# Small effective population sizes in a widespread selfing species, *Lymnaea truncatula* (Gastropoda: Pulmonata)

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## Abstract

**We present here a spatial and temporal population genetic survey of a common freshwater snail, also a predominantly selfing species, *Lymnaea truncatula*. The rate of genetic diversity loss was quantified by estimating the effective size ($N_e$) of the snail populations, using two different methods. A temporal survey allowed estimation of a variance effective size of the populations, and a spatial survey allowed the estimation of an inbreeding effective size, from two-locus identity disequilibria estimates. Both methods were consistent and provided low $N_e$ values. Drift due to (i) high amounts of selfing and (ii) fluctuations in population sizes because of temporary habitats, and also selection coupled to genome-wide linkage disequilibria, could explain such reductions in $N_e$. The loss of genetic diversity appears to be counterbalanced only very partially by low apparent rates of gene flow.**

*Keywords*: effective size estimate, genetic drift, microsatellite markers, population genetics, selfing, spatial and temporal study

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## Introduction

The maintenance of genetic diversity is a key question in evolution, as genetic diversity is the base material for selection, adaptation and/or divergence leading to speciation. However, in natural populations, some species appear to be genetically impoverished, e.g. 4% of plant taxa and 4.7% of animal species display no allozyme polymorphism (Nevo *et al.* 1984; Hamrick & Godt 1989; Amos & Harwood 1998). Such a low level of intrapopulation genetic diversity is usually found in rare and endangered species, whereas a genetic impoverishment is seldom reported in common and widespread species (Amos & Harwood 1998).

Further assessment of such cases may help understanding if and at which scale genetic variation may be maintained. The freshwater snail species *Lymnaea truncatula* is a choice model to address these issues. *L. truncatula* populations have already been shown to be genetically impoverished, as inferred from allozymes (Jabbour-Zahab *et al.* 1997) and microsatellite markers (Trouvé *et al.* 2000; Meunier *et al.* 2001).

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*L. truncatula* is a predominantly selfing species (Meunier *et al.* 2004; Trouvé *et al.* 2003), and lives mainly in temporary habitats (e.g. in ponds, ditch margins or any depression in the ground storing water) (Morel-Vareille 1973; Smith 1981).

Several factors, including drift, selection, migration and metapopulation dynamics, may affect the amount of genetic diversity (Barton & Whitlock 1997; Amos *et al.* 1998; Pannell & Charlesworth 2000). In selfing species, the effective population size ($N_e$) is reduced compared to outcrossing species (Jarne 1995; Tachida 1996), thereby enhancing drift. Directional selection may also enhance drift, because of a reduced effective recombination across loci (linkage disequilibria): in such cases, genetic hitchhiking (Hedrick 1980) and/or background selection (Charlesworth *et al.* 1993) may cause neutral genetic diversity losses analogous to the effect of drift (Caballero 1994).

Thus, the effective population size ($N_e$) allows one to summarize all effects of drift into one parameter, and to quantify the rate of genetic diversity loss. As $N_e$ is affected most strongly by periods of reduced population size (Caballero 1994; Hartl & Clark 1997), estimating $N_e$ should also allow quantification of drift associated with population dynamics features of the considered species.

We estimated effective population sizes in *L. truncatula* as part of a population genetics survey of a total of 17 samples of this snail species in France, using seven variable microsatellite loci. Combining both a temporal and a spatial study allowed us to use two independent methods for the estimation of the population effective sizes. Moreover, temporal studies provide insights into population dynamics, e.g. the occurrence of bottleneck events, and into patterns of dispersal (see Viard *et al*. 1997). This, in turn, helps understanding of the spatial structuring of the genetic variability, addressing the issue of migration–drift equilibrium in such populations. Having quantified the amount of drift, and thereby the rate of genetic diversity loss within populations, we addressed the question of the maintenance of diversity at higher, i.e. metapopulation, scales.

## Materials and methods

### Sampling

Most of the sampling was conducted in the Limousin region (Central France) (Adriers, Migné, St Marcel, Chateauponsac, St Priest, Fig. 1). We sampled one location in Southern France, St Paul et Valmalle, distant from all the others by 600 km. All populations were sampled along side-of-the-road ditches except the St Priest population, which is a river population, and St Marcel B, which is a pond population. In each sampling site, we sampled across only *c*. 30 m along the ditch or the river to avoid sampling biases (e.g. Wahlund effects) by sampling across a larger scale. The absence of spatial differentiation between samples 10 m apart (StMA and B, not shown) provides an a posteriori confirmation of this choice of scale. Snail densities were sufficient to ensure a sampling of 50–100 snails, among which we chose randomly a subsample of snails to be genotyped (Table 1).

We performed both spatial and temporal sampling (Fig. 1). The spatial survey was undertaken at two scales, a small scale in 1998 (Fig. 1B) and a larger scale in 2000 (Fig. 1A,B). The temporal survey allowed the testing of our sampling scheme: possible biases may indeed arise from incomplete sampling of the different families in a site if mating among relatives is frequent. However, what we named a 'population' showed some temporal stability (see Results), indicating that sampling was, each time, almost complete in terms of breeding groups.

### Microsatellite analysis

DNA extraction protocols, description of microsatellite loci and genotyping methods have already been described in Trouvé *et al*. (2000). The snails were genotyped at seven variable microsatellite loci (9, 16, 21, 24, 36, 37, 43, GenBank Accession nos: AF226976, AF226978, AF226980, AF226981,

**Table 1** $F_{IS}$ and Nei's gene diversities ($H_E$) per sample, averaged over loci. s: selfing rate inferred as $s = 2F_{IS}/(1 + F_{IS})$. CP, Chateauponsac, Ad, Adriers, StM, St Marcel, StP, St Priest, PV, St Paul et Valmalle, Mi, Migné. A, B, C: subpopulations in Adriers and St Marcel (see Fig. 1); Aut: autumn, Spr: spring

| Pop | Ad A | Ad A | Ad A | Ad B | Ad C | CP | CP | Mi | Mi | Mi | StM A | StM B | StM C | PV | PV | StP | StP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Aut. 98 | Spr. 00 | Aut. 00 | Aut. 98 | Aut. 98 | Aut. 00 | Aut. 01 | Aut. 98 | Spr. 00 | Aut. 00 | Aut. 98 | Aut. 98 | Aut. 98 | Aut. 00 | Aut. 01 | Aut. 00 | Aut. 01 |
| N | 30 | 31 | 25 | 30 | 14 | 28 | 70 | 20 | 29 | 21 | 16 | 30 | 30 | 20 | 30 | 44 | 30 |
| $F_{IS}$ | 0.88 | 0.64 | 0.85 | 0.93 | 0.89 | 0.92 | 0.86 | 0.94 | 0.89 | 1 | 0.84 | 0.94 | 0.81 | 0.93 | 0.80 | 1 | 0.81 |
| s | 0.93 | 0.78 | 0.92 | 0.96 | 0.94 | 0.96 | 0.92 | 0.97 | 0.94 | 1 | 0.92 | 0.97 | 0.89 | 0.96 | 0.89 | 1 | 0.89 |
| $H_E$ | 0.27 | 0.22 | 0.04 | 0.41 | 0.27 | 0.14 | 0.10 | 0.12 | 0.05 | 0.05 | 0.35 | 0.32 | 0.15 | 0.42 | 0.49 | 0.26 | 0.31 |

(A)

Limousin region

St Paul et Valmalle
1. F00 (20)
2. F01 (30)

(B)

Migné
1. F98 (20)
2. S00 (29)
3. F00 (21)

St Marcel A
1. F98 (16)

St Marcel B
1. F98 (30)

St Marcel C
1. F98 (30)

St Marcel

2 km

Adriers A
1. F98 (30)
2. S00 (31)
3. F00 (25)

Adriers B
1. F98 (30)

Adriers C
1. F98 (14)

250 m

Adriers

Chateauponsac
1. F00 (28)
2. F01 (70)

St Priest
1. F00 (44)
2. F01 (30)

20 km
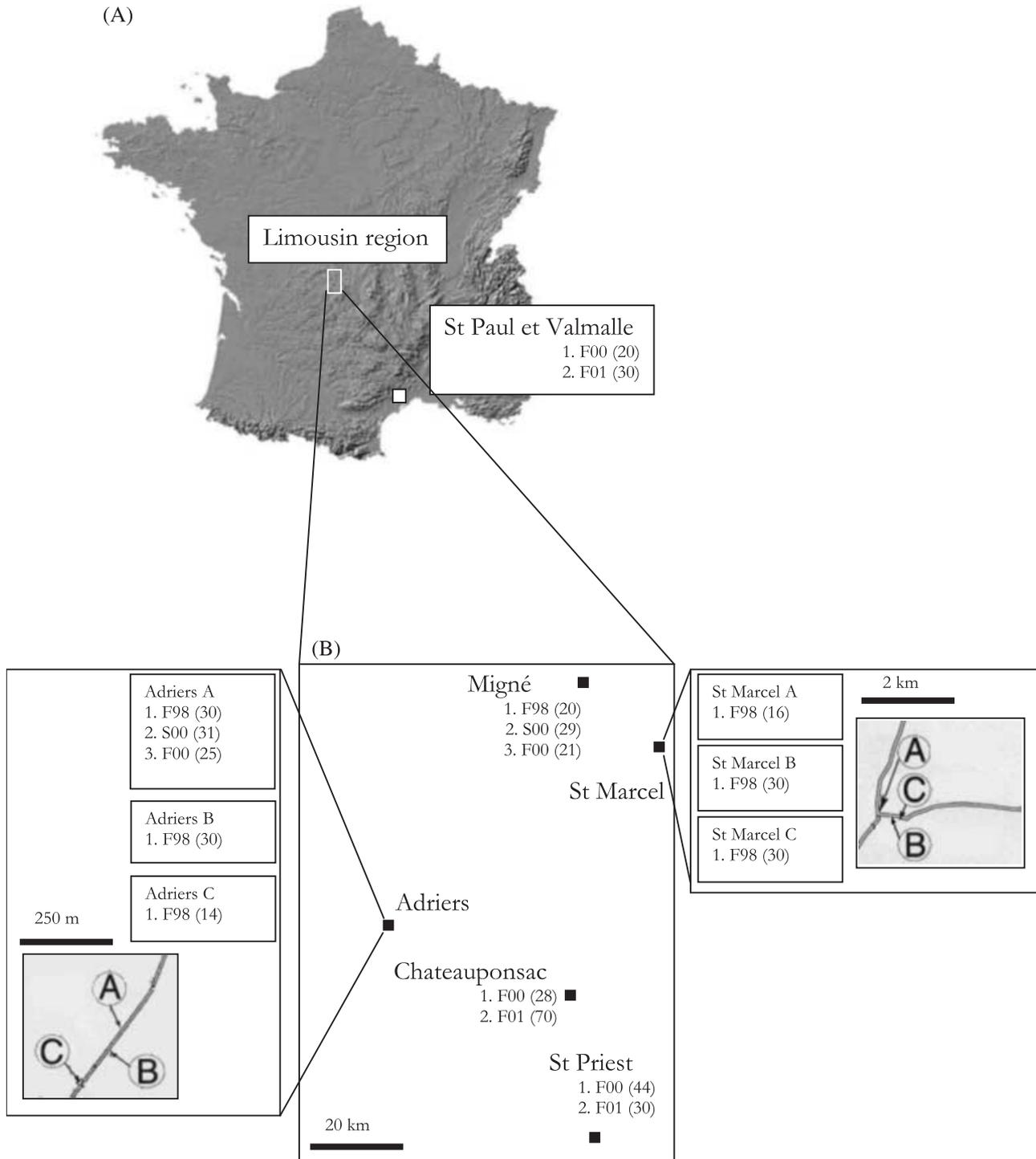
**Fig. 1** Map of the sampled sites. (A) Map of France indicating the two regions of sampling. (B) Detail of the Limousin region, indicating the five locations sampled in this region. Further details of the two subdivided populations (Adriers and St Marcel): A, B, C: locations of the sampled subsites are also indicated. Sampling dates: F98: autumn 98. S00: spring 00. F00: autumn 00. F01: autumn 01. Sample sizes are indicated in parentheses.

AF226984, AF226985, AF226986, respectively). For unknown technical reasons, at locus 24 only 61% and 25% of the snails amplified in the 2000 and 2001 samples, respectively. However, this amplification problem did not bring out any bias in our analyses (not shown).

### Spatial study; level of structuring

Nei's genetic diversities ($H_E$), $F_{IS}$ and $F_{ST}$ estimates were computed using FSTAT 2.9.3. (Goudet 1995, 2001), which provides the unbiased estimators $f$ (for $F_{IS}$) and $θ$ (for $F_{ST}$) defined in Weir and Cockerham (1984). Departures from Hardy–Weinberg expectations were tested within sample, randomizing alleles among individuals within samples, and using the statistic $f$ to classify tables of randomized data sets.

We also tested for genotypic disequilibrium between all pairs of loci overall samples using FSTAT, and applying Bonferroni corrections to the *P*-values obtained. We adjusted *P*-values experimentally for a testwise *P*-value of 0.05.

Bootstrapped values of $θ$ over loci were obtained for the populations of Adriers and St Marcel (each of which is sub-divided, see Fig. 1B), and for all populations sampled in 1998 or 2000. This allowed us to compare the level of structuring at a small scale (one subdivided population) to the structuring at the whole spatial scale of the study. As bootstrapped values are potentially biased by the use of a small number of loci, we also tested differentiation among samples using the *G*-based global test on $θ$ in FSTAT, not assuming Hardy–Weinberg equilibrium within samples (Goudet *et al.* 1996).

Friedmann rank sum tests were performed over populations sampled at the same date, in order to test for spatial heterogeneity in genetic diversity ($H_E$). Spatial variation in $H_E$ may indeed impede the detection of isolation by distance (Goodman *et al.* 2001). To test for isolation by distance also allows testing for migration–drift equilibrium (see Hutchinson & Templeton 1999). This requires performing Mantel tests between pairwise $ρ = F_{ST}/(1 − F_{ST})$ and the logarithm of the distance (ln d) between populations (Rousset 1997), and also to test for an increase of the variance in genetic distances with geographical distance. A Mantel test was therefore performed between (ln d) and the residuals of the regression between $ρ$ and (ln d).

### Temporal study; consistency of the estimated parameters through time

We tested for temporal differentiation in genotypic frequencies also performing a *G*-based global test, as above. We also tested for change in $H_E$ in the same population over time: we used the FSTAT 'biased dispersal menu' to test for differences in gene diversity between sampling dates (date 1 and date 2). First, the procedure estimates the observed value of 'diff $H_E$' = [$H_E$ (date 1) − $H_E$ (date 2)] in the data set. Then the labels 'date 1' and 'date 2' were assigned randomly to individuals in the same sample and 'diff $H_E$' was estimated for all randomized data sets. The *P*-value refers to the probability of obtaining a value of 'diff $H_E$' larger or equal to that observed in the randomized data set.

### Estimation of effective sizes

Two different methods allowed us to estimate effective sizes: first, the temporal study allowed us to estimate the variance effective sizes ($N_e$) of five populations studied (see Fig. 1, repetitive sampling) (Waples 1989). We used the software MACLEEPS 1.1 (Anderson *et al.* 2000) which performs maximum-likelihood estimates for different $N_e$ using the allele frequencies shifts between generations. The computation assumes that selection, migration and mutation are negligible in changing allelic frequencies, compared to drift. We assumed two generations per year in *L. truncatula* (Morel-Vareille 1973). We used at least $10^5$ iterations for the computation of the log-likelihood of each $N_e$ values. A 95% confidence interval (CI) was estimated using the values of $N_e$ at which the log-likelihood has decreased of two units from its maximum (Anderson *et al.* 2000).

Second, the spatial study allowed us to estimate the inbreeding effective size of the populations using the software ESTIM 1.2 (Vitalis & Couvet 2001a). This software performs estimates of the two-locus identity disequilibria, $η$, within populations, together with a single locus parameter

$$F = \frac{Q_{1,i} − Q_2}{1 − Q_2}, \text{where } Q_{1,i} \text{ is the probability of identity of a}$$

pair of genes in subpopulation *i*, and $Q_2$ the probability of identity for two genes in two different subpopulations (Vitalis & Couvet 2001c). These two parameters, F and $η$, both depend on local $N_e$ and *m*, the immigration rate, but not on 'nuisance' parameters such as the mutation rate or mutation model (Vitalis & Couvet 2001b,c). However, the selfing and recom-bination rates must be known. In our study case, selfing rates have already been estimated by progeny arrays in two populations of the Limousin region (Meunier *et al.* 2004). Thus we have a range of probable values for selfing rates (between 0.75 and 0.90). Choosing to estimate $N_e$ along such a range also allows buffering against possible overestimations of selfing rates in some populations, due to ignored Wahlund effects or local inbreeding. We fixed the recombination rate between loci to 0.5, assuming their physical independence (the estimations rely on the extant of physical, not statistical, linkage; see helpfile in ESTIM). We computed effective sizes jointly for populations sampled at the same date and report estimates only for which we obtained reliable CI (i.e. different from [0−∞]).

## Results

### Genetic diversities

Nei's $H_E$ computed for every location are spatially and temporally variable (range 0.05–0.49, Table 1). Most populations exhibit low $H_E$ (Table 1), except the populations of St Paul et Valmalle and Adriers site B, which display more genetic diversity. The Friedmann test for spatial variation in $H_E$ was significant in 1998 and autumn 2000 ($P = 0.04$, $P = 0.02$, respectively).

### Heterozygote deficiencies

All populations display significant departures from Hardy–Weinberg equilibrium (global test, $P \leq 0.001$). $F_{IS}$ values are displayed in Table 1. Neglecting Wahlund effects and apparent selfing due to mating among relatives, these $F_{IS}$ values translate into very high values of selfing rates (Table 1, range 0.78–1).

### Linkage disequilibrium

We find significant linkage disequilibrium within populations in 10 cases of 21 comparisons between locus pairs (not shown). Locus 36 is in linkage disequilibrium with all six other loci.

### Spatial structure: scaling gene flow

θ estimates reach very high values (Fig. 2). Contrasting subpopulation and whole study scales does not provide evidence for a reduced structuring at a small scale (Fig. 2). The population of St Marcel is significantly less structured
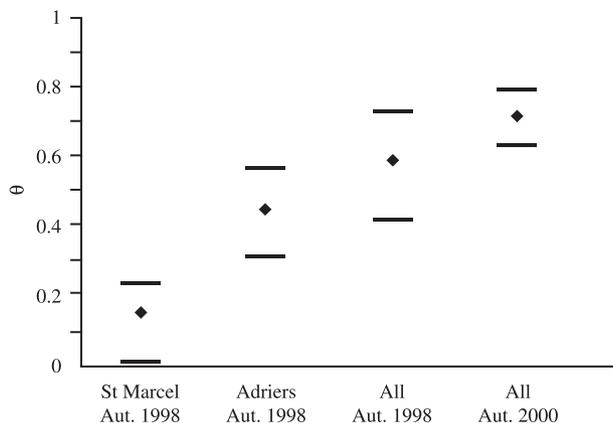
than Adriers and the whole data set in 1998 and 2000 (see bootstraps CI in Fig. 2). Whatever the scale, population differentiation is always highly significant (global test, $P \leq 0.001$ for both surveys and in Adriers and St Marcel).

In the 1998 survey, there was a weak positive effect of geographical distances ($R^2 = 0.22$, $P = 0.03$, Fig. 3A) on $\rho = F_{ST}/(1 - F_{ST})$ when simple Mantel tests are undertaken. However, the Mantel test between residuals of the former regression and geographical distances is not significant ($P = 0.13$; $R^2 = 0.11$).

At a larger scale (12–600 km in 2000 vs. 0.01–69 km in 1998) there is no significant isolation by distance ($P = 0.33$, Fig. 3B).

### Temporal study; allelic frequencies and gene diversity shifts

Gene diversity may vary within a single population through time (see Tables 1 and 2). We observe a significant increase in $H_E$ in the population of St Paul et Valmalle (Tables 1 and 2), but significant losses in gene diversity in two populations: in Adriers in the two consecutive samples from 2000 and in Migné between 1998 and spring or autumn 2000 (Tables 1 and 2). These gene diversity losses correlate with a significant temporal structuring in Adriers and Migné, respectively (Table 2). We also noted a weak but significant differentiation between temporal samples in Chateauponsac. However, temporal $F_{ST}$ values were consistently lower than spatial $F_{ST}$ (mean: 0.11, 0.59, respectively, see Table 2 and Fig. 2).
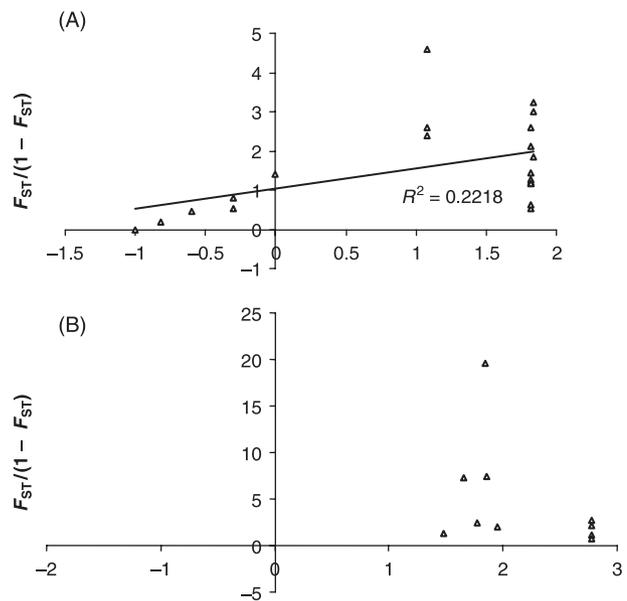


**Fig. 2** θ-values and 95% bootstrap confidence interval for these values (bars). St Marcel, Adriers: θ estimated in these subdivided populations (see Fig. 1). All: θ estimated in the whole data set for the 1998 and 2000 surveys, Aut: autumn.



**Fig. 3** Linear regressions of $F_{ST}/(1 - F_{ST})$ with ln (d) (geographical distance). Regression line and $R^2$ are added when the regression is significant. (A) Survey in autumn 1998. (B) Survey in autumn 2000.

| Population | Parameter tested | Samples 1/2 | Samples 2/3 | Samples 1/3 |
|---|---|---|---|---|
| Adriers A | $\theta$ | 0.03 NS | 0.17*** | 0.15*** |
| | $H_E$ | NS | *** | *** |
| Migné | $\theta$ | 0.002 NS | 0.31*** | 0.47*** |
| | $H_E$ | ** | NS | * |
| Chateauponsac | $\theta$ | 0.04* | — | — |
| | $H_E$ | NS | — | — |
| St Paul et Valmalle | $\theta$ | −0.01 NS | — | — |
| | $H_E$ | * | — | — |
| St Priest | $\theta$ | 0.02 NS | — | — |
| | $H_E$ | NS | — | — |

**Table 2** Temporal dynamics of the snail samples. Results of the test for population differentiation in FSTAT ($\theta$) and results of the 'biased dispersal' tests ($H_E$) (see Material and methods) between temporal samples of the same location. Samples 1, 2, 3 are temporal samples of the same location (see Fig. 1). Samples 1/2, 2/3, 1/3: pairwise comparisons for $\theta$ or $H_E$ between temporal samples. $\theta$-values are given together with *P*-values of the test for population differentiation, after Bonferroni corrections; NS, *, **, ***: nonsignificant, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively

| Population | Variance effective size | | Inbreeding effective size | | | |
|---|---|---|---|---|---|---|
| | ML | 95% CI | s | $N_e$ | 95%CI | m |
| Adriers A[a] | 16 | [9; 30] | 0.75 | 16.9 | [0; 148] | 0.02 |
| | | | 0.80 | 24.3 | [0; 201] | 0.01 |
| | | | 0.85 | 37 | [0; 180] | 0.01 |
| | | | 0.90 | − > 0 | [0; 0] | — |
| St Marcel A[a] | — | — | 0.75 | 11.3 | [0; 102] | 0.04 |
| | | | 0.80 | 17.9 | [0; 107] | 0.03 |
| | | | 0.85 | − > 0 | [0; 0] | — |
| | | | 0.90 | − > 0 | [0; 0] | — |
| St Marcel B[a] | — | — | 0.75 | − > 0 | [0; 37] | — |
| | | | 0.8 | − > 0 | [0; 35] | — |
| | | | 0.85 | 12.2 | [0; 52] | 0.03 |
| | | | 0.90 | − > 0 | [0; 0] | — |
| Chateauponsac[b] | 20 | [14; 44] | 0.75 | − > 0 | [0; 117] | — |
| | | | 0.8 | 5.36 | [0; 155] | 0.02 |
| | | | 0.85 | 9.97 | [0; 221] | 0.01 |
| | | | 0.90 | 17.75 | [0; 161] | 0.01 |
| St Priest[b] | 27 | [14; 135] | 0.75 | 10.65 | [0; 82] | 0.03 |
| | | | 0.8 | 16.46 | [0; 112] | 0.02 |
| | | | 0.85 | − > 0 | [0; 117] | — |
| | | | 0.90 | − > 0 | [0; 0] | — |
| Migné | 4 | [3; 10] | — | — | — | — |
| St Paul et Valmalle | 28 | [12; 174] | — | — | — | — |

**Table 3** Computed variance effective size and computed inbreeding effective size of *L. truncatula* populations. $N_e$: effective size. m: immigration rate. s: range of tested selfing rates. ML: maximum likelihood $N_e$ estimate. 95% CI: 95% confidence interval for the estimates. For the inbreeding effective size, only estimates with most accurate CI are shown. —: not computed. [a,b] joint estimations of inbreeding $N_e$ for populations with the same letter, using the 1998 and 2000 sampling, respectively

## Estimating effective sizes

The variance effective sizes computed for the five locations where we had temporal sampling were generally low (Table 3). Using the second method (inbreeding effective sizes), we report here the estimates for five populations (Table 3), for which the CI were reliable. These $N_e$ values depend strongly on the selfing rate, but were again very low. These inbreeding effective sizes were in the same order of magnitude as the variance effective sizes when comparisons were possible (Table 3).

## Discussion

### Selfing vs. local inbreeding vs. Wahlund effects

We observed extremely high $F_{IS}$ values in *L. truncatula* populations (range 0.64–1, see Table 1). Population genetics inferences make it difficult to disentangle selfing, mating among relatives and Wahlund effects in their contribution to $F_{IS}$ values (Städler & Jarne 1997, but see also Goudet *et al.* 1994), unless fine-scale genetic analyses involving measures of relatedness and spatial distances between individuals

are conducted (Vekemans & Hardy 2004). Quantitative, direct assessments of selfing rates (e.g. breeding experiments and progeny arrays) may also help to distinguish between selfing and mating among relatives (Ritland 2002). Such direct studies have already been conducted in this species, and there is now consistent evidence from earlier works that here selfing contributes much more to the high $F_{IS}$ we observed than other factors (Meunier *et al.* 2001; Trouvé *et al.* 2003; Meunier *et al.* 2004). Finally, also consistently with high selfing rates, we detected linkage disequilibrium between microsatellite loci within populations.

Selfing reduces $N_e$ by a factor $(1 + F_{IS})$, and, thus, here the mating system contributed to a loss in gene diversity. Moreover, in selfing species directional and background selection, as well as hitchhiking effect, can lead to a genome-wide reduction in genetic diversity, owing to extant linkage disequilibria between selected and neutral markers (Caballero 1994; Green *et al.* 2001). This effect would be analogous to drift and also reduce $N_e$ estimations.

However, gene diversities here were lower than other estimates in freshwater snails, e.g. the mixed-mating species *Biomphalaria glabrata* (mean $H_E$ of 0.6, Mavarez *et al.* 2002), or the highly selfing species *Bulinus truncatus* ($H_E$ generally above 0.5, Viard *et al.* 1996). Comparisons between species may be difficult because of possible varying mutation rates of the microsatellite markers used (Jarne & Lagoda 1996; Hancock 1999). However, some of our results point to additional causes of gene diversity loss, i.e. bottleneck events.

### Model of population structure

The population structure model (migration-drift equilibrium or metapopulation model) has to be taken into account when addressing the issue of the maintenance of genetic diversity. Tests of isolation by distance and examination of the scatterplot led to the rejection of a regional equilibrium between migration and drift (see also Hutchinson & Templeton 1999), even though we observed some degree of association between geographical and genetic distances for very close neighbouring demes (see Fig. 3).

Further results seem to point towards an alternative model of metapopulation structure: first, we observed a significant spatial heterogeneity in $H_E$ values. This would explain partly why geographical relationships seem to be a poor predictor of genetic distances. Differences here in gene diversity (i.e. differences in $N_e$) may explain more readily $F_{ST}$ values (see Hedrick 1999). High differences in $N_e$ may arise if events of extinction/recolonization are frequent. Thus, in this study we could have indirect evidence for fluctuations in population size.

Second, furthermore, although our populations show an overall temporal stability (Table 2), our temporal survey also showed evidence of some fluctuations in $H_E$ over time. In two populations (Adriers and Migné, Table 2), we observed a significant decrease in $H_E$: this result may reflect bottleneck events. Random sampling of individuals and founder effects after bottlenecks would also be consistent with the significant temporal structure we found in these two populations (Table 2). Freshwater snail populations may indeed be disturbed frequently, depending on the stability of the habitat, i.e. on the permanence of water amount in the sites (Jarne 1995; Städler *et al.* 1997). Earlier direct observations had also shown extinction followed by recolonization of some sites over time (Roberts 1950; Moens 1991; Vareille-Morel *et al.* 2002). Such events could also explain partly the extent of linkage disequilibrium we observed, due to population admixtures during recolonizations.

These snail populations, thus, seem connected partly with gene flow on one hand and on the other hand also undergoing extinction–recolonization dynamics. The most probably population structure model is thus a metapopulation one (*sensu* Hanski & Simberloff 1996).

### Quantifying genetic drift: estimation of effective sizes

$N_e$ estimates provide a measure of the effects causing or analogous to drift in populations: in this study, we have shown that selfing and probable fluctuations in population size may contribute to gene diversity losses.

We quantified drift in estimating effective sizes in some of the studied populations using two different methods. In the first method (variance effective size), neglecting migration and selection may seem a restrictive hypothesis. However, the effect of directional, background selection and hitchhiking on neutral markers, especially in selfing species, may be considered as analogous to drift (Caballero 1994). When comparison is possible, the estimated sizes are within the same order of magnitude between the two independent methods, which make different assumptions on migration. Therefore, the assumption of negligible migration in the temporal method may also seem acceptable.

In the ESTIM method, the estimation-underlying model is a Wright–Fisher island model, which assumes that migrants all originate from a random sample of all demes. On the contrary, *L. truncatula* more probably disperse locally (see previous section). However, when the total migration rate is low, the differentiation between adjacent populations is close to that expected in an island model (Kimura & Maruyama 1971; Rousset 2000). The estimation is also relatively robust with respect to finite deme number (Vitalis, Couvet 2001b). More problematic may be the assumption of migration–drift equilibrium. Bottlenecks events would lead possibly to increased pairwise $F_{ST}$ between populations (Chakraborty & Nei 1977; see also previous section). However, Vitalis & Couvet (2001b) showed the relative robustness of their estimation method to bias arising from nonequilibrium situations: while simulating sampling of individuals 10–50 generations

after the initial state, they showed that after only 50 generations bias and variance of $N_e$ were as small as after 1000 generations.

Furthermore, the estimation of $N_e$ relies on the comparison of observed *F*- and η-values to expected values (Vitalis & Couvet 2001b). Expected values take into account fixed loci in their computation, whereas the observed values of these parameters are computed on polymorphic loci. Consequently, the expected values for *F* and η, and therefore also $N_e$ values, may be overestimated (R. Vitalis, pers. comm.).

The estimated effective sizes are here generally low (range 4–37, Table 3). As a comparison, Arnaud & Laval (2004) estimated variance effective sizes in another gastropod, the outcrossing snail *Helix aspersa.* Depending on the method used, they found $N_e$ values ranging from 6 to ∞, and explained low $N_e$ values by extinction–recolonization dynamics in transient habitats.

### *Maintenance of genetic diversity at the metapopulation scale*

In these snail populations, we therefore noted high rates of loss of genetic variation through drift, due to selfing and population dynamics. We also noted low rates of gain of genetic diversity, due to the low migration rates inferred. This situation should lead to an increase in the effective size at the metapopulation level, and thereby to gene diversity storing at this higher scale. Subdivided metapopulations retain more genetic diversity than panmictic ones of the same size, assuming limited dispersal rates and constant deme sizes (Barton *et al.* 1997; Wang & Caballero 1999). However, when there are differences in reproductive success among demes, of which an extreme case is extinction–recolonization dynamics, as our results seem to indicate, the metapopulation amount of genetic diversity always decreases (Barton *et al.* 1997; Pannell & Charlesworth 1999).

However, despite its apparent genetic impoverishment this snail species may not lack evolutionary potential, as its large distribution range would indicate. Adaptive variation may indeed be maintained despite reduced neutral variation (see, e.g. McKay *et al.* 2001), and studies have shown a possible lack of correlation between genetic diversity at neutral and quantitative markers (Reed & Frankham 2001).

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