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Evolutionary aspects of population structure for molecular and quantitative traits in the freshwater snail *Radix balthica*

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Q_{ST};
Radix balthica.

Abstract

Detecting the action of selection in natural populations can be achieved using the Q_{ST}–F_{ST} comparison that relies on the estimation of F_{ST} with neutral markers, and Q_{ST} using quantitative traits potentially under selection. Q_{ST} higher than F_{ST} suggests the action of directional selection and thus potential local adaptation. In this article, we apply the Q_{ST}–F_{ST} comparison to four populations of the hermaphroditic freshwater snail *Radix balthica* located in a floodplain habitat. In contrast to most studies published so far, we did not detect evidence of directional selection for local optima for any of the traits we measured: Q_{ST} calculated using three different methods was never higher than F_{ST}. A strong inbreeding depression was also detected, indicating that outcrossing is probably predominant over selfing in the studied populations. Our results suggest that in this floodplain habitat, local adaptation of *R. balthica* populations may be hindered by genetic drift, and possibly altered by uneven gene flow linked to flood frequency.

Introduction

Various evolutionary forces including selection, mutation, migration and drift shape the genetic divergence of natural populations. Measuring the relative importance of these different mechanisms is crucial for understanding the evolutionary processes driving the structure of populations. The use of neutral molecular markers can give some insights into the effects of gene flow, drift, and, to a lesser extent, mutations, on the observed structure of populations. Detecting the action of selection in wild populations is more difficult but can be achieved through various indirect approaches. First, studies of local adaptation based on common garden and reciprocal transplant experiments can demonstrate the action of local selective pressures (Kawecki & Ebert, 2004). Secondly, long-term studies of wild populations combining the annual recording of life history traits on marked individuals with the inference of extended pedigrees, allow to apply powerful quantitative genetics tools like the 'animal model'. This model can in turn be used to infer, for instance, the response to selection (review in Kruuk, 2004). Thirdly, detecting the action of selection in natural populations can be based on the measurement of morphological and life history traits in individuals kept in the laboratory for several generations. Quantifications of the traits considered, which were possibly under selection, are then used to infer the structure of populations. Such an approach relies on the calculation of Q_{ST}, an index analogous to F_{ST} (Wright, 1931), but based on quantitative traits (Spitze, 1993). To apply this method, a measure of genetic divergence for neutral markers is required and takes the form of F_{ST}. Then three conclusions are possible according to the differences observed between F_{ST} and Q_{ST}: (i) if $Q_{ST} > F_{ST}$, directional selection and potentially local adaptation are involved in the differentiation of the trait considered, (ii) if $Q_{ST} < F_{ST}$, uniform selection best explains the genetic similarities found between populations and (iii) if $Q_{ST} = F_{ST}$ the amount of divergence observed can be explained by drift alone. The Q_{ST}–F_{ST} method has several advantages over alternative approaches. First, it gives information on the nature of selection (directional vs. uniform, when

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detected) and it uses a comparison with a 'null model' of differentiation by drift and gene flow. Secondly, this method does not require the measurement of traits combined with the inference of pedigrees in natural populations.

Reviews of empirical data (Merilä & Crnokrak, 2001; McKay & Latta, 2002) showed that Q_{ST} is usually higher than F_{ST}, leading to the conclusion that the observed divergence for quantitative traits should be mainly attributed to differential selection for local optima (but see Petit *et al.*, 2001; Lee & Frost, 2002; Edmands & Harrison, 2003; Koch *et al.*, 2004). However, the statistical methods used to calculate the components of variance and the confidence interval (CI) around Q_{ST} can influence the conclusions of Q_{ST}–F_{ST} experiments (Morgan *et al.*, 2005; Waldmann *et al.*, 2005). O'Hara & Merilä (2005) showed that point estimates of components of variance obtained with Restricted Maximum Likelihood (REML) ANOVA can differ from the estimates provided by Bayesian analyses. These authors also demonstrated that the width of CI around Q_{ST} could differ among the most frequently used methods: non-parametric bootstrap, Delta method or Bayesian analysis. In addition, the relationship between Q_{ST} and F_{ST} relies on the assumption that additive genetic variance (V_A) is being accurately extracted from phenotypic variance by using for instance a paternal half-sib design (Koskinen *et al.*, 2002; Edmands & Harrison, 2003), but this condition is not met in most studies (Merilä & Crnokrak, 2001). In practice, a full-sib design is easier to set up for many organisms but the covariance between full sibs includes not only an additive genetic component but also some dominance and epistasis effects as well as a common environment component (Lynch & Walsh, 1998). Even in situations where V_A has been accurately measured, it has been theoretically shown that non-additive genetic factors can modify the relation Q_{ST}–F_{ST} (Whitlock, 1999; Lopez-Fanjul *et al.*, 2003; Goudet & Büchi, 2006). Dominance in particular is worth considering as it is well known to influence the genetic architecture of fitness-related traits (Crnokrak & Roff, 1995). The amount of dominance is difficult to measure but one can at least detect its presence (if under selection) by carrying out inbreeding depression experiments. If the fitness of inbred individuals is lower than that of outcrossed individuals, there is evidence for inbreeding depression necessarily associated with some degree of directional dominance. Such experiments give some insights into the genetic architecture of traits but also some indications on the mating system of the study population in case of hermaphroditic species (Husband & Schemske, 1996).

In the present study, we investigated the Q_{ST}–F_{ST} relationship among four populations of the hermaphroditic freshwater snail *Radix balthica* and we measured inbreeding depression in each population. We used the palette of available Q_{ST} estimators and tested whether they provide similar results. Our goal was to test whether directional selection could be detected at a local scale in a heterogeneous floodplain environment.

Methods

Species and populations studied

Radix balthica (L. 1758), formerly *R. ovata* or *Lymnaea ovata*, is a temperate freshwater snail inhabiting lakes, ponds and marshes. This hermaphroditic species is capable of both self- and cross-fertilization but is considered to be a preferential outcrosser (Coutellec-Vreto *et al.*, 1997). The four populations studied are located in three cut-off meanders within the floodplain of the Ain river upstream from Lyon, France (Fig. 1). The study sites have contrasted environmental characteristics that are likely to influence *R. balthica* populations (Table 1). Regular field observations from 2000 to 2003 revealed that PL, BX1 and PN are quasi-permanent pools that only dried out during the exceptionally dry summer 2003 whereas BX2 can be considered as a temporary pool (Table 1). In addition, while none of these sites are permanently connected to the

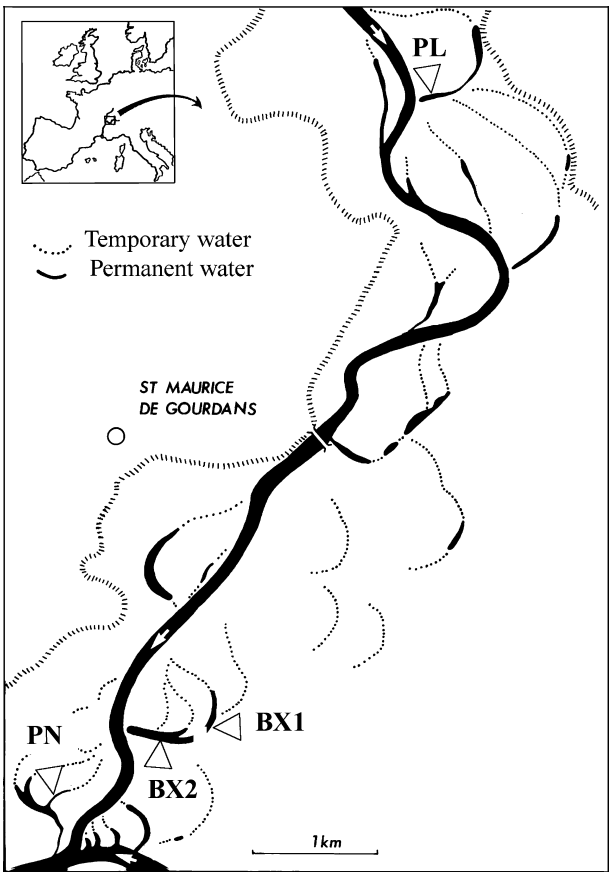

Fig. 1 Map of the study sites located along the Ain river floodplain (south-eastern France).

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Table 1 Environmental characteristics of the floodplain sites where *Radix balthica* populations were studied. Connection and drought frequencies were estimated for the period 2000–2003.

	PL	BX1	BX2	PN
Frequency of connection with the river	0.50	0.004	0.25	0.06
Drought frequency	<0.02	<0.02	0.35	0.02
Organic content of the sediment (%)	9.0	7.2	7.8	3.1

Sediment data are taken from Antoine (2002).

Ain River, they can all get connected to this river for various discharges (Hérouin & Piégay, 2000) and thus not at the same frequency: BX1 is the least often connected, PN has an intermediate frequency, and PL and BX2 are the most frequently connected (Table 1). Sediment from PN has a very low organic content (Antoine, 2002), which suggests that floods have a strong influence on this site (Rostan *et al.*, 1987). Connection and drought frequencies were calculated using discharge data from the Ain river (available from the HYDRO database: http://hydro.rnde.tm.fr/accueil.html). First, to compute drought frequencies, we used 15 dates between September 1999 and September 2003, for which field measurements were available, and fitted a linear regression of the water level in each site as a function of the river discharge. Discharge of the Ain River explained between 85% and 98% of the variance of water levels in the different sites (*P* values between 0.002 and 0.001). These significant relationships enabled us to consider the river discharge as a surrogate for the water levels in the study sites. We then used the maximum river discharge associated with an observed absence of surface water in the sites, as a threshold under which they were considered 'dry'. Similarly, to compute connection frequencies, we used the data by Hérouin & Piégay (2000) to determine the minimum discharge associated with a connection between the river and each site. Above this threshold, a site was estimated 'connected'. Then, the numbers of days during which the different sites were dried out or connected were calculated for the period April 2000–April 2003 and expressed as frequencies (Table 1).

Molecular analyses

To infer molecular genetic variation, 30 snails were sampled in each population and genotyped using the AFLP technique (Amplified Fragment Length Polymorphism, Vos *et al.*, 1995). A foot sample was collected on individuals called G0 (see Fig. 2) once they had laid eggs, and DNA was extracted using Qiagen column procedure following the manufacturer's instructions (Qiagen, Valencia, CA, USA). AFLP procedure was performed using the AFLP Plant Mapping kit (Applied Biosystems, Foster City, CA, USA) following the protocol by Vos *et al.* (1995). Restriction-ligation of 5.5 μL of genomic DNA (200 ng on average) was made according to kit instructions except that the mixture was diluted 10 times in $TE_{0.1}$ buffer before

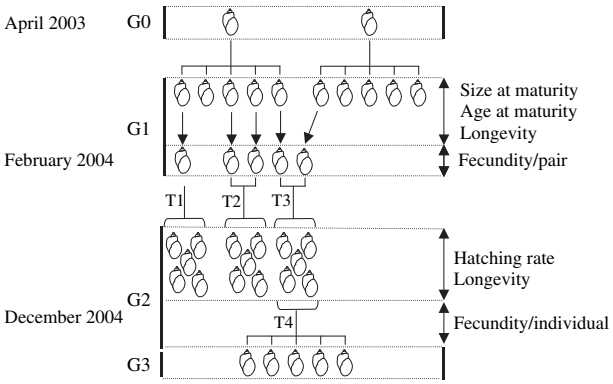

Fig. 2 Design of the experiment carried out in each population. G0, G1, G2 and G3 represent the individuals from first, second, third and fourth generations, respectively. The traits measured at each generation are indicated on the right (see Methods section for details on cross treatments T1, T2, T3 and T4).

the next step. Preselective polymerase chain reaction (PCR) was performed using 2 μL of the precedent mixture added to 7.5 μL of AFLP Core Mix (Applied Biosystems), 0.25 μL *Mse*I primer and 0.25 μL *Eco*RI primer, both at 10 μM. PCR reactions followed kit instructions. The amplification product was diluted 20 times in $TE_{0.1}$ buffer. Selective PCR was performed with 2 μL of diluted product of the previous step added to 7 μL of AFLP Core Mix, 0.5 μL of fluorescent *Eco*RI primer (5 μM), 0.5 μL *Mse*I primer (1 μM). PCR reactions were profiled according to kit instructions. Two primer pairs were used for selective amplification: E-ACT × M-CTG and E-AGG × M-CAT. Then 6 μL of PCR product were evaporated before the addition of a 2.5 μL mixture made of formamide, loading buffer and Rox 500 size standard. After denaturation at 95 °C for 3 min electrophoresis was run on an automated sequencer ABI 377 (Applied Biosystems) for 5 h. Fragments were visualized with GENESCAN 3.1.2 (Applied Biosystems) and scored using the software BINTHERE (N. Garnhart & T.D. Kocher, University of New Hampshire, Department of Zoology, Durham, NC, USA). We used automatic scoring because manual scoring is weakly reproducible between different observers (Bonin *et al.*, 2004). BINTHERE generates spreadsheets with loci scored within 1 bp size-specific bins and we analysed bins within the range 100.5–499.5 bp to avoid size homoplasy, which is more frequent in small fragments (Vekemans *et al.*, 2002). To avoid merging adjacent loci in the same bin, we calculated for each bin the frequency of loci whose size was outside the interval [bin − 0.4, bin + 0.4], i.e. between 0.5 and 0.6 or between 0.4 and 0.5. We discarded bins having such loci at a frequency higher than 5%.

AFLP data analyses

For each population, genetic diversity was quantified in terms of mean expected heterozygosity (H_E) and

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proportion of polymorphic loci, both averaged over primer combinations using the software HICKORY 1.0 (option full model, Holsinger *et al.*, 2002). F_{ST} was first estimated with θ_B using the Bayesian approach implemented in HICKORY by running a full model (simultaneous estimation of f and θ_B). A second estimate of θ_B was calculated using an f-free model in HICKORY. For comparison purposes, we also computed three other estimates of F_{ST}: Φ_{ST} using ARLEQUIN 2.0 (Schneider *et al.*, 2000), F_{ST} assuming Hardy–Weinberg equilibrium in all populations and F_{ST} assuming full inbreeding ($f = 1$) in all populations using POPGEN 1.32 (Yeh & Boyle, 1997). While θ_B is close to θ, a widely used estimator of F_{ST} (Weir & Cockerham, 1984), Φ_{ST} when calculated with dominant markers is conceptually different because it requires the treatment of the multilocus phenotype as a haplotype. Numbers of pairwise differences between haplotypes are quantified in an analysis of molecular variance framework, and Φ_{ST} is computed as the ratio of the component of variance due to differences among populations over the total variance (AMOVA, Excoffier *et al.*, 1992; Stewart & Excoffier, 1996).

Quantitative traits measurement on G1 individuals

The whole experimental design repeated for each population is presented in Fig. 2. For this experiment, individuals kept alone and groups of snails were reared in plastic cups filled with 200 mL filtered water from lake Geneva. Snails were fed *ad libitum* with defrozen lettuce and water was changed twice a week for isolated snails and every 2 days for groups of individuals. The position of all families was randomized in the laboratory.

The experiment started when 30 mature snails called G0 were sampled in each population (April 2003). These G0 individuals were supposed to have stored sperm from previous matings in the field (Coutellec-Vreto *et al.*, 1997). It is important to point here that we did not apply a strict half-sib design because this would be very difficult to set up with *R. balthica* as copulations cannot be easily controlled in this hermaphroditic species. The first clutches from G0s were collected and 10 individuals per clutch were randomly sampled 1 week after hatching. These 10 G1 individuals were kept together 12 weeks after which they were separated in groups of five individuals for three additional weeks in order to avoid the negative effects of high density on growth (Coutellec-Vreto *et al.*, 1998). During this period, survival of G1 snails was monitored once a week. Then (at 15 weeks), five G1s were randomly collected in each family and isolated (July 2003). At this stage, we checked every 2 days for the presence of clutches in individual cups in order to determine age at maturity and incidentally, individual survival. Size at maturity (measured as shell height, Coutellec-Vreto *et al.*, 1998) was recorded to the nearest 0.01 mm using a digital calliper. Fecundity was then measured according to three different inbreeding treatments described in the following section.

Inbreeding depression

Radix balthica is considered as a preferential outcrosser (Coutellec-Vreto *et al.*, 1997) but to check this assumption in our study populations we measured inbreeding depression in each population, as Husband & Schemske (1996) showed that species with a high outcrossing rate also have a high inbreeding depression. This assumption has been rarely tested in animals but Wiehn *et al.* (2002) found that in a *R. balthica* population, family-level resistance to parasites increased with outcrossing rate, suggesting that selfing could lead to inbreeding depression. In addition, Wiehn *et al.* (2002) did not find such a relationship in three other populations with higher inbreeding coefficients and Coutellec-Vreto *et al.* (1998) found a high inbreeding depression in a population having an almost null selfing rate. These results suggest that inbreeding depression is negatively related to selfing rate in *R. balthica* populations. Fecundity measurements were initiated when 80% of G1s had reached maturity under isolation as described in Coutellec-Vreto *et al.* (1998). At this time (February 2004), three types of crosses were made on each family of five individuals: one snail remained isolated and could thus only self-fertilize (treatment T1, Fig. 2), two full-sib individuals were paired (T2) and the two remaining snails were paired with unrelated individuals from the same population (T3). For each treatment the number of eggs laid during 4 weeks and the hatching rate of the first three clutches were recorded. This measure of fecundity on G1 individuals does not reflect inbreeding depression but can give an indication on self-fertilization depression in these *R. balthica* populations. As fecundity of G1s measured according to treatments T2 and T3 was also potentially influenced by density effects, we did not use these data to infer Q_{ST} but those, unbiased, measured on G2s (see the following section). In addition to hatching rate of G2s, the second trait for which inbreeding depression was measured was the longevity of G2 hatchlings. Four weeks after hatching, five G2s were randomly sampled in each family of all three treatments and isolated, survival being checked twice a week afterwards.

Fecundity measurement on G2 individuals

Using individuals from T3 cross, we measured the individual fecundity of G2s under outcrossing to estimate Q_{ST} for this trait, as only fecundity for pairs of snails was available for G1s (T3) and thus eggs could not be assigned to one or the other individual due to the inbreeding depression design. To avoid the problem of late maturity observed in G1s kept isolated (a phenomenon likely related to 'delayed selfing', see Tsitrone *et al.*, 2003), we crossed the G2s before 80% of them had reached

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maturity under isolation (criterion used for G1s). As a result, the treatment (T4, Fig. 2) was initiated when 80% of G2s had reached the average size at maturity recorded in G1s (December 2004). We paired unrelated individuals similarly to treatment T3 except that to obtain the individual fecundity, G2s were paired for 3 days and then isolated for 1 week, during which the number of eggs laid was recorded. This process was repeated four times, which gave a measure of fecundity for 4 weeks.

Quantitative traits and inbreeding depression data analyses

For age and size at maturity of G1s, hierarchical ANOVAS were carried out with families nested within populations. Survival of G1s was analysed at 15 weeks and before the crosses, using generalized linear models (GLMs) with a binomially distributed error and logistic link function. Longevity (days) of G1s was also analysed with a hierarchical ANOVA with Box-Cox transformed data. Fecundity by pair of G1s (T3) and by individual G2 (T4) were analysed using a GLM with a quasi-Poisson-distributed error to account for overdispersion.

Given the high outcrossing rate of this species, progeny from G0 individuals were more likely to be half-sibs than full-sibs (Coutellec-Vreto *et al.*, 1997) but as we cannot certify this was always the case, we explored both possibilities in estimating Q_{ST}. Q_{ST} was computed according to the formula by Spitze (1993) assuming random mating:

$$Q_{ST} = \frac{\sigma_b^2}{\sigma_b^2 + 2\sigma_w^2},$$

where σ_b^2 is the component of additive genetic variance between population and σ_w^2 the component within population. σ_b^2 was estimated by V_p, the observed component of variance between populations and σ_w^2 was estimated by V_f the observed component of variance between families under two hypotheses: (i) $2V_f$ in the case G1 families were considered as made of full-sibs and (ii) $4V_f$ if these families were thought to be made of half-sibs (Lynch & Walsh, 1998).

Heritability in each population was estimated using the following formulae: $H^2=2V_f/(V_f+V_i)$ broad-sense heritability for a full-sib design; $h^2=4V_f/(V_f+V_i)$ narrow-sense heritability for a half-sib design; where V_i is the observed component of variance between individuals. We also estimated coefficients of genetic variation (CV) for each trait as the square root of $2V_f$ or $4V_f$ divided by the trait mean according to a full or half-sib design, respectively. Houle (1992) suggested that CVs are more adequate measures of the ability of a population to respond to selection than heritability.

We have computed V_p, V_f, V_i, h^2, H^2, CV and Q_{ST} using three different methods. First, components of variance were calculated with the classical hierarchical ANOVA approach using an algorithm correcting for unequal sample sizes, and allowing for several levels of nesting (Yang, 1998). Secondly, we used the REML approach implemented in the NLME package of R (R Development Core Team, 2004), which is based on a linear mixed effects model allowing for random nested effects as described in Pinheiro & Bates (2000). Thirdly, a Bayesian analysis was carried out following the procedure described in Waldmann *et al.* (2005) using WINBUGS 1.4 (Spiegelhalter *et al.*, 1999). Gamma distributions (0.001, 0.001) were used as priors for (the inverse of) the variances ($1/V_p$, $1/V_f$ and $1/V_i$). We also tested Uniform distributions (0, 10 000) as priors as it was recently suggested that these distributions may have better frequentist properties than Gamma distributions (Gelman, 2006; O'Hara & Merilä, 2005). For each Bayesian analysis, two chains were run after a burn-in of 5000 iterations; every five of the next 25 000 iterations were taken to give a total of 10 000 draws from the posterior distribution (Palo *et al.*, 2003). In comparison with the other methods, a mechanistic partitioning of the components of variance was used for the Bayesian estimates of heritability to keep these estimates between 0 and 1: in the case of full sib families, V_f was decomposed into $V_A/2$ and V_i into $V_A/2 + V_{env}$, and in the case of half-sib families, V_f became $V_A/4$ and V_i was equal to $3V_A/4 + V_{env}$, where V_{env} designated the residual variance made of environmental and dominance effects. Then heritability was computed as $V_A/(V_A + V_{env})$. 95% CI were obtained by (i) nonparametric bootstrap for the classical ANOVA analyses where whole families were sampled with replacement and kept within their original populations and (ii) Bayesian analysis using 95% coverage of the posterior distributions (Palo *et al.*, 2003).

Inbreeding depression data were analysed using GLMs with quasi-Poisson- and quasibinomial-distributed errors for fecundity and hatching rate, respectively.

Results

Molecular variation

The two primer combinations provided 190 polymorphic loci (109 for E-ACT × M-CTG and 81 for E-AGG × M-CAT), indicating a relatively high number of bands per combination in comparison with the results from other studies on gastropods (Miller *et al.*, 2000; Wilding *et al.*, 2001). Overall θ_B and Φ_{ST} are 0.181 (95% CI: 0.157–0.205) and 0.168 ($P < 10^{-5}$), respectively. θ_B computed without parallel inference of f (f-free model) was 0.166 (95% CI: 0.137–0.198). F_{ST} computed assuming either Hardy–Weinberg equilibrium or full inbreeding ($f = 1$) were 0.117 and 0.156, respectively. Pairwise θ_B (full model) and Φ_{ST} presented in Table 2 reveal that PN and PL are more weakly differentiated than all other pairs of populations. Overall, pairwise θ_B and Φ_{ST} are strongly correlated ($r = 0.99$, $P < 0.001$) but there is no relation-

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Population	PL	PN	BX1	BX2
PL		0.044 (0.030–0.062)	0.264 (0.217–0.315)	0.253 (0.212–0.301)
PN	0.048		0.190 (0.150–0.235)	0.185 (0.148–0.227)
BX1	0.233	0.160		0.153 (0.118–0.195)
BX2	0.238	0.170	0.137	

Table 2 Pairwise Φ_{ST} and θ_B between populations, below and above diagonal, respectively (values for Φ_{ST} are all significant at 10^{-5} level and 95% CI are given for θ_B values).

Table 3 Estimators of molecular diversity (genetic diversity H_E and number of polymorphic loci) and mean (SD/sample size) of age and size at maturity, longevity and fecundity (G2s, T4) within the different populations.

	Gene diversity	Polymorphic loci	Size at maturity (cm)	Age at maturity (days)	Longevity (days)	Fecundity (eggs)
PL	0.233 (0.005)	158	9.64 (1.1/128)	176.43 (29.2/128)	313.71 (69.3/144)	7.71 (19.5/7)
PN	0.237 (0.005)	169	10.21 (1.1/121)	177.55 (29.6/121)	319.29 (66.3/147)	9.0 (18.1/24)
BX1	0.181 (0.004)	104	10.35 (1.2/119)	179.40 (33.2/119)	315.68 (67.9/142)	8.86 (15.5/29)
BX2	0.187 (0.004)	115	10.56 (1.2/103)	195.14 (33.5/103)	323.28 (65.7/147)	12.83 (22.9/24)

Table 4 Results from hierarchical ANOVA for three traits measured on G1 individuals and from generalized linear model for fecundity of G1 pairs (T3) and of G2 individuals (T4).

	Population			Family		
	d.f.	*F*	*P*	d.f.	*F*	*P*
Age at maturity	3	9.31	10^{-4}	116	1.41	0.009
Size at maturity	3	16.76	10^{-9}	116	2.09	10^{-6}
Longevity	3	0.64	0.59	116	1.67	10^{-4}
Fecundity G1s	3	1.61	0.19	–	–	–
Fecundity G2s	3	0.36	0.78	22	1.29	0.21

ship between either pairwise θ_B or Φ_{ST} with geographical distance ($r = -0.08$, $P = 0.9$ and $r = -0.13$, $P = 0.8$, respectively). PN and PL have a higher genetic diversity and more polymorphic loci than BX1 and BX2 (Table 3).

Quantitative variation and Q_{ST}–F_{ST} comparison

For age and size at maturity, there are significant differences both at the population and family levels (Tables 3 and 4). Survivals at 15 weeks and before treatment do not differ at the population or at the family levels (data not shown). Longevity of G1s differs between families but not populations (Table 4). For both measures of fecundity (G1s and G2s), populations and families do not differ, but sample sizes are lower than for the other traits (Tables 3 and 4).

The results concerning heritability within each population are given in Table 5. Both frequentist methods gave very similar results and revealed that traits have moderate to high heritabilities. Bayesian estimates are similar to those of REML and classical ANOVA for the case of full-sib families but slightly different in the situation of half-sib families (in particular for fecundity, the trait with the lowest sample size, Table 3). The results are presented for analyses using Uniform distributions as priors because posterior distributions had a more regular shape and the convergence was better than with Gamma distributions. For fecundity, values are aberrant for PL (−8.2) and PN (−1), certainly because of too low sample sizes in these populations (see Tables 5 and 3, respectively). The values −8.2 and −1 were rounded from −8.21 and −0.98 respectively (Table 5). Overall, as sample sizes are low for fecundity, heritability values for this trait are only given for information and should be considered with caution (and in the hierarchical ANOVA detailed above, the family effect was not significant for fecundity). Importantly, only size at maturity shows relatively constant heritability values across populations. Coefficient of variation (CVs) are less variable than heritability values but similarly, only size at maturity has identical CVs between populations.

Q_{ST} obtained by assuming that G1s are either full-sibs or half-sibs are presented in Fig. 3. Concerning Bayesian analyses, the results are given for size at maturity and longevity with Gamma distributions as priors because (in contrast with heritability) they gave better results than Uniform distributions (see also Discussion section). The results for fecundity and age at maturity are not presented because the posterior distributions were asymmetrical with CIs for Q_{ST} ranging from 0 to 1 using both types of priors. This is certainly linked to the low sample size for fecundity and to the heterogeneity of within-population variance for age at maturity (see values for heritability and CVs). The results were similar between REML and classical ANOVA analyses and Q_{ST} in case of half-sib G1 families are logically about twice lower than when these individuals are considered full-sibs. Clearly, there is no evidence that Q_{ST} is higher than F_{ST}. However, Q_{ST} varies between traits: in the full-sib situation, size and age at maturity have a Q_{ST} of about 0.1 while Q_{ST} is null for individual fecundity and longevity. 95% CI are wide and overlapping both among traits, and between traits and θ_B (Fig. 3).

Table 5 Heritability and coefficient of variation (below) of four traits computed for each population using classical ANOVA, REML and Bayesian analyses according to a full-sib or half-sib design.

	ANOVA				REML				Bayesian			
	PL	PN	BX1	BX2	PL	PN	BX1	BX2	PL	PN	BX1	BX2
Half-sib (h^2)												
Age at maturity	0.49	0.25	0.08	0.69	0.37	0.26	0.01	0.63	0.38	0.34	0.24	0.48
	0.12	0.08	0.05	0.14	0.10	0.09	0.02	0.10	0.11	0.10	0.09	0.12
Size at maturity	0.85	0.88	0.85	0.92	0.85	0.90	0.85	0.82	0.64	0.66	0.61	0.58
	0.11	0.10	0.11	0.11	0.11	0.11	0.11	0.10	0.09	0.09	0.09	0.09
Fecundity (G2)	−8.21	−0.98	0.38	0.22	0.002	2.23	0.61	0.32	0.49	0.41	0.54	0.51
	–	–	1.08	0.84	0.11	0.05	1.38	1.02	4.44	1.6	1.47	1.53
Longevity	0.28	0.46	0.87	0.53	0.28	0.45	0.86	0.52	0.33	0.42	0.65	0.49
	0.12	0.14	0.20	0.15	0.12	0.14	0.20	0.15	0.13	0.14	0.18	0.15
Full-sib (H^2)												
Age at maturity	0.25	0.13	0.04	0.35	0.18	0.13	0.007	0.31	0.22	0.18	0.13	0.32
	0.08	0.06	0.04	0.10	0.07	0.06	0.02	0.14	0.08	0.07	0.07	0.10
Size at maturity	0.42	0.44	0.42	0.46	0.43	0.45	0.42	0.41	0.44	0.45	0.40	0.39
	0.07	0.07	0.08	0.08	0.08	0.07	0.08	0.07	0.08	0.08	0.08	0.07
Fecundity (G2)	−4.1	−0.49	0.19	0.11	0.001	1.11	0.31	0.16	0.48	0.34	0.48	0.48
	–	–	0.76	0.59	0.08	0.03	0.97	0.72	4.64	1.57	1.48	1.64
Longevity	0.14	0.23	0.43	0.27	0.14	0.23	0.43	0.26	0.17	0.23	0.44	0.28
	0.08	0.10	0.14	0.11	0.08	0.10	0.14	0.10	0.10	0.10	0.15	0.11

We also investigated the relationships between molecular and quantitative measures of genetic diversity. Between genetic variance (V_f, inferred from classical ANOVA) and genetic diversity, correlations were not significant: $r = -0.78$, -0.16 and -0.90, for longevity, age and size at maturity, respectively. Similarly, correlations between Q_{ST} and F_{ST} were not significant: $r = 0.44$, 0.21 and 0.39, respectively.

Inbreeding depression

Fecundity of G1s and hatching rate of G2s according to the type of crossing (selfing, full-sib mating or nonsib mating) and for each population are presented in Fig. 4. Fecundity differs significantly both between populations and types of cross (Table 6). In each population, fecundity is always higher for $F = 0$ than for $F = 0.5$. However, as these fecundity data are measured on G1s, they do not directly reflect inbreeding depression but rather, they show that individuals lay more eggs when they mate with an unrelated partner than when they self-fertilize or mate with a full-sib. Hatching rate also varies significantly between populations and types of cross (Table 6) but while Fig. 4b suggests that this trait does not always decreases with inbreeding according to the population considered, the interaction between the population and type of cross effects is not significant (Table 6). These differences observed between populations are probably due to a very low number of selfing crosses in PL and BX2 because when data from the four populations are pooled (Fig. 5), a clear inbreeding depression is detected for hatching rate: $\delta = 0.56$. In addition, for this trait there is a nonlinear decrease of fitness as a function of the inbreeding coefficient (Fig. 5). For longevity of G2s, differences are also significant between populations and type of cross (Table 6), and inbreeding depression calculated for the whole data set is equal to 0.27.

Discussion

Q_{ST} vs. F_{ST}: methodological issues

We did not find any evidence of selection for different optima in the populations studied. The divergence found for all traits can be explained by drift alone, as Q_{ST} was never significantly different from F_{ST}, either calculated according to a full or a half-sib design. Importantly, these conclusions only hold if Q_{ST} was calculated without any methodological bias. In practice, this is rarely the case and we will discuss below how several statistical or biological issues can influence our results and more generally the outcome of Q_{ST}–F_{ST} experiments.

First, the number of populations studied is relatively low even though many families (30 per population) and individuals (total of 600) were used for the quantitative analyses. O'Hara & Merilä (2005) showed that the bias and the variance in the estimation of Q_{ST} are especially large when few populations are studied. However, the bias and the variance in Q_{ST} due to the number of populations decrease for low values of Q_{ST}. Goudet & Büchi (2006) found similar results in a simulation study, with a marked decrease in the variation of Q_{ST} for values around 10% and below, which is in the magnitude of our results.

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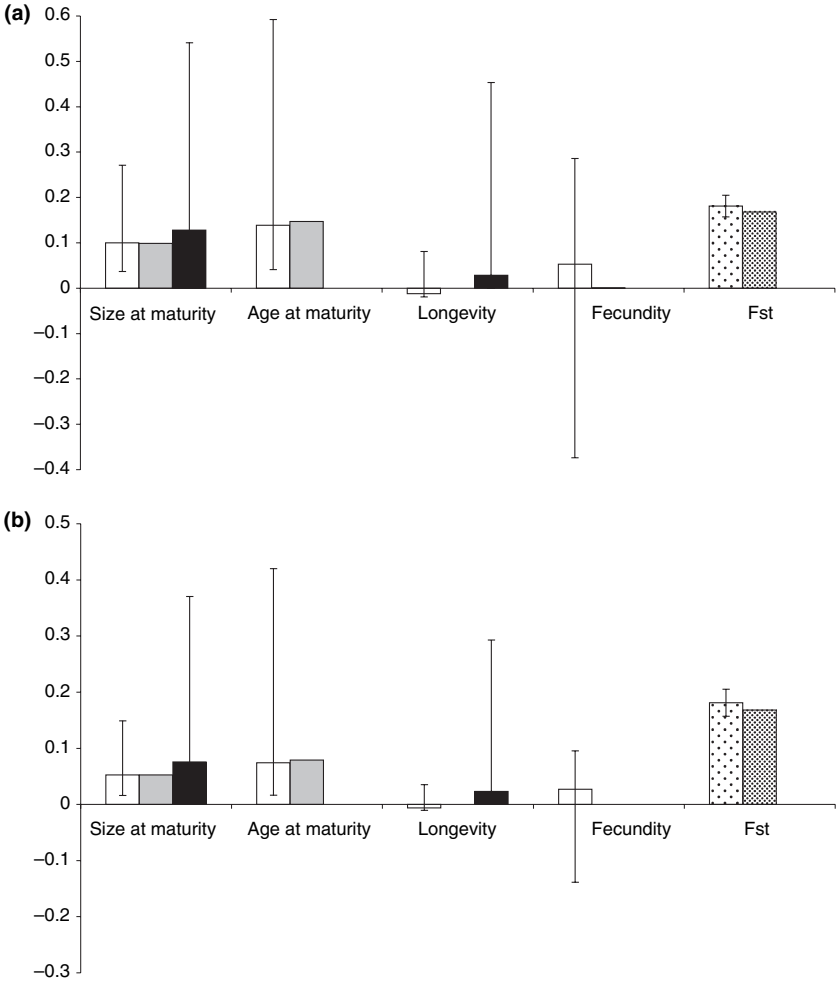

Fig. 3 Comparison of F_{ST} (Φ_{ST} and θ_B in dotted and light dotted, respectively) with Q_{ST} obtained using ANOVA (white), REML (grey) and Bayesian (black) analyses for four traits and assuming either a full-sib (a) or a half-sib design (b). Vertical bars represent 95% CI. Results of Bayesian analyses are given only for size at maturity and longevity (see text) and values of Q_{ST} for fecundity and longevity based on REML analyses are not visible as they are very close to zero.

A second point to consider is the type of statistical tool used to analyse quantitative traits. The most widely used method to extract components of genetic variance from phenotypic data is the classical hierarchical ANOVA based on either the method of moments or REML analyses. These two frequentist approaches gave similar results. The outcomes from Bayesian analyses were more difficult to interpret because for two traits out of four, posterior distributions had odd shapes with CI for Q_{ST} between 0 and 1. This problem can be explained by (i) low sample size for fecundity and (ii) heterogeneity of within-population variance for age at maturity (Waldmann *et al.*, 2005). Furthermore, the shape of the posterior distributions was sensitive to the prior used (Gamma vs. Uniform) and this is still unclear which prior should be used for inferences on Q_{ST}: Waldmann *et al.* (2005) found similar results using Gamma and Uniform distributions, while O'Hara & Merilä (2005) showed that the latter had better frequentist properties. We found that for heritability a Uniform distribution performed better than a Gamma, but for Q_{ST} we observed the reverse. To further investigate this issue we made some simulations with a setting based on our sampling scheme (600 individuals distributed in four populations with 30 families each) and according to the method described in Goudet & Büchi (2006). For a trait with a purely additive basis and an expected value for F_{ST} of 0.18, we found a better estimate of Q_{ST} using a Gamma distribution than a Uniform distribution. However, this was only true for the number of populations corresponding to our sampling design because with more populations the two priors performed similarly.

Importantly, the three statistical methods used gave similar results for size at maturity, which suggests that they perform equally well when the genetic variance is evenly distributed within the different populations and even when few populations are present. The results obtained with frequentist and Bayesian analyses have rarely been compared in quantitative genetics studies but recently O'Hara & Merilä (2005) found similar estimates for Q_{ST} using REML and Bayesian methods, except a slight downward bias with REML for high Q_{ST} values. Differences in variance between families (reflected by

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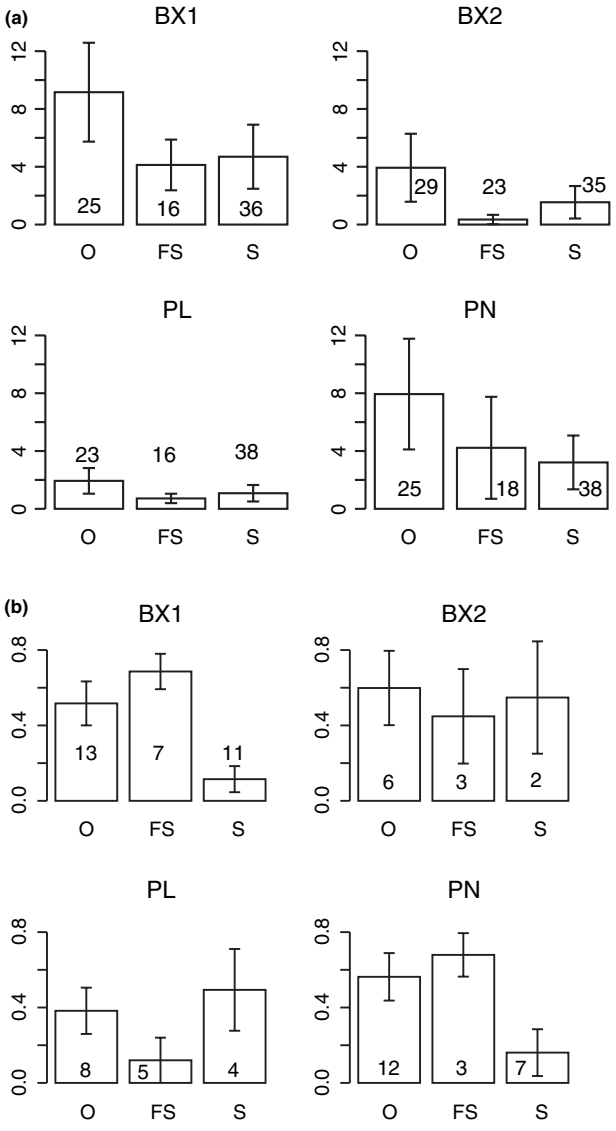

Fig. 4 Fecundity averaged by individual G1s (number of eggs ± SE) in each population (a) according to three treatments: selfing (S), full-sib mating (FS), mating between unrelated individuals (O). Hatching rate (±SE) of G2s in each population (b) according to the three types of cross. Sample size is given within or above the bars.

Table 6 Results from generalized linear models for fecundity of G1s ($n = 322$), hatching rate of G2s ($n = 81$) and from ANOVA (with Box-Cox transformation) for longevity of G2s ($n = 189$) in each population and according to three types of cross: selfing, full-sib mating and mating between unrelated individuals.

	d.f.	% of deviance or variance explained	*F*	*P*
Fecundity				
Population	3	7.7	4.33	0.005
Cross	2	4.1	3.44	0.033
Population × cross	6	0.8	0.22	0.97
Hatching rate				
Population	3	11.5	4.64	0.005
Cross	2	25.9	15.74	10^{-5}
Population × cross	6	8.9	1.80	0.11
Longevity				
Population	3	34.8	6.71	0.0003
Cross	2	45.2	8.71	0.0002
Population × cross	5	14.8	2.85	0.02

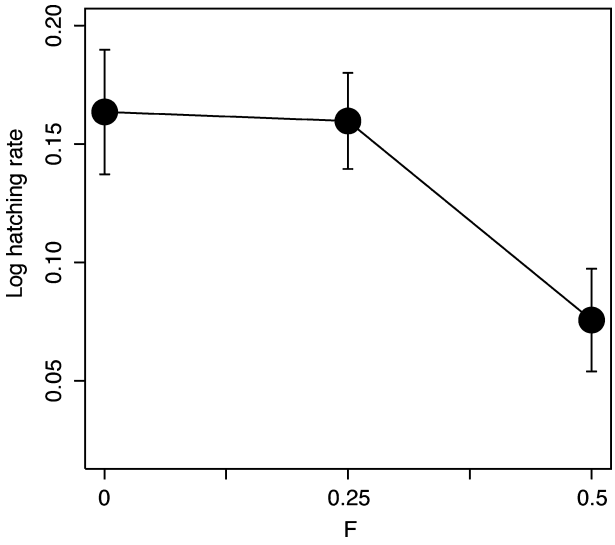

Fig. 5 Hatching rate (log-transformed) of G2s (±SE) averaged over four populations as a function of the inbreeding coefficient (*F*).

CVs and heritability values) among populations can result in very wide CIs for Q_{ST}, even for a trait with reasonable sample size like age at maturity. CIs calculated by bootstrap over families appear narrower than Bayesian ones, which is in accordance with the results of O'Hara & Merilä (2005) who showed that the precision of CI for Q_{ST} is highly variable depending on the method used, the bootstrap of families (according to dam or sire) leading to narrower CI than for instance, Bayesian CI. However, these authors also found a better coverage of Bayesian CI compared with other methods. For all traits, it is also worth considering that when expressed as evolvability (CV), the within-population variance is more homogeneous among populations than heritability. In the literature, values for within-population variances are rarely given (Waldmann *et al.*, 2005); it is thus unclear how often these values could be heterogeneous.

A third methodological issue to consider is the fact that we started the experiment with G0 individuals supposed previously outcrossed in the field. There are two potential biases related to this approach. First, depending on whether these individuals mated with one or several partners, the progenies were then made of full-sibs or

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half-sibs. However, in both cases the conclusion of our study is similar: Q_{ST} for survival and fecundity are not significantly different from zero and Q_{ST} for age and size at maturity do not differ from F_{ST}. Secondly, it is also possible that some G0s may have selfed instead of outcrossed. It could be taken into account by using a non-null F_{IS} value in the calculation of Q_{ST} (Bonnin *et al.*, 1996). With F_{IS} values of 0.1 and 0.2, which represent selfing rate of 0.18 and 0.33, the conclusions of our study are not affected. Thus, even with a selfing rate as high as 30%, Q_{ST} is never higher than F_{ST} for any trait. Also, as we detected a strong inbreeding depression for hatching rate, outcrossing is probably predominant over selfing in our study populations but a molecular analysis will be necessary to confirm this assumption. In addition, the nonlinear decrease of fitness as a function of inbreeding might indicate an influence of epistasis on the genetic architecture of hatching rate (Willis, 1993). However, as we do not know whether individuals from treatment T2 are full- or half-sibs, we cannot conclude on this point.

Another important issue is the potential influence of nongenetic maternal effects on the three traits that were measured on G1 individuals rather than on G2s. However, maternal effects are known to act more strongly on precocious traits like early growth and survival (Mousseau & Fox, 1998; Pakkasmaa *et al.*, 2003), whereas all traits used here to infer Q_{ST} were measured relatively late in the life cycle. Nonadditive genetic factors and common environment effects may also have influenced these traits as they were only discarded in the case of half-sib families. Common environment effects are supposed to be relatively low as relatives were kept isolated most of the time during the experiment. In contrast, dominance variance is known to be high in fitness-related traits and could have increased V_f and thus diminished Q_{ST}. However, it is worth considering that even in an ideal situation where additive variance is perfectly estimated, theoretical studies have demonstrated that epistasis and dominance effects can lower Q_{ST} (Whitlock, 1999; Lopez-Fanjul *et al.*, 2003; Goudet & Büchi, 2006). This could potentially explain the very low Q_{ST} we found for fecundity and longevity. In the case of fecundity, no conclusion can be drawn because of low sample size. For longevity, we found a clear inbreeding depression ($\delta = 0.27$), which strongly suggests the action of dominance on the genetic architecture of this trait. However, in a recent simulation study, Goudet & Büchi (2006) found that dominance was likely to deflate Q_{ST} relative to F_{ST} but the effect was only strong for high levels of structure ($F_{ST} > 30\%$). As F_{ST} was 0.18 in the present study, dominance should not have greatly influenced the results. Nevertheless, it is quite clear that the Q_{ST}–F_{ST} approach shows its limitations in situations where stabilizing selection is suspected but the action of dominance (and possibly other nonadditive factors) cannot be neglected (Toro & Caballero, 2005). In contrast, when $Q_{ST} > F_{ST}$, the conclusion of the action of directional selection for different local optima is robust to the effect on nonadditive gene actions (Goudet & Büchi, 2006).

Relation between molecular and quantitative genetic variation

In this study, we also noticed the absence of relation between molecular and quantitative genetic variations. This result holds for genetic variance and genetic diversity as well as for Q_{ST} and F_{ST} but the statistical power is low due to the number of populations studied. Theory predicts a positive correlation between molecular and quantitative levels (Falconer, 1960; Soulé & Yang, 1973) but this prediction was only partially confirmed by empirical studies. Using meta-analyses, Reed & Frankham (2001) showed that the relation is weak at the within- and between-population levels and McKay & Latta (2002) found also a nonsignificant positive relationship at the between-populations level (Q_{ST} and F_{ST}). Contrastingly, Merilä & Crnokrak (2001) found a strong positive correlation between Q_{ST} and F_{ST}.

Absence of local adaptation

At this scale, we did not detect any evidence of local adaptation despite a high environmental heterogeneity between well-differentiated populations ($F_{ST} = 0.18$). However, demonstrating the occurrence of local adaptation is difficult because, as stated by Kawecki & Ebert (2004), this process can be 'hindered by gene flow, confounded by genetic drift, opposed by natural selection due to temporal variability, and constrained by lack of genetic variation or by the genetic architecture of underlying traits'. Among these factors, gene flow and genetic drift are worth considering because they are likely to have a major influence on molecular and quantitative genetic structure in a floodplain context. For gene flow, this is illustrated by the striking differences among pairwise F_{ST}: 15% between BX1 and BX2, which are spatially the closest populations (500 m), while F_{ST} is <5% between PN and PL, which are 6700 m far from each other. All other pairwise comparisons are found between 15% and 25%. If one assumes that dispersal of freshwater snails is passive and occurs from upstream to downstream (Cellot & Bournaud, 1988; Cellot, 1996), these results suggest that the level of gene flow from PL to PN is higher than from PL to any other sites. This is consistent with the fact that PL is located upstream from the other sites and frequently connected to the river, while PN is rarely connected but strongly influenced by floods as reflected by the very low organic content from its sediments. In contrast, BX2 is frequently connected to the river but it is unlikely to receive migrants from the river (and thus from PL) because of its geographical setting (Citterio & Piégay, 2000). However, gene flow may occur from BX1 to BX2, BX1 being located upstream

from BX2, but this must be very rare as they are rarely connected to each other (and there is no current between the two sites), and F_{ST} appears relatively high between these sites. Another feature of floodplain habitats is the temporary character of certain pools, which can be linked to high demographic fluctuations that increase the effects of genetic drift. For instance, BX2 has dried out for a total of 13 months between September 2000 and September 2003. The genetic diversity in this site is reduced compared with PN and PL, but it is similar to the one found in BX1 that rarely dried out during the same period. This suggests that other factors should be taken into account to explain the observed genetic diversity. For example, population size could be low in BX1, or few individuals may have founded this population, as BX1 is the most isolated of all sampled sites. The action of genetic drift and gene flow cannot be definitely disentangled in the present study but given the floodplain context, our results suggest that these forces strongly influence *R. balthica* populations by shaping their neutral genetic structure and potentially counteracting their local adaptation. Then, it would be interesting to investigate the temporal fluctuations in population size and genetic structure in order to measure the relative importance of genetic drift and gene flow in the evolution of these populations.

Conclusions

In contrast to most Q_{ST}–F_{ST} comparisons carried out so far, we did not find $Q_{ST} > F_{ST}$, which implies that directional selection cannot explain the quantitative genetic divergence observed among the populations of *R. balthica* we studied. Our results also demonstrate that frequentist and Bayesian methods provide similar estimates for Q_{ST} if the amount of additive variance is similar within each population. In case of heteroscedasticity, CVs are more constant across populations than heritability. Despite a high environmental heterogeneity at a local scale, we did not detect any evidence of directional selection for local optima, which could be hindered by genetic drift, and to a lesser extent by gene flow in the floodplain habitat considered.

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