



HAL
open science

Interaction of climate, demography and genetics: a ten-year study of *Brassica insularis*, a narrow endemic Mediterranean species

Florence Noël, Sandrine Maurice, Agnès Mignot, Sylvain Glémin, David Carbonell, Fabienne Justy, Isabelle Guyot, Isabelle Olivieri, Christophe Petit

► To cite this version:

Florence Noël, Sandrine Maurice, Agnès Mignot, Sylvain Glémin, David Carbonell, et al.. Interaction of climate, demography and genetics: a ten-year study of *Brassica insularis*, a narrow endemic Mediterranean species. *Conservation Genetics*, 2010, 11 (2), pp.509-526. 10.1007/s10592-010-0056-1 . halsde-00467232

HAL Id: halsde-00467232

<https://hal.science/halsde-00467232>

Submitted on 31 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Interaction of climate, demography and genetics: a ten-year study of**

2 ***Brassica insularis*, a narrow endemic Mediterranean species**

3

4 Florence Noël ‡, Sandrine Maurice‡*, Agnès Mignot‡*, Sylvain Glémin‡, David Carbonell‡,
5 Fabienne Justy‡, Isabelle Guyot#, Isabelle Olivieri‡^Δ and Christophe Petit‡.

6

7 ‡ Université Montpellier2, Institut des Sciences de l'Evolution de Montpellier (ISEM), UMR
8 5554 CNRS, 34095 Montpellier cedex05, France

9 # Conservatoire du littoral, 3 rue Luce de Casabianca, 20200 Bastia, France

10 * These authors have contributed equally to this work

11

12 Corresponding author, email: Isabelle.Olivieri@univ-montp2.fr, tel.: +33(0)467143750; Fax:
13 +33(0)467143622

14

15

16 **Abstract**

17 Long-term demographic surveys, needed to obtain accurate information on population
18 dynamics and efficiently manage rare species, are still very scarce. Matrix population
19 models are useful tools to identify key demographic transitions and thus help setting up
20 conservation actions. Furthermore, the combination of ecological, demographic and genetic
21 data is likely to improve the identification of the threats acting upon populations and help
22 conservation decisions. In this paper we illustrate the power of this approach on *Brassica*
23 *insularis*, a Mediterranean endemic plant species, rare and endangered in Corsica (France).
24 In four populations of this species, a long-term demographic survey (2000-2009), genetic
25 analyses (in 2000 and 2009) and survey of ecological variables (climatic variables,
26 competition and herbivory) were performed. By using both deterministic and stochastic
27 matrix model analyses, we assessed the viability of each population and tested for both
28 spatial and temporal variations in demographic vital rates. Populations exhibited differing
29 demographic behaviours and environmental stochasticity occurred in populations.
30 Significant correlations between climatic variables and vital rates were detected. Stochastic
31 simulations suggested that three out of the four populations studied might present a high
32 risk of extinction on the short-term and should actively be managed, or at least surveyed. It
33 could be, however, that two of these populations are experiencing density-dependent
34 regulation, rather than being declining. Microsatellite diversity was slightly reduced in a
35 single population and similar in the three others, consistently with expectations based on
36 population census size and geographic area, as well as with diversity at the S-locus
37 observed in 2000. The combination of all data led to specific recommendations for
38 managing each population. We discuss the implications for conservation of such a general
39 approach.

40

41 KEYWORDS: demography; genetic monitoring; conservation; matrix models; environmental
42 stochasticity; extinction probability

43

44 **Introduction**

45

46 To efficiently manage rare and threatened species, it is important to understand their
47 population dynamics and identify the current threats acting on them (Oostermeijer et al.
48 1996). Long-term demographic surveys are needed to obtain accurate information on
49 species life-history and identify biotic or abiotic factors that affect population dynamics
50 (Waite and Hutchings 1991; Fieberg and Ellner 2001; Fréville et al. 2004; Pfeifer et al.
51 2006). Assessing the relative impact of these factors is a central issue in ecology (Harper
52 1977; Horvitz et al. 1997; Caswell 2001). However, for rare and endangered species, the
53 number of long-term studies, especially on plants, is still low while crucially needed for
54 their conservation (Fréville et al. 2004; Jacquemyn et al. 2007). Matrix population models
55 are powerful tools to identify key demographic transitions (Hayward and Mc Donald 1997;
56 Jimenez-Sierra et al. 2007), to assess the impact of environments (Ehrlén et al. 2005;
57 Ghimire et al. 2008), to perform viability analyses (PVA; Menges 2000; Reed et al. 2002)
58 and to suggest conservation actions (Enneson and Litzgus 2008; Lubben et al. 2008).
59 Moreover, construction of deterministic and stochastic models (Caswell 2001; Morris and
60 Doak 2002) allows making projections of demographic trajectory.

61 The effects and contributions of the different life-history parameters to the variation of
62 population growth rate can be identified using life-table response experiments (LTRE), *i.e.*
63 variance decomposition into contributions to the change of asymptotic growth rate (Caswell

64 1996, 2001). Classic deterministic projection matrices, variance decomposition and
65 analyses of variation of the vital rates (log-linear models and generalized linear models,
66 Caswell 1996, 2001) allow investigating the occurrence of spatial and/or temporal
67 variability of demographic vital rates. One can thus assess the impact of environmental
68 variation and the relevance of stochastic demographic projection models (Morris and Doak
69 2002).

70 Demographic analyses are sometimes not sufficient to fully understand the processes
71 acting on population dynamics. For rare species which often occur as small and isolated
72 populations, genetic stochasticity could have severe demographic consequences
73 (Oostermeijer et al. 2003). Drift load, that is the random increase of frequency of
74 deleterious alleles or their random fixation, may lead to decreased fitness components
75 (individual survival rates and fecundities) at the population level. For a self-incompatible
76 species, the decrease of the number of S-alleles through genetic drift could lead to an Allee
77 effect leading to reduced fecundity (Willi et al. 2005; Wagenius et al. 2007; Leducq et al.,
78 2010). Finally, on the long-term, genetic drift could reduce standing variation and thus
79 evolutionary potential. However, the importance of genetic impacts on extinction risks is
80 still debated in the literature (Lande 1988; Caughley 1994; Spielman et al. 2004; Frankham
81 2005) and few studies have tried to integrate both demography and genetic approaches
82 (Newman and Pilson 1997; Menges and Dolan 1998; Brook et al. 2002; Jacquemyn et al.
83 2003; Fréville et al. 2004; Proctor et al. 2004; Endels et al. 2007a; Brito et al. 2008)
84 whereas they likely constitute the best approach to efficiently manage endangered species
85 (Menges 2000; Asquith 2001). Unfortunately, the only two software-packages allowing to
86 integrate genetic effects in population viability analyses, Vortex (Lacy 1993) and RAMAS
87 (Akçakaya and Root 2002) only consider age-structured, rather than stage-structured

88 populations. They thus cannot be used for most long-lived perennial plants, let alone plants
89 with particular mating systems such as self-incompatibility. Until such a software is
90 developed, one has to analyse the two kinds of information separately, or develop one's
91 own model (as done by Kirchner et al. 2006 and Hoebee et al. 2008).

92 Finally, very few studies have tried to combine data on demography and genetics with
93 ecological data to assess the respective impact of these factors on population dynamic of a
94 rare plant species (Burgman and Lamont 1992, Oostermeijer 2000). In the present study, we
95 aim at documenting the genetic (microsatellites and S-locus) and demographic status of four
96 populations of a protected species, and at explaining the observed differences among years
97 and among populations in transition parameters by various factors, including diversity at the
98 S-locus, ecological characteristics and climate. Furthermore, we monitor microsatellite
99 diversity at a ten-year interval to document the amount of differentiation among populations
100 and its evolution, confirm the spatial pattern of local genetic diversity, and document
101 potential bottlenecks.

102 In Corsica, about 13% of plant species are endemic (Jeanmonod and Gamisans
103 2007) and most of them need management for their conservation. *Brassica insularis* Moris
104 (Brassicaceae) is listed in the French Red Book (Olivier et al. 1995) of endangered species
105 and protected by European "Habitat Directive". This species is endemic to the
106 Mediterranean region. Demographic surveys of four populations in Corsica were initiated in
107 2000. Using a combination of demographic analyses, projection models and genetic
108 analyses (performed in 2000 and 2009; Petit et al. 2001; Glémin et al. 2005, 2008; present
109 study), the aim of the present study is to assess the viability of these four populations and
110 evaluate the most efficient management for each of them. From the ten-year demographic
111 dataset available, we used deterministic and stochastic matrix models combined with

112 genetic, ecological and climatic data to answer the following questions: (1) Do populations
113 have different demographic dynamics? (2) Is there significant variation in vital rates among
114 populations and years? (3) Is there any relationship between vital rates and environmental
115 variables? (4) Is there any evidence for an impact of genetic stochasticity through time?
116 And finally, (5) can populations persist in the future? We then suggest potential
117 management strategies for each population of this rare and endemic species.

118

119

120 **Material and Methods**

121

122 **Study species, demographic and environmental data**

123

124 *Brassica insularis* (Brassicaceae) is a rare Mediterranean species of the *Brassica*
125 *oleracea* group. Its distribution is restricted to small, isolated populations in Corsica (with 9
126 known populations), Sardinia (17 populations), Tunisia (5), Algeria (1) and in Pantelleria
127 island, near Sicily (Snogerup et al. 1990). The species is found primarily on limestone or
128 schist cliffs at elevations of 300-1000m. It is a perennial, insect pollinated species, which
129 flowering period extends from March to May with only a small proportion of individuals
130 flowering in any given year. Seedlings emerge in autumn. As in other *Brassica* sp., there is
131 a sporophytic self-incompatibility system (see Hiscock and McInnis 2003 for a review).

132 We studied four Corsican populations localised from north to southeast (Fig. 1):
133 Teghime, Punta Corbaghiola (hereafter called Corbaghiola), Inzecca and Punta Calcina
134 (Calcina). The demographic survey was initiated in 2000 and in this paper we analyse the
135 2000-2009 data set. Within each site, 32 to 54 permanent plots (quadrats) of various sizes (1

136 to 10m², depending on local density and accessibility) were set up in 2000, covering all
137 individuals that were safely accessible. Within each quadrat, individuals were localised by
138 at least two coordinates using fixed points. Once a year, during the flowering season, we
139 recorded the stage of each individual present within each quadrat. The total number of
140 individuals studied in each population since 2000 varied from 151 to 554 individuals with
141 on average 131.3 individuals per population and year (Table 1). Based on estimations of
142 population sizes in 2003 (Glémin et al. 2005), we estimated that we have been following
143 about 12.5% of all the individuals in Teghime, 20% in Corbaghiola, 27% in Inzecca, and
144 100% in Calcina.

145 To assess ecological differences among populations, in 2007 we measured the magnitude
146 of intra and interspecific competition experienced by each individual both semi-
147 quantitatively (as “null”, “low”, “medium” or “high”) and qualitatively (competition with
148 herbaceous plants, woody plants or both). Because the type of substrate may differ in
149 moisture and stability, it was recorded and three categories were defined: bare soil, grassy
150 soil and scree. The presence of herbivorous aphids was also recorded since 2000; aphid
151 attacks occurred mainly on inflorescences and could have an impact on fecundity.

152 Finally, daily data from April 1999 to May 2009 obtained from four meteorological
153 stations (Météo France) localised in the vicinity of each population (less than 10 km away)
154 were collected and we corrected the data for the altitude (subtracting or adding one Celsius
155 degree per 100m when the altitude of the meteorological station and the site differed). The
156 meteorological variables described the basic climatic conditions at each site (i.e.
157 temperatures and precipitations). For each time period, these variables were: (a) the
158 minimum of mean day temperature per month for the winter months (from October to
159 March), (b) the maximum of mean day temperature per month for the summer months

160 (from April to September), (c) the cumulative degree day for the year (in Celsius degree),
161 (d) the cumulative rainfall for the year (in mm), (e) the total number of rain events > 0.1
162 mm and < 1 mm per year and (f) the total number of rain events > 1 mm per year.

163

164 **Demographic analyses**

165

166 We analyzed our demographic data using two different softwares, which handle
167 environmental stochasticity in different ways. For most demographic analyses, we used the
168 POPBIO package (Stubben and Milligan 2007) from the R statistical freeware (R version
169 2.6.0). For viability analyses, we also used RAMAS/Metapop (Akçakaya and Root 2002),
170 so that we could compare the results from the two analyses (see below for the specific
171 description of each analysis).

172

173 *Matrix model construction and asymptotic growth rates* - Based on survival and fecundity
174 data, we built a projection matrix model (Caswell 2001) and a three stages life-cycle with a
175 one-year time step: (1) seedlings (< 1 -year old), (2) vegetative adults (always at least 1-year
176 old) and (3) flowering plants (always at least 2-year old) (Fig. 2). We estimated the
177 transition probabilities probabilities a_{ij} from survival and flowering probabilities (see Fig.
178 2). Matrix elements were calculated per population and per year. No individual fecundity or
179 early juvenile survival was available (f and s_0 , Fig. 2). Thus, fecundity and seedling
180 establishment and survival could not be analysed separately, and the matrix element a_{13}
181 only contains a single vital rate 'reproduction': the ratio of the number of seedlings observed
182 a given year divided by the total number of flowering plants observed in the same
183 population the previous year. No data on the soil seed bank or dormancy was available.

184 However, because we estimated the fecundity term a_{13} from seedlings in $t+1$ and flowering
 185 plants in t , the presence of a seed bank should not affect our demographic projections,
 186 unless no flowering plant occurred in a given year t , a situation which never happened in
 187 this 10-year study. Furthermore, for a long-lived species such as *B. insularis*, soil seed bank
 188 is likely to have a low impact on population dynamics (Olano et al. 2005; García et al.
 189 2008). Therefore, we did not include a seed stage in the life-cycle (as, e.g., in Fréville et al.
 190 2004; Jongejans et al. 2008). Our model did not include either any seed dispersal among
 191 populations, as genetic differentiation and geographical distances both suggest that such
 192 dispersal events were very unlikely (Petit et al. 2001; Glémin et al. 2005; present study).
 193 For each population and each pair of years, the asymptotic growth rate λ_a was calculated as
 194 the dominant eigenvalue of the corresponding matrix (Caswell 2001). For each population,
 195 we also calculated the mean asymptotic growth rate λ_a for the 10-year dataset, using a mean
 196 matrix obtained by averaging each transition parameter over 10 years.

197

198 *Contribution of vital rates variation to the variance of λ_a* – To assess how variation in the
 199 vital rates contributed to the variation of asymptotic growth rate λ_a among years and
 200 populations, a life-table analysis response (LTRE) was performed (Caswell 2001). We used
 201 the 36 values of λ_a corresponding to all combinations of population (4) * pairs of years (9)
 202 combinations. In terms of higher level vital rates a_{ij} , the variance of λ_a is expressed as:

203
$$V(\lambda_a) \approx \sum_{ij} \sum_{kl} C(a_{ij}, a_{kl}) (\delta \lambda_a / \delta a_{ij}) (\delta \lambda_a / \delta a_{kl})$$

204 with $C(a_{ij}, a_{kl})$ the covariance of the vital rates a_{ij} and a_{kl} , and δx representing small variation
 205 in variable x . In terms of the lower-level vital rates, the variance of λ_a is expressed as:

206
$$V(\lambda_a) \approx \sum_{ij} C(x_i, x_j) (\delta \lambda_a / \delta x_i) (\delta \lambda_a / \delta x_j)$$

207 with $C(x_i, x_j)$ the covariance of the lower-level vital rates x_i , and x_j . In both analyses, the
208 contribution of each vital rate variation was calculated as the sum of the contributions for all
209 the covariances involving that rate (Horvitz et al. 1997; Caswell 2001).

210

211 *Population stable stage distributions* – To test for differences in population stage
212 distribution, and to test the hypothesis that each population was at demographic
213 equilibrium, the stable stage distribution was calculated based on the arithmetic mean
214 matrix over the 10 years (Caswell 2001). We then compared the expected stable stage-
215 distribution to the observed stage-structure for each population and each year using a G-test
216 (Sokal and Rohlf 1995).

217

218 *Spatio-temporal variation: log-linear analysis and generalized linear models* –To
219 investigate the statistical significance of spatio-temporal variation in transition probabilities,
220 we performed log-linear analyses. All plants were classified into stage classes each year,
221 including an extra class for dead plants. Loglinear analyses were performed on a four-way
222 contingency table defined by the following factors: state **S** (stage of each individual at time
223 t), population **P** and year **Y**, and fate **F** (stage of the individual at time $t+1$). In our model, **P**,
224 **Y** and **S** were considered as explanatory variables, and **F** as the response variable (Caswell
225 2001). The (sequential) effects of year **Y**, population **P** and their interaction on transition
226 probabilities were assessed by a model selection approach using the Akaike's information
227 criterion (AIC, Burnham and Anderson 1998; Caswell 2001). To analyse the pattern of
228 variations of lower-level vital rates, survival (s_x) and flowering (p_y) probabilities, we
229 performed generalized linear models using a logit-link function and specifying binomial
230 error. The relevance of adding the effects of year **Y**, population **P** and their interaction to

231 explain the variations of these vital rates were tested by comparing the models based on
232 AIC selection criteria. The tests for population differences in fecundity (f) and in early
233 juvenile survival probability (s_0) were not performed due to the absence of individual
234 values.

235

236 *Population stochastic growth rates and quasi-extinction probability* – We used computer
237 simulations to calculate a stochastic growth rate (λ_s , Morris and Doak 2002). To generate
238 environmental stochasticity, we used the POPBIO package in R (Stubben and Milligan
239 2007) which allowed choosing randomly one of the matrices for each year (assuming equal
240 probability among the 9 matrices) and multiplied it by the most recent population vector. It
241 allowed calculating, for each population, the stochastic lambda for $t = 50$ years with 5000
242 replicates to estimate the 95% CI.

243 The quasi-extinction threshold is the population size below which the population is
244 likely to be critically and immediately imperilled (Ginzburg et al. 1982). For a whole
245 population, a population size below $n = 50$ could be considered highly endangered
246 assuming that under this threshold the demographic stochasticity becomes particularly
247 important (Menges 1991, 1992; Lande 1993, 1998). However because such threshold would
248 be arbitrary and could depend of many other factors (e.g., the species life span, its mating
249 system, its incompatibility system), using POPBIO we calculated for each population the
250 cumulative probability of extinction (CPE) for a short-time $t = 50$ years and different quasi-
251 extinction thresholds ($n = 50, 30, 20, 10, 5$ and 1). Following Morris and Doak (2002) we
252 performed one run with 5000 replicates for each population. Estimates of CPE per
253 population were performed with the stage distribution of individuals corresponding to the
254 mean stage distribution for the 10 years for each population. The initial population sizes we

255 used were 2000, 500, 300 and 100 for Teghime, Inzecca, Corbaghiola and Calcina
256 respectively. These values were consistent with population sizes estimated by Glémin et al.
257 (2005).

258

259 We also ran simulations with RAMAS/Metapop, which uses a different method to
260 model environmental stochasticity, under various scenarios. Instead of randomly sampling
261 matrices as does POPBIO, RAMAS samples transition probabilities from a log-normal
262 distribution with mean value equal to the estimated mean parameter value. Total survival
263 for a given stage is the sum of all non-reproductive transitions from that stage to all other
264 stages. If this sum is close to 1 and the standard deviations are also large, the sampled
265 survival rates may add up to a number larger than 1. Since survival rates are constrained to
266 be less than or equal to 1, survival rates will be reduced to make the sum equal to 1. If such
267 truncations happen frequently during a simulation, the simulated survival rates will be
268 smaller than what was entered in the model. To fix this problem, we imposed (as suggested
269 in the Help file of RAMAS) a correlation of -1 between the largest stage-specific survival
270 rate and other survival rates in the same column. This option is akin to assuming that the
271 (environmental) correlation among survival rates is not perfect. In addition, we assumed
272 that fecundity and survival rates were correlated, with the result that largest survival rates
273 would also be negatively correlated with fecundities. For each parameter set, we ran 5000
274 replicate simulations during 200 time steps (years).

275 We first assumed that none of the populations was regulated by density, and used
276 the same initial population sizes as above. We assumed an extinction threshold of 1, thus
277 neglecting a likely Allee effect due to self-incompatibility. Because for each population the
278 mean asymptotic growth-rate was close to 1, we then hypothesized that each population was

279 in fact regulated by density. We modeled a scramble type of density-dependence, assuming
280 that the fecundity term was regulated by the number of flowering adults (and hence also by
281 the number of competing seedlings) the previous time step. We also assumed an Allee
282 effect with a threshold value of three individuals. We ran simulations with same initial
283 population sizes as above, with carrying-capacity and maximum growth-rates more or less
284 consistent with the observed stage matrix. We used two values of the maximum growth
285 rates: 1.01 (consistent with the observed stage matrix) and 1.09 (less consistent). We
286 considered that in populations Calcina, Corbaghiola and Inzecca, populations were slightly
287 over their carrying-capacity K (hence their observed asymptotic growth-rate lower than 1),
288 whereas Teghime was below (hence its growth-rate above 1). We assumed initial
289 population sizes (and carrying-capacities) of 100 ($K=80$), 300 ($K=280$), 500 ($K=480$) and
290 2000 ($K=2500$) for Calcina, Corbaghiola, Inzecca and Teghime, respectively, with an initial
291 stable stage distribution corresponding to the mean matrix.

292

293 **Environmental analyses**

294

295 *Climatic data* - We first compared climatic data between years and between populations
296 using non parametric analyses of variance and Kruskal-Wallis tests implemented in Proc
297 Npar1way (SAS 9.1, SAS Institute 2005). We then tested for the effect of climatic
298 conditions on demographic vital rates. Because the weather probably affects the
299 demographic vital rates over more than one year, correlations between the survival rates,
300 flowering probabilities and fecundity term observed for the annual transition from spring of
301 year $t-1$ to spring of year t and the meteorological data from April $t-1$ to March t and from
302 April $t-2$ to March $t-1$ were tested. Because of a lack of normality, we only computed and

303 tested Spearman rank correlations. We first considered the whole data set (36 combinations
304 of population * year), and then considered each population separately (9 years for each
305 population).

306

307 *Differences in ecological variables* - To assess if there was significant variation among
308 populations in the other ecological variables (aphid attacks, competition and type of
309 substrate), we performed Chi-square tests for the presence/absence of aphids, for the
310 qualitative intra and inter-competition data and for the substrate data and Kruskal-Wallis
311 tests on the semi-quantitative intra and inter-competition data.

312

313 **Genetic data analyses**

314

315 To assess potential genetic problems in the populations over the ten-year period of the
316 study, we used the genetic data available from individuals sampled in 2000 (Glémin et al.
317 2005) on microsatellite loci and the S-locus (self-incompatibility). We then re-sampled the
318 same populations in 2009, and performed new analyses of the microsatellite loci.
319 Individuals were sampled and genotyped as in Glémin et al. (2005), using similar sampling
320 sizes as in 2000 (40, 41, 41 and 70 for Calcina, Corbaghiola, Inzecca and Teghime
321 respectively). For one population (Teghime) sampling in 2009 concerned only a subset of
322 the population, so that for comparison we re-estimated from the 2000 dataset genetic
323 parameters corresponding to the same subset. We performed genotyping of the 2009
324 individuals at 11 microsatellite markers (as done in Glémin et al. 2005). Extraction, PCR
325 and gel migration protocols are described in appendix A. For most loci, allele sizes that
326 were read in 2009 differed from those read in 2000, presumably because the protocols and

327 the size markers (and readers) were different (see Appendix A for details). However, using
328 a few DNA samples from 2000, we could recode without ambiguity the genotype of 2000
329 samples at 3 out of 11 loci (26A, 59A and AT129). We did not try to re-genotype the
330 samples from 2000 at the other loci. However, for 5 other loci (D02, 38A, H09, B01, and
331 B06), there was a systematic bias of a 1 to 4 bp, which was easily corrected by comparing
332 allele frequencies at the overall (Corsican) level, under the assumption that highly frequent
333 alleles in 2000 would still occur at reasonable frequencies in 2009, whereas very rare alleles
334 could not have become highly frequent in such a short period of time. For one locus (F07), a
335 new and very different allele (allele 168) appeared at high frequency in a single population
336 (Inzecca). Re-genotyping and re-reading gels from 2000 clearly showed that allele 168 had
337 in fact mistakenly been taken for a parasitic band in 2000. We thus recoded all 2000
338 samples for this locus. We then recovered very similar allele frequencies between 2000 and
339 2009, as was observed at the other loci in Inzecca population. Finally, for two loci (D08 and
340 E06), reading difficulties, very large number of alleles, and lack of any allele occurring at
341 high frequency in any population, prevented us from comparing the two data sets. These
342 two loci were therefore removed from all the genetic analyses presented here.

343 Population genetic parameters (Heterozygosity (H_e = proportion of heterozygotes
344 expected under random mating), Fixation index (F_{IS} = relative deficit of heterozygotes
345 compared to H_e), mean number of alleles per locus) were calculated using GENETIX
346 software (Belkhir 2004). We also estimated the parameter θ , which is related to population
347 effective size, in the same way as performed by Glémin et al. (2005 – note that there is a
348 mistake in the formula given in this paper – but not in the calculated values), from the
349 Heterozygosity (H_e) under the stepwise mutation model (Kimura and Ohta 1978),

350 $\hat{Q} = \frac{1}{2} \frac{\sum_{i=1}^L H_i}{\sum_{i=1}^L H_i} \cdot \frac{1}{\theta}$. θ was estimated as the mean of the θ_i estimated for each locus. We

351 tested for the effect of population, year and locus on the number of alleles, Heterozygosity
352 and \hat{Q} by using Proc ANOVA with Duncan's multiple range test for comparing years,
353 populations, or loci (SAS 9.1, SAS Institute, 2005). Bootstraps over loci implemented in
354 GENETIX provided confidence intervals for F_{IS} .

355 Using the software BOTTLENECK (Cornuet and Luikart 1996), we tested for evidence of a
356 bottleneck in each population in 2000 and in 2009. The test compares the actual
357 Heterozygosity (H_e , the expected proportion of heterozygotes under panmictic equilibrium)
358 to the one expected at mutation – drift equilibrium, given the observed number of alleles.
359 Because H_e is mostly determined by frequent alleles, which are less likely to disappear
360 following a bottleneck, it is less sensitive than the number of alleles to a recent bottleneck.
361 Thus a signature of a bottleneck is detected whenever there is a significant excess of H_e
362 compared to what is expected at mutation – drift equilibrium given the number of alleles.

363 Population genetic structure was analysed by estimating global F_{ST} , F_{ST} between pairs of
364 population, and F_{ST} between years within populations. As for F_{IS} , confidence intervals for
365 each value were provided by bootstrapping over the 9 loci. Mantel test (also implemented in
366 GENETIX) was performed to test for significance of the correlation (Pearson correlation
367 coefficient) between the 2000 and 2009 F_{ST} matrices.

368

369 **Results**

370

371 **Demographic vital rates, asymptotic growth rates and contribution of vital rates**
372 **variation to variation of lambda**

373

374 Arithmetic means, standard deviation and elasticities for demographic vital rates are given
375 in Table 2. The lowest transition probability was observed for the transition from vegetative
376 to flowering stages (a_{32}) with only 24.5 % of vegetative individuals reaching the flowering
377 stage. This low transition was due to the low probability of flowering for vegetative
378 individuals ($p_2 = 0.296$) rather than to a low survival at the vegetative state ($s_2 = 0.829$). The
379 fecundity term (a_{13}) was also low (0.331) with a high standard deviation (0.424); it was
380 highly variable among populations and years (see Appendix B). The largest transition rate
381 that was observed was the probability for a flowering plant to survive and flower the
382 following year ($a_{33} = 0.615$). The probability for a flowering individual to return to a
383 vegetative stage the following year was also high: $a_{23} = 0.311$. The survival probability of
384 flowering individuals (s_3) showed the lowest variation (0.053).

385 Mean asymptotic growth rates λ_a ranged from 0.89 (Corbaghiola) to 1.02 (Teghime).
386 Asymptotic growth rates per year and per population ranged from 0.5 to 1.21 (Table 3) and
387 were particularly sensitive to the survival probabilities of vegetative and flowering
388 individuals (s_2 and s_3 , see elasticities in Table 2).

389 For Teghime population, the variation of λ_a was mainly due to variation of the
390 probability of flowering for the vegetative individuals (p_2) and of their survival rate (s_2). For
391 the three other populations, the variation of λ_a was mostly due to variation of survival rates
392 of vegetative individuals (s_2) (Fig. 3).

393

394 **Spatial and temporal variations in population stage structure, transitions probabilities**
395 **and lower-level vital rates**

396

397 The comparison of the stable and the observed stage-distributions (SSD and OSD) per
398 population and per year showed that Teghime population was almost every year at its SSD
399 (except in 2007, 2008 and 2009, Fig. 4). This population showed a large proportion of
400 vegetative individuals and low proportions of seedling and flowering individuals,
401 corresponding to a low probability of flowering and a large survival rate of vegetative
402 individuals ($p_2 = 0.132$, $s_2 = 0.884$, see Appendix B). This was consistent with the high
403 transition a_{22} (0.755) in this population. In 2007, 2008 and 2009, lower recruitment rates or
404 excess of flowering plants may explain the deviation from the SSD (Appendix B).
405 Conversely, the three other populations were significantly different from their SSD in 5 to 7
406 out of the 10 years. Compared to Teghime population, they had more flowering individuals
407 and less vegetative individuals and seedlings due to higher flowering probabilities and
408 lower recruitment rates. For most populations and years, the years that deviated from the
409 SSD showed less vegetative and more flowering individuals compared to the stable-stage
410 distribution.

411 For the whole dataset, the variations in transition probabilities a_{ij} were best supported by
412 the “population + year” model which accounts for additional population and year effects
413 (Appendix C). All the other models had no support ($\Delta AIC > 7$).

414 Variation in seedling survival (s_1) was best explained by the model including
415 year*population effect but there was some support for the model with effects of year +
416 population (Appendix C). Variation in vegetative survival rate (s_2) was best explained by
417 the model including year + population effects. Variation in survival of flowering individuals
418 (s_3) was best explained by the year model with some support also for the model including
419 year + population. Finally, variations in both flowering probabilities (p_2 and p_3) were best
420 explained by the model including year*population effects (Appendix C).

421

422 **Stochastic growth rates, quasi-extinction probabilities and stochastic simulations**

423

424 Only the Teghime population had a stochastic growth rate λ_s larger than 1 (Table 3). Rates
425 of population growth predicted by deterministic and stochastic models were similar (Table
426 3).

427 Figure 5 presents the quasi-extinction probabilities (P_{ext}) for each population at 50
428 years for the mean stage-distribution for the 10 years. The results show an extinction risk at
429 50 years constant and null for Teghime whatever the quasi-extinction threshold (none of the
430 simulation trajectories led to extinction). For the three other populations, extinction
431 probabilities were positive and increased with the quasi-extinction threshold. For quasi-
432 extinction thresholds below 30, Corbaghiola population always showed the highest
433 extinction probabilities at 50 years, followed by Inzecca and Calcina (Fig 5).

434

435 The simulations performed with RAMAS/Metapop with a threshold of 1 over a
436 period of 100 years, assuming initial population size close to census size and no density-
437 dependence gave similar results, in that Teghime population was the only one unlikely to go
438 extinct (Table 4A). However, unlike with POPBIO, the three other populations had similar
439 time to extinction (35 – 42 years on average). It could be that the smaller standard errors of
440 vital rates in Inzecca and Corbaghiola favour these populations compared to Calcina in
441 these simulations where (unlike those with POPBIO) vital rates are individually sampled
442 from a log-normal distribution. Assuming initial population size of 2000 individuals (as in
443 Teghime) in each population increased the time to extinction to 52 and 54 in Corbaghiola
444 and Inzecca, and to 86 in Calcina, suggesting that if this population could be reinforced it

445 might actually persist for a longer time compared to Corbaghiola and Inzecca (Table 4B).
446 Assuming scramble density-dependence with a maximal growth rate of 1.01, initial
447 population sizes equal to census size, and an Allee effect with a threshold density of 3
448 individuals (a really low value for a self-incompatible species) increased the persistence
449 time of Inzecca and Corbaghiola but not of Calcina, even though we assumed an initial
450 population size larger than the carrying-capacity (Table 4C, to compare to Table 4A). This
451 latter effect on Calcina is likely to be due to the Allee effect in these simulations. When the
452 maximum growth rate was set to 1.09, Calcina population also quickly went extinct,
453 presumably because of the strong Allee effect experienced in such a small population. All
454 three other populations persisted for 100 generations under these overly optimistic
455 conditions (Table 4d).

456

457 **Comparison of meteorological and ecological data among years and populations**

458

459 There were no significant differences of temperature among years ($df = 8$, $P > 0.05$ for all
460 comparisons). Conversely the total amount of rainfall varied significantly among years
461 ($\chi^2=17.20$, 8 df, $P=0.028$), with years 2001, 2003 and 2009 being particularly wet. The
462 number of rain events $> 0.1\text{mm}$ also varied among years, with year 2002 being particularly
463 dry ($\chi^2=17.5$, 8 df, $P=0.025$).

464

465 All temperature variables showed significant differences among populations ($df = 3$,
466 $P < 0.04$ for all comparisons). Teghime and Corbaghiola had the lowest values for all three
467 temperature variables, whereas Inzecca population had the largest values. The amount of
468 rainfall also varied significantly among populations, with Inzecca significantly wetter than

469 the three other populations for the total amount of rainfall as well as the number of rain
470 events > 0.1 mm, and Corbaghiola and Teghime significantly drier for the number of rain
471 events > 1 mm (Appendix D). Overall, Corbaghiola and Teghime experienced relatively
472 cold and dry climatic conditions, Inzecca experienced hot and wet conditions, whereas
473 Calcina experienced hot and slightly wet conditions relative to Corbaghiola and Teghime,
474 but dry relative to Inzecca.

475 Both the semi-quantitative intra and inter-competition variables tested showed a
476 significant population effect ($df = 3$, $P < 0.0001$ for all values, Table 5). The differences
477 among populations were significant for aphid herbivory ($df = 3$, $P < 0.0001$) and the type of
478 inter-specific competition and the soil substrate were also highly significant ($df = 6$, $P <$
479 0.0001 for both effects). Thus the four populations showed significant differences for all the
480 ecological variables tested. The most obvious difference among populations was the amount
481 of attack by aphids, which was largest in Calcina and lowest in Teghime. Further, Inzecca
482 and Corbaghiola were characterized by a scree type of soil substrate and a limited amount
483 of intra-specific competition.

484

485 **Correlations of vital rates with and environmental variations**

486

487 We first computed Spearman correlations coefficients using the 36 combinations of
488 population * year. The recruitment rate (transition parameter a_{13}) was negatively correlated
489 with the maximum mean summer temperature of years $t-1$ and $t-2$ ($r=-0.35$, $P=0.04$ and $r=-$
490 0.38 , $P=0.02$, respectively). The survival rate of juveniles (parameter s_1) was negatively
491 correlated with both variables concerning the number of rain events (>0.1 mm or >1 mm) at
492 $t-1$ ($r=-0.41$, $P=0.01$ and $r=-0.40$, $P=0.02$, respectively). The survival rate of non flowering

493 adults (parameter s_2) was similarly negatively correlated with the number of rain events >
494 1mm at $t-1$ ($r=0.35$, $P=0.03$). The survival rate of flowering adults (parameter s_3) was
495 negatively correlated with three climatic variables at $t-2$: the total amount of rain ($r=-0.49$,
496 $P=0.002$), the number of rain events > 1mm ($r=-0.50$, $P=0.002$), and the maximum mean
497 summer temperature ($r=-0.34$, $P=0.04$). Flowering probabilities were not correlated with
498 any climatic variable, with the exception of p_2 (probability of a rosette to flower), which
499 was positively correlated with the maximum mean summer temperature at $t-2$ ($r=0.35$,
500 $P=0.04$). Note that none of these correlation coefficients would remain significant after
501 Bonferroni correction to take into account the type 2 error rate.

502 We then computed correlations between meteorological variables and the demographical
503 vital-rates within each population.

504 In Calcina population, the survival rate of flowering adults (parameter s_3) was negatively
505 correlated with the maximum mean summer temperature of year $t-1$ ($r=-0.83$, $P=0.005$), as
506 well as with the number of rain events > 1mm at $t-2$ ($r=-0.82$, $P=0.007$). The survival rates
507 of the other stages were not correlated to any climatic variable. With regards to flowering
508 probabilities, both flowering probabilities of rosette (p_2) and of flowering individuals the
509 previous year (p_3) were negatively correlated with the minimum mean winter temperature at
510 $t-1$ ($r=-0.72$, $P=0.03$ and $r=-0.82$, $P=0.007$ for p_2 and p_3 , respectively). To sum up, for the
511 most part only temperature variable affected demographic parameters in Calcina, with cold
512 winters favouring flowering, and cool, dry summers favouring adult survival.

513 In Corbaghiola population, most vital rates were not correlated to any climatic variable.
514 The flowering probability of rosettes was negatively correlated with both numbers of rain
515 events at $t-1$ ($r=-0.70$, $P=0.02$) and positively correlated with the maximum mean summer
516 temperature at $t-2$ ($r=+0.71$, $P=0.03$). The flowering probability of flowering adults was

517 negatively correlated with the number of rain events $> 1\text{mm}$ at $t-1$ ($r=-0.67$, $P=0.05$).

518 Overall, dry years with hot summers favored flowering in Corbaghiola population.

519 In Inzecca population, juvenile survival (s_1) decreased as the total amount of rainfall in
520 year $t-1$ increased ($r=-0.67$, $P=0.05$). The survival rate of rosettes (s_2) was positively
521 correlated with all three temperature variables (total degree days, maximum summer and
522 minimum winter temperatures) at $t-1$ ($r=0.69$ to 0.77 , $P=0.02$ to 0.04). The flowering
523 probability of rosettes (p_2) decreased with increasing amount of rainfall at $t-2$ ($r=-0.70$,
524 $P=0.04$) and increasing minimum mean winter temperature at $t-2$ ($r=-0.77$, $P=0.02$). Overall,
525 in Inzecca survival and later flowering were both favoured by dry years, whereas only
526 survival was enhanced by warm winters, and later flowering was favoured by cool winters.

527 Finally, in Teghime population, both the juvenile (s_1) and the rosette (s_2) survivals
528 decreased with increasing number of rain events $> 0.1\text{mm}$ ($r=-0.75$ and -0.80 , $P=0.02$ and
529 0.01 , for s_1 and s_2 respectively). Both probabilities of flowering p_2 and p_3 increased with the
530 mean maximum summer temperature at $t-2$ ($r=0.72$, $P=0.03$ and $r=0.87$, $P=0.002$ for p_2 and
531 p_3 , respectively). Overall, dry years favoured survival and hot summers favoured flowering
532 in Teghime population.

533 Overall, the probabilities of flowering (p_2 and p_3) tended to correlate with
534 temperature. However the effect of temperature on flowering depended on the population,
535 consistently with the analyses described in Appendix C. Warmer summer temperatures
536 favoured flowering in Teghime and Corbaghiola (the populations with the lowest summer
537 temperatures), whereas colder winter temperature favoured flowering in Inzecca and
538 Calcina (the populations with the highest temperatures, see Appendix D). Survival
539 probabilities tended to correlate negatively with one of the rainfall variables for all
540 populations but Corbaghiola.

541

542

543 **Genetic monitoring**

544

545 Results of genetic analyses for 2000 and 2009 on 9 loci are compiled in Tables 6 and 7. The
546 number of alleles depended on the population and the locus considered. The mean number
547 of alleles per locus was not significantly different in 2009 compared to 2000 (3.47 in 2000
548 *versus* 3.14 in 2009, $df = 1$, $F = 0.4331$, $P = 0.51$). It was significantly smaller in Calcina
549 population compared to the three others (1.61 in Calcina, *versus* 3.72 - 4.16 in the three
550 other populations, see Table 7; $df = 3$, $F = 6.36$, $P = 0.0007$). Two loci out of the 9 analysed
551 (D02 and H09) displayed significantly larger number of alleles compared to the seven
552 others (5.87 and 6.37, respectively, *versus* 2.00 – 2.87 for the other loci; $df = 8$, $F = 8.73$, P
553 < 0.0001). Expected heterozygosity (and θ , results not shown) depended on population ($df =$
554 3 , $F = 9.19$, $P < 0.0001$) and locus ($df = 8$, $F = 3.70$, $P < 0.0013$), but did not vary between
555 2000 and 2009 ($df = 1$, $F = 0.0041$, $P = 0.95$). Expected heterozygosity was significantly
556 lower in Calcina compared to the three other populations (0.10 *versus* 0.42-0.47). Within
557 populations fixation index (F_{IS}) did not vary between 2000 and 2009: mean F_{IS} was 0.10
558 (bootstrap over loci confidence interval: 0.01-0.20) in 2000 and 0.14 (0.06– 0.24) in 2009.
559 F_{IS} was positive and significantly different from 0 in Corbagiola and Teghime in both years,
560 whereas it was negative in Calcina in 2000 (see confidence intervals obtained by
561 bootstrapping over loci in Table 6). Population pairwise F_{ST} ranged from 0.19 to 0.55 in
562 2000 (Table 7A) and from 0.18 to 0.60 in 2009 (Table 7B). Among-population F_{ST} values
563 of 2000 and 2009 were significantly correlated (Mantel test, $r = 0.98$, $P=0.001$). Overall
564 differentiation among populations was similar in 2000 and 2009 ($F_{ST} = 0.34$ in both years)

565 (confidence intervals from bootstrapping over loci: 0.26 - 0.41 and 0.26 - 0.44 in 2000 and
566 2009, respectively). Differentiation among years was low and significantly different from 0
567 in Calcina population only (Table 7C). Differentiation within populations among years was
568 much lower than among populations within years (cf. Table 7A-B and 7C). Thus, overall no
569 change occurred from 2000 to 2009, apart for the smallest population, Calcina.

570 The main evidence for a recent bottleneck concerned Teghime population in the
571 2009 data set, for which 6 out of 8 polymorphic loci had an excess of Heterozygosity
572 compared to the value expected from the number of alleles (One-tail Wilcoxon tests for
573 heterozygote excess: $P = 0.02$ assuming IAM mutation model, $P = 0.10$ assuming TPM, P
574 $= 0.47$ assuming SMM, see Cornuet and Luikart 1996).

575

576

577 **Discussion**

578

579 **Demography: evidence for environmental stochasticity**

580

581 In this study, we have compared the demographic behaviour of four populations of
582 the rare endemic species *Brassica insularis* in Corsica. This species is a long-lived
583 perennial and demographic transitions between adult stages (vegetative to flowering,
584 flowering to flowering and flowering to vegetative) are similar to those of other long-lived
585 perennials (e.g. Picó et al. 2002; Endels et al. 2007b), with survival rates of 0.8 for
586 vegetative adults and 0.9 for reproductive adults.

587 The observed stage distribution (OSD) for each population and each year revealed
588 contrasting patterns among populations and years. Teghime showed a stage distribution

589 with mainly vegetative individuals and more juveniles in comparison to the other
590 populations, which showed mainly flowering individuals and less vegetative and juvenile
591 individuals. The comparison between the observed and the stable stage distribution (OSD
592 vs. SSD) suggested that demographic rates varied among years: for all populations, the OSD
593 was significantly divergent from the SSD at least for three years. All populations have been
594 known to exist for several decades, so temporal variation is the most likely explanation for
595 the divergence between SSD and OSD (Colling and Matthies 2006; Jiménez-Sierra et al.
596 2007) rather than a recent origin of the populations. Moreover, results on demographic
597 transitions and lower-vital-rates showed significant population and year effects as well
598 (Appendix C). So, vital rates exhibited both spatial and temporal variations: environmental
599 stochasticity occurs.

600

601 **Genetic monitoring suggests no or limited change of diversity over 10 years**

602

603 The genetic analyses showed that overall allele frequencies did not change between 2000
604 and 2009 and neither did population structure. The only significant differentiation between
605 years was observed for Calcina, the smallest population. Using the software Bottleneck
606 (Cornuet and Luikart 1996), we hardly detected any evidence for a recent bottleneck, in
607 either 2000 or 2009. Despite stochastic demographic variation, perenniality may buffer
608 genetic variation in this species. Note however that the small number of loci (nine) of the
609 present study prevents powerful testing. There was some signature for Teghime population.
610 This can be related to the occurrence of a large fire in part of this population in 2003. Dolan
611 et al. (2008) recently showed an increase, rather than a decrease, of genetic diversity after
612 fire in another perennial species, *Hypericum cumulicola*, as a consequence of recolonisation

613 from an extensive seed-bank. However, we have no evidence of such a seed bank in
614 *Brassica insularis*, so that an increase of genetic diversity after a fire would be unlikely.
615 Albeit this fire and the slight evidence for a bottleneck, Teghime remained the most
616 genetically diverse population for microsatellites. According to Glémin et al. (2005, 2008),
617 it was also the most diverse population with regards to the number of alleles at the S-locus
618 (estimated numbers according to the lowest and most probable scenario were 15, 11, 12 and
619 5 for Teghime, Inzecca, Corbaghiola and Calcina respectively; Glemin et al 2005).
620 Conversely, Calcina appeared as the most genetically threatened with the lowest genetic
621 diversity in both 2000 and 2009, and, in 2000, the lowest diversity at the S-locus. These
622 figures correspond well to estimated census adult (vegetative and flowering individuals)
623 population sizes observed in 2000 (Teghime: 2000, Inzecca: 500, Cobaghiola: 300, Calcina:
624 80) (Glemin et al. 2005).

625

626 **What determines variation in vital rates among populations?**

627

628 We found significant differences among populations for climatic and (biotic) ecological
629 conditions, which might be driving spatial variation for demographic transition parameters.
630 In particular, in Teghime recruitment rate and rosette flowering probability were larger than
631 in the three other populations; recruitment rate in Calcina was particularly low; rosette
632 survival rate was lower in Corbaghiola; and flowering adults survival rate was lower in
633 Corbaghiola and Inzecca (see Appendix B). Lower temperatures, in particular summer ones,
634 could explain the lower flowering probabilities of rosettes and still higher recruitment rate
635 observed in Teghime compared to Calcina and Inzecca (Appendix B). Corbaghiola and
636 Teghime have very similar climatic conditions, but it could be that the slightly less cold

637 winter in Teghime provides significantly better conditions. However, we did not find any
638 significant effect of the minimum winter temperature on the recruitment rate in
639 Corbaghiola. It could be that the large recruitment rate in Teghime compared to
640 Corbaghiola is enhanced by its large population size and concomitantly its large genetic
641 diversity, in particular at the S-locus, as well as by the lack of herbivory attack by aphids.

642 The particularly low recruitment rate in Calcina (mean $a_{13} = 0.11$, see Appendix B),
643 conversely, could be partly explained by it being heavily attacked by aphids. Aphids were
644 found on juveniles, but also, some years, totally destroyed flowering stems (pers. obs.).
645 Aphids, by preventing plants to reproduce, might actually decrease the strength of
646 frequency-dependent selection acting upon the S-locus in Calcina population, leading to a
647 further decrease of genetic diversity at this locus, thus engaging the population in a Vortex
648 of extinction through the interaction of environmental (aphids) and genetic (S-locus)
649 factors. If, indeed, reproductive events are rare in this population, it is likely that selection
650 pressure to resist aphids is also low.

651 The lower adult survival rate in Corbaghiola could also, like recruitment rates, be
652 explained by the colder winters in this population. The lower flowering adult survival rate
653 in Inzecca compared to Calcina and Teghime might be related to the high annual rainfall in
654 this population. This is supported by the fact that, within Inzecca, flowering probability is
655 enhanced by former years of low rainfall.

656 Overall, mainly climatic factors seem to be influencing among-population variance
657 in adult survival and flowering probability, whereas other factors, such as biotic ecological
658 and genetic ones, might also be involved in determining recruitment rates.

659

660 **Could populations persist in the future and what could be done to prevent extinction ?**

661

662 Our analysis suggests that environmental variation acts on demographic behaviour.
663 Populations experience different environments and temporal variations within these
664 environments. Applying stochastic demographic models is thus more relevant to incorporate
665 this environmental variation (Morris and Doak 2002; Ghimire et al. 2008).

666 Teghime appears to be the only growing population (stochastic $\lambda_s > 1$), with no risk
667 of extinction at 50 years whatever the quasi-extinction threshold chosen. Results of
668 extinction probabilities based on demographical matrices for the last 10 years are consistent
669 with genetic analyses. Teghime showed the highest genetic diversity and highest
670 recruitment rates. It was the only non-threatened population. Even the occurrence of a fire
671 in 2003 (pers. obs.) did not appear to have much consequence (other than a slight decrease
672 of genetic diversity), and from Table 3 it is unlikely to explain the demographic growth of
673 this population. Presumably, its large size allows this population to be very healthy, from
674 both genetic and demographic points of view. Some preliminary results of Glémin et al.
675 (2002, 2006) suggested that strong inbreeding depression and low heterosis occur for adult
676 survival in this population. Since the population is quite large, it is unlikely that running a
677 population viability analysis incorporating the random fixation of slightly deleterious
678 mutations and inbreeding load would much affect our conclusion about the viability of this
679 population, especially given that such mutations seem to be rare (low heterosis). However,
680 if this population was to experience a sudden and strong demographic bottleneck, the
681 occurrence of lethal or deleterious mutations (as suggested by the occurrence of inbreeding
682 depression) could lead to a large loss of fitness, and hence of viability. Overall, Teghime
683 population does not appear to be threatened from the genetic or environmental conditions,

684 at least on the short-term and in the absence of further catastrophic events such as fire.
685 Thus, monitoring the site is a sufficient measure to adopt.

686 For the three other populations (Inzecca, Calcina and Corbaghiola) the stochastic
687 analysis assuming density-independence suggests that these populations might go extinct in
688 the near future. Inzecca and Corbaghiola populations are smaller than Teghime, and have
689 lower recruitment rates. Differences observed between Inzecca and Corbaghiola in the slope
690 of extinction probability curves could be explained by their different population sizes, as
691 Corbaghiola is slightly smaller than Inzecca, and as our comparison between results of
692 RAMAS simulations starting with different initial sizes suggest (compare Table 4A and
693 4B). Alternatively, it could be that these populations are at carrying-capacity, so that the
694 demographic transition parameters estimated in the present study do not reflect recruitment
695 and survival rates at low density. Assuming that the probabilities of extinction which we
696 computed under the hypothesis of density-independence reflect the likely future of each
697 population, one can ask whether projected changes in climatic conditions will increase or
698 decrease the likelihood that some of these populations go extinct. Since 1980,
699 Mediterranean areas experienced warmer and drier conditions (Dufresne et al. 2006). If this
700 trend continues, and assuming that the large amount of rainfall at Inzecca and the low
701 winter temperature at Corbaghiola are the main determinants of their low growth-rate, this
702 trend will favour these two populations. In terms of short-term conservation, one option
703 would be to use these populations to create new populations elsewhere, in climatic
704 conditions more similar to those of Teghime. Given the type of habitat of the species, i.e.
705 rocks, creating new populations might be difficult. The difficulty will be in finding suitable
706 sites where introductions could have some chance to succeed. Transplants usually provide
707 greater initial success than do sown seeds (Menges 2008), but this might not be possible in

708 rocky environments, whereas seeds could be more easily introduced (e.g., see Colas et al.,
709 2008, for successful translocation of monocarpic perennial *Centaurea corymbosa* using
710 seeds in a rocky environment). Alternatively, projected climatic changes might be sufficient
711 to increase the viability of each of these two populations. Furthermore, we will first have to
712 conduct detailed analyses about the likelihood that these two populations are actually
713 regulated by density rather than declining.

714 Finally, for Calcina population, extinction probability based on demographic data
715 was large under the assumption of density-independence. However, at least with POPBIO
716 simulations, it was surprisingly low compared to Inzecca and Corbaghiola, given its lowest
717 population size and genetic diversity at both S- and microsatellite loci. It could be that local
718 climatic conditions, with mild winters and abundant rainfall, and albeit hot summers, favour
719 adult survival rates enough to decrease the extinction risks. The survivorship of adults in
720 this population could allow it to cope with high variations in reproductive output, as
721 observed in other species (Enneson and Litzgus 2008). Comparisons of Table 4A and B,
722 whereby we increased the initial population size of Calcina, suggest that indeed
723 demographic stochasticity might be a major problem in this population. Indeed, census size
724 and genetic diversity, including at the S-locus, are very low. Given its large survival rates
725 and low recruitment rates, finding ways to increase the recruitment rate would be useful.
726 This might be done in two ways: acting upon genetic diversity, in particular at the S-locus,
727 and acting upon aphid attack. Adding individuals with different genetic make-up will likely
728 increase the number of S-alleles and therefore potentially increase the reproductive success
729 in this population. Adding individuals will also help decrease the impact of demographic
730 stochasticity and, according to our viability analysis, strongly increase the time to extinction
731 (compare again Tables 4A and 4B). It will be easy to introduce genetic diversity in Calcina

732 population from the other Corsican populations. Because Teghime is the healthier, largest
733 and most diverse population, we recommend introducing a few individuals from there.
734 Note, however, that reinforcement could be problematic in *Brassica insularis* for which the
735 occurrence of sub-species, varieties and/or local adaptation are still not clear (Petit et al.
736 2001). Further considerations are necessary prior to such management actions (Lubben et al.
737 2008). Concerning aphids, pesticide treatment is not something one would want to
738 recommend, but biocontrol using ladybugs could be an option.

739 Thus, the combination of ecological, demographic and genetic analyses used in this
740 study have allowed us to determine the main threats acting on four Corsican populations of
741 *Brassica insularis* and suggest management options for their conservation, as summarized
742 by Table 8.

743

744

745 **Conclusion : Integration of demographic analyses, climatic data, biotic**
746 **ecological factors, and genetic analyses: implications for conservation**

747

748 Few plant studies have integrated demographic, genetic and ecological data
749 (Newman and Pilson 1997; Menges and Dolan 1998; Fréville et al. 2004; Endels et al.
750 2007a). However, such integrative studies are necessary to assess factors threatening
751 protected species (Schemske et al. 1994; Menges 2000; Reed et al. 2002; Oostermeijer et al.
752 2003). Overall, our results suggest that limiting the conservation study to only one kind of
753 analyses (demographic or genetic or ecological) would have led to incomplete assessment
754 of those factors threatening each population and possibly miscalculate the management or
755 restoration to apply, as suggested by Robert *et al.* (2007). Furthermore, it is clear that for

756 populations of long-lived perennials with asymptotic growth-rates close to one, purely
757 demographic analyses at the population level must be complemented by detailed analyses of
758 density-dependent factors acting on vital rates. Such detailed demographic analyses would
759 be sufficient to assess population viability. However, in order to actively manage
760 populations, it is necessary to understand the factors influencing vital rates and population
761 viability. Depending on whether the main factors involved are, e.g., climatic or biotic or
762 genetic, management options will be quite different. If climatic differences among
763 populations are found to be the main explanatory factor, it is clear that reinforcement of
764 threatened populations will not be very useful, and translocation of those populations in
765 more suitable environments (such as those of healthy populations) would be a better option.
766 If conversely or complementary, genetic factors are involved, such as a lack of diversity at
767 the S-locus, or because of the accumulation of deleterious mutations (as would be suggested
768 by strong heterosis among populations), reinforcement of threatened populations by
769 individuals from other populations would be most useful. Finally, biotic factors such as
770 herbivores, pathogens, and competitors, if shown to threaten a population, could lead to
771 particular targeted management actions. All these factors are likely to interact, so that it is
772 necessary to analyse them simultaneously.

773 Last, it would be important to understand the generality for the apparent suitability
774 of local ecological factors, that is to assess the species niche and its variation among
775 populations. If particular climatic conditions are systematically associated with declining
776 populations, it is likely that the species is unable to adapt to these conditions. In this case,
777 reinforcing such declining populations, occurring in unsuitable conditions, will probably be
778 useless. However, if variation in climatic factors does not explain variation in vital rates, in
779 particular if there is no significant variation of climatic factors among sites, it could be that

780 declining populations are simply less well-adapted to their own environment. In this case,
781 reinforcing such populations might allow them to adapt. Further, unlike climatic factors,
782 biotic factors are themselves influenced by the level of local adaptation of populations. A
783 population might be heavily attacked by a given herbivore because of local climatic and
784 edaphic conditions, or because it lacks genes for resistance to this herbivore (or both).
785 Reinforcement might be efficient at reducing herbivory in the latter, but not the former
786 situation. Clearly, genetic monitoring of traits involved in local adaptation to ecological
787 factors would be helpful. Unfortunately, even neutral genetic diversity is not properly
788 considered in most conservation strategic plans (Laikre *et al.*, 2009). It will take some time
789 before non neutral genetic variation, at the basis of phenotypic evolution and adaptation, is
790 taken into account.

791

792 **Acknowledgements**

793

794 We thank Agnès Vivat and Sheila Luijten for their help in the field, Eric Imbert for his help
795 in the field and for his comments on a first version of the manuscript. We also thank Olivier
796 Gimenez for his help and discussion on statistical analyses. We thank two anonymous
797 reviewers for very helpful comments and suggestions. We benefited from discussions of the
798 NESCENT-NCEAS Working Group on *Genetic Monitoring*. Finally, we had insightful
799 discussions about this work at several meetings of the ESF Science Networking Programme
800 *ConGen*. This is publication ISEM- 2010-001 of the Institut des Sciences de l'Evolution.

801

802

803 **References**

804

805 Akçakaya HR, Root W (2002) RAMAS Metapop: Viability Analysis for Stage-structured
806 Metapopulations (version 4.0). Applied Biomathematics, Setauket, New York.

807 Asquith NM (2001) Misdirections in conservation biology. *Conservation Biology* 15:345-
808 352.

809 Belkhir K (2004) GENETIX, logiciel sous Windows™ pour la génétique des populations..
810 Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de
811 Montpellier II, Montpellier (France).

812 Brito, DC, Grelle EV, Boubli JP (2008) Is the Atlantic Forest protected area network
813 efficient in maintaining viable populations of *Brachyteles hypoxanthus*?
814 *Biodiversity and Conservation* 17:3255-3268.

815 Brook BW, Tonkyn DW, O'Grady JJ, Frankham R (2002) Contribution of inbreeding to
816 extinction risk in threatened species. *Conservation Ecology* 6:16.

817 Burgman MA, Lamont BB (1992) A stochastic model for the viability of *Banksia cuneata*
818 populations: environmental, demographic and genetic effects. *Journal of Applied*
819 *Ecology* 29:719-727.

820 Burnham KP, Anderson DR (1998) Model selection and inference: a practical information-
821 theoretic approach. Springer-Verlag, New York.

822 Caswell H (1996) Analysis of life table response experiments II. Alternative
823 parameterizations for size- and stage structured models. *Ecological Modelling*
824 88:73–82.

825 Caswell H (2001) Matrix population models: construction, analysis and interpretation, 2nd
826 edn. Sinauer Associates, Sunderland, Massachusetts.

827 Caughley G (1994) Directions in conservation biology. *Journal of Animal Ecology* 63:215-
828 244.

829 Colas B, Kirchner F, Riba M, Olivieri I, Mignot A, Imbert E, Beltrame C, Carbonell D,
830 Fréville H. (2008) Restoration demography: a 10-year demographic comparison
831 between introduced and natural populations of endemic *Centaurea corymbosa*
832 (Asteraceae). *Journal of Applied Ecology* 45, 1468-1476.

833 Colling G, Matthies D (2006) Effects of habitat deterioration on population dynamics and
834 extinction risk of an endangered long-lived perennial herb (*Scorzonera humilis*).
835 *Journal of Ecology* 94:959-972.

836 Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting
837 recent population bottlenecks from allele frequency data. *Genetics* 144:2001-2014.

838 Dolan RW, Quintana-Ascencio PF, Menges ES (2008) Genetic change following fire in
839 populations of a seed-banking perennial plant. *Oecologia* 158:355-360.

840 Dufresne J-L, Salas y Melia D, Denvil S, Tyteca S, Arzel O, Bony S, Braconnot P,
841 Brockmann, P, Cadule P, Caubel A, Chauvin F, Déqué M, Douville H, Fairhead, L,
842 Fichet, T, Foujols, M-A, Friedlingstein, P, Grandpeix, J-Y, Guérémy, J-F,
843 Hourdin, F, Idelkadi, A, Krinner, G, Lévy, C, Madec, G, Marquet, P, Marti, O,
844 Musat, L, Planton, S, Royer, J-F, Swingedouw, D, Voltaire, A (2007) Simulation du
845 climat récent et futur par les modèles du CNRM et de l'IPSL. *La Météorologie*
846 55:45-59.

847 Endels P, Jacquemyn H, Brys R, Hermy M (2007a) Genetic erosion explains deviation from
848 demographic response to disturbance and year variation in relic populations of the
849 perennial *Primula vulgaris*. *Journal of Ecology* 95:960-972.

850 Endels P, Jacquemyn H, Brys R, Hermy M (2007b) Reinstatement of traditional mowing
851 regimes counteracts population senescence in the rare perennial *Primula vulgaris*.
852 *Applied Vegetation Science* 10:351-360.

853 Enneson JJ, Litzgus JD (2008) Using long-term data and a stage-classified matrix to assess
854 conservation strategies for an endangered turtle (*Clemmys guttata*). *Biological*
855 *Conservation* 141:1560-1568.

856 Ehrlén J, Syrjänen K, Leimu R, Begoña-Garcia M, Lehtilä K (2005) Land use and
857 population growth of *Primula veris*: an experimental demographic approach. *Journal*
858 *of Applied Ecology* 42:317-326.

859 Fieberg J, Ellner SP (2001) Stochastic matrix models for conservation and management: a
860 comparative review of methods. *Ecology Letters* 4:244-266.

861 Frankham R (2005) Genetics and extinction. *Biological Conservation* 126:131-140.

862 Fréville H, Colas B, Riba M, Caswell H, Mignot A, Imbert E, Olivieri I (2004) Spatial and
863 temporal demographic variability in the endemic plant species *Centaurea*
864 *corymbosa* (Asteraceae). *Ecology* 85:694–703.

865 García MB, Picó FW, Ehrlén J (2008) Life span correlates with population dynamics in
866 perennial herbaceous plants. *American Journal of Botany* 95:258-262.

867 Ghimire SR, Gimenez O, Pradel R, McKey D, Aumeeruduy-Thomas Y (2008)
868 Demographic variation and population viability in a threatened Himalayan
869 medicinal and aromatic herb (*Nardostachys grandiflora*): matrix modeling of
870 harvesting effects in two contrasting habitats. *Journal of Applied Ecology* 45:41-51.

871 Ginzburg LL, Slobodkin B, Johnson K, Bindman AG (1982) Quasi-extinction probabilities
872 as a measure of impact on population growth. *Risk Analysis* 2:171–181.

873 Glémin S, Petit C, Maurice S, Mignot A (2008) Consequences of low mate availability in
874 the rare self-incompatible species *Brassica insularis*. *Conservation Biology* 22:216-
875 221.

876 Glémin S, Vimond L, Ronfort J, Bataillon T, Mignot A (2006) Marker-based investigation
877 of inbreeding depression in the endangered species *Brassica insularis*. *Heredity*
878 97:304-311.

879 Glémin S, Gaude T, Guillemin ML, Lourmas M, Olivieri I, Mignot A (2005) Balancing
880 selection in the wild: Testing population genetics theory of self-incompatibility in
881 the rare species *Brassica insularis*. *Genetics* 171:279-289.

882 Glémin S (2002) Dépression de consanguinité, systèmes de reproduction et biologie de la
883 conservation. Approches théoriques et expérimentales chez *Brassica insularis*
884 Moris. PhD thesis, University of Montpellier 2, Montpellier.

885 Harper JL (1977) *Population Biology of Plants*. Academic press, London.

886 Hayward GD, McDonald DB (1997) Matrix population models as a tool in development of
887 habitat models, *in*: Duncan, J.R., Johnson, D.H. & Nicholls, T.H. (Eds.), *Biology*
888 *and Conservation of Owls of the Northern Hemisphere*, General Technical Report
889 NC-190, US Forest Service, Winnipeg, Manitoba, Canada, pp. 205-212.

890 Hiscock SJ, McInnis SM (2003) Pollen recognition and rejection during the sporophytic
891 self-incompatibility response: *Brassica* and beyond. *Trends in Plant Science*
892 8:606–613.

893 Hoebee SE., Thrall PH, Young AG (2008) Integrating population demography, genetics and
894 self-incompatibility in a viability assessment of the Wee Jasper *Grevillea* (*Grevillea*

- 895 *iaspicula* McGill., Proteaceae). Conservation Genetics 9:515-529.
- 896 Horvitz CC, Schemske DW, Caswell H (1997) The 'relative' importance of life-history
897 stages to population growth: prospective and retrospective analysis, in: Tuljapurkar,
898 S. & Caswell, H. (Eds.), Structured population models in marine, terrestrial and
899 freshwater systems, Chapman & Hall, New York, pp. 247–272.
- 900 Jacquemyn H., Van Rossum F, Brys R, Endels P, Hermy M, Triest L, de Blust G (2003)
901 Effects of agricultural land use and fragmentation on genetics, demography and
902 population persistence of the rare *Primula vulgaris*, and implications for
903 conservation. Belgian Journal of Botany 136:5-22.
- 904 Jacquemyn H, Brys R, Hermy M, Willems JH (2007) Long-term dynamics and population
905 viability in one of the last populations of the endangered *Spiranthes spiralis*
906 (Orchidaceae) in the Netherlands. Biological Conservation 134:14-21.
- 907 Jeanmonod D, Gamisans J (2007) Flora Corsica. Edisud, Aix-en-provence.
- 908 Jiménez-Sierra C, Mandujano MC, Eguiarte LE (2007) Are populations of the candy barrel
909 cactus (*Echinocactus platyacanthus*) in the desert of Tehuacán, Mexico at risk?
910 Population projection matrix and life-table response analysis. Biological
911 Conservation 135:278-292.
- 912 Jongejans E, de Vere N, de Kroon H (2008) Demographic vulnerability of the clonal and
913 endangered meadow thistle. Plant Ecology 198:225-240
- 914 Kirchner F, Robert A, Colas B (2006) Modelling the dynamics of introduced populations in
915 the narrow-endemic *Centaurea corymbosa*: a demo-genetic integration. Journal of
916 Applied Ecology 43:1011-1021.
- 917 Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies

918 in a finite population. Proc Natl Acad Sci USA 75:2868-2872 1978.

919 Lacy RC (1993) Vortex - a Computer-Simulation Model for Population Viability Analysis.
920 Wildlife Research 20: 45-65.

921 Laikre LA, Allendorf FW, Aroner LC, Baker CS, Kendall KC, McKelvey K, Neel MC,
922 Olivieri I, Ryman N, Schwarz MK, Short Bull R, Stetz JB, Tallmon A, Taylor BL,
923 Vojta CD, Waller DM, Waples S (2009) Neglect of Genetic Diversity in
924 Implementation of the Convention on Biological Diversity. Conservation Biology.
925 Conservation Biology (Published Online: 16 Dec 2009).

926 Lande R (1988) Genetics and demography in biological conservation. Science 241:1455-
927 1460.

928 Lande R (1993) Risks of population extinction from demographic and environmental
929 stochasticity and random catastrophes. The American Naturalist 142:911-927.

930 Lande R (1998) Anthropogenic, ecological and genetic factors in extinction and
931 conservation. Researches on Population Ecology 40:259–269.

932 Leducq J.-B., Gosset C, Poiret M, Hendoux F, Vekemans X, Billiard S (2010) An
933 experimental study of the S-Allee effect in the self-incompatible plant *Biscutella*
934 *neustriaca*. Conservation Genetics (****)

935 Lubben J, Tenhumberg B, Tyre A, Rebarber R (2008) Management recommendations based
936 on matrix projection models: the importance of considering biological limits.
937 Biological Conservation 141:517-523.

938 Menges ES (1991) The application of minimum viable population theory to plants, *in*: Falk,
939 D.A. & Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants, Oxford
940 University Press, New York, pp. 450-461.

- 941 Menges ES (1992) Stochastic modelling of extinction in plant populations, *in*: Fiedler, P.L.
942 & Jain, S.K. (Eds.), Conservation Biology: the Theory and Practice of Nature
943 Conservation, Preservation and Management, Chapman & Hall, New York, pp. 253-
944 276.
- 945 Menges ES, Dolan RW (1998) Demographic viability of populations of *Silene regia* in
946 midwestern prairies: relationships with fire management, genetic variation,
947 geographic location, population size and isolation. *Journal of Ecology* 86:63–78.
- 948 Menges ES (2000) Population viability analyses in plants: challenges and opportunities.
949 *Trends in Ecology and Evolution* 15:51–56.
- 950 Menges ES (2008) Restoration demography and genetics of plants: when is a translocation
951 successful? *Australian Journal of Botany* 56:187-196.
- 952 Morris WF, Doak DF (2002) Quantitative conservation biology: theory and practice of
953 population viability analysis. Sinauer, Sunderland, MA.
- 954 Newman D, Pilson D (1997) Increased probability of extinction due to decreased genetic
955 effective population size: experimental populations of *Clarkia pulchella*. *Evolution*
956 51:354–362.
- 957 Olano JM, Caballero I, Loidi J, Escudero A (2005) Prediction of plant cover from seed bank
958 analysis in a semi-arid plant community on gypsum. *Journal of Vegetation Science*
959 16:215-222.
- 960 Olivier L, Galland JP, Maurin H, Roux JP (1995) Livre Rouge de la Flore Menacée de
961 France. Tome 1. Espèces prioritaires. Paris, France: Muséum National d’Histoire
962 Naturelle.

963 Oostermeijer JGB, Brugman ML, de Boer ER, den Nijs HCM (1996) Temporal and spatial
964 variation in the demography of *Gentiana pneumonanthe*, a rare perennial herb.
965 Journal of Ecology 84:153–166.

966 Oostermeijer, JGB (2000) Population viability analysis of the rare *Gentiana pneumonanthe*:
967 the importance of genetics, demography and reproductive biology. Pages 313–334
968 in A. G. Young and G. M. Clarke, editors. Genetics, demography and viability of
969 fragmented populations. Cambridge University Press, Cambridge, United Kingdom.

970 Oostermeijer JGB, Luijten SH, den Nijs HCM (2003) Integrating demographic and genetic
971 approaches in plant conservation. Biological Conservation 113:389–398.

972 Petit C, Fréville H, Mignot A, Colas B, Riba M, Imbert E, Hurtrez-Bousses S, Virevaire M,
973 Olivieri I (2001) Gene flow and local adaptation in two endemic plant species.
974 Biological Conservation 100:21–34.

975 Pfeifer M, Wiegand K, Heinrich W, Jetschke G (2006) Long-term demographic fluctuations
976 in an orchid species driven by weather: implications for conservation planning.
977 Journal of Applied Ecology 43:313-324.

978 Picó FX, De Kroon H, Retana J (2002) An extended flowering and fruiting season has few
979 demographic effects in a mediterranean perennial herb. Ecology 83:1991-2004.

980 Proctor MF, Servheen C, Miller SD, Kasworm WF, Wakkinen WL (2004) A comparative
981 analysis of management options for grizzly bear conservation in the US-Canada
982 trans-border area. Ursus 15:145-16

983 R Development Core Team (2007) R: A Language and Environment for Statistical
984 Computing. R Foundation for Statistical Computing, Vienna, Austria, URL
985 <http://www.R-project.org/>.

986 Reed MJ, Mills LS, Dunning JB, Menges ES, McKelvey KS, Frye R, Beissinger SR,
987 Anstett MC, Miller P (2002) Emerging issues in population viability analysis.
988 Conservation Biology 16:7–19.

989 Robert A, Couvet D, Sarrazin F (2007) Integration of demography and genetics in
990 population restorations. Ecoscience 14:463-471.

991 Schemske DW, Husband BC, Ruckelshaus MH, Goodwillie C, Parker IM, Bishop JG
992 (1994) Evaluating approaches to the conservation of rare and endangered plants.
993 Ecology 75:584–606.

994 Snogerup S, Gustafsson M, Bothmer RV (1990) Brassica sect. Brassica (Brassicaceae). I.
995 Taxonomy and variation. Willdenowia 19:271–365.

996 Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological
997 research. 3rd edn. Freeman, W.H. & Company, New York.

998 Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction
999 before genetic factors impact them. Proceedings of the National Academy of
1000 Sciences 101:15261-15264.

1001 Stubben C, Milligan B (2007) Estimating and analyzing demographic models using the
1002 popbio package in R. Journal of Statistical Software 22, issue 11.

1003 Wagenius S, Lonsdorf E, Neuhauser C (2007) Patch Aging and the S-Allee Effect:
1004 Breeding System Effects on the Demographic Response of Plants to Habitat
1005 Fragmentation. The American Naturalist 169:383-397.

1006 Waite S, Hutchings MJ (1991) The effects of different management regimes on the
1007 population dynamics of *Ophrys sphegodes*: analysis and description using matrix
1008 models, in: Wells, T.C.E & Willems, J, Population Ecology of Terrestrial Orchids,
1009 SPB Academic Publishing, The Hague, pp. 161-175.

1010 Willi Y, Van Buskirk J, Fischer M (2005) A threefold genetic allee effect: population size
1011 affects cross-compatibility, inbreeding depression and drift load in the self-
1012 incompatible *Ranunculus reptans*. *Genetics* 169: 2255-65.

1014 **Captions for Figures**

1015

1016 **Figure 1.** Localization of the four study populations of *Brassica insularis* in Corsica. In the
1017 text, Punta Calcina and Punta Corbaghiola are referred to as Calcina and Corbaghiola,
1018 respectively.

1019 **Figure 2.** Life-cycle of *Brassica insularis*. Three stages are considered: seedlings,
1020 vegetative and flowering individuals. The time-step is one year. The upper-vital rates (a_{ij})
1021 correspond to the transitions between stages, the lower-vital rates (s_i) correspond to survival
1022 probabilities ($i=1, 2, 3$), (p_i) to flowering probabilities ($i=2, 3$), and f is the fecundity term.

1023 **Figure 3.** Contribution (*100) of the lower vital rates variation (s_i and p_j) to asymptotic
1024 growth rate λ_a variation per population.

1025 **Figure 4.** Observed stage structure (OSD) per population and year and stable stage structure
1026 (SSD) per population. The percentages of each of the three stages are shown in light grey
1027 (seedlings), black (vegetative individuals) and dark grey (flowering individuals). The stars
1028 indicate years when the stage-structure are significantly different (after sequential
1029 Bonferroni correction) from the corresponding stable stage-structure (last distribution on the
1030 right) calculated from the mean matrices over all years per population.

1031 **Figure 5.** Quasi-extinction probabilities per population (P_{ext}) after 50 years for different
1032 quasi-extinction thresholds n assuming the average stage distribution over the 10 years. The
1033 extinction probabilities were computed from 5000 replicates each. Initial population sizes
1034 for the simulations for each population were 300 (Corbaghiola), 100 (Calcina), 500
1035 (Inzecca) and 2000 (Teghime).

1036 Table 1: Summary of sample size for demographic data (2000 – 2009) for each
1037 population of *Brassica insularis*.

| 1038 | Population | Teghime | Inzecca | Calcina | Corbaghiola |
|------|------------------|---------|---------|---------|-------------|
| 1039 | Year | | | | |
| 1040 | 2000 | 199 | 113 | 77 | 54 |
| 1041 | 2001 | 252 | 167 | 92 | 36 |
| 1042 | 2002 | 218 | 100 | 88 | 47 |
| 1043 | 2003 | 263 | 162 | 92 | 69 |
| 1044 | 2004 | 240 | 151 | 88 | 60 |
| 1045 | 2005 | 256 | 160 | 95 | 68 |
| 1046 | 2006 | 233 | 128 | 95 | 63 |
| 1047 | 2007 | 260 | 125 | 87 | 65 |
| 1048 | 2008 | 265 | 121 | 86 | 59 |
| 1049 | 2009 | 247 | 113 | 89 | 71 |
| 1050 | Mean/year | 243.3 | 134 | 88.9 | 59.2 |
| 1051 | Total since 2000 | 554 | 360 | 169 | 151 |
| 1052 | Approximate | | | | |
| 1053 | Population size | 2000 | 500 | 100 | 300 |

Table 2: Arithmetic mean values and standard deviation (SD) of the upper-level (a_{ij}) and the lower-level (s_x and p_y) vital rates, calculated over the 36 population-year matrices. The elasticities were calculated for the arithmetic mean matrix.

| <i>Upper-Level Vital rates</i> | | | | | | |
|--------------------------------|----------|----------|----------|----------|----------|----------|
| <i>Parameter</i> | a_{13} | a_{21} | a_{22} | a_{23} | a_{32} | a_{33} |
| <i>Mean</i> | 0.331 | 0.581 | 0.583 | 0.311 | 0.245 | 0.615 |
| <i>SD</i> | 0.424 | 0.209 | 0.166 | 0.181 | 0.168 | 0.179 |
| <i>Elasticities</i> | 0.068 | 0.068 | 0.272 | 0.105 | 0.173 | 0.314 |
| <i>Lower-Level Vital rates</i> | | | | | | |
| <i>Parameter</i> | s_1 | s_2 | s_3 | p_2 | p_3 | |
| <i>Mean</i> | 0.581 | 0.829 | 0.926 | 0.296 | 0.664 | |
| <i>SD</i> | 0.209 | 0.085 | 0.053 | 0.196 | 0.190 | |
| <i>Elasticities</i> | 0.068 | 0.445 | 0.419 | 0.058 | 0.106 | |

Table 3: Stochastic growth rates λ_s and mean asymptotic growth rate λ_a per population. The λ_a (00-09) over all years was calculated from the arithmetic mean matrix per population. CI λ_a is the confidence interval for asymptotic λ_a calculated by bootstrapping over the years. Asymptotic growth-rate corresponding to individual population matrices are also given for each pair of years.

| <i>Population</i> | λ_s (00-09) | λ_a (00-09) | CI λ_a | λ_a (00-01) | λ_a (01-02) | λ_a (02-03) | λ_a (03-04) | λ_a (04-05) | λ_a (05-06) | λ_a (06-07) | λ_a (07-08) | λ_a (08-09) |
|--------------------|---------------------|---------------------|----------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| <i>Corbaghiola</i> | 0.885 | 0.891 | 0.712-0.921 | 0.831 | 0.896 | 1.017 | 0.500 | 1.003 | 0.880 | 0.865 | 0.873 | 0.812 |
| <i>Calcina</i> | 0.942 | 0.946 | 0.875-0.997 | 0.940 | 1.032 | 0.895 | 0.885 | 1.053 | 0.975 | 0.765 | 1.031 | 0.884 |
| <i>Inzecca</i> | 0.899 | 0.900 | 0.866-0.930 | 0.850 | 0.881 | 0.956 | 0.927 | 0.858 | 0.814 | 0.956 | 0.950 | 0.920 |
| <i>Teghime</i> | 1.005 | 1.017 | 0.921-1.102 | 1.201 | 0.970 | 1.046 | 0.843 | 1.208 | 0.910 | 1.088 | 1.054 | 0.784 |

1 Table 4. Results from RAMAS simulation during 100 time steps (years): proportion of time
 2 steps during which each population remain extant (Minimum, Average, Maximum, and + or
 3 - 1 standard deviation, for 5000 replicates). A) No density-dependence, initial population
 4 sizes are observed sizes (300, 100, 500 and 2000 for Corbaghiola, Calcina, Inzecca and
 5 Teghime, respectively). B) No density-dependence, initial population size = 2000 in each
 6 population. For each population the initial conditions correspond to the stable age structure
 7 of the mean matrix. C) Scramble competition with an Allee effect (threshold level=3
 8 individuals), initial conditions as in A, respective carrying-capacity = 280, 80, 480 and
 9 2500, Maximum growth-rate 1.01). D) Same as C with maximum growth-rate=1.09.

10

| 11 | A. Population | Number of Time Steps | | | | |
|----|---------------|----------------------|---------|---------|---------|---------|
| 12 | | Minimum | -1 S.D. | Average | +1 S.D. | Maximum |
| 13 | Corbaghiola | 19.0 | 29.5 | 35.4 | 41.3 | 62.0 |
| 14 | Calcina | 18.0 | 29.3 | 36.5 | 43.8 | 79.0 |
| 15 | Inzecca | 25.0 | 35.7 | 41.7 | 47.7 | 73.0 |
| 16 | Teghime | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

17

| 18 | B. Population | Number of Time Steps | | | | |
|----|---------------|----------------------|---------|---------|---------|---------|
| 19 | | Minimum | -1 S.D. | Average | +1 S.D. | Maximum |
| 20 | Corbaghiola | 35.0 | 45.2 | 51.6 | 58.1 | 85.0 |
| 21 | Calcina | 63.0 | 78.7 | 86.5 | 94.4 | 100.0 |
| 22 | Inzecca | 38.0 | 48.3 | 54.4 | 60.6 | 81.0 |
| 23 | Teghime | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

24

| 25 | C. Population | Number of Time Steps | | | | |
|----|---------------|----------------------|---------|---------|---------|---------|
| 26 | | Minimum | -1 S.D. | Average | +1 S.D. | Maximum |
| 27 | Corbaghiola | 21.0 | 36.5 | 49.1 | 61.7 | 100.0 |
| 28 | Calcina | 7.0 | 13.5 | 17.0 | 20.5 | 35.0 |
| 29 | Inzecca | 34.0 | 57.3 | 72.7 | 88.1 | 100.0 |
| 30 | Teghime | 70.0 | 97.4 | 99.7 | 101.9 | 100.0 |

31

| 32 | D. Population | Number of Time Steps | | | | |
|----|---------------|----------------------|---------|---------|---------|---------|
| 33 | | Minimum | -1 S.D. | Average | +1 S.D. | Maximum |
| 34 | Corbaghiola | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 35 | Calcina | 9.0 | 16.9 | 23.4 | 29.9 | 61.0 |
| 36 | Inzecca | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 37 | Teghime | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

38

39

41 Table 5. Summary of the ecological data measured in the field in our study (only in 2007
 42 for the competition measures and soil substrate). The semi-quantitative competition values
 43 are the average of the individual values as 0 for “null”, 1 for “low”, 2 for “medium” or 3 for
 44 “high”; the qualitative measures of competition, soil substrate and plant aphids attack are
 45 expressed in percentage for each population. SD = standard deviation.

| <i>Population</i> | <i>Teghime</i> | <i>Inzecca</i> | <i>Corbaghiola</i> | <i>Calcina</i> |
|--|----------------|----------------|--------------------|----------------|
| <i>Semi-quantitative competition</i> | | | | |
| <i>Intra-specific quantitative competition</i> | 0.750 | 0.367 | 0.375 | 1.110 |
| <i>SD</i> | 0.906 | 0.600 | 0.655 | 1.080 |
| <i>Inter-specific quantitative competition</i> | 1.496 | 1.102 | 1.672 | 0.989 |
| <i>SD</i> | 0.992 | 0.840 | 1.055 | 0.876 |
| <i>Inter-specific qualitative competition</i> | | | | |
| <i>Herbaceous</i> | 0.685 | 0.438 | 0.703 | 0.604 |
| <i>Woody</i> | 0.142 | 0.313 | 0.125 | 0.077 |
| <i>Herbaceous-woody</i> | 0.008 | 0.000 | 0.047 | 0.000 |
| <i>Null</i> | 0.165 | 0.250 | 0.125 | 0.319 |
| <i>Soil substrate</i> | | | | |
| <i>Grassy</i> | 0.627 | 0.297 | 0.516 | 0.451 |
| <i>Bare</i> | 0.369 | 0.438 | 0.266 | 0.549 |
| <i>Scree</i> | 0.000 | 0.258 | 0.219 | 0.000 |
| <i>ND</i> | 0.004 | 0.008 | 0.000 | 0.000 |
| <i>Plant aphids attack</i> | | | | |

Yes

0.069

0.219

0.391

0.681

46 Table 6. Summary of genetic data (mean and standard deviation or, for F_{IS} , bootstrap confidence interval) obtained from individuals
 47 sampled in 2009 (this study) and in 2000 (described in Glemin et al. 2005) in each population. Abbreviations correspond to Mean
 48 number of alleles per locus (Nb_All), unbiased Heterozygosity (H_e), Fixation index (F_{IS}) and Theta ($\theta = 4N_e\mu$ estimated assuming a
 49 stepping mutation model, see text). Significant F_{IS} values are in bold.

| Year Population | Nb_All | | H_e | | F_{IS} | | θ | |
|--------------------|----------------|----------------|----------------|----------------|--------------------------------|----------------------------|----------------|----------------|
| | 2000 | 2009 | 2000 | 2009 | 2000 | 2009 | 2000 | 2009 |
| <i>Calcina</i> | 1.67 (1.32) | 1.56 (0.73) | 0.13 (0.23) | 0.07 (0.13) | -0.10 -0.22 - -0.007 | 0.12 -0.04 - 0.25 | 0.53 (1.31) | 0.12 (0.24) |
| <i>Corbaghiola</i> | 3.89 (1.45) | 3.56 (1.42) | 0.47 (0.23) | 0.48 (0.22) | 0.14 0.03 - 0.22 | 0.20 0.07 - 0.30 | 2.19 (1.91) | 2.20 (1.74) |
| <i>Inzecca</i> | 3.89 | 3.56 | 0.40 | 0.44 | -0.05 | 0.05 | 2.71 | 2.83 |

Teghime

| | | | | | | | |
|--------|--------|--------|--------|--------------|--------------|--------|--------|
| (2.31) | (2.65) | (0.31) | (0.30) | -0.14 - 0.02 | -0.06 - 0.13 | (3.46) | (3.14) |
| 4.44 | 3.89 | 0.46 | 0.45 | 0.20 | 0.17 | 3.80 | 4.38 |
| (2.55) | (2.47) | (0.26) | (0.30) | 0.12 - 0.25 | 0.09 - 0.23 | (5.71) | (6.72) |

50 Table 7. Fst matrix between pairs of populations within each year (*A*, *B*) and between the
 51 two years within each population (*C*). Mean and confidence intervals (bootstrap over loci)
 52 are given. Values significantly different from 0 are in bold characters. Among-population
 53 matrices of 2000 and 2009 were significantly correlated ($r = 0.98$, Mantel test $P = 0.001$).

54 **A. 2000**

| | Corbaghiola | Inzecca | Teghime |
|----|-------------|-------------|--------------|
| 55 | | | |
| 56 | | | |
| 57 | Calcina | 0.55 | 0.27 |
| 58 | | (0.41-0.67) | (0.20-0.53) |
| 59 | | | (0.06- 0.40) |
| 60 | Corbaghiola | 0.37 | 0.32 |
| 61 | | (0.21-0.58) | (0.24-0.42) |
| 62 | | | |
| 63 | Inzecca | | 0.19 |
| 64 | | | (0.12- 0.25) |
| 65 | | | |

66 **B. 2009**

| | Corbaghiola | Inzecca | Teghime |
|----|-------------|-------------|--------------|
| 67 | | | |
| 68 | | | |
| 69 | Calcina | 0.60 | 0.30 |
| 70 | | (0.44-0.72) | (0.24- 0.48) |
| 71 | | | (0.21-0.39) |
| 72 | Corbaghiola | 0.35 | 0.34 |
| 73 | | (0.24-0.51) | (0.20-0.53) |
| 74 | | | |
| 75 | Inzecca | | 0.18 |
| 76 | | | (0.12-0.28) |
| 77 | | | |

78 **C. 2000 versus 2009**

| | F _{ST} | (CI) |
|----|-----------------|--------------------------|
| 79 | | |
| 80 | Calcina | 0.07 (0.01- 0.09) |
| 81 | Corbaghiola | 0.02 (-0.003-0.05) |
| 82 | Inzecca | 0.03 (-0.002 - 0.08) |
| 83 | Teghime | 0.04 (-0.001 - 0.10) |

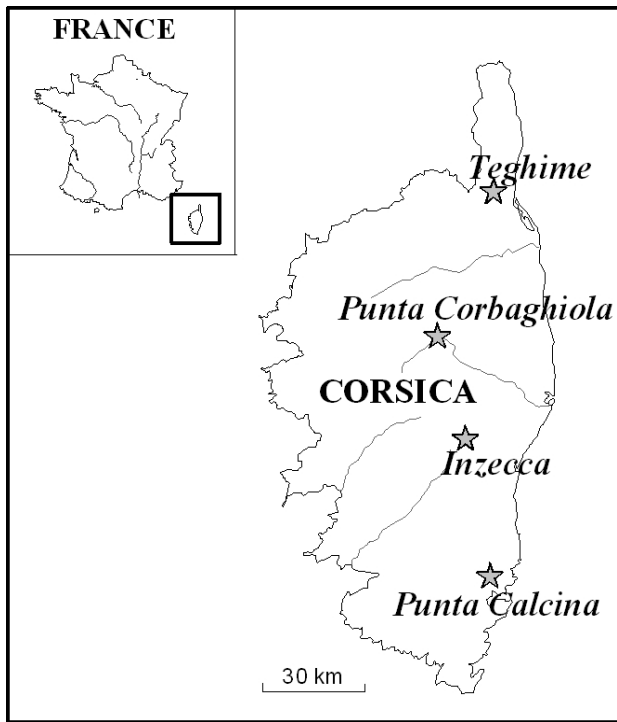
84 Table 8. Summary of population characteristics

85

| | Teghime | Inzecca | Calcina | Corbaghiola |
|--------------------------|----------------------------|--------------------------------|---------------------------|---------------------------------|
| Population size | Large (2000) | Moderate (500) | Very small (100) | Small to Moderate (300) |
| S-locus Diversity | Large | Moderate | Low | Moderate |
| Microsatellite Diversity | Moderate | Moderate | Low | Moderate |
| Genetic structure | strong | none | none | strong |
| Temperature | Cold winter Cool summer | Mild winter Very hot summer | Mild winter Hot summer | Very cold winter Cool summer |
| Rainfall | Low to Moderate | Very large | Moderate | Moderate |
| Herbivory | Safe | Moderate Aphid herbivory | Heavy Aphids herbivory | Moderate Aphid herbivory |
| Survival rates | Large | Moderate | Moderate | Low to Moderate |
| Flowering probabilities | Low | Moderate | Large | Large |

| | | | | |
|--|-----------|-------------------------------------|--------------------------|-------------------------------------|
| Recruitment rates | Large | Moderate | Low | Moderate |
| Time to extinction (assuming no density- dependence) | infinite | 42 years | 37 years | 35 years |
| Time to extinction (assuming scramble density dependence) | 100 years | 73 – 100 years | 17 – 23 years | 37-100 years |
| Recommendations | Surveys | Surveys + possibly translocation | Surveys + reinforcements | Surveys + possibly translocation |

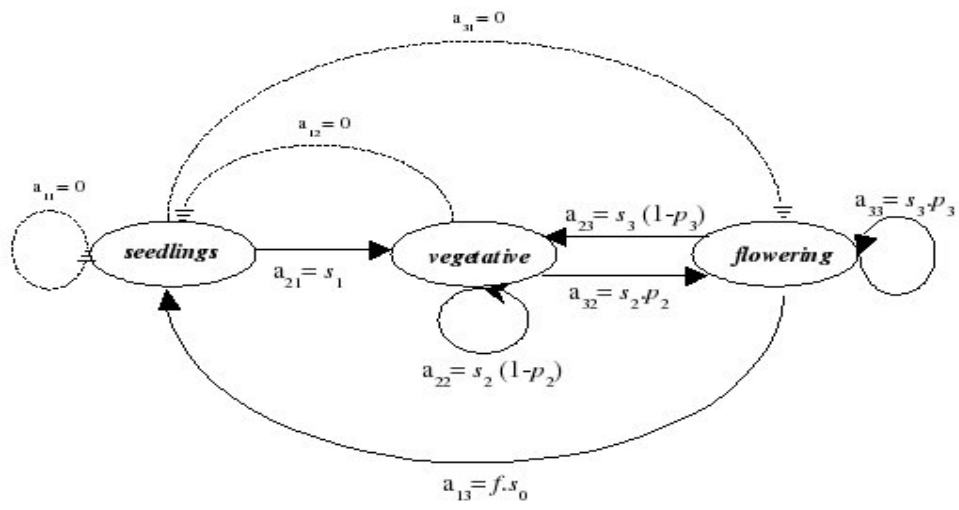
86



87

88 Figure 1

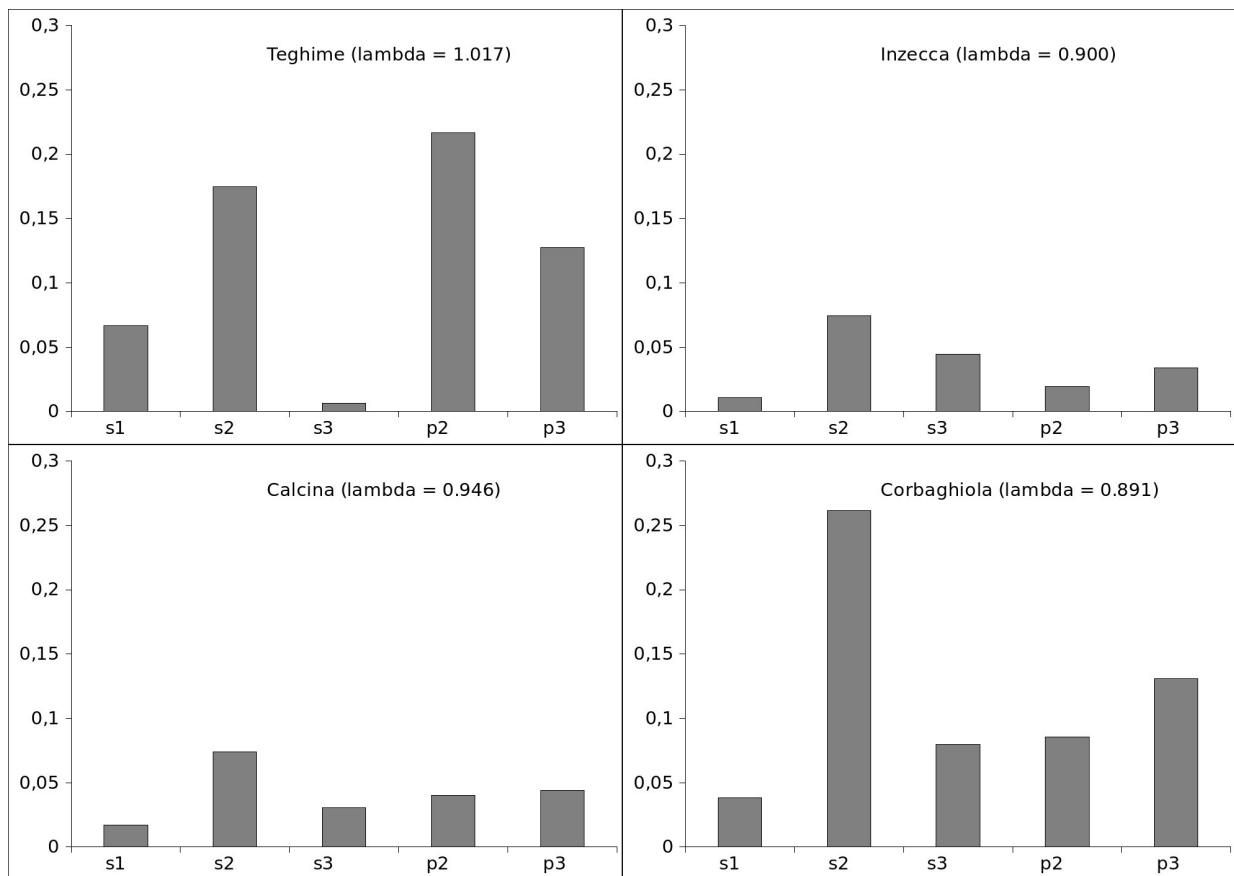
90



91

92 Figure 2.

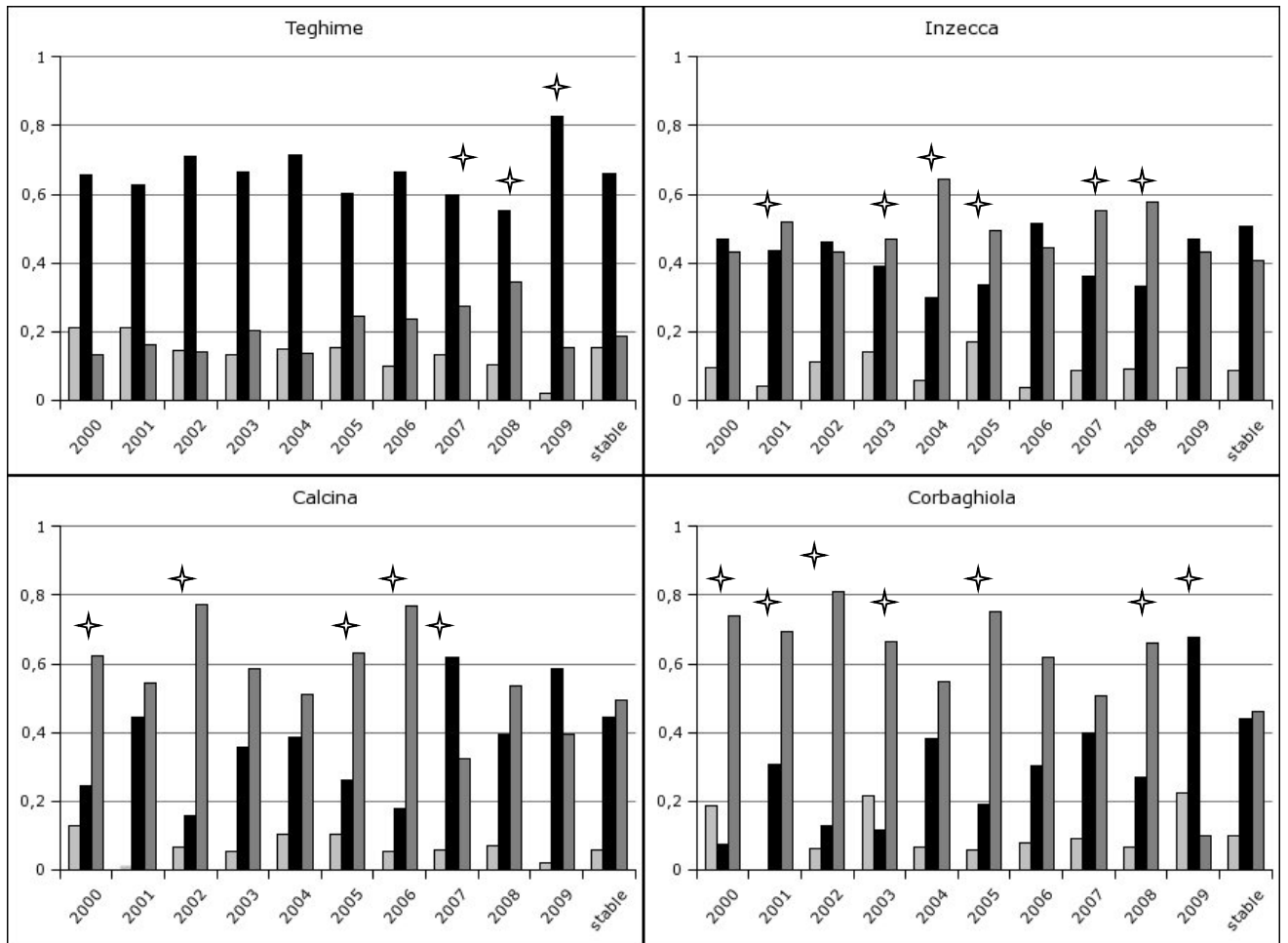
94 Figure 3.



95

96

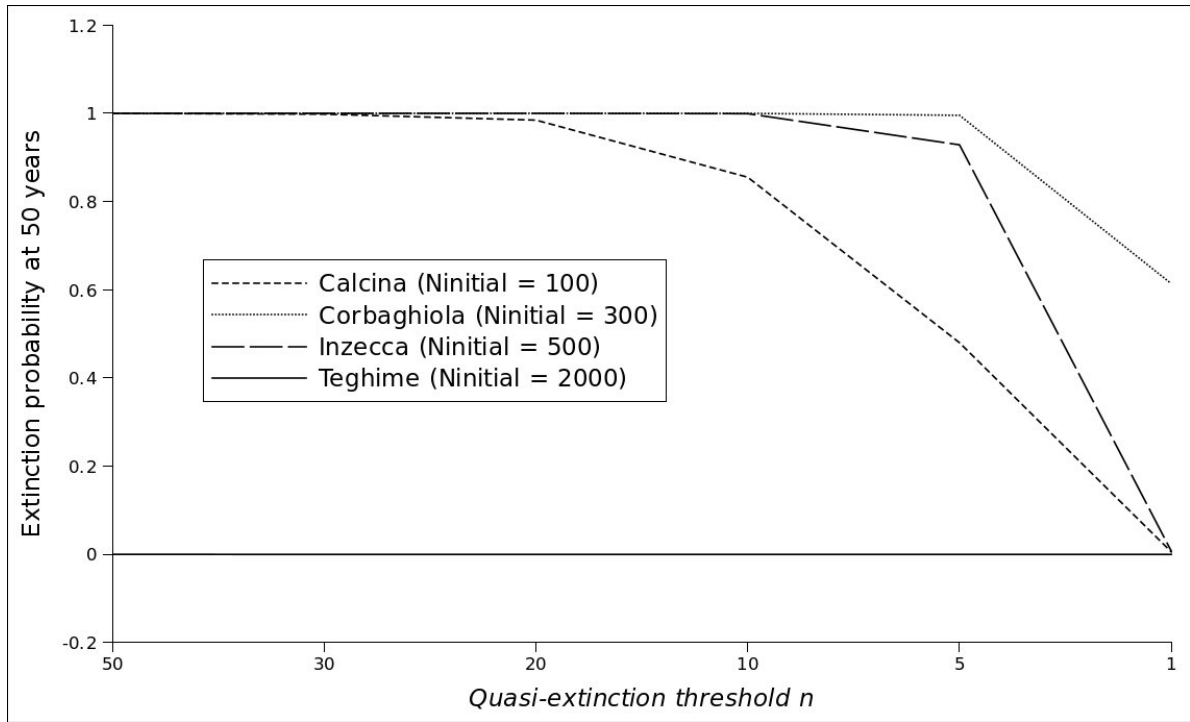
98 Figure 4.



99

100

102 Figure 5



103

104

105 **SUPPLEMENTARY MATERIAL**

106 **Appendix A – Sampling protocol and genotyping of microsatellite markers**

107

108 In 2009, individuals from the four populations studied were sampled for genetic analysis. A
109 total of 238 individuals were randomly sampled (94 in Teghime, 55 in Inzecca, 49 in
110 Corbaghiola and 40 in Calcina). DNA from all individuals was extracted using the method
111 of Doyle and Doyle (1987). We used 11 microsatellite markers (38A, 10-B01, 10-B06, 10-
112 D08, 10-E06, 10-F07, 12-D02, AT129, 59A, 26A, 11-H09; see Glémin et al 2005 for
113 references). Amplifications were done for a total of 192 individuals (in 96 PCR plates, so
114 96*2): Teghime 70, Corbaghiola 41, Calcina 40 and Inzecca 41. The number of individuals
115 analysed per population in 2009 is similar to the sampling used in Glémin et al (2005).

116

117 Two different genotyping protocols were used between 2000 and 2009. In 2000, reverse
118 microsatellite primers were labelled with \blacksquare -³³P. The size of the amplification fragments
119 were then determined by radiography after migration on 8% acrylamide-bisabrylamide/
120 TBE gels for loci ,300 bp and on 8% acrylamide-bisabrylamide/TTE gels for the other loci..
121 The exact sizes of the fragments were determined using the puc 19 sequence (EMBL no.
122 L09137) as a reference. In 2009, the reverse microsatellite primers used were labelled with
123 a fluorescent dye. PCR amplifications were carried out in a total volume of 10 µl,
124 containing the PCR buffer DreamTaq Fermentas [20mM Tris-HCl (pH 8.0), 1mM DTT, 0.1
125 mM EDTA, 100mM KCl, 0.5% Nonidet P40, 0.5% Tween 20 and 50% glycerol]; 10 ng of
126 genomic DNA; 0.5 unit of Taq DNA polymerase; 0.2 mM of each dNTP; 0.7 µM of each
127 primer labelled with 6-FAM, Cy5 or Cy3 for forward primers; and 1mM (38A, B01, B06,

128 D08, E06, F07, D02), 2mM (AT129, 59A, 26A) or 0.5mM (H09) of MgCl₂. PCR
129 amplifications were carried out on a PTC-100 of MJ Research and samples were amplified
130 as follows: 2 min at 94°; 30 cycles of 30 sec at 94°, 30 sec at annealing temperature of each
131 locus, 50 sec at 72°; and 2 min at 72°; or a “touchdown” program for loci (10-B06, 10-D08,
132 10-E06, 10-F07, 11-H09 and 12-D02) that did not amplified with the standard protocol.
133 After PCR, products were separated by electrophoresis on 6% acryl-bisacrylamide gels, and
134 sized relative to an 82 molecular size marker (Internal Lane Standard 600, Promega).
135 Genotyping was performed on the Hitachi FMBio scanner and photographs of gels were
136 analysed with the FMBio Analysis 8.0 software.

137

138 **References:**

139 Doyle, J. J., and J. L. Doyle, 1987 A rapid DNA isolation procedure for small quantities of
140 fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.
141 Glémin, S., Gaude, T., Guillemin, M.L., Lourmas, M., Olivieri, I., Mignot, A., 2005.
142 Balancing selection in the wild: Testing population genetics theory of self-incompatibility
143 in the rare species *Brassica insularis*. *Genetics* 171, 279-289.

144 **Appendix B. Vital rates per population and year**

145

146 **1. Upper-level vital rates**

147

| Population | Year | <i>a13</i> | <i>a21</i> | <i>a22</i> | <i>a23</i> | <i>a32</i> | <i>a33</i> |
|--------------------|-------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Corbaghiola | 00-01 | 0.000 | 0.714 | 0.250 | 0.312 | 0.500 | 0.563 |
| | 01-02 | 0.143 | 0.516 | 0.364 | 0.048 | 0.545 | 0.762 |
| | 02-03 | 0.536 | 0.667 | 0.499 | 0.286 | 0.333 | 0.607 |
| | 03-04 | 0.114 | 0.400 | 0.500 | 0.457 | 0.000 | 0.457 |
| | 04-05 | 0.129 | 0.500 | 0.304 | 0.097 | 0.434 | 0.903 |
| | 05-06 | 0.111 | 0.500 | 0.538 | 0.222 | 0.308 | 0.622 |
| | 06-07 | 0.158 | 0.600 | 0.474 | 0.316 | 0.263 | 0.579 |
| | 07-08 | 0.129 | 0.000 | 0.423 | 0.129 | 0.346 | 0.774 |
| | 08-09 | 0.421 | 0.750 | 0.812 | 0.815 | 0.000 | 0.132 |
| | mean | 0.170 | 0.557 | 0.418 | 0.248 | 0.340 | 0.642 |
| | SD | 0.169 | 0.109 | 0.112 | 0.140 | 0.182 | 0.146 |
| Calcina | 00-01 | 0.021 | 0.900 | 0.579 | 0.458 | 0.316 | 0.521 |
| | 01-02 | 0.120 | 1.000 | 0.341 | 0.179 | 0.586 | 0.780 |
| | 02-03 | 0.074 | 0.500 | 0.571 | 0.309 | 0.215 | 0.662 |
| | 03-04 | 0.167 | 0.400 | 0.576 | 0.296 | 0.212 | 0.630 |
| | 04-05 | 0.222 | 0.556 | 0.441 | 0.111 | 0.441 | 0.889 |
| | 05-06 | 0.083 | 0.400 | 0.280 | 0.084 | 0.640 | 0.867 |
| | 06-07 | 0.068 | 0.400 | 0.765 | 0.548 | 0.000 | 0.356 |
| | 07-08 | 0.214 | 0.800 | 0.518 | 0.321 | 0.371 | 0.679 |
| | 08-09 | 0.044 | 0.333 | 0.647 | 0.489 | 0.206 | 0.444 |
| | mean | 0.113 | 0.588 | 0.524 | 0.311 | 0.332 | 0.648 |
| | SD | 0.073 | 0.248 | 0.150 | 0.165 | 0.202 | 0.183 |
| Inzecca | 00-01 | 0.070 | 0.300 | 0.634 | 0.260 | 0.200 | 0.590 |
| | 01-02 | 0.138 | 0.714 | 0.658 | 0.575 | 0.164 | 0.375 |
| | 02-03 | 0.548 | 0.636 | 0.500 | 0.524 | 0.283 | 0.405 |
| | 03-04 | 0.127 | 0.545 | 0.540 | 0.113 | 0.317 | 0.774 |
| | 04-05 | 0.284 | 0.556 | 0.578 | 0.242 | 0.155 | 0.621 |
| | 05-06 | 0.067 | 0.481 | 0.482 | 0.319 | 0.296 | 0.493 |
| | 06-07 | 0.193 | 0.600 | 0.454 | 0.193 | 0.379 | 0.719 |
| | 07-08 | 0.162 | 0.545 | 0.533 | 0.132 | 0.288 | 0.794 |
| | 08-09 | 0.157 | 0.364 | 0.825 | 0.214 | 0.100 | 0.629 |
| | mean | 0.204 | 0.547 | 0.578 | 0.286 | 0.256 | 0.568 |
| | SD | 0.169 | 0.132 | 0.114 | 0.163 | 0.085 | 0.152 |
| Teghime | 00-01 | 2.120 | 0.848 | 0.840 | 0.401 | 0.122 | 0.560 |
| | 01-02 | 0.800 | 0.811 | 0.880 | 0.450 | 0.038 | 0.500 |
| | 02-03 | 1.129 | 0.312 | 0.766 | 0.161 | 0.117 | 0.839 |
| | 03-04 | 0.692 | 0.486 | 0.742 | 0.519 | 0.040 | 0.481 |
| | 04-05 | 1.182 | 0.833 | 0.724 | 0.061 | 0.182 | 0.878 |
| | 05-06 | 0.371 | 0.667 | 0.693 | 0.436 | 0.131 | 0.483 |
| | 06-07 | 0.630 | 0.913 | 0.723 | 0.277 | 0.191 | 0.666 |
| | 07-08 | 0.431 | 0.806 | 0.677 | 0.246 | 0.207 | 0.739 |
| | 08-09 | 0.055 | 0.571 | 0.753 | 0.626 | 0.021 | 0.330 |

| | | | | | | | |
|--------------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|
| | mean | 0.823 | 0.694 | 0.755 | 0.353 | 0.117 | 0.608 |
| | SD | 0.603 | 0.201 | 0.066 | 0.180 | 0.070 | 0.184 |
| Mean matrix | | 0.331 | 0.581 | 0.583 | 0.311 | 0.245 | 0.615 |
| sensitivity | | 0.196 | 0.112 | 0.445 | 0.322 | 0.673 | 0.487 |
| elasticity | | 0.068 | 0.068 | 0.272 | 0.105 | 0.173 | 0.314 |

148

149

150

2. Lower-level vital rates

| Population | Year | s1 | s2 | s3 | p2 | p3 |
|--------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Corbaghiola | 00-01 | 0.714 | 0.750 | 0.875 | 0.667 | 0.643 |
| | 01-02 | 0.516 | 0.909 | 0.810 | 0.600 | 0.941 |
| | 02-03 | 0.667 | 0.832 | 0.893 | 0.400 | 0.680 |
| | 03-04 | 0.400 | 0.500 | 0.914 | 0.000 | 0.500 |
| | 04-05 | 0.500 | 0.738 | 1.000 | 0.588 | 0.903 |
| | 05-06 | 0.500 | 0.846 | 0.844 | 0.364 | 0.737 |
| | 06-07 | 0.600 | 0.737 | 0.895 | 0.357 | 0.647 |
| | 07-08 | 0.000 | 0.769 | 0.903 | 0.450 | 0.857 |
| | 08-09 | 0.750 | 0.812 | 0.947 | 0.000 | 0.139 |
| | mean | 0.557 | 0.759 | 0.890 | 0.425 | 0.722 |
| SD | 0.109 | 0.131 | 0.060 | 0.225 | 0.155 | |
| Calcina | 00-01 | 0.900 | 0.895 | 0.979 | 0.353 | 0.532 |
| | 01-02 | 1.000 | 0.927 | 0.959 | 0.632 | 0.813 |
| | 02-03 | 0.500 | 0.786 | 0.971 | 0.274 | 0.682 |
| | 03-04 | 0.400 | 0.788 | 0.926 | 0.269 | 0.680 |
| | 04-05 | 0.556 | 0.882 | 1.000 | 0.500 | 0.889 |
| | 05-06 | 0.400 | 0.920 | 0.951 | 0.696 | 0.912 |
| | 06-07 | 0.400 | 0.765 | 0.904 | 0.000 | 0.394 |
| | 07-08 | 0.800 | 0.889 | 1.000 | 0.417 | 0.679 |
| | 08-09 | 0.333 | 0.853 | 0.933 | 0.242 | 0.476 |
| | mean | 0.588 | 0.852 | 0.956 | 0.389 | 0.700 |
| SD | 0.248 | 0.070 | 0.032 | 0.240 | 0.190 | |
| Inzecca | 00-01 | 0.300 | 0.834 | 0.850 | 0.240 | 0.694 |
| | 01-02 | 0.714 | 0.822 | 0.950 | 0.200 | 0.395 |
| | 02-03 | 0.636 | 0.783 | 0.929 | 0.361 | 0.436 |
| | 03-04 | 0.545 | 0.857 | 0.887 | 0.370 | 0.873 |
| | 04-05 | 0.556 | 0.733 | 0.863 | 0.211 | 0.720 |
| | 05-06 | 0.481 | 0.778 | 0.812 | 0.380 | 0.607 |
| | 06-07 | 0.600 | 0.833 | 0.912 | 0.455 | 0.788 |
| | 07-08 | 0.545 | 0.821 | 0.926 | 0.351 | 0.857 |
| | 08-09 | 0.364 | 0.925 | 0.843 | 0.108 | 0.746 |
| | mean | 0.547 | 0.806 | 0.886 | 0.317 | 0.645 |
| SD | 0.132 | 0.043 | 0.048 | 0.099 | 0.177 | |
| Teghime | 00-01 | 0.848 | 0.962 | 0.961 | 0.127 | 0.583 |
| | 01-02 | 0.811 | 0.918 | 0.950 | 0.041 | 0.526 |
| | 02-03 | 0.312 | 0.883 | 1.000 | 0.133 | 0.839 |
| | 03-04 | 0.486 | 0.782 | 1.000 | 0.051 | 0.481 |
| | 04-05 | 0.833 | 0.906 | 0.939 | 0.201 | 0.935 |
| | 05-06 | 0.667 | 0.824 | 0.919 | 0.159 | 0.526 |

| | | | | | | |
|--------------------|-------------|--------------|--------------|--------------|--------------|--------------|
| | 06-07 | 0.913 | 0.914 | 0.943 | 0.209 | 0.706 |
| | 07-08 | 0.806 | 0.884 | 0.985 | 0.234 | 0.750 |
| | 08-09 | 0.571 | 0.774 | 0.956 | 0.027 | 0.345 |
| | mean | 0.694 | 0.884 | 0.959 | 0.132 | 0.657 |
| | SD | 0.201 | 0.061 | 0.031 | 0.066 | 0.175 |
| Mean matrix | | 0.581 | 0.829 | 0.926 | 0.296 | 0.664 |
| sensitivity | | 0.112 | 0.512 | 0.431 | 0.188 | 0.152 |
| elasticity | | 0.068 | 0.445 | 0.419 | 0.058 | 0.106 |

151

152

153 **Appendix C. Spatio-temporal variations of vital rates**

154

155 **1- Log-linear analysis of the upper-level vital rates**

156 Spatio-temporal variations and log-linear analysis of the upper-level vital rates (i.e.
157 transitions a_{ij}). The matrices were compared excluding fecundity and adding death as a fate.
158 A constant 0.5 was added to all cells prior to analysis. The explanatory variables are
159 population (P) and Year (Y). The null model is a model with the response variable
160 corresponding to the fate F of individuals at time $t+1$ conditional to the state S at time t with
161 no additional effects of populations or years. The best model is marked in bold.

| <i>Model</i> | <i>G2</i> | <i>df</i> | <i>AIC</i> | <i>ΔAIC</i> |
|-----------------------------|--------------|------------|---------------|-------------|
| <i>all populations</i> | | | | |
| null model | 940.2 | 384 | 172.2 | 221.1 |
| null model + Y | 641.2 | 288 | 65.2 | 114.1 |
| null model + P | 601.9 | 288 | 25.9 | 74.8 |
| null model + (P + Y) | 383.1 | 216 | - 48.9 | 0 |
| null model + (P * Y) | 0 | 0 | 0 | 48.9 |

162

163 **2- Generalized linear model for the lower-level vital rates**

164 Spatio-temporal variations of lower-level vital rates. Results of the generalized linear model
165 using a logit link function and specifying binomial error, for the survival of seedlings (s_1),
166 vegetative (s_2) and flowering individuals (s_3), and the probability of vegetative (p_2) and
167 flowering individuals (p_3) to flower the next year. For each parameter, the best model and

168 the alternative good model ($\Delta AIC \leq 2$) is indicated. The explanatory variables are
169 population (P) and Year (Y).

| <i>Parameter</i> | best model | good model |
|------------------|-------------------|-------------------|
| s_1 | $Y * P$ | $Y + P$ |
| s_2 | $Y + P$ | - |
| s_3 | Y | $Y + P$ |
| p_2 | $Y * P$ | - |
| p_3 | $Y * P$ | - |

Appendix D: Meteorological variables per year and population. ‘Year’ corresponds to a period of overlapping years (00-01 refers to data from April 2000 to March 2001). Rainfall : Total amount of rainfall (mm) over the period. TTD : Total number of degree-days (°C). MaxST : Maximum value of mean day temperature per summer month (April to September). MinWT: Minimum value of mean day temperature per winter month (October to March). Rain>1: total number of rain events >1mm per period. Rain>0.1: total number of rain events >0.1mm and < 1mm per year.

| Population | Year | Rainfall | TTD | MaxST | MinWT | Rain>1 | Rain>0.1 |
|------------|-------|----------|--------|-------|-------|--------|----------|
| Calcina | 00-01 | 868.6 | 5959.3 | 24.5 | 9.3 | 137 | 71 |
| Calcina | 01-02 | 463.4 | 5804.8 | 25.5 | 7.7 | 113 | 53 |
| Calcina | 02-03 | 1015.2 | 5581.5 | 23.6 | 5.7 | 158 | 91 |
| Calcina | 03-04 | 632.0 | 6006.9 | 28.2 | 8.3 | 138 | 70 |
| Calcina | 04-05 | 956.8 | 5493.3 | 24.2 | 5.7 | 162 | 81 |
| Calcina | 05-06 | 877.0 | 5552.4 | 24.6 | 6.6 | 164 | 72 |
| Calcina | 06-07 | 559.2 | 5969.9 | 26.6 | 9.6 | 152 | 48 |
| Calcina | 07-08 | 953.0 | 5667.0 | 24.2 | 8.3 | 146 | 72 |

| | | | | | | | |
|-------------|-------|--------|--------|------|------|-----|-----|
| Calcina | 08-09 | 1233.6 | 5682.5 | 25.0 | 7.8 | 150 | 87 |
| Corbaghiola | 00-01 | 878.1 | 3786.1 | 19.3 | 3.2 | 95 | 83 |
| Corbaghiola | 01-02 | 476.6 | 3762.2 | 20.4 | 1.2 | 58 | 53 |
| Corbaghiola | 02-03 | 832.4 | 3612.6 | 18.5 | 0.1 | 108 | 91 |
| Corbaghiola | 03-04 | 819.4 | 3935.3 | 22.3 | 2.5 | 105 | 93 |
| Corbaghiola | 04-05 | 956.6 | 3543.2 | 18.9 | 0.2 | 100 | 88 |
| Corbaghiola | 05-06 | 850.4 | 3538.6 | 19.0 | 0.5 | 102 | 80 |
| Corbaghiola | 06-07 | 472.2 | 3928.7 | 20.6 | 2.9 | 115 | 60 |
| Corbaghiola | 07-08 | 856.7 | 3910.1 | 18.8 | 3.1 | 155 | 74 |
| Corbaghiola | 08-09 | 1230.7 | 3551.6 | 18.8 | 1.5 | 173 | 97 |
| Inzecca | 00-01 | 1588.0 | 6687.2 | 28.2 | 10.9 | 169 | 107 |
| Inzecca | 01-02 | 945.4 | 6543.4 | 27.9 | 9.1 | 130 | 70 |
| Inzecca | 02-03 | 1333.2 | 6316.9 | 25.6 | 8.1 | 182 | 114 |
| Inzecca | 03-04 | 1122.7 | 6951.7 | 31.0 | 9.8 | 140 | 97 |
| Inzecca | 04-05 | 1139.0 | 6378.7 | 26.7 | 7.5 | 152 | 99 |

| | | | | | | | |
|---------|-------|--------|--------|------|------|-----|-----|
| Inzecca | 05-06 | 1255.2 | 6403.0 | 26.9 | 9.0 | 160 | 99 |
| Inzecca | 06-07 | 935.7 | 6911.6 | 28.8 | 11.7 | 154 | 84 |
| Inzecca | 07-08 | 1173.5 | 6650.0 | 27.1 | 10.8 | 138 | 92 |
| Inzecca | 08-09 | 1750.3 | 6450.9 | 27.4 | 9.4 | 174 | 116 |
| Teghime | 00-01 | 809.8 | 4153.1 | 18.2 | 5.1 | 98 | 70 |
| Teghime | 01-02 | 390.6 | 3959.0 | 19.2 | 3.6 | 67 | 40 |
| Teghime | 02-03 | 820.8 | 3850.6 | 17.8 | 2.0 | 118 | 79 |
| Teghime | 03-04 | 674.5 | 4219.0 | 22.1 | 4.4 | 105 | 76 |
| Teghime | 04-05 | 802.5 | 3796.4 | 19.0 | 1.7 | 101 | 77 |
| Teghime | 05-06 | 909.9 | 3813.1 | 18.8 | 2.7 | 108 | 70 |
| Teghime | 06-07 | 549.2 | 4267.2 | 20.8 | 5.8 | 77 | 47 |
| Teghime | 07-08 | 904.0 | 3158.7 | 17.2 | 3.2 | 112 | 59 |
| Teghime | 08-09 | 1325.4 | 3633.7 | 18.6 | 2.8 | 152 | 84 |