

Population biology of the invasive freshwater snail *Physa acuta* approached through genetic markers, ecological characterization and demography

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Appendix A1

1. Segregation of alleles at microsatellite loci

Mendelian segregation of alleles was tested using selfed progenies. Thirteen immature snails originating from five populations around Montpellier were kept isolated in the laboratory until they self-fertilised. Twelve to 15 hatchlings per family were raised during six weeks. Parents and offspring were placed in 90° alcohol. DNA extraction was performed on parents and seven haphazardly chosen offspring according to the method presented in Sourrouille *et al.* (2003). Allelic segregation was analyzed at 10 microsatellite loci. The characterization of variability was performed as described in Sourrouille *et al.* (2003). The loci studied have been described by Monsutti & Perrin (1999) and Sourrouille *et al.* (2003). PCR coamplification was possible for some groups of loci (Pac1, Pac2 and Pac5; Pac4 and Pac7; AF108762 and AF108764). PCR products were run in two groups (Pac1, Pac2, Pac5, Pac4, Pac7, AF108761, AF108762 and AF108764; Pac14 and AF108758) on an ABI Prism 310 Genetic Analyzer. When the parent was heterozygous at a given locus, the genotypic distribution among progenies was tested for deviation against both 1:1 (homozygote : heterozygote) and 1:2:1 (homozygote : heterozygote : homozygote) ratios using Fisher exact test.

Homozygous individuals always engendered homozygous offspring with the parental allele (Web Table 1). Forty six situations were detected in which the parent was heterozygous at one locus at least. The distribution of alleles among offspring was not significantly different from the expected 1:1 and 1:2:1 ratio ($P > 0.05$ in Web Table 1), with a single exception (family L18 at locus AF108762). Locus AF108758 showed an unexplained pattern. Two bands of size 140 and 147 systematically co-occurred, and were transmitted as a single allele to offspring. They were collectively referred to as allele 407 (Web Table 1). The other allele had size 144. Sequencing of two homozygous individuals indicated that allele 144 has the same flanking region than allele 146 in Monsutti & Perrin (1999). They only differed by one microsatellite repeat. On the other hand, allele 140 showed a six-base pairs deletion in the 5' flanking region. It remains unclear why this allele amplified as two bands (140 and 147).

In conclusion, although microsatellite loci have been heavily used in population biology (see e.g. Goldstein & Schlötterer 1999), Mendelian segregation of alleles is not often tested. This is yet a basic assumption of most models in population genetics. We did not observe significant deviation from Mendelian expectations beyond what is expected by chance. The two potential forces generating segregation distortion, namely null alleles and selection (see e.g. Bierne *et al.* 1998; Hodgetts *et al.* 2001), apparently played no role in the 13 families studied.

2. The role of null alleles

Allelic frequencies are given per locus in Web Table 2. A few null homozygotes (no PCR product) were observed at loci Pac2 (1 individual), Pac4 (21), Pac5 (3), Pac7 (26), AF108758 (1) and AF108762 (1), out of 488 individuals studied. Individual / loci combinations that failed to amplify were analyzed again. Amplification failure was never observed at all loci of the same individual, discarding the possibility of poor DNA quality. Several nulls were detected in Vio2, FMB and FVR (Pac4) and Vio1, FB and FVR (Pac7). However excluding Pac4 and Pac7 quantitatively modified our results to a limited extent only. With regard to deviations from Hardy-Weinberg equilibrium, we detected 22 multilocus deviations (out of 32 tests), all indicating heterozygote deficiencies, when using all loci, and 15 once Pac4 and Pac7 have been removed. Significantly non-null estimates of F_{IS} ranged from 0.02 to 0.46 with all loci and from 0.06 to 0.44 without Pac4 and Pac7. The corresponding estimates of S ranged from 0.04 to 0.63 and from 0.12 to 0.61 respectively. The average F_{IS} estimate over all populations decreased from 0.16 to 0.11 (not statistically different; Mann-Whitney $U = 537$, $n_1 = 32$, $n_2 = 32$, $P = 0.74$), and the selfing rate from 0.27 to 0.20. Furthermore, there was

no correlation between the frequencies of null homozygotes at Pac4 and Pac7 and F_{IS} estimates at local scale, at global scale and when considering populations outside the Montpellier area. Including Pac4 and Pac7 resulted in a higher number of significant demographic tests conducted using the methodology of Cornuet & Luikart (1996). Note that all tests reported in the main text remained significant. Heterozygosity deficits were also detected in Sal2, FMB and FVR under the stepwise mutation model, and heterozygosity excesses, indicative of bottlenecks, were detected in Vio1 (t_2), Vio2 (t_2), Vio12 (t_2), Mos6 (t_1), FML, FMR and FPB under the infinite allele model. It remains possible that the occurrence of null alleles artificially inflates the number of significant tests detected with the methodology of Cornuet & Luikart (1996), although this has not been theoretically evaluated. Estimates of paired F_{ST} (Web Table 3) were only slightly affected when including Pac4 and Pac7, and there was no change with regard to environmental descriptors (same significant differences than in Table 4 of main text). Results relating to isolation by distance were not affected.

3. Studying migration using assignment and exclusion methods

In addition to the analysis based on F_{ST} , migration among populations was also approached from genetic data using the assignment and exclusion methods described in Cornuet *et al.* (1999). Based on the identification of rare multilocus genotypes, they were used to identify immigrants and their origin (Cornuet *et al.* 1999) using the program GENECLASS (Cornuet JM & Piry S (1999) GeneClass version 1.0.02 (<http://www.ensam.inra.fr/urib/GENECLASS>). Individuals were excluded when their probability of belonging to a population was below 0.05 and reassignment was done only for probabilities higher than 0.05. The assignment probability was based on Cavalli-Sforza's chord distance (Cavalli-Sforza & Edwards 1967), as no assumption on Hardy-Weinberg equilibrium is required. Samples in the eight populations sampled twice were pooled prior to analysis. Similar results were obtained when they were considered separately.

At local scale, 50 individuals (mean fraction per population 0.23, *s.e.* = 0.28) were excluded from the population in which they were sampled (Web Figure 1), corresponding to 28 and 18 individuals in ponds and rivers respectively (Web Table 4). The four individuals from Mos6 were not considered because of too small sample size. The reassignment probability of 24 individuals (13 in closed and 11 in open habitats) was too low ($P < 0.05$) for the population of origin to belong to one of the populations sampled. Out of the 26 remaining individuals, three were reassigned to the nearest pond and six to the same river. All but one of the remaining individuals (16, 12 of which originated from ponds) were reassigned to a river. This is not explained by geographic distance, because the geographic distance between the populations from which individuals were excluded to all ponds (6.0 ± 3.5 km) was shorter (Mann-Whitney, $U = 3.0$, $n_1 = n_2 = 6$, $P = 0.02$) than that with rivers (12.5 ± 1.5 km). When several individuals were excluded from a population, they were generally not reassigned to the same population. A significant positive relationship (Spearman ranks order correlation coefficient, $r_s = 0.98$, $N = 6$, $P = 0.003$) was observed between the number of individuals reassigned in a population and the mean number of alleles of this population.

Predictions were made with regard to habitat openness and water regime, as for other aspects of the genetic structure and demography (Table 2 in main text). We expected less exclusion and reassignment in ponds than in rivers, but no difference between permanent and temporary habitats. The latter was observed, though not the former: exclusion and reassignment probabilities did not differ significantly between ponds and rivers, although the difference goes in the predicted direction (Web Table 4). A possible explanation for reassignment probabilities is higher F_{ST} estimates in ponds than in rivers (see Cornuet *et al.* 1999). Also the populations in ponds were closer to one another and surrounded by rivers (Fig. 1 in main text). A more appropriate design should avoid effects of geographic distance (see also Mavárez *et al.* 2002b). A further result is that most excluded / reassigned individuals were reassigned to rivers, whatever their population of origin. This cannot be explained by geographic distance. Possible explanations are asymmetric gene flow, rivers serving as source of migrants, and higher genetic variation in rivers (Cornuet *et al.* 1999) as shown by the significant positive relationship between the probability of exclusion-reassignment and the genetic variation per population.

4. The influence of water regime (permanent versus temporary habitats)

As mentioned in the main text, demographic and genetic parameters were contrasted between permanent and temporary habitats. The comparisons were made with regard to the four factors studied, namely population

size (number of alleles, gene diversity and harmonic mean density), bottlenecks (number of genetic signature of bottlenecks, occurrence of low density events, temporal genetic differentiation) and migration (spatial genetic differentiation and isolation by distance). No significant differences were detected, and the results were therefore relegated here. Predictions are given in Table 2, and results in Web Table 5.

5. Bottlenecks

Results of the tests on bottlenecks implemented using the method of Cornuet & Luikart (1996) are presented in Web Table 6.

6. References

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Table S1 Analysis of allelic segregation at ten microsatellite loci in 13 selfed families (seven offspring). Some (L2, L3, L8, L10, L12, L15, L18) were second laboratory generation offspring of individuals collected in March 2002 in a pond located on the CNRS campus in Montpellier. Other individuals were collected in March 2002 at the egg stage either in streams (Lam1a, Lam1b, Lez5), or in ponds (Vio8a, Vio8b, Vio12) located within a radius of 20 km around Montpellier (Table 1, Fig. 1 of main text). The parental genotype (allele sizes) is given per family and locus on the first line. The second line indicates allelic segregation among offspring (homozygotes for the smallest allele : homozygotes for the largest allele: heterozygotes). ***indicates significant deviation from Mendelian expectations.

Family	Locus									
	Pac1	Pac2	Pac4	Pac5	Pac7	Pac 14	AF108761	AF108762	AF108764	AF108758
Lam1a	100 112 (2:0:5)	165 (7:0:0)	249 253 (1:3:3)	265 (7:0:0)	344 (7:0:0)	280 (7:0:0)	136138 (1:3:3)	150 (7:0:0)	141 149 (3:0:4)	144 (7:0:0)
Lam1b	100 (7:0:0)	165 (7:0:0)	241 249 (0:2:4)	261 (7:0:0)	344 (7:0:0)	280 (7:0:0)	138 (7:0:0)	152 (7:0:0)	141 (7:0:0)	144 407 (2:0:5)
Lez5	118 (7:0:0)	161 165 (0:3:4)	245 (7:0:0)	261 (7:0:0)	340 344 (2:2:3)	280 (7:0:0)	138 (7:0:0)	150 164 (2:1:4)	141 (7:0:0)	407 (7:0:0)
Vio8a	112 (7:0:0)	165 181 (1:0:6)	241 245 (0:2:5)	261 (7:0:0)	314 318 (3:1:3)	280 (7:0:0)	136 (7:0:0)	150 (7:0:0)	141 (7:0:0)	144 (7:0:0)
Vio8b	112 (7:0:0)	165 181 (1:2:4)	241 (7:0:0)	261 (7:0:0)	318 344 (1:2:4)	280 (7:0:0)	136 (7:0:0)	164 (7:0:0)	149 (7:0:0)	144 (7:0:0)
Vio12	112 (7:0:0)	165 181 (3:0:4)	241 245 (3:3:1)	261 (7:0:0)	314 (7:0:0)	280 (7:0:0)	134 (7:0:0)	150 (7:0:0)	141 (7:0:0)	144 (7:0:0)
L2	100 112 (2:0:4)	161 165 (0:3:3)	245 253 (2:0:5)	265 (7:0:0)	340 (7:0:0)	280 (7:0:0)	138 (7:0:0)	152 (7:0:0)	147 149 (2:2:3)	144 407 (0:5:1)
L3	100 112 (2:3:2)	161 (7:0:0)	245 249 (0:3:4)	265 (7:0:0)	340 (7:0:0)	268 280 (2:3:2)	138 (7:0:0)	152 (7:0:0)	147 149 (3:1:3)	144 407 (2:2:3)
L8	100 112 (2:1:4)	181 (7:0:0)	245 (7:0:0)	261 265 (2:1:4)	314 344 (0:1:6)	268 280 (3:1:3)	138 (7:0:0)	150 (7:0:0)	147 (7:0:0)	407 (7:0:0)
L10	100 112 (1:1:5)	165 181 (3:3:1)	253 (7:0:0)	261 265 (0:4:3)	340 (7:0:0)	280 (7:0:0)	138 (7:0:0)	152 164 (2:1:4)	145 147 (2:0:5)	144 (7:0:0)
L12	100 112 (0:3:4)	165 (7:0:0)	245 253 (2:1:4)	261 265 (0:1:6)	340 (7:0:0)	280 (7:0:0)	138 (7:0:0)	150 (7:0:0)	147 149 (4:2:1)	407 (7:0:0)
L15	100 112 (0:2:5)	165 181 (2:2:3)	249 253 (1:2:4)	261 265 (2:2:3)	340 (7:0:0)	280 (7:0:0)	138 (7:0:0)	164 (7:0:0)	149 (7:0:0)	144 (7:0:0)
L18	112 (7:0:0)	165 181 (3:2:2)	253 (7:0:0)	261 (7:0:0)	314 (7:0:0)	280 (7:0:0)	138 (7:0:0)	152 164 (6:1:0)***	147 (7:0:0)	144 407 (1:4:2)

Table S2 Allelic frequencies at eight microsatellite loci in the 24 populations of *Physa acuta* analysed. Values at Pac4 and Pac7 were obtained once null homozygotes were discarded. For locus AF108758, allele 407 indeed represents two bands of size 140 and 147 respectively (see 1 in the text above for details). Sampling dates are given in Table 1 of main text.

Population		Locus Pac1			Locus Pac2					Locus Pac4										Locus Pac5											
		100	112	118	157	161	165	173	181	205	209	213	229	233	237	241	245	249	253	257	261	297	331	241	253	257	261	265	269		
Vio11	t ₁	0.53	0.47	0	0	0	1	0	0	0	0	0	0	0	0	0.81	0.19	0	0	0	0	0	0	0	0	0	0	0.67	0.33	0	
	t ₂	0.50	0.50	0	0	0	1	0	0	0	0	0	0	0	0.03	0.89	0.06	0.03	0	0	0	0	0	0	0	0	0	0.63	0.37	0	
Vio2	t ₁	0.36	0.22	0.42	0	0.47	0.42	0	0.11	0	0	0	0	0	0	0.09	0.25	0.66	0	0	0	0	0	0	0	0	0	1	0	0	
	t ₂	0.45	0.18	0.38	0	0.55	0.35	0	0.10	0	0	0	0	0	0	0.31	0.69	0	0	0	0	0	0	0	0	0	1	0	0		
Vio7	t ₁	0	1	0	0	0.03	0.97	0	0	0	0	0	0	0	0.03	0.39	0.58	0	0	0	0	0	0	0	0	0	1	0	0		
	t ₂	0.03	0.98	0	0	0	0.95	0	0.05	0	0	0	0	0	0.03	0.53	0.45	0	0	0	0	0	0	0	0	0	1	0	0		
Vio1	t ₁	0.29	0.29	0.41	0	0.71	0.06	0	0.24	0	0.03	0	0	0	0	0.03	0.41	0.53	0	0	0	0	0	0	0.03	0	0	0.97	0	0	
	t ₂	0.45	0.21	0.34	0	0.71	0.03	0	0.26	0	0	0	0	0	0	0.63	0.37	0	0	0	0	0	0	0	0	0	0	0.97	0.03	0	
Vio12	t ₁	0.21	0.79	0	0	0	0.38	0	0.62	0	0	0	0	0	0.21	0.35	0.24	0.21	0	0	0	0	0	0	0	0	0	0.53	0.47	0	
	t ₂	0.11	0.89	0	0	0.03	0.58	0	0.39	0	0	0	0	0	0.28	0.47	0.22	0.03	0	0	0	0	0	0	0	0	0	0.53	0.47	0	
Vio8	t ₁	0	1	0	0	0	0.92	0	0.08	0	0	0	0	0	0.68	0.07	0	0	0.25	0	0	0	0	0	0	0	0	0.96	0.04	0	
	t ₂	0	1	0	0	0	0.87	0	0.13	0	0	0	0	0	0.68	0.13	0	0	0.18	0	0	0	0	0	0	0	0	0.97	0.03	0	
Mos1	t ₁	0.61	0.36	0.03	0	0.28	0.53	0	0.19	0	0.17	0	0	0	0.28	0.25	0.28	0.03	0	0	0	0	0	0	0	0	0	0.83	0.17	0	
	t ₂	0.55	0.36	0.10	0	0.24	0.64	0	0.12	0	0.07	0	0	0	0.36	0.24	0.24	0.10	0	0	0	0	0	0	0	0	0	0.81	0.19	0	
Sal2	t ₁	0.63	0.32	0.05	0	0.11	0.61	0	0.29	0	0	0	0	0	0.08	0.16	0.39	0.32	0.03	0	0.03	0	0	0	0	0	0	0.71	0.29	0	
	t ₂	0.67	0.31	0.03	0	0.06	0.56	0	0.39	0	0	0	0.08	0.06	0.06	0.44	0.25	0.08	0.03	0	0	0	0	0	0	0.03	0.78	0.19	0		
Lam1	t ₁	0.31	0.69	0	0	0	0.59	0.13	0.28	0	0	0	0	0.03	0.25	0.34	0.34	0.03	0	0	0	0	0	0	0	0	0	0.91	0.09	0	
Lam12	t ₁	0.38	0.62	0	0	0	0.79	0.03	0.18	0	0	0	0	0	0.26	0.21	0.29	0.24	0	0	0	0	0	0	0	0	0	0.94	0.06	0	
Mos6	t ₁	0.88	0.13	0	0	0.25	0.25	0	0.50	0	0.13	0	0	0	0.13	0.25	0.25	0.25	0	0	0	0	0	0	0	0	0	0	0.67	0.33	0
FMB		0.21	0.67	0.13	0	0.29	0.63	0	0.08	0	0.11	0	0	0	0.06	0.44	0.39	0	0	0	0	0	0	0.04	0	0.08	0.83	0	0.04	0	
FML		0.46	0.54	0	0	0.21	0.63	0	0.17	0	0	0	0	0	0.29	0.21	0.13	0.25	0.13	0	0	0	0	0	0	0	0	0.92	0.08	0	
FMR		0.23	0.77	0	0	0	0.69	0.12	0.19	0	0	0	0	0	0.27	0.27	0.31	0.15	0	0	0	0	0	0	0	0	0	0.85	0.15	0	
FV		0.29	0.25	0.46	0	0.25	0.33	0	0.42	0	0	0	0	0	0.04	0.96	0	0	0	0	0	0	0	0	0	0	0	0.96	0.04	0	
FC		0.33	0.21	0.46	0.25	0.46	0.29	0	0	0.08	0.71	0.04	0	0	0	0.17	0	0	0	0	0	0	0	0.04	0	0	0	0.88	0.08	0	
FT		0	0.75	0.25	0	0.5	0.25	0	0.25	0	0	0	0	0	0.15	0.60	0.15	0.10	0	0	0	0	0	0	0	0	0	0.71	0.29	0	
FS		0.46	0.46	0.08	0	0.33	0.17	0	0.50	0	0	0	0	0	0	0.75	0.25	0	0	0	0	0	0	0	0	0	0	0.88	0.13	0	
FL		0.54	0.42	0.04	0	0.58	0.29	0	0.13	0	0	0	0	0.09	0.45	0.41	0.05	0	0	0	0	0	0	0	0	0	0	0.75	0.25	0	
FB		0.08	0.38	0.54	0.04	0.63	0.13	0	0.21	0	0	0	0	0	0.10	0.70	0.20	0	0	0	0	0	0	0	0	0	0	0.95	0.05	0	
FPB		0.46	0	0.54	0	0.08	0.83	0	0.08	0	0	0	0	0	0.25	0	0.33	0	0.38	0	0	0.04	0	0	0	0	0	0.63	0	0.38	
FVR		0.08	0.71	0.21	0	0.08	0.67	0	0.25	0	0.06	0	0.06	0.06	0.17	0.61	0	0.06	0	0	0	0	0	0	0.04	0	0	0.92	0.04	0	
FR		0.46	0.42	0.13	0	0.67	0.08	0	0.25	0	0	0	0	0	0.13	0.13	0.67	0.08	0	0	0	0	0	0	0	0	0	0.67	0.33	0	
FRJ		0.33	0.17	0.50	0	0.88	0.08	0	0.04	0	0	0	0	0	0	0	0	0	0.46	0	0.54	0	0	0	0	0	1	0	0		

Web Table 2 – Bousset *et al.* (continued)

Population		Locus Pac7															AF108758											
		310	314	318	322	326	332	336	340	344	348	370	372	390	432	438	472	512	129	133	144	151	153	157	163	407		
Vio11	t ₁	0.03	0.69	0.25	0	0	0	0	0	0.03	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
	t ₂	0	0.67	0.22	0	0	0	0	0	0.11	0	0	0	0	0	0	0	0	0	0	0.97	0	0	0	0	0	0.03	
Vio2	t ₁	0.03	0.81	0.06	0	0	0	0	0	0.11	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
	t ₂	0.03	0.62	0.03	0	0	0	0	0	0.32	0	0	0	0	0	0	0	0	0	0	0.98	0	0	0	0	0	0.03	
Vio7	t ₁	0.37	0.13	0	0.24	0	0	0	0	0.26	0	0	0	0	0	0	0	0	0	0	0.32	0	0	0	0	0	0.68	
	t ₂	0.35	0.18	0	0.13	0	0	0	0	0.35	0	0	0	0	0	0	0	0	0	0	0.48	0	0	0	0	0	0.53	
Vio1	t ₁	0.14	0.75	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.06	0.85	0	0	0.09	0	0	
	t ₂	0.36	0.25	0.21	0	0	0	0	0	0.18	0	0	0	0	0	0	0	0	0	0	0.05	0.76	0	0	0.18	0	0	
Vio12	t ₁	0.06	0.71	0.03	0.12	0	0	0	0	0.09	0	0	0	0	0	0	0	0	0	0	0.76	0	0	0	0	0	0.24	
	t ₂	0.03	0.61	0.08	0.17	0.03	0	0	0	0.08	0	0	0	0	0	0	0	0	0	0	0.81	0	0	0	0	0	0.19	
Vio8	t ₁	0	0.39	0.50	0	0	0	0	0	0.11	0	0	0	0	0	0	0	0	0	0	0.96	0	0	0	0	0	0.04	
	t ₂	0	0.39	0.42	0	0	0	0	0	0.18	0	0	0	0	0	0	0	0	0	0	0.97	0	0	0	0	0	0.03	
Mos1	t ₁	0	0.19	0.14	0.08	0	0.03	0	0	0.53	0.03	0	0	0	0	0	0	0	0	0	0.03	0	0.44	0	0	0	0	0.53
	t ₂	0	0.33	0.07	0.05	0.02	0	0	0.02	0.38	0.05	0	0	0.07	0	0	0	0	0	0	0.07							

Table S4 Relationship between the mean (standard deviation) proportions of individuals excluded and reassigned per population and ecological characterization of habitats. See main text for details on habitat characterization. The ‘Test’ column indicates Mann-Whitney U test (n_1 ; n_2 ; U); P is the associated probability and = is followed by ‘#’ when the predictions were fulfilled. Samples in the eight populations sampled twice (at t_1 and t_2) were pooled prior to analysis.

	<u>Openness</u>				<u>Water availability</u>			
	Ponds	Rivers	Test	P	Permanent	Temporary	Test	P
Exclusion	0.13 (0.08)	= 0.20 (0.16)	U (4; 6; 6)	0.26	0.13 (0.08)	= # 0.18 (0.08)	U (5; 5; 11)	0.84
Assignment	0.07 (0.08)	= 0.10 (0.14)	U (4; 6; 9)	0.61	0.08 (0.08)	= # 0.09 (0.13)	U (5; 5; 11)	0.84

Table S5 Comparisons of the genetic and demographic parameters (defined in Table 3) between permanent and temporary habitats. Values are means with standard deviations into parentheses. See main text for details on the parameters estimated and habitat characterization. The tests were conducted at local scale only, since water regime was not determined at global scale. The ‘test’ column indicates either Mann-Whitney U test (n_1 ; n_2 ; U) or Fisher exact test F (n_1 ; n_2), and P is the associated probability. ‘nt’ holds for no test, either when testing was irrelevant (Mantel and $D\sigma^2$) or not possible (no demographic data at global scale, not enough data for bottlenecks and extinctions). $>$, $<$ and $=$ are followed by ‘#’ when the predictions of Table 2 of main text were fulfilled. Values in the ‘bottlenecks’ row indicate the number of significant tests under the infinite allele model (IAM), two-phase model (TPM) and stepwise mutation model (SMM), and the total number of tests performed. ‘km’ in the ‘differentiation’ row gives the mean geographic distance (standard deviation) between population pairs. Slope (a) values in the isolation by distance row are provided with their 95% confidence intervals generated by bootstrapping.

	Permanent		<u>Temporary</u>	Test	P
<u>Genetic structure</u>					
<u>Polymorphism</u>					
<u>N_{all}</u>	2.80 (0.78)	=	2.59 (1.46)	U (9; 10; 41)	0.78
<u>H_e</u>	0.39 (0.12)	=	0.39 (0.10)	U (9; 10; 45)	1
<u>Bottleneck (nb of tests)</u>					
(IAM/TPM/SMM/total)	2/2/1/10		1/1/1/9	nt	
<u>Differentiation</u>					
<u>paired F_{ST}</u>	0.25 (0.12)	=	0.26 (0.10)	U (33; 40; 1405)	0.41
<u>km</u>	12.1 (5.9)	>	8.2 (4.3)	U (33; 40; 420)	7.10^{-3}
<u>Isolation by distance</u>					
<u>Mantel (P)</u>	0.05		6.10^{-4}	nt	
<u>slope a</u>	-0.27		0.33	<u>irrelevant</u>	
<u>[95% CI]</u>	[-0.45;0.36]		[0.17;0.92]		
<u>$D\sigma^2$</u>	<u>irrelevant</u>		0.30	nt	
<u>Temporal variation</u>					
<u>paired F_{ST}</u>	0.002 (0.001)	=	0.01 (0.02)	U (3; 5; 2)	0.14
<u>Demography</u>					
<u>Density (harmonic mean)</u>	1.50 (1.81)	=	4.12 (6.41)	U (7; 5; 2)	0.46
<u>Low density (% events)</u>	26%	=	35%	F (112; 123)	16%
<u>Extinction (nb)</u>	0		3	nt	

Table S6 Tests of mutation-drift equilibrium under the infinite allele model (IAM), the two-phase model (TPM) and the stepwise mutation model (SMM). The table gives the probability associated with Wilcoxon signed ranks test of the null hypothesis of equilibrium. ‘Excess’ and ‘Deficit’ (when $P \leq 0.05$) indicate respectively a bottleneck and an expansion.

Population		Mutation model			
		IAM	TPM	SMM	
Vio11	t ₁	0.13	0.13	0.88	
	t ₂	0.81	1	1	
Vio2	t ₁	0.13	0.13	0.13	
	t ₂	0.16	1	0.81	
Vio7	t ₁	0.31	1	0.88	
	t ₂	0.63	0.81	1	
Vio1	t ₁	0.84	0.56	0.05	<u>Deficit</u>
	t ₂	0.44	0.84	0.44	
Vio12	t ₁	0.44	0.44	0.44	
	t ₂	0.06	0.06	0.16	
Vio8	t ₁	1	0.81	0.81	
	t ₂	0.81	1	1	
Mos1	t ₁	0.02	<u>Excess</u>	0.02	<u>Excess</u>
	t ₂	0.02	<u>Excess</u>	0.02	<u>Excess</u>
Sal2	t ₁	0.44	0.44	1	
	t ₂	0.69	0.69	0.16	
Lam1	t ₁	0.03	<u>Excess</u>	0.03	<u>Excess</u>
Lam12	t ₁	0.11	0.56	0.84	
Mos6	t ₁	0.11	0.44	0.56	
FMB		1	0.44	0.11	
FML		0.44	0.84	0.84	
FMR		0.16	0.44	1	
FV		0.08	0.16	0.84	
FC		0.56	0.84	1	
FT		0.03	<u>Excess</u>	0.08	0.84
FS		0.05	<u>Excess</u>	0.08	0.69
FL		0.03	<u>Excess</u>	0.11	0.44
FB		0.69	0.84	0.44	
FPB		0.08	0.84	1	
FVR		0.84	0.56	0.08	
FR		0.16	0.44	1	
FRJ		0.63	1	1	

Fig. S1 Probability of exclusion/reassignment in populations studied at local scale, located within a radius of 20 km around Montpellier (Table 1, Fig. 1 of main text). Full bars indicate individuals not excluded from the population in which they were sampled, empty bars indicate individuals excluded and not reassigned to the populations studied, and dashed bars indicate individuals excluded and reassigned to one of the populations included in the study. Samples from June (t_1) and October (t_2) were pooled before analysis.

