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ORIGINAL PAPER

Phenotypic plasticity and genetic isolation-by-distance in the freshwater mussel *Unio pictorum* (Mollusca: Unionoida)

A. Zieritz · J. I. Hoffman · W. Amos · D. C. Aldridge

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Abstract Freshwater mussels (Unionoida) show high intraspecific morphological variability, and some shell morphological traits are believed to be associated with habitat conditions. It is not known whether and which of these ecophenotypic differences reflect underlying genetic differentiation or are the result of phenotypic plasticity. Using 103 amplified fragment length polymorphism (AFLP) markers, we studied population genetics of three paired *Unio pictorum* populations sampled from two different habitat types (marina and river) along the River Thames. We found genetic differences along the Thames which were consistent with a pattern of isolation by distance and probably reflect limited dispersal via host fish species upon which unionoid larvae are obligate parasites. No consistent genetic differences were found between the two different habitat types suggesting that morphological differences in the degree of shell elongation and the shape of dorso-posterior margin are caused by phenotypic plasticity. Our study provides the first good evidence for phenotypic plasticity of shell shape in a European unionoid and illustrates the need to include genetic data in order properly to interpret geographic patterns of morphological variation.

Keywords Amplified fragment length polymorphisms (AFLPs) · Morphological variation · Morphometrics · Phenotypic plasticity · Unionidae

Introduction

Many species of freshwater mussels of the order Unionoida display great variability in shell morphology, some of which appear to be associated with differences in habitat (Ortmann 1920; Agrell 1948; Eagar 1978; Watters 1994; Zieritz and Aldridge 2009). These so-called ecomorphotypes may arise through two mechanisms. First, they may simply reflect phenotypic plasticity, the ability to change phenotype in response to variation in the environment (Via et al. 1995). The alternative mechanism involves genetic variability. If a

A. Zieritz (✉) · J. I. Hoffman · W. Amos · D. C. Aldridge
Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK
e-mail: alexandra.zieritz@gmx.net

species produces very large numbers of offspring, it is possible that those that manage to settle in any given habitat represent a biased subset of all offspring, specifically those able to do best under those conditions. The unionoid life cycle potentially allows for both mechanisms to operate. Thus, the larvae of almost all unionoid species are obligate fish parasites, making the habitat in which the juvenile mussel excysts from the host highly unpredictable, and hence putting a premium on the ability to thrive under diverse habitats. Equally, females brood eggs in marsupia which can be fertilised by several males via the inhalant current, potentially producing genetically diverse offspring (e.g. Kat 1984). Which of these two mechanisms dominates in nature remains open to debate.

Few genetic studies have explicitly investigated the role of genetic versus phenotypic variability on morphological variation in unionoids, and these have typically used relatively conservative DNA markers that lacked adequate resolution to detect genetic differences among populations (e.g. Soroka and Zdanowski 2001). Nevertheless, previous publications have generated mixed support for the two hypotheses. For example, reciprocal transplant experiments on two unionoid species between Canadian lakes showed that shell height to length ratios usually change as a function of the environment, indicating phenotypic plasticity of shell form. On the other hand, overall growth rates appear to be under genetic control (Hinch et al. 1986; Hinch and Green 1989).

Transplant experiments offer a direct test of whether phenotypic plasticity operates, but are often undesirable ecologically. Alternative tests are based on searches for a correlation between phenotype and genotype. For example, Buhay et al. (2002) and Machordom et al. (2003), studied morphologically distinct *Epioblasma* and *Margaritifera* “subspecies” respectively, but failed to find differences based on allozymes and/or mitochondrial genes. On the other hand, Serb et al. (2003) found that mtDNA sequence data supported the validity of several *Quadrula* taxa that had originally been classified on the basis of shell morphology but were later lumped with other species on the basis that this simply reflected phenotypic plasticity. Moreover, in their study on genetics of central European freshwater pearl mussels (*Margaritifera margaritifera* Linneaus), Geist and Kuehn (2005) observed that, while in some cases morphologically atypical mussels showed a strong genetic divergence from other populations, in other populations a link between genetic status and shell shape was not evident.

Such inconsistent findings hint at a deeper problem. In looking for a correlation between phenotype and genotype it is natural to select individuals from opposite ends of the observed morphological range. These will often be drawn from habitats that are not only divergent in ecological properties, but also widely separated geographically. Consequently, the resulting samples may appear genetically different simply through a long-term lack of gene flow, rather than the presence of discrete lineages adapted to each habitat. Since most of the work on unionoid population genetics has so far focused on systematics and phylogeny (e.g. Davis and Fuller 1981; Serb et al. 2003; Källersjö et al. 2005), conservation genetics (e.g. Mulvey et al. 1997; Buhay et al. 2002; Geist and Kuehn 2005) and evolutionary history (e.g. Nagel 2000; Huff et al. 2004; Elderkin et al. 2007), morphological differences between populations in these studies were usually accompanied with geographic distance. This makes it difficult to assess if genetic differences between such populations are merely a result of isolation by distance (i.e. evidence for phenotypic plasticity) or actually reflect differences in morphology (i.e. evidence for genetically induced morphological differences).

Here, we attempt to distinguish between phenotypic plasticity and genetic adaptation by exploiting an unusual system in which large habitat differences can be found over very short geographic distances. *Unio pictorum* (Linnaeus) is an abundant species in the River

Thames and can be found living both in the river itself, and in adjacent marinas (Fig. 1). By sampling from paired sites along the river, we are able to ask whether any genetic differences are due primarily to a simple isolation by distance model, or to the existence of two genetically distinct morphs, one adapted to the marina habitat and one to the river. Genetic analysis was conducted using amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995; Blears et al. 1998), a technique that reveals large numbers of variable traits capable of resolving finescale differentiation within a population (Mueller and Wolfenbarger 1999).

Materials and methods

Sampling

From May to October 2007, a total of 146 *U. pictorum* specimens were collected by hand from three river and three adjacent marina sites of the River Thames (Fig. 1). A foot tissue sample was removed from each mussel and stored in 96% ethanol for subsequent genetic analysis. Marinas were directly connected to the river and the paired sampling sites were no more than 800 m apart, whereas geographic distances between the three sampling locations ranged from about 30–100 km. The River Thames in the stretch studied has an average width of ca. 50 m, and maximum depth of ca. 4 m. Flow is regulated by a series of weirs, such that mean summer and winter discharges approximate 40–80 $m^3 s^{-1}$, respectively (data from UK Environment Agency). Marina sites were typical lentic systems with no flow, surface areas of 10–80 ha and maximum depths up to 4 m. The river sites typified a UK lowland lotic system. Zieritz and Aldridge (2009) showed that water temperatures and phytoplankton densities in River Thames marinas were consistently higher than in the adjacent main river channel.

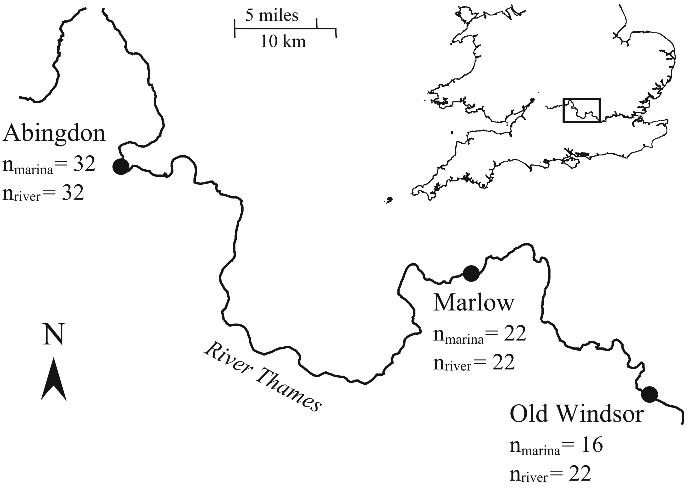


Fig. 1 Sampling locations along the River Thames. Sample sizes are shown for each marina and river site (see methods section for details). Map© Crown Copyright/database right 2008, an Ordnance Survey/EDINA supplied service

Morphological analysis

Shell length (*L*; maximum diameter) and shell height (*H*; maximum diameter perpendicular to *L*) were measured in each individual to ±0.1 cm using a vernier calliper. To estimate relative influence of habitat and location on the degree of shell elongation (*H:L*-ratio), we constructed a general linear model (GLM; Minitab 15 for Windows). For this, *H* was the response variable with *L* and *geographic distance* (measured as distance from most upstream site, i.e. Abingdon Marina, to ±10 m) fitted as covariates and *habitat* as a factor with two levels. To find the minimum adequate model, the full model was fitted and then simplified by sequentially removing non significant predictor variables (Crawley 2002).

For a more detailed analysis of shell morphology, we used Fourier shape analysis, as developed and explained by Crampton and Haines (1996). This method decomposes xy-coordinates of an outline into a number of harmonics, each of which is in turn explained by two Fourier coefficients, which can be analysed statistically like any other traits. Digital photographs of all specimens were taken and digitised using the program IMAGEJ (Rasband 2008). The digitised outlines were then subjected to fast Fourier transformation using the program HANGLE, applying a smoothing normalisation of 20 to eliminate high-frequency pixel noise. Preliminary analysis indicated that the first 10 harmonics described the outlines with sufficiently high precision. Discarding of the first harmonic, not containing any shape information, by the program resulted in a set of 18 Fourier coefficients per individual. After rotating outlines to maximum overlap by program HMATCH, principal component analysis (PCA) was performed on the 18 Fourier coefficients using program PAST (Hammer and Harper 2006). Number of principal components to be retained was determined by use of the broken stick model of the scree plot. Synthetic outlines of "extreme shell forms" were drawn using program HCURVE as explained in Crampton and Haines (1996).

To test for statistical significance of habitat and location on sagittal shell shape, GLMs of all significant principal components were carried out, fitting *habitat* (as a factor with two levels) together with *geographic distance* as predictors, and sequentially dropping non-significant factors from the model (Crawley 2002). Morphological distances in overall sagittal shell outline between the six populations were estimated by performance of discriminant function analysis (DFA) and subsequent calculation of Mahalanobis's D^2 distances of each population pair using Minitab 15 for Windows. Age and sex were not included as predictors in GLMs because sexual dimorphism and allometric growth during ontogeny exert a negligible influence on relative shell elongation and overall sagittal shell shape in the six populations studied (Zieritz and Aldridge 2009). Similarly, the degree of shell inflation (relative shell width) was not considered, in this case because, in contrast to shell elongation and sagittal shell shape, this morphological character is strongly influenced by ontogenetic growth in unionoids (Zieritz and Aldridge 2009).

Genetic analysis

Total genomic DNA was extracted from a small piece of foot tissue using a high salt method (Watts 2001). AFLP genotyping was based on the protocol of Vos et al. (1995) and is described in detail by Dasmahapatra et al. (2009). Briefly, 100–400 ng of genomic DNA was first digested using *Taq*I (5 U in a 10 µl volume at 65°C for 2 h) and then with *Eco*RI (5 U in a 20 µl volume at 37°C for 2 h). *Taq*I and *Eco*RI and adapters (Ajmone-Marsan et al. 1997) were then ligated onto the digested DNA using T4 DNA ligase (1 U in a 50 µl volume at 37°C for 3 h), and the resulting products diluted by a factor of ten in 10 mM

Tris–HCl and EDTA (0.1 mM, pH 8.0). For the pre-amplification, 5 µl of ligation mix was added to a 50 µl PCR reactions containing Tris–HCl (10 mM, pH 8.3), $MgCl_2$ (1.5 mM), KCl (50 mM), dNTPs (0.2 mM), *Taq* polymerase (1 U) and 50 ng each of the *Taq*I-C and *Eco*RI-A pre-amplification primers (the primer sequences were 5′-GATGAGTCCTGACCGAC-3′ and 5′-GACTGCGTACCAATTCA-3′ respectively). Following 30 pre-amplification cycles (30 s at 94°C, 60 s at 50°C and 60 s at 72°C) the products were diluted 10 times with 10 mM Tris–HCl and EDTA (0.1 mM, pH 8.0).

For the selective amplification, 2.5 µl of the diluted pre-amplification product was added to a 12.5 µl reaction containing Tris–HCl (10 mM, pH 8.3), $MgCl_2$ (1.5 mM), KCl (50 mM), dATPs, dTTP and dGTP (0.2 mM each), dCTP (0.04 mM), α^{33}P-dCTP, *Taq* polymerase (0.2 U), *Taq*I selective primer (30 ng) and *Eco*RI selective primer (5 ng). Samples were subjected to 13 selective amplification cycles (30 s at 94°C, 60 s at 65°C, reducing by 0.7°C each cycle, and 60 s at 72°C), followed by a further 23 cycles (30 s at 94°C, 60 s at 56°C and 60 s at 72°C). Eight different selective primer combinations were used (Table 1). PCR products were resolved by electrophoresis on standard 6% polyacrylamide sequencing gels and detected by autoradiography. Exposed X-ray films were assessed and if required, a second exposure was made for an adjusted time period. Gels were scored manually.

Quantification of the genotyping error rate

Although AFLPs tend to be reproducible due to the use of highly specific restriction endonucleases coupled with stringent PCR conditions (Vos et al. 1995; Mueller and Wolfenbarger 1999; Bonin et al. 2004), genotyping errors can nevertheless accrue, with potential sources of error including DNA contamination (Dyer and Leonard 2000), restriction artefacts (Polisky et al. 1975), human error (Bonin et al. 2004) and variation in DNA quality among samples. Consequently, we estimated the genotyping error rate for our dataset by independently re-extracting, re-genotyping and blind-scoring 14 (9.6%) of the samples following Hoffman and Amos (2005). To ensure broad coverage of the dataset in this regenotyping exercise, two

Table 1 Primer combinations used for the AFLP selective amplification and numbers of monomorphic AFLP loci, and unambiguously scoreable and poorly resolved AFLP polymorphisms generated for 146 *U. pictorum* individuals

*Taq*I primer (5′–3′)	*Eco*RI primer (5′–3′)	Monomorphic[a]	Polymorphic scored	Polymorphic poorly resolved[a]
GATGAGTCCTGACCGACAC	GACTGCGTACCAATTCACA	16	10	13
GATGAGTCCTGACCGACAG	GACTGCGTACCAATTCACA	27	24	11
GATGAGTCCTGACCGACAG	GACTGCGTACCAATTCATG	36	6	38
GATGAGTCCTGACCGACCA	GACTGCGTACCAATTCAGC	28	14	17
GATGAGTCCTGACCGACGA	GACTGCGTACCAATTCAGC	25	10	11
GATGAGTCCTGACCGACGA	GACTGCGTACCAATTCATG	15	7	33
GATGAGTCCTGACCGACTG	GACTGCGTACCAATTCAAC	23	15	11
GATGAGTCCTGACCGACTG	GACTGCGTACCAATTCAGC	15	17	26
Total		185	103	160

[a] As with all AFLP analyses these values are somewhat arbitrary since inevitably there are bands which occur above the resolution of the gel end and cannot be scored

to three individuals were selected at random from each of the six populations. The error rate per reaction was then quantified as the number of mismatching genotypes divided by the number of polymorphic bands compared (Bonin et al. 2004).

Genetic data analysis

All gels were independently scored by two observers and genotypes recorded as '1' = band present or '0' = band absent. Data were entered into a spreadsheet. We used the program AFLP-SURV v1.0 (Vekemans 2002) to calculate estimated heterozygosity values for each population and pairwise F_{ST} values between the six populations (Weir and Cockerham 1984) following the approach of Lynch and Milligan (1994). This program was also used to conduct a permutation test for overall genetic differentiation using 10,000 permutations of the dataset.

In order to test whether any of the AFLP loci scored could be subject to divergent selection relating to habitat type and thus, potentially associated with shell morphology, we used the Dfdist program package (Beaumont and Balding 2004; Beaumont 2008) following the approach of Beaumont and Nichols (1996). Dfdist implements the Bayesian method of Zhivotovsky (1999) to estimate allelic frequencies from the proportion of recessive phenotypes (absent bands) and then compares F_{ST} values estimated for each locus against a theoretical null distribution of genetic differentiation conditional upon heterozygosity in a subdivided population using the coalescent and an island model of migration. Loci that fall outside specified confidence limits can be identified as having significantly elevated or reduced F_{ST} values relative to expectations under the assumption of selective neutrality. This analysis was conducted separately on four population pairs: (1) Pooled marina *vs.* river, (2) Abingdon marina vs. Abingdon river, (3) Marlow marina vs. Marlow river and (4) Old Windsor marina vs. Old Windsor river.

For each analysis, parameters $a = 0.25$ and $b = 0.25$ were used for the beta-distributed prior of the Bayesian allele frequency estimator and the trimmed mean F_{ST} was computed by removing the 30% highest and lowest of the observed F_{ST} values. A null distribution was generated based on 50,000 simulated loci, the parameter $4\ \mathrm{N}\mu$ set to 0.5 and the target-neutral F_{ST} determined using the program pv2. Simulation models were run using different small baseline F_{ST} values until the "correct" F_{ST} for the simulations was obtained, i.e. half the neutral points were greater than/less than the median and no trend in the proportion of *P*-values greater than 0.5 with increasing heterozygosity was observable (Beaumont 2008). The robustness of the results to variations in the simulation parameters was further evaluated by repeating the analyses with varying values of $4\ \mathrm{N}\mu$ (i.e. 0.2 and 1.0). Loci with F_{ST} values above the 99% quantile were inferred as being potentially under directional selection. This approach bypasses the joint problem that trimmed mean F_{ST} (as recommended by Caballero et al. (2008) was slightly negative in all cases, while a zero F_{ST} cannot be used because it prevents the coalescent from determining a common ancestor (see Miller et al. 2007).

Results

Morphological analysis

We began by constructing general linear models to explore which factors are significantly associated with shell shape, summarised in Table 2. When shell height was the response

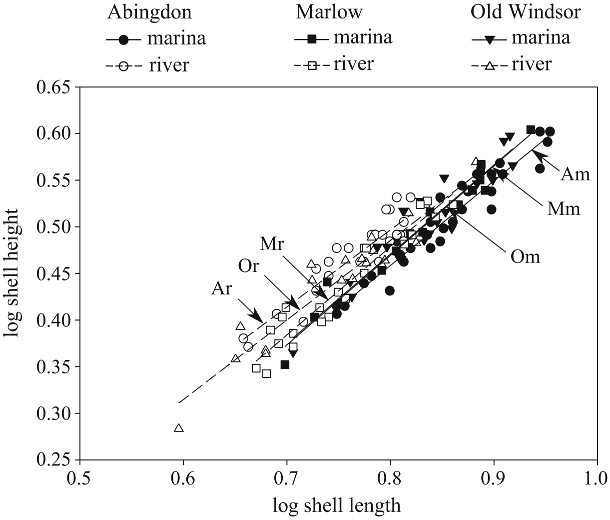
Table 2 Results of general linear models (GLMs) of shell height and the four significant principal components obtained by Fourier shape analysis, comparing the six *U. pictorum* populations

Response variable	Source	*df*	SS	*F*	*P*
Shell height	Habitat	1	0.621	39.04	<0.0001
$R^2 = 0.91$	Geographic distance	–	–	–	n.s.
	Shell length	1	19.421	1219.98	<0.0001
	Error	143	2.276		
PC1 (Fourier)	Habitat	1	0.033	133.03	<0.0001
$R^2 = 0.49$	Geographic distance	1	0.002	9.34	0.003
	Error	143	0.035		
PC2 (Fourier)	Habitat	–	–	–	n.s.
$R^2 = 0.02$	Geographic distance	–	–	–	n.s.
	Error	–	–		
PC3 (Fourier)	Habitat	–	–	–	n.s.
$R^2 = 0.28$	Geographic distance	1	0.010	57.44	<0.0001
	Error	143	0.025		
PC4 (Fourier)	Habitat	–	–	–	n.s.
$R^2 = 0.00$	Geographic distance	–	–	–	n.s.
	Error	–	–		

Values correspond to the minimal adequate models (Crawley 2002)

variable, habitat but not geographic distance was retained as a significant predictor, indicating a strong morphological difference between mussels from the marina and linked river habitats. This difference is also evidenced by the pronounced clustering of marina and river individuals in a log height-length plot (Fig. 2). To learn about other aspects of shape we summarised the 18 Fourier coefficients using a PCA analysis and then fitted four further GLMs, one each using the first four principal components as the response variable

Fig. 2 Log shell length vs. shell height scatterplots of six *U. pictorum* populations sampled along the River Thames. Upper case indicates the location, lower case the habitat of the site: *A*, Abingdon, *M*, Marlow, *O*, Old Windsor; *m*, marina, r, river



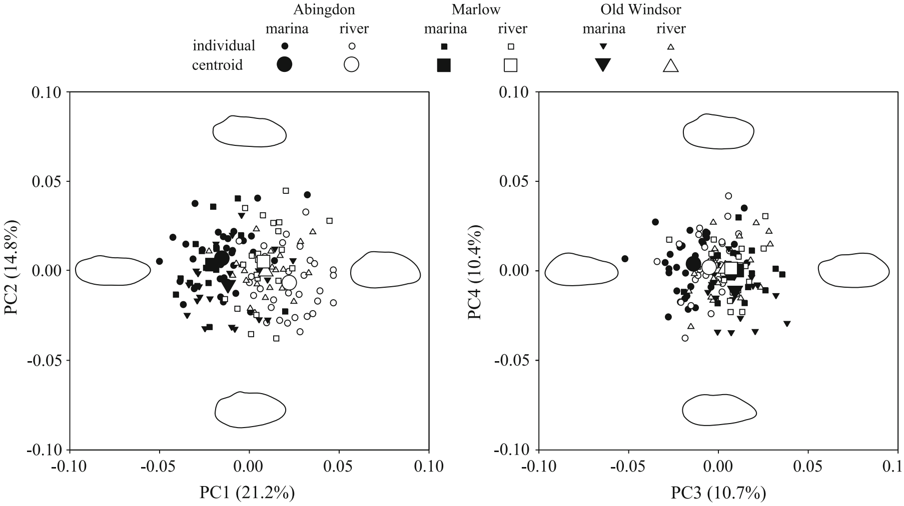


Fig. 3 Principal component scores for the first four PC axes obtained by PCA on 18 Fourier coefficients. Synthetic shell outlines of "extreme" morphotypes are displayed with the posterior margin facing to the right and the dorsal margin to the top of the page

(Table 2). These four components were selected using the broken stick model and together explain 57.1% of the total variance in sagittal shell shape.

PC1 scores were significantly influenced by both habitat type and geographic distance (Table 2). However, whereas fitting the GLM without the term 'geographic distance' resulted in a decrease of only 2.6% of variance explained by the model, 46% of the variance explained was lost following exclusion of the term 'habitat'. This strong habitat-shell shape association is also reflected by the pronounced clustering of river and marina individuals in Fig. 3, which shows that marina mussels tended to have more pointed posterior margins whereas river shells displayed more arched dorso-posterior margins. While the second and fourth principal components were not significantly influenced by any of the two factors, PC3 was significantly correlated with *geographic distance* but not *habitat* (Table 2). With respect to extreme shell outlines along this axis (Fig. 3), this suggests that the posterior region of *U. pictorum* from the Marlow and Old Windsor populations was broader than those from the two Abingdon sites.

Population structure

By genotyping a total of 146 individuals at eight different selective primer combinations, we obtained 103 AFLP loci that could be scored unambiguously across most of the samples (Table 1). The AFLP dataset analysed consisted of 13, 917 binary characters representing the presence and absence of bands. The genotyping error rate was 2.4% (35 mismatches observed out of 1,442 comparisons), which is broadly consistent with a range of previously reported values for studies using AFLPs (Bensch and Åkesson 2005; Bonin et al. 2007).

Estimated heterozygosity values were similar in all six populations (Table 3). Pairwise F_{ST}- and P-values among the six populations (Table 4a) show that almost all of the populations were significantly different from each other except Abingdon marina from

Table 3 Number of polymorphic loci (out of 103 scored) and expected heterozygosity values for the six *U. pictorum* populations

Population	Number of polymorphic loci	Expected heterozygosity ± SE
Am	94	0.3274 ± 0.0149
Ar	91	0.3312 ± 0.0155
Mm	94	0.3400 ± 0.0147
Mr	97	0.3389 ± 0.0137
Om	100	0.3556 ± 0.0142
Or	94	0.3379 ± 0.0148

Upper case indicates the location, lower case the habitat of the site: *A* Abingdon, *M* Marlow, *O* Old Windsor, *m* marina, *r* river

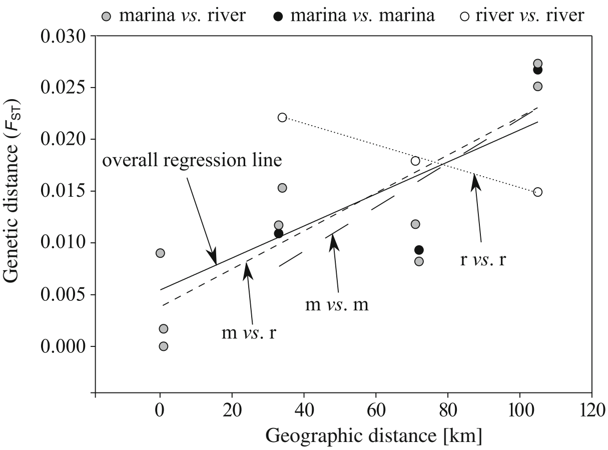
Table 4 (a) Genetic distance matrix (F_{ST}-values bottom left corner, *P*-values upper right corner), (b) Mahalanobis' D^2 distance matrix obtained by DFA on 18 Fourier coefficients (*F*-values bottom left corner, *P*-values upper right corner)

	Am	Ar	Mm	Mr	Om	Or
(a) Genetic distance						
Am		0.8864	0.0058	0.0012	<0.0001	<0.0001
Ar	0		0.0142	<0.0001	<0.0001	0.0012
Mm	0.0093	0.0082		0.1643	0.0092	0.0033
Mr	0.0118	0.0179	0.0017		0.0009	<0.0001
Om	0.0267	0.0251	0.0109	0.0153		0.0205
Or	0.0273	0.0149	0.0117	0.0221	0.009	
(b) Morphological (Mahalanobis' D^2 distance)						
Am		<0.0001	<0.0001	0.0004	0.0008	<0.0001
Ar	11.4		<0.0001	0.0208	<0.0001	0.0180
Mm	12.8	14.5		0.180	1	0.0090
Mr	7.4	4.5	8.6		0.0064	0.7664
Om	9.9	16.1	3.6	7.2		0.0091
Or	8.5	5.5	7	2.5	7.8	

Upper case indicates the location, lower case the habitat of the site: *A* Abingdon, *M* Marlow, *O* Old Windsor, *m* marina, *r* river

Abingdon river and Marlow marina from Marlow river. In contrast, pairwise comparisons of overall sagittal shell outline measured using Mahalanobis' D^2 distances (Table 4b) reveal that all population pairs except Marlow and Old Windsor marina and Marlow and Old Windsor river were significantly different from each other. Moreover, the geographically closest pairs of populations (i.e. the two respective populations of each location) were also genetically the closest, whereas comparison of geographically most distant ones (i.e. Abingdon *vs.* Old Windsor) resulted in comparatively high F_{ST} values, indicating genetic isolation by distance (Fig. 4). Although there are too few sites to test this pattern statistically using a Mantel test to control for non-independence, discrimination by type of comparison (Fig. 4; marina vs. river, marina vs. marina and river vs. river) reveals that this isolation-by-distance trend was largely driven by the paired river—marina and marina—marina comparisons. In contrast, the river—river comparisons all show rather similar genetic distances and the trend they yield is negative. Thus, if an isolation by distance pattern does exist, it appears stronger in the marina—river and marina—marina comparisons than in the river—river comparisons.

Fig. 4 Geographic vs. genetic distance (F_{ST} values obtained by 103 AFLP loci) between six *U. pictorum* populations sampled along the River Thames



We next asked whether shell shape differences between the river and marina habitats could be associated with individual AFLP loci, as might be the case if one or a small number of loci were influenced by selection. To do this, we compared heterozygosity and F_{ST} values estimated for each locus against a theoretical null distribution (Fig. 5). For the comparison of pooled marina *vs.* pooled river mussels, in only one of the three simulations (i.e. when using $4\ N\mu = 1.0$) one locus was significantly above the 99% CI of the theoretical null distribution (grey circle in Fig. 5a). The only other evidence for possible selection between the two habitats was found at the Marlow populations, where two different loci consistently showed significantly elevated F_{ST} values (Fig. 5c). However, none of these three loci were found to be significantly different between the two habitats at the remaining two locations, Abingdon and Old Windsor (Fig. 5b and c). This lack of consistency provides further support for phenotypic plasticity.

Discussion

The question of if and how much of the intraspecific morphological variation in freshwater mussels (Unionoida) is attributable to genotype as opposed to phenotypic plasticity has been the subject of considerable debate. Here we provide evidence for phenotypic plasticity of two shell shape characters, the degree of shell elongation and shape of the dorso-posterior margin, across six River Thames populations of *U. pictorum*. Furthermore, a genetic isolation-by-distance pattern over moderate geographic distances (i.e. 30–100 km), indicates that the AFLP markers offered sufficient genetic resolution to detect subtle differences in genetic composition within the populations under study.

Genetic population structure

Overall our data suggest a trend of increasing genetic distance with increasing geographic distance over a scale of about 100 km, which is presumably driven by appreciable numbers of larvae (glochidia) settling out close to where they were born. However, an isolation by distance pattern was stronger across marina—marina comparisons of sites than across river—river comparisons. One possibility is that such a pattern could arise entirely from chance. Alternatively however, it might reflect a key aspect of the unionoid life cycle in

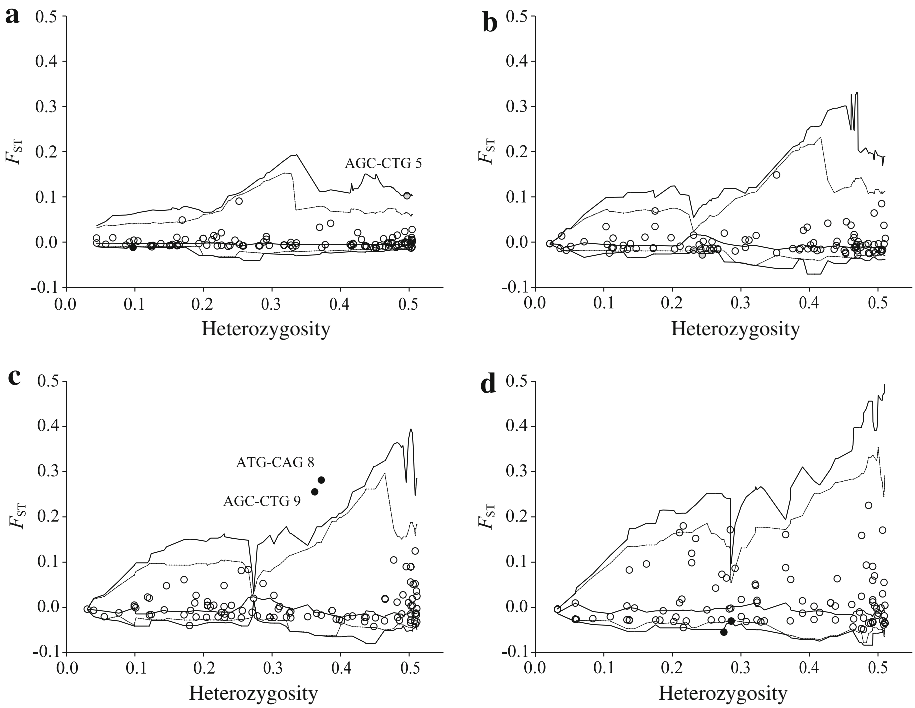


Fig. 5 Plots of F_{ST} vs. heterozygosity for 103 AFLP markers and four respective pairs of *U. pictorum* populations from the River Thames: (**a**) pooled marina *vs.* pooled river specimens (baseline $F_{ST} = 0.003$), (**b**) Abingdon marina vs. Abingdon river specimens (baseline $F_{ST} = 0.0005$), (**c**) Marlow marina vs. Marlow river specimens (baseline $F_{ST} = 0.001$), and (**d**) Old Windsor marina vs. Old Windsor river specimens (baseline $F_{ST} = 0.015$). *Solid lines* indicate the mean and 99% CI, and *dotted lines* represent 95% CI for selectively neutral loci as determined by simulation. *Black circles* correspond to loci falling outside of the 99% CI in all simulation models performed per population pair using different 4 Nμ values, grey circles indicate loci outside the 99% CI in one of the simulation models. Loci with significantly elevated F_{ST} are labelled with their identifying code

which the prevalent means of dispersal is thought to be via parasitic larvae that attach to fish (Kat 1984). *U. pictorum* exploits a variety of different host fish species (Berrie and Bioze 1985; Aldridge 1997; Blažek and Gelnar 2006). In the River Thames, Berrie and Bioze (1985) observed ten fish species to be infested by glochidia of the two British *Unio* species, but the three-spined stickleback (*Gasterosteus aculeatus* Linnaeus) and perch (*Perca fluviatilis* Linnaeus) together carried about 90% of all *Unio* glochidia. While both fish species are widespread and common within the River Thames (UK Environment Agency, pers. comm.), the relatively small-sized three-spined stickleback is more commonly associated with lentic localities such as marinas (Maitland and Campbell 1992) from which populations will show predominantly localised dispersal (Bolnick et al. 2009). Localised dispersal is also implied from a study of lentic (lake) perch (Bergek and Björklund 2007) where cryptic barriers to dispersal restricted gene flow. Conversely, host fishes, especially perch, within the river can be expected to be both actively and passively more mobile (Tudorache et al. 2008). If the dominant host fishes within the marinas show relatively low dispersal compared with the river, this would explain the weaker isolation by distance observed for riverine mussel samples, with their vagile hosts dispersing excysted juvenile mussels some distance from their parents. Thus, the striking difference in IBD trends between marina and river populations demonstrates that the evolutionary consequences of a parasite–host interaction (genetic divergence of parasite populations) could be dependent on the habitat where the interaction takes place.

A pattern of genetic isolation-by-distance has been observed in other species of freshwater mussel, for example, the North American species *Quadrula quadrula* Rafinesque (Berg et al. 1998), but is by no means universal. Thus, other studies have failed to find evidence of isolation by distance across similar or even greater geographic distances in, for example, *Lexingtonia dolabelloides* (Lea) (Grobler et al. 2006), *M. margaritifera* (Machordom et al. 2003; Bouza et al. 2007) and *Amblema plicata* (Say) (Elderkin et al. 2007). Reasons for such differences between species and/or water bodies are likely to be complicated and, besides differences in the vagility of their fish hosts, probably include confounding patterns of pre- and postglacial colonisation that may “overshadow” recent dispersal events and anthropogenic influences relating to fish stocking measures.

Phenotypic plasticity of shell form

By comparing geographically distant, replicate population pairs of two respective habitats, the present study has shown that individuals sampled from the same habitat were morphologically more similar but genetically more distinct from one another compared with those from different but geographically adjacent habitats. This suggests that the degree of relative elongation, i.e. shell height to length ratio, and the shape of the dorso-posterior margin, two shell characters that are strongly and consistently different between marina and river sites, are unlikely to be under genetic control in *U. pictorum*. Further evidence for phenotypic plasticity of shell shape in *U. pictorum* is provided by the fact that not a single locus could be identified that showed convincing evidence of natural selection among the different habitats we sampled, and thus, could potentially be associated with shell shape differences of ecomorphotypes.

Our findings support observations by Hinch et al. (1986), who, on the basis of reciprocal transplant experiments, found that relative shell height to length growth in the North American unionoid *Elliptio complanata* (Lightfoot) was determined by phenotypic plasticity. Besides providing molecular evidence for the same pattern in the European species *U. pictorum*, we also show that the shape of the dorso-posterior margin, which is a more consistent ecophenotypic shell character in unionoids than relative shell elongation (Zieritz and Aldridge 2009), is also probably not under genetic control. This knowledge fills a gap in the scarce molecular evidence for phenotypic plasticity of shell morphology in unionoids, which has so far consisted mainly of studies reporting a lack of match of intraspecific morphological and genetic patterns (Buhay et al. 2002; Machordom et al. 2003; Molina 2004; Geist and Kuehn 2005). However, since most of these previous studies were not primarily focused on determining the basis of phenotypic plasticity, they failed to include a joint analysis of morphology, genetics and geographic distance of populations, and therefore generally fail to make a convincing case.

Evidence for phenotypic plasticity of shell morphology has been found in a number of other mollusc taxa that occupy heterogenous habitats and have high dispersal potential, most notably marine gastropods with planktonic larvae (e.g. *Littorina striata* (King), de Wolf et al. 1998; *Nodilittorina australis* (Gray), Yeap et al. 2001; *Nacella concinna* (Strebel), Hoffman et al. online early). Conversely, local adaptation in other gastropod species might be capable of driving genetic divergence even within continuous populations (e.g. Tregenza and Butlin 1999; Doebeli and Dieckmann 2003). Potential examples for such ecological nonallopatric speciation include *Littorina saxatilis* (Olivi) (Johannesson and Johannesson 1996; Panova et al. 2006; Conde-Padín et al. 2007) and *Nucella lapillus* (Linnaeus) (Guerra-Varela et al. 2009). However, extensive gene flow is predicted to prevent local adaptation (Johannesson 2003) so that these species are usually direct developers with restricted larval dispersal. Moreover, despite some evidence for divergent selection acting on marine broadcast spawners with long-lived pelagic larvae (Luttikhuizen et al. 2003; de Aranzamendi et al. 2008), the dispersal of unionoid larvae by their fish hosts apparently leads to a relatively even redistribution of larval genotypes across different habitats. Consequently, morphological adjustments are possible only through a plastic phenotype.

The unionoid shell ecophenotypes observed might have adaptive significance which, along with their possible functional morphologies, are discussed in detail by Zieritz and Aldridge (2009) and references therein. In short, hydrological parameters such as water movement (e.g. mean and/or maximum current velocities) are probably the main factors determining the sagittal shape of these unionoids' shells. Dorso-posterior arching of river morphotypes, for example, results in heavier weight and increased pedal gape of the shell, allowing the foot to extend further into the sediment. These features would increase initial probing force, anchorage, and the stability of the bivalve when subject to lifting forces resulting from turbulent water (Eagar 1978). Alternatively, differences in shell shapes could have no adaptive value whatsoever but be caused by a non-functional reaction of the mussel to the environment (e.g. via distortion of the shell secreting mantle margin as a result of water movement). If the observed phenotypic plasticity of shell form is adaptive (i.e. results in an improvement in growth, survival or reproduction; Stearns 1989), non-adaptive or even maladaptive remains to be tested.

Finally, our study potentially has implications for mussel classification. Previous work has debated the extent to which morphotypes reflect phenotypic plasticity versus genuine differentiation leading to speciation (e.g. Mulvey et al. 1997; Serb et al. 2003). This problem is exacerbated by the problem that contrasting morphologies were often sampled from geographically distant sites, increasing the chance that morphological and genetic isolation will be correlated (Davis et al. 1981; Davis 1983, 1984; Baker et al. 2003). A further issue is that, over and above any phenotypic plasticity, some molluscan shell characteristics are known to be under genetic control (e.g. smoothed versus ribbed shell sculpture in the gastropod *Oncomelia hupensis* Gredler; Davis and Ruff 1974). Although limited in range, our study illustrates the value of looking at genetics and morphology over a range of geographic scales in order to interpret which factors are most important in driving divergence and provides what we believe is the first good evidence for phenotypic plasticity of shell shape in *U. pictorum*. Further similar studies on other unionoid species using similar markers will be needed in order to establish the generality of what we have found.

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