.387
	C	0.013	0.014	0.015	0.026	0.013	0.000	0.026	0.025
	D	0.675	0.600	0.652	0.513	0.526	0.663	0.641	0.575
	E	0.000	0.000	0.000	0.039	0.000	0.000	0.013	0.000
	F	0.050	0.043	0.030	0.013	0.053	0.050	0.026	0.013
	H_{obs}	0.450	0.543	0.455	0.500	0.447	0.450	0.385	0.525
	H_{exp}	0.479	0.520	0.483	0.568	0.554	0.483	0.520	0.518
	p-χ^2	0.837	1.000	0.677	0.001	0.364	0.394	0.207	0.492
	p-ext	0.763	1.000	0.811	0.012	0.331	0.343	0.083	0.607
MPI	(N)	39	35	38	38	40	40	40	40
	A	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.115	0.129	0.105	0.053	0.112	0.138	0.063	0.125
	C	0.885	0.843	0.895	0.947	0.887	0.863	0.938	0.875
	D	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000
	H_{obs}	0.231	0.286	0.211	0.105	0.225	0.275	0.075	0.250
	H_{exp}	0.204	0.273	0.188	0.100	0.200	0.237	0.117	0.219
	p-χ^2	0.635	0.121	1.000	1.000	0.688	0.609	0.099	0.611
	p-ext	1.000	0.321	1.000	1.000	1.000	1.000	0.124	1.000
PGD	(N)	37	33	39	38	39	40	40	40
	A	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000
	B	0.973	0.970	0.974	0.974	0.962	0.962	0.988	0.962
	C	0.000	0.030	0.000	0.026	0.000	0.038	0.000	0.000
	D	0.027	0.000	0.026	0.000	0.026	0.000	0.013	0.038
	H_{obs}	0.054	0.061	0.051	0.053	0.077	0.075	0.025	0.075
	H_{exp}	0.053	0.059	0.050	0.051	0.075	0.072	0.025	0.072
	p-χ^2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	p-ext	1.000	1.000	1.000	1.000	1.000	1.000	na	1.000
MDH	(N)	36	39	40	40	40	40	40	40
	A	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.013
	B	1.000	1.000	1.000	0.975	1.000	1.000	1.000	0.987
	H_{obs}	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.025
	H_{exp}	0.000	0.000	0.000	0.049	0.000	0.000	0.000	0.025
	p-χ^2	na	na	na	1.000	na	na	na	1.000
	p-ext	na	na	na	1.000	na	na	na	na

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