

Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae) I. Pattern of pollen dispersal

O. J. HARDY,*† S. C. GONZÁLEZ-MARTÍNEZ,†‡ H. FRÉVILLE,†§ G. BOQUIEN,†
A. MIGNOT,† B. COLAS¶ & I. OLIVIERI†

*Laboratoire de Génétique et Ecologie Végétales, Université Libre de Bruxelles, Brussels, Belgium

†Institut des Sciences de l'Évolution de Montpellier, Université de Montpellier 2, Montpellier, France

‡Unidad de Genética Forestal, Centro de Investigación Forestal, Instituto Nacional de Investigación Agraria y Tecnología Alimentaria, Madrid, Spain

§Département Ecologie et Gestion de la Biodiversité, UMR 5176 CNRS-MNHN, Brunoy, France

¶Laboratoire Fonctionnement et Evolution des Systèmes Ecologiques, Université de Paris VI, Paris, France

Keywords:

Centaurea;
dispersal kernel;
endemic plants;
paternity analysis;
pollen dispersal;
selfing.

Abstract

Pollen dispersal was characterized within a population of the narrowly endemic perennial herb, *Centaurea corymbosa*, using exclusion-based and likelihood-based paternity analyses carried out on microsatellite data. Data were used to fit a model of pollen dispersal and to estimate the rates of pollen flow and mutation/genotyping error, by developing a new method. Selfing was rare (1.6%). Pollen dispersed isotropically around each flowering plant following a leptokurtic distribution, with 50% of mating pairs separated by less than 11 m, but 22% by more than 40 m. Estimates of pollen flow lacked precision (0–25%), partially because mutations and/or genotyping errors (0.03–1%) could also explain the occurrence of offspring without a compatible candidate father. However, the pollen pool that fertilized these offspring was little differentiated from the adults of the population whereas strongly differentiated from the other populations, suggesting that pollen flow rate among populations was low. Our results suggest that pollen dispersal is too extended to allow differentiation by local adaptation within a population. However, among populations, gene flow might be low enough for such processes to occur.

Introduction

Assessing the pattern of gene dispersal in natural populations is a central topic of evolutionary biology because (a) gene flow is a key determinant of the pattern of genetic variation of both neutral and selected genes, (b) dispersal capabilities of zygotes and gametes condition the demographic behaviour of populations and (c) dispersal rates are themselves under selection (e.g. Olivieri *et al.*, 1995). In plant species, gene flow is especially complex because it represents two levels of dispersal (pollen and seed). Pollen dispersal is often the major contributor to gene flow, spatial genetic structure being frequently a result of limited seed dispersal (Schnabel, 1998; Miyazaki & Isagi, 2000). New tools

and methods based on genetic markers allow a more detailed description of the pattern of gene flow in natural populations. In particular, the development of highly polymorphic genetic markers, such as microsatellites, now permits accurate paternity analyses (e.g. Dow & Ashley, 1996; Streiff *et al.*, 1999; Konuma *et al.*, 2000; Miyazaki & Isagi, 2000; methods reviewed in Jones & Ardren, 2003), and thus direct estimates of effective pollen dispersal. In this study, we applied such methods to a rare plant species for which assessment of gene dispersal is useful for conservation purposes (Lande, 1988; Richards, 2000; Hedrick, 2001). The rarity of this plant is also an opportunity to study patterns of pollen movement in great detail because almost all potential pollen donors can be identified within a population.

Our model is *Centaurea corymbosa* Pourret (Asteraceae), which is known from only six populations that are found within a 3 km² area along the French Mediterranean coast (Colas *et al.*, 1996). It occurs only in an extreme

Correspondence: Olivier Hardy, Laboratoire de Génétique et Ecologie Végétales, Chaussée de Wavre 1850, 1160 Bruxelles, Belgium.
Tel.: +32 2 650 9169; fax: +32 2 650 9170;
e-mail: ohardy@ulb.ac.be

habitat: the top of limestone cliffs where few other plant species survive. Besides extreme habitat specialization, the very narrow distribution of this species can also be attributed to its limited seed dispersal capabilities and the difficulties of successful establishment in new sites due to the monocarpic life cycle (i.e. each plant flowers only once) and the self-incompatibility system (Colas *et al.*, 1997, 2001; Fréville *et al.*, 2001). Since 1994, this species has been the focus of thorough multidisciplinary investigations regarding its ecology, demography and genetics. Genetic studies have mainly focused on the extent and partition within and among populations of the genetic variation of allozyme (Colas *et al.*, 1997) and microsatellite (Fréville *et al.*, 2001) markers, and of quantitative traits (Petit *et al.*, 2001).

Genetic markers revealed that populations are highly differentiated despite their geographic proximity, indicating that genetic drift is strong relative to gene flow. Genetic variation fitted an isolation-by-distance model, suggesting that gene flow is most likely to occur (or to have occurred in the past) between adjacent populations. Accordingly, genotype assignment analyses suggested that gene exchanges between populations are very rare, except between the two closest populations (Fréville *et al.*, 2001; Wilson & Rannala, 2003). Although very informative, the pattern of population differentiation depends on the product of effective population size (N) and (past) gene migration rate among populations (m), so that genetic drift and gene flow are not distinguished. In contrast, paternity analyses (like the assignment analyses of Wilson & Rannala, 2003) can estimate directly (contemporary) m , at least for the pollen-mediated component of gene flow, providing complementary information.

Although seed and pollen dispersal are thought to be also limited within populations (Colas *et al.*, 1997), so that Wahlund effects may be expected to occur, no departure from expected genotypic frequencies under random mating has ever been detected at the population level, even when using very polymorphic markers (Fréville *et al.*, 2001). Explanations related to inbreeding depression or the self-incompatibility system were proposed to account for this pattern (Colas *et al.*, 1997; Fréville *et al.*, 2001), but the significance of these processes and their necessity to explain the absence of heterozygote deficit within populations have not yet been proven. Investigations are thus necessary to find the genetic functioning of a single population and assess whether gene dispersal is sufficiently limited within a population to cause significant genetic structuring and affect genetic drift. Here again, paternity analyses can provide the necessary information for the pollen-mediated component of gene dispersal.

The present paper is the first of several papers reporting results from a study aiming at characterizing fine-scale genetic structure and gene flow within one of the six populations of *C. corymbosa* using microsatellite genetic

markers. Complementing investigations of the population demography (Colas *et al.*, 1997, 2001) and local adaptation (Petit *et al.*, 2001), such a study may help in the design of conservation strategies (e.g. Richards, 2000). This paper focuses on results from paternity analyses and addresses the following questions: (a) What is the rate of selfing? (b) What is the distribution of distances between mates (effective pollen dispersal)? (c) What is the shape of the pollen dispersal distribution around each plant (dispersal kernel)? (d) What is the rate of pollen flow from other populations? Evolutionary implications in terms of the potential for local adaptation will be discussed.

Material and methods

Study organism

Centaurea corymbosa is thought to be derived from the widespread taxon *C. maculosa* ssp. *maculosa* Lam. after a founder effect or a bottleneck event on the border of its distribution range, followed by ecological specialization and morphological differentiation (Fréville *et al.*, 1998). In the six known natural populations, a total of 343–632 individuals were found to reproduce each year between 1995 and 2002. The life cycle begins with a rosette stage. After at least 2 years (on average 5.5 years), the plant produces on several stems a total of from 1 to 200 (on average 30) capitula, flowering from early May to mid-July with a peak in June (B. Colas *et al.*, unpublished results). Pollination is insect-mediated. Flowers are visited mainly by Coleoptera, Hymenoptera, Lepidoptera, Diptera and Thysanoptera (S. Luijten, pers. comm.), but the relative importance of these insects in pollination is yet unknown. *Centaurea corymbosa* has a self-incompatibility system, though selfing events have been reported (H. Fréville & A. Mignot, unpublished results). The species being monocarpic, plants die after seed dispersal.

According to direct measurements in the surroundings of isolated plants, most seeds disperse less than 0.5 m (average distance = 32 cm, Colas *et al.*, 1997). A negative correlation found between the fertilization rate of capitula and the distance to the closest flowering individual suggested that pollen dispersal is also localized (Colas *et al.*, 2001). Nevertheless, fertilization (at a low rate) of plants 30–150 m away from flowering individuals suggested that relatively long-range pollen dispersal may sometimes occur (Colas *et al.*, 1997), although exceptional selfing events could also have accounted for these observations.

Individual sampling

We studied one medium size population (population 'A' in Colas *et al.*, 1997) located along the top of a 50-m-high northwest exposed cliff. The choice of this population was in part motivated by the accessibility of most plants,

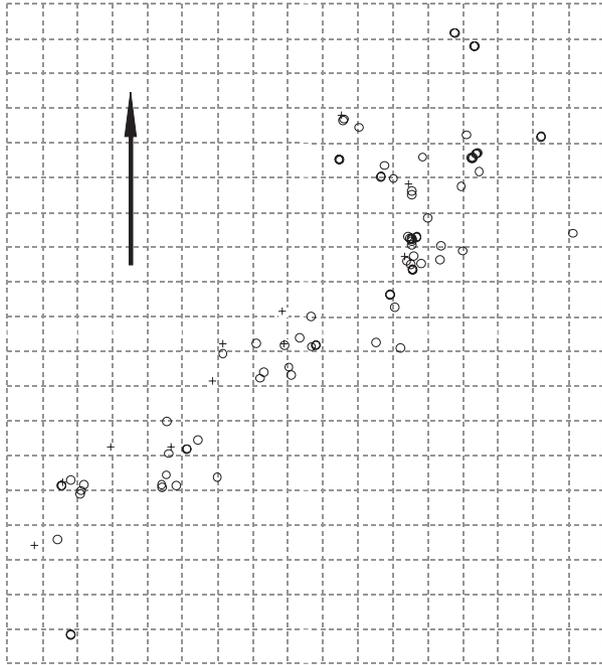


Fig. 1 Vertical projection map of population A representing reproducing *C. corymbosa* individuals sampled (○) and not sampled (+) in summer 2000. Squares are $10 \times 10 \text{ m}^2$. The arrow points to the north. The population is situated on the top of a 50-m-high cliff that borders its north-western side. All individuals represented lie within a range of altitude of 23 m. Note that several nearby individuals cannot be distinguished.

an important criterion in carrying out paternity analysis. It is probably a very old population, as *C. corymbosa* was first described in this site in 1783 (Pourret, 1788). We visited the population on 1, 6 and 22 June 2000 and mapped, in three dimensions, all individuals that were found to reproduce that year, totalling 96 plants (Fig. 1). Leaf samples for DNA extraction were collected on 85 individuals and stored in liquid nitrogen. The remaining 11 reproducing plants were not accessible, being situated on clefts a few meters below the top of the cliff. We might have missed a few flowering plants hidden behind rocks or bushes along the vertical cliff (possibly around ten according to B. Colas, who has monitored this population since 1994). Down the valley, approximately 150 m to the east of the cliff, two additional flowering individuals were found and collected, but later observations showed that three more plants had flowered in this area (B. Colas, pers. obs.). Unless stated, this isolated subgroup was not considered in most analyses. The number of capitula at each stage (bud, flowering, senesced) was recorded for each accessible plant.

Sib families were obtained on 49 reproducing plants from the main part of the population (capitula from 12 plants collected on 1 June, 13 on 6 June and 24 on 22 June), as well as on the two sampled individuals

from the subgroup in the valley. To limit human impact, we usually collected one capitulum per plant, except for five plants for which two or three capitula were sampled. Seeds were germinated and we collected leaves on each young plant for DNA extraction. Genomic DNA was extracted using a CTAB-based protocol (CTAB 2%, Tris-HCl 100 mM, EDTA 20 mM, NaCl 1.4 M, β -mercapto-ethanol 0.2% v/v). In total, DNA material was obtained for 372 offspring from the main part of the population, plus six offspring from the isolated subgroup.

Genetic markers

We used nine microsatellite loci: 21D9, 12B1, 28A7, 13B7, 13D10, 17E3, 13A9, 35C3, and 37F7_AC. The first six loci are described in Fréville *et al.* (2000), the last three were developed specifically for this study and their technical specifications are given in Table 1. Genotypes were assessed for 87 (85 on the cliff + 2 down the valley) reproducing individuals from the natural population and for 378 (372 + 6) offspring collected on the 49 (47 + 2) different mother plants.

Data analyses

Diversity at each locus was assessed by the number of alleles and the gene diversity (Nei, 1987). The average inbreeding coefficients of reproducing adults (F_I) were computed by the software GENEPOP, ver 3.2a (Raymond & Rousset, 1995), which also tested heterozygote deficit for each locus, as well as genotypic linkage disequilibrium for each pair of loci.

Paternity analyses

Paternity analyses were carried out by (a) an exclusion approach and (b) a likelihood approach, using the software CERVUS ver 2.0 (Marshall *et al.*, 1998). Paternity exclusion probabilities (EP) per locus and for all loci were computed following Jamieson & Taylor (1997). These paternity analyses considered the 85 adults and 372 offspring sampled in the population situated on the cliff. In some analyses, the two adults and six offspring sampled in the subgroup situated in the valley were added to check if pollen flow could be detected between these entities. The exclusion and likelihood approaches proved to be equally efficient for paternity assignments, leading to very similar results. Hence, for clarity and brevity, we will describe and report only the exclusion-based approach for which we also developed new simulation-based inference procedures. Details about the results obtained using the likelihood approach can be obtained from O. J. Hardy.

The exclusion approach consists in identifying for each genotyped offspring, given its mother genotype, which genotype among the reproducing individuals collected (including the mother) is compatible as father. The

Locus	Primer pairs (5' → 3')	Repeat motif	Annealing (°C)	Expected size (bp)
13A9	F: CCTGCGAGATGGCTAT R: TATCCCTACACCTCACTAACCC	T ₅ (CT) ₄ T ₃ (GT) ₄ (CT) ₂ T ₂ (CT) ₁₂	55	125
35C3	F: CCCTTCCACTTCTGATTTGAA R: TGTTTCTTCCGTTAACCTCA	(CA) ₁₆	55	119
37F7_AC	F: CTCGTAATAATTAGCATTCT R: TTGCTTGAACCTAATTCTAT	(AC) ₁₃	49.5	106

Table 1 Characteristics of the primers of the three new microsatellite loci developed for this study.

following cases were distinguished: (a) offspring with no compatible father, indicative of pollen flow from non-sampled individuals (within or outside the population); (b) offspring with just one compatible father, revealing pollen dispersal events; (c) offspring with several compatible fathers, for which paternity is unresolved; (d) offspring with a genotype incompatible with the maternal genotype, indicative of mutations, genotyping errors or null alleles. In addition, the proportion of offspring for which the mother was compatible as father provided an upper limit of the selfing rate. Though very simple, the exclusion approach has some disadvantages: (a) it does not take into account the possibility that offspring with a single compatible candidate father might actually have been sired by a nonsampled individual; (b) it does not resolve paternity when there are several candidate fathers, even though one candidate might be much more likely than the other ones; (c) the possible occurrence of genotyping errors (or mutation or null alleles) is not taken into account, possibly leading to the rejection of the true father following an apparent mismatch. We will show later how these difficulties can be overcome by a simulation procedure.

Pollen dispersal within the population

Pollen dispersal events monitored by paternity analyses were used to fit a function characterizing pollen movements within the population: the pollen dispersal kernel, $f(x, y)$. It represents the probability that a pollen grain from one individual moves to a position x, y , the father being at the origin. Pollen was assumed to disperse isotropically around each adult plant following a two-dimensional exponential power function (Tufto *et al.*, 1997; S. Oddou-Muratorio, E. Klein & F. Austerlitz, pers. comm.):

$$f(\alpha, \beta; x, y) = \frac{\beta}{2\pi \Gamma(2/\beta)} \exp\left(-\left(\frac{\sqrt{x^2 + y^2}}{\alpha}\right)^\beta\right) \quad (1)$$

where Γ is the gamma function and α, β are parameters to be fitted. The parameter β determines the shape of the distribution (the tail is longer for lower β) whereas, for a given β value ($\beta > 0$), the α parameter expresses the global extent of dispersal distances. The mean distance travelled by pollen grains under this kernel is given by

$\delta_k = \alpha[\Gamma(3/\beta)/\Gamma(2/\beta)]$ (S. Oddou-Muratorio, E. Klein & F. Austerlitz, pers. comm.), so that the kernel is fully characterized by β and δ_k . The kernel reduces to the bivariate exponential distribution when $\beta = 1$, and to the bivariate normal distribution when $\beta = 2$. Thus, following this model, the probability that a pollen grain lands on some position is proportional to $\exp(-r/\alpha)^\beta$, where r is the distance crossed. The third dimension (altitude z) was neglected here because our population could be approximated as extending on a two-dimensional space folded at the edge of the cliff. To compute inter-individual distances, r was approximated as $\sqrt{x^2 + y^2 + z^2}$. Because of the occurrence of a self-incompatibility system in our plant model, self-pollination was not considered (i.e. $f(\alpha, \beta; 0, 0) = 0$). The probability that adult i is the father of a progeny collected on a given mother j under given α, β parameters is the proportion of the pollen received by j that originates from i :

$$P(i|\alpha, \beta, j) = \frac{w_{if}(\alpha, \beta; r_i)}{\sum_k w_{kf}(\alpha, \beta; r_k)} \quad (2)$$

where r_k is the distance between j and k , w_k represents the total amount of pollen produced by individual k and the summation is over all potential fathers. Note that the parameters α and β are assumed equal for all adults.

A maximum likelihood approach permits fitting of the parameters α and β on observed dispersal events. The latter are represented by a set of N pollination events inferred by paternity analysis: offspring n has mother j_n and father i_n . In practice, we used offspring with only one compatible father and removed selfing events. Equation 2 provides the probability of occurrence of the n th pollination event under the model for a given mother and given values of the parameters α and β , $P(i_n|\alpha, \beta, j_n)$, so that the log-likelihood of the N events is

$$L(\alpha, \beta) = \sum_{n=1}^N \log(P(i_n|\alpha, \beta, j_n)) \quad (3)$$

Parameters α and β can thus be estimated by maximizing eqn 3. When computing $P(i_n|\alpha, \beta, j_n)$, only genotyped adults were considered in eqn 2 because no mating event involving a nongenotyped father could have been identified by paternity analysis. We assumed either that (a) all adults produced the same amount of pollen ($w_k = 1$ for

all k) or (b) the quantity of pollen produced was proportional to the number of capitula ($w_k =$ number of capitula counted on individual k), as suggested by an on-going analysis of male reproductive success (S. C. González-Martínez & O. J. Hardy, unpublished results). The dispersal model used here assumed isotropic dispersal. To test if this assumption holds in reality, the numbers of observed dispersal events following each of eight directions (N, N-W, W, S-W, S, S-E, E, N-E) were counted and compared with the numbers expected under the model (for the best fitting α, β parameters) using a chi-square test.

Estimation of parameters α and β by maximizing eqn 3 does not provide confidence intervals on α and β , and does not permit to test if the model is sufficient to explain the data. To test the consistency of the model and get confidence intervals, we performed simulations consisting of independent realizations of the dispersal process under the model and tested, for a wide range of α, β parameter values, if the data fit the dispersal model well.

Pollen flow from outside of the population

A proportion of the fertilizing pollen may come from (a) nonsampled adults from the population studied (i.e. unseen adults or adults mapped but inaccessible for sampling) or (b) other populations. To estimate the contributions of these sources of pollen, mating events were simulated in order to calibrate the results from paternity analyses to actual levels of pollen flow. To this end, we developed a new approach based on paternity exclusion which (a) estimates concomitantly the true pollen flow and the mutation/genotyping error rate and (b) provides confidence intervals on these estimates. We tried to maximize the use of the available information by accounting for (a) the genotypes of the sampled adults and offspring, (b) the variability of fathering probabilities among adults (assumed proportional to the number of capitula counted) and (c) the limited pollen dispersal within the population (dispersal kernel), using the spatial information relative to the sampled offspring and adults, including the 11 nonsampled but detected adults.

Each simulation run produced 372 offspring according to the family sizes and maternal genotypes of our sample. Offspring genotypes were constructed by combining one maternal allele and one paternal allele at each locus, allowing typing error or mutation at a rate ε (in case of typing error/mutation, the allele is chosen randomly among the existing alleles). The paternal alleles could come from one of the 96 adults found in the main part of the population (probability $1 - m_p$), or from an immigrant pollen (probability m_p), which represents pollen from the five other populations, pollen from the subgroup of the valley or pollen from undetected adults in the population (note that m_p does not involve pollen from detected but nongenotyped adults from the main

part of the population). In the case of nonmigrant pollen ($1 - m_p$), a father was chosen stochastically among the 96 adults according to the model of pollen dispersal defined previously (using the best fitting α, β parameters and accounting for the number of capitula produced per plant). When the selected father was one of the 85 genotyped adults, for each of the nine loci, the paternal allele was taken randomly among the two present in the father, allowing again for typing error or mutation at rate ε per locus. If the selected father was one of the 11 nongenotyped but detected adults, the paternal alleles were drawn randomly according to the allele frequencies found among the 85 sampled adults. In the case of immigrant pollen (m_p), the paternal alleles were chosen randomly according to the average allele frequencies found in the pollen cloud of offspring with no compatible father (these frequencies do not provide the exact allelic composition of migrant pollen, as it may include alleles coming from the detected but not sampled plants, but they are the best available estimates). Once genotypes were defined for all 372 offspring, each one was tested against each candidate father (i.e. the 85 sampled adults), recording the following cases: (a) genotypic mismatch with mother; (b) no compatible father found; (c) one compatible father found; (d) several compatible fathers found. Moreover, we computed, for case (c), the proportion of offspring whose single compatible father is the true father, and for case (b) the proportion of offspring fertilized by immigrant pollen. For a wide range of m_p and ε values, 200 replicates of simulation runs were produced and, for each, the numbers of the different cases noted above were compared with the numbers observed by exclusion analysis. Therefore, a pair of m_p, ε values was considered compatible with the data when the observed numbers of cases (a)–(c) were all within the respective 95% central ranges of values obtained by simulation.

Offspring without a compatible candidate father have potentially been fertilized by immigrant pollen. To get more insight about the origin of this pollen, genetic differentiation between this pollen pool and each of the six populations of *C. corymbosa* was assessed by computing pairwise F_{ST} values using the software SPAGED1 (Hardy & Vekemans, 2002). To this end, pollen genotypes of offspring without a compatible candidate father were assessed by subtracting the maternal contribution from the offspring genotypes (see Smouse *et al.*, 2001). F_{ST} values between this pollen pool and each of the six populations were computed using adults sampled in 1999 and genotyped at six loci by Fréville *et al.* (2001). An additional F_{ST} estimate between the pollen pool and population 'A' was computed using all nine loci and the adults sampled for the present study (sampled in 2000). The same procedure was applied on the pollen pool having fertilized offspring showing at least one compatible candidate father. We also checked whether some allele present among the offspring was absent from the

sampled adults, which might indicate pollen immigration events from a population where such an allele is frequent.

Results

Marker diversity

Among the nine microsatellite loci, five showed relatively low levels of diversity, having two or three alleles and gene diversities (H_e) ranging from 0.12 to 0.55, whereas the four others were more polymorphic, having five to 11 alleles and H_e ranging from 0.67 to 0.78 (Table 2). Reproducing individuals had inbreeding coefficients very close to zero at all loci, with a multilocus estimate $F_i = 0.028$ (Table 2). Heterozygote deficiency was, however, detected at two loci (12B1, $P < 0.05$ and 13A9, $P < 0.05$), and the multilocus test was marginally significant (Table 2, $P = 0.058$). These results likely reflect a weak level of inbreeding but might also be due to the presence of null alleles. Tests of linkage disequilibrium were significant at the 5% level for only one over the 36 pairs of loci, a number close to that expected under the null hypothesis (1.8).

Paternity analysis

The paternity exclusion probability was high ($EP = 0.976$) when all loci were considered (Table 2). Paternity assignments gave the following results: among 372 offspring, 65 (17%) had no compatible father among the reproducing individuals collected, 123 (33%) had only one compatible father, 178 (48%) had more than one compatible father and five (1.3%) showed a mismatch with the genotype of the mother at one locus. Mismatches occurred at different loci, and null alleles could explain them in only two cases (i.e. cases where

mother and offspring appeared homozygous at different alleles). The frequency of these mismatches was used to estimate the rate of genotyping error/mutation at each locus, accounting for the probability of detecting a mismatch, and the average value computed by CERVUS was 0.0027. Typical average mutation rates for microsatellites might be high enough (10^{-3} – 10^{-5} per locus and reproduction event; Ellegren, 2000) to explain this level of mismatches without considering genotyping mistakes.

Paternity was assigned to the mother of two offspring, providing a selfing rate estimate of 1.6%. Moreover, only 16 offspring were potentially compatible with a selfing event, giving an upper limit of the selfing rate at 4.3%.

When the two adults and six offspring from the subgroup of the valley were added to the paternity analyses, none of the 372 offspring was assigned uniquely to one of the two added adults, and none of the six added offspring were assigned to a single candidate father from the cliff, so that there is no evidence of pollen flow between the main population and the subgroup. Moreover, four of the six added offspring had no compatible father according to the exclusion analysis, suggesting that they might have been sired by some of the nonsampled adults from the subgroup.

Pollen dispersal distance distribution was estimated by considering the 123 offspring that had only one compatible father. The resulting frequency distributions of pollen dispersal distances can be compared with the expected distribution under random mating (i.e. random adult–offspring pairs). It appears that pollen dispersal distances are limited relative to random mating (Figs 2 and 3). We found that 20% of mates were separated by less than 1.5 m and 50% by less than 11 m, but 20% were separated by more than 43 m (Fig. 3). The average distance between mates was 21.6 m, and the average squared distance between mates was 1166 m² (this quantity will be used to characterize the neighbourhood size).

Table 2 Number of alleles (A), gene diversity (H_e), paternity exclusion probability (EP) and inbreeding coefficient (F_i), at each locus and the multilocus average (A , H_e , F_i) or multilocus estimate (EP), using the genotypes of 85 reproducing individuals.

Locus	A	H_e	EP	F_i
13B7	2	0.122	0.034	-0.0633ns
17E3	3	0.216	0.103	-0.0374ns
13D10	3	0.335	0.161	-0.0184ns
28A7	5	0.721	0.492	0.0873ns
21D9	7	0.773	0.570	-0.0501ns
12B1	11	0.776	0.632	0.0144*
13A9	5	0.667	0.383	0.1537*
35C3	3	0.341	0.147	-0.0019ns
37F7_AC	3	0.552	0.228	0.0197ns
Multilocus	4.7	0.500	0.976	0.0280#

Results of exact tests of heterozygote deficit are given next to F_i values.

Significance levels: ns, non significant; # $P < 0.10$; * $P < 0.05$.

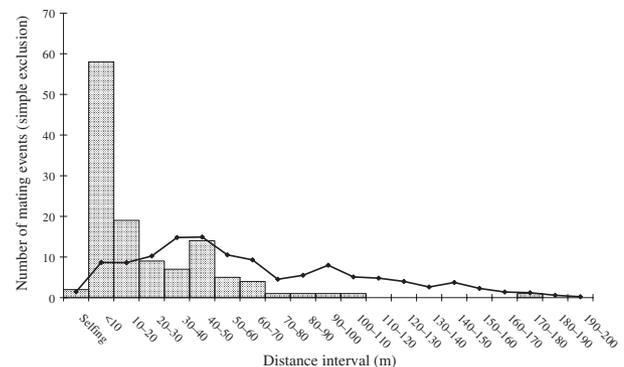


Fig. 2 Numbers of mating events assessed by the exclusion approach (i.e. offspring with just one compatible candidate father) for each class of distance between mates. The line represents the numbers expected under random mating.

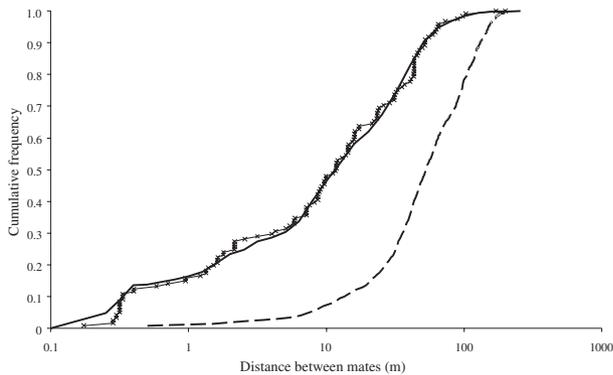


Fig. 3 Cumulative frequency distributions of effective pollen dispersal distances estimated considering: (a) 123 paternity assignments by simple exclusion (×); (b) the distribution expected under the exponential power dispersal model for the best-fitting parameters (plain line). The broken line represents the distance distribution expected under random mating (includes random selfing).

Modelling pollen dispersal within the population

The pollen dispersal model assuming an exponential power function was fitted to the 121 allogamous dispersal events (i.e. discounting two selfing events) assessed by simple exclusion. The best-fitting parameters were $\alpha = 2.30$ and $\beta = 0.44$ ($L = -420.4$, mean distance travelled by pollen grains $\delta_k = 91$ m) when the quantity of pollen produced was assumed to be proportional to the number of capitula, and $\alpha = 2.10$ and $\beta = 0.43$ ($L = -429.3$, $\delta_k = 100$ m) when all adults were assumed to produce the same quantity of pollen. As expected *a priori*, a better fit was obtained (higher L) when the number of capitula was taken into account, but the estimates of the α , β parameters were robust, being little affected by assumptions concerning the amount of pollen produced per adult. According to the parameters of this kernel, the average contribution of the nonsampled individuals to paternity was estimated to be 9%, which is less than their percentage in the population (14%) because they are on average more distant from the plants on which offspring were sampled than random individuals (Fig. 1).

To check the consistency of the model (i.e. given α , β parameters) with actual data, we defined a quantity expressing the deviation between the data and the expectations under the model. This quantity was the area between the observed cumulated frequency distribution of dispersal events and the expected distribution under the model (cf. Fig. 3). Confidence intervals of this between-curves area under the model were obtained by simulations, whereby fathers of the N sampled offspring were chosen stochastically according to their relative probabilities of siring as defined by eqn 2, using 200 simulation replicates per α , β parameter values. When the observed between-curves area was within the range

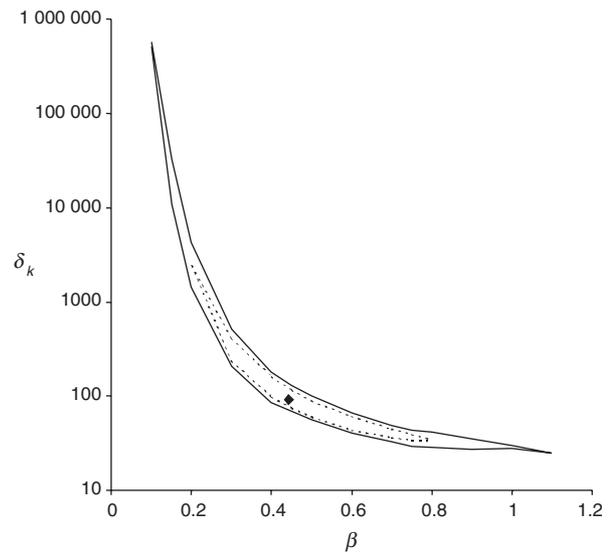


Fig. 4 Ranges of the β and δ_k parameters of the exponential power pollen dispersal model that fit the data (paternity exclusion) with a confidence level of 95% (plain line) and 80% (broken line). Parameter β expresses the degree of leptokurtosis of the pollen dispersal function and δ_k is the mean distance travelled by pollen grains under the model. The confidence levels were assessed by simulating mating events in the population. The diamond symbol shows the best-fitting parameter values (maximum likelihood estimates).

of the 95 or 80% lowest between-curves areas assessed by simulations, the α , β parameters were considered consistent with the data.

The range of α , β parameters consistent with the data varied over a narrow space and permitted estimation of confidence intervals on β and δ_k (Fig. 4). Interestingly, the range of the β parameter varied between 0.2 and 0.8 at a confidence level of 80%, and was lower than 1.1 at a 95% confidence level (the lower bound was here undeterminable). Hence, pollen dispersal is much more leptokurtic than the bivariate normal distribution ($\beta = 2$), which can be rejected, and seems also more leptokurtic than the bivariate exponential distribution ($\beta = 1$), which can be rejected with less confidence.

As 6–9% of the dispersal events assessed by simple exclusion should be erroneous (see results from next section), there is a risk that part of these false mating events correspond to long-distance dispersal events (because they should correspond approximately to random offspring–adult pairs), causing the inferred dispersal kernel to appear more leptokurtic than it is in reality. To check that the leptokurtic nature of the kernel was not an artefact, we repeated the analysis after the removal of the five (ten) longest inferred dispersal events, and obtained as best-fitting parameters $\beta = 0.62$ (0.69) and $\delta_k = 44$ m (34 m). Although the resulting kernel was somewhat less leptokurtic, it was still much more

leptokurtic than either the bivariate normal or exponential distributions.

The observed (expected) numbers of dispersal events assessed by simple exclusion in each direction were equal to N, 21 (19.6); N-W, 10 (12.1); W, 16 (9.4); S-W, 16 (20.2); S, 23 (19.0); S-E, 14 (11.8); E, 4 (11.4); NE, 17 (17.4). The difference between observed and expected numbers was not significant ($P = 0.1$) so that there is no evidence that pollen disperses anisotropically.

Pollen flow

Simulations permitted determination of the range of values of the error rate ϵ (genotyping errors or mutations) and the pollen flow m_p (from other populations or undetected adults in the population) that were compat-

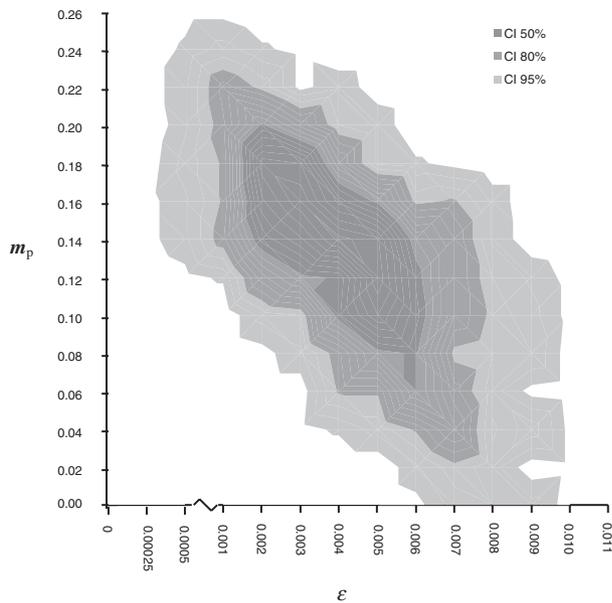


Fig. 5 Ranges of pollen immigration rate (m_p) and genotyping error/mutation rate (ϵ) that fit the data (paternity exclusion) with a confidence level of 95% (light grey), 80% (intermediate grey) and 50% (dark grey). These confidence levels were assessed by simulating mating events in the population.

ible with the data (Fig. 5). The *a priori* probabilities of paternity in the present simulations were defined by the number of capitula produced and the distances to the mother following eqns 1 and 2, with $\alpha = 2.30$ and $\beta = 0.44$ (the best-fitting parameters). The values showing best compatibility with the data were $m_p = 14\%$ and $\epsilon = 0.004$, and their 95% confidence intervals were $m_p = (0-25\%)$ and $\epsilon = (0.00025-0.01)$. It is worth noting that the data can be explained with the absence of pollen flow if the error rate is relatively high (0.005–0.010), but if the error rate is assumed lower (e.g. ≤ 0.001), the pollen flow from other populations must be between 13 and 25% (Fig. 5). Hence, assumptions made on the error rate can have a strong influence on estimates of pollen flow. In the range of ϵ and m_p values compatible with data, simulations showed that, among offspring with a single compatible father, the latter was the true father in 91–94% of cases, these percentages giving thus confidence levels for the paternity analysis.

The pollen pool having fertilized offspring without a compatible candidate father was little (but statistically significantly) differentiated from the adults of the population ($F_{ST} = 0.023$ for the adults sampled in 2000 and $F_{ST} = 0.030$ for the adults sampled in 1999), whereas differentiation was much more pronounced with respect to the other populations of *C. corymbosa* ($F_{ST} = 0.150-0.280$ for individuals sampled in 1999; Table 3). Hence, most of this pollen pool must originate from population 'A'. The levels of differentiation of the other populations with respect to this pollen pool were somewhat lower than with respect to the pollen pool having fertilized offspring showing at least one compatible candidate father in population 'A' (Table 3), which might indicate that a small fraction of the former pollen pool originates from the other populations (this result might, however, be an artefact due to the use of the same data to compute F_{ST} and perform the paternity analysis).

Five offspring carried an allele not present among the genotyped adults (allele 135 of locus 21D9), which might suggest long-distance pollen flow as this allele was found at a high frequency (0.49) only in population 'Pe' (data from Fréville *et al.*, 2001 for samples collected in 1999). However, the latter data also showed that this allele was present in population 'A' (the current

	Populations, date of sampling and number of loci scored						
	2000 (nine loci)	1999 (six loci)					
Pollen cloud having fertilized offspring	A	A	E1	E2	Pe	Po	Cr
Without any compatible candidate father from A	0.023	0.030	0.150	0.181	0.205	0.280	0.168
With compatible candidate father(s) from A	0.014	0.002	0.189	0.207	0.226	0.275	0.190

Population names follow Colas *et al.* (1997).

Table 3 Pairwise F_{ST} values between each of the six populations of *C. corymbosa* and the pollen cloud having fertilized offspring in population A in 2000, distinguishing offspring with or without a compatible candidate father in population A. Italicized values are significant (bold italics: $P < 0.05$; plain italics: $P < 0.001$).

population) at a frequency of 2.4%, so that it is also very likely that this allele was actually present among the flowering plants of population 'A' that were not sampled in 2000, and that the five offspring were sired by these adults.

In summary, most offspring showing no compatible candidate father appear to result from (a) the fertilization by pollen from nonsampled adults (detected or undetected) from the population and (b) genotyping errors or mutations causing mismatch with the unrecognized actual father. According to simulations, genotyping errors/mutations account for 10–35% of the cases assuming $m_p = 14\%$ and $\varepsilon = 0.004$.

Discussion

Our results confirm that *C. corymbosa* is essentially an outbreeder, although selfing events can sometimes occur, and that pollen dispersal is limited and follows an isotropic leptokurtic distribution. At most 25% of pollen came from outside the population, but the actual amount of pollen immigration is probably much lower. In the following, we will first compare our marker-based estimates with previous knowledge on pollen dispersal in *C. corymbosa* and other species, and then discuss the implications of our results for the evolution and conservation of *C. corymbosa* populations.

Mating system and pollen dispersal within the population

Experimental crosses in the field showed that *C. corymbosa* is strongly self-incompatible (Colas *et al.*, 1996), although many artificially selfed plants were able to produce a few seeds in the greenhouse (H. Fréville & A. Mignot, pers. obs.). Such leaky self-incompatibility has been reported in many self-incompatible species (e.g. Les *et al.*, 1991; Reinartz & Les, 1994; Levin, 1996; Lipow *et al.*, 1999). Nevertheless, selfing events appear to be rare at least in population 'A' of *C. corymbosa*, our data suggesting a selfing rate of approximately 1.6% and, in any case, not larger than 4.3%.

Previous investigations in populations of *C. corymbosa* suggested limited pollen dispersal (Colas *et al.*, 1997, 2001), but also reported long-distance dispersal events, up to 150 m (supposing self-incompatibility, Colas *et al.*, 1997). The effective pollen dispersal curve described in the present study (Figs 2 and 3) confirms these previous results, showing that half of the fertilizing pollen moves less than 11 m, but 20% disperses beyond 43 m. Other studies based on paternity analysis reported pollen dispersal events of a few tens or a few hundreds of meters in wind-pollinated species (e.g. Dow & Ashley, 1996; Streiff *et al.*, 1999; Sork *et al.*, 2002), but also in insect-pollinated species (e.g. Meagher, 1986; Stacy *et al.*, 1996; Kwak *et al.*, 1998; Konuma *et al.*, 2000). These results contrast somewhat with the previous belief that

insect pollinators cause very localized pollen dispersal because inter-plant flight distances are usually short (Levin & Kerster, 1974).

The pollen dispersal kernel best fitting the data had a very leptokurtic distribution ($\beta = 0.44$). This 'fat-tailed' dispersal distribution explains why most dispersal events occur over a short range whereas long-range dispersal can also be observed. Actually, leptokurtosis might be underestimated because long-distance dispersal events between populations could not be included when fitting the kernel function. In another study fitting pollen dispersal in an insect-pollinated tree species (*Sorbus torminalis*), a low exponent of the exponential power function was also found ($\beta = 0.16$ – 0.71 according to models; S. Oddou-Muratorio, E. Klein & F. Austerlitz, pers. comm.). Leptokurtic dispersal distributions were also reported in studies that directly monitored pollen movements in insect-pollinated plants (e.g. Levin, 1981; Lavigne *et al.*, 1998; Marr *et al.*, 2000).

Some caution must be taken in the interpretation of the pollen dispersal kernel. For example, the mean distance travelled by pollen grains under the best-fitting kernel was equal to $\delta_k = 91$ m, which is much larger than the mean distance between mates ($\delta_e = 21.6$ m). Actually, δ_e depends on pollen that fertilized ovules (effective dispersal), whereas δ_k is not conditional on ovule fertilization. If the population extends much further than pollen movements in two-dimensional space, and individuals are distributed at random, δ_e and δ_k should be equal. In reality, our population extends over a limited range and individuals are aggregated (Fig. 1), so that pollen dispersed over short distances has a higher chance of landing on a stigma, explaining why $\delta_e < \delta_k$ (if individuals were overdispersed rather than aggregated, we would expect $\delta_e > \delta_k$). In the case of wind-dispersed pollen, the kernel can be interpreted as the average density function of the pollen cloud liberated by each individual. Such a physical interpretation does not hold well for animal-pollination, where pollen is not distributed continuously through space but only where pollinators land, i.e. mostly on flowers. Consequently, the spatial distribution of plants can strongly affect the dispersal kernel for animal-pollination, but not for wind-pollination (except if the presence of plants modifies wind flow). Hence, the leptokurtic kernel reported could result from the aggregated spatial distribution of the individuals, forcing many pollinators to move frequently among nearby individuals and rarely among clusters of individuals.

Pollen flow

As the studied population is highly differentiated from neighbouring populations (pairwise F_{ST} values between this population and the five others ranged from 0.27 to 0.46 with allozyme markers, Colas *et al.*, 1997; and from 0.19 to 0.33 with microsatellite markers, Fréville *et al.*,

2001), pollen migration among populations should *a priori* be limited. Further evidence of limited gene flow comes from assignment tests (Fréville *et al.*, 2001) and Bayesian inference of recent migration rates using multilocus genotypes (Wilson & Rannala, 2003).

The new exclusion-based simulation approach developed in this study suggested that pollen flow from other populations and/or undetected adults within the population could occur at a rate comprised between 0 and 25% when genotyping errors/mutations were accounted for and simultaneously estimated. Hence, estimation of pollen flow suffered from the low power of the paternity analysis. This lack of power is at least partially due to the nonexhaustive sampling of the adults from the population. But we have also shown that the assumed mutation/genotyping error rate is very critical, so that a wide range of combinations of rates of pollen flow and of mutation/genotyping error were found consistent with the data. Despite this problem, the approach has several advantages: limited pollen dispersal and the flowering success of each plant were accounted for, nongenotyped but detected adults could also be accounted for and confidence intervals could be estimated for pollen flow and the mutation/genotyping error rate.

More insights about pollen flow came from the observation that the pollen having fertilized offspring for which no candidate father was found was little differentiated (though statistically significantly) from the population under study and was highly differentiated from the other populations. Hence, we can conclude that most of this pollen came from nonsampled individuals in the population, and little or no pollen came from other populations.

Evolutionary implications

Colas *et al.* (1997, 2001) observed a reduction of seed fertilization rate for isolated individuals more than 4 m away from their nearest neighbours, suggesting the occurrence of pollen limitation. Very short pollen dispersal distances were proposed to explain pollen limitation (Colas *et al.*, 1997). If pollen dispersal was limited to a few meters, given that seed dispersal is even more limited (Colas *et al.*, 1997), the population should be strongly subdivided genetically, leading to biparental inbreeding, and differential selection might cause local adaptation within the population. Contrary to these expectations, previous investigations found no inbreeding at the population level (Colas *et al.*, 1997; Fréville *et al.*, 2001), motivating in part the present study. Our results indicate that 70% of mates are separated by more than 4 m, and 20% by distances comparable to the scale of the population. Hence, it is not surprising that inbreeding is so weak ($F_I = 0.028$).

Wright's neighbourhood model is often considered to assess the minimal scale at which genetic differentiation might occur (Wright, 1943). Although the relevance of

the neighbourhood concept has been debated (e.g. Rousset, 1997; Fenster *et al.*, 2003), it might still serve as a rough guideline. For plants, the neighbourhood area is $Na = 4\pi(\sigma_p^2/2 + \sigma_s^2)$, where σ_p^2 and σ_s^2 are half the mean square dispersal distances of pollen and seeds, respectively (Crawford, 1984). Neglecting seed dispersal, we get $Na = 3661 \text{ m}^2$, which is the area included within a circle of 34 m of radius. Hence, a single 'neighbourhood' would enclose about one-third of the whole population. Consequently, even if different parts of the population were subject to differential selection pressures, gene flow is likely to be too high to permit local adaptation at this scale.

Is gene flow among populations sufficiently low to permit local adaptation at this higher level? The result depends on the ratio of the rate of gene flow among populations (m) and selection coefficients (Slatkin, 1973). According to a new method developed by Wilson & Rannala (2003), estimates of recent migration rates are very low among the populations of *C. corymbosa* (about 1%), except between the two closest ones separated by only 0.3 km (E1 and E2). The paternity analysis conducted here was not powerful enough to provide precise estimates of the pollen-mediated component of m (m_p). We remain with qualitative indications that m_p must be low but we cannot assess the selection coefficients required to overcome gene flow. In a previous comparison of the levels of population genetic differentiation between molecular markers (F_{ST}) and quantitative traits (Q_{ST}), similar values were found (Petit *et al.*, 2001) so that there was no evidence that these quantitative traits were subject to differential or unifying selection.

Implications for conservation

The rarity and very narrow geographical range displayed by *C. corymbosa* are thought to result from the combined effect of (a) extreme specialization in a habitat occurring in scattered patches and (b) limited dispersal capabilities (Colas *et al.*, 1997). If a seed was dispersed a long distance to land in an empty suitable site, the establishment of a new population would remain unlikely because of self-incompatibility, restricted pollen dispersal and monocarpy. We have shown that one of five matings occurs between plants separated by more than 43 m, showing that the rate of such long-distance pollen dispersal is much larger than suggested in an earlier study (Colas *et al.*, 1997). The best-fitting pollen dispersal model also suggests that, if the population were to occupy a larger area, the distance between mates could be substantially larger than observed here because $\delta_k > \delta_e$. Pollen limitation should thus not be a major problem for the extension of a population at the limits of its range as long as habitat remains suitable. Note, however, that the pollen dispersal kernel was characterized within a continuous population (neglecting the aggregated distribution of individuals). It is not known how gaps 50–100 m

wide would affect pollinator behaviour and hence pollen dispersal.

Our analyses did not allow us to estimate the rate of very long distance pollen flow (i.e. among populations and more generally among suitable sites), but this rate must be low. As self-fertilization also occurs rarely, pollen limitation should be a major concern for the very early establishment of a new isolated population. Two selfing events were detected on two mothers that produced, respectively, four and seven other offspring that were all incompatible with selfing events. Hence, there is no evidence that some individuals self-pollinated more easily (breakdown of self-incompatibility system). Leaky self-incompatibility appears a more likely explanation for these selfing events. This phenomenon might contribute to early population establishment but, in the absence of a breakdown of the self-incompatibility system, further population expansion would be hindered by a lack of variability at self-incompatibility loci (a minimum of four alleles are required under a sporophytic self-incompatibility system, whereas a single founder plant brings only two alleles). Thus, at the minimum, the migration of two seeds, or one seed and two pollen grains, is required to initiate the colonization of a new suitable site, something that has a very low probability of occurrence.

Acknowledgments

Sophie Gerber, Eric Imbert, Doyle McKey, François Rousset, Xavier Vekemans, and two anonymous reviewers provided useful comments on a previous draft of the manuscript. The Belgian National Fund for Scientific Research (FNRS) and the Communauté française de Belgique provided financial support to O.J.H. for his postdoctoral work at the University of Montpellier 2. Part of the data were analysed while O.J.H. was carrying out a post-doctorate at INRA Montpellier, with financial support from the European Science Foundation (Programme Plant Adaptation, chaired by O. Savolainen). S.C.G.-M. was supported by the programme TMR Fragland headed by I. Hanski. This work was supported by the Ministère de l'Aménagement du Territoire et de l'Environnement (Contract MATE n°98153 to I.O.) through the National Programme Diversitas, Fragmented Populations network, and by the E.U. Programme 'Plant Dispersal' attributed to Ben Vosman. This is contribution number N°ISEM 2003-065 of the Institut des Sciences de l'Evolution de Montpellier (UMR 5554 CNRS).

References

- Colas, B., Riba, M. & Molina, J. 1996. Statut démographique de *Centaurea corymbosa* Pourret (Asteraceae), *Hormatophylla pyrenaica* (Lapeyr.) Cullen & Dudley (Brassicaceae), et *Marsilea strigosa*. Willd (Marsileaceae-Pteridophyta), trois plantes rares dans le sud de la France. *Acta Bot. Gallica* **143**: 191–198.
- Colas, B., Olivieri, I. & Riba, M. 1997. *Centaurea corymbosa*, a cliff-dwelling species tottering on the brink of extinction: a demographic and genetic study. *Proc. Natl. Acad. Sci.* **94**: 3471–3476.
- Colas, B., Olivieri, I. & Riba, M. 2001. Spatio-temporal variation of reproductive success and conservation of the narrow-endemic *Centaurea corymbosa* (Asteraceae). *Biol. Cons.* **99**: 375–386.
- Crawford, T.J. 1984. What is a population? In: *Evolutionary Ecology* (B. Shorrocks, ed.), pp. 135–173. Blackwell, Oxford.
- Dow, B.D. & Ashley, M.V. 1996. Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa*. *Mol. Ecol.* **5**: 615–627.
- Ellegren, H. 2000. Microsatellite mutations in the germline: implications for evolutionary inference. *Trends Genet.* **16**: 551–558.
- Fenster, C.B., Vekemans, X. & Hardy, O.J. 2003. Quantifying gene flow from spatial genetic structure data in a metapopulation of *Chamaecrista fasciculata* (Leguminosae). *Evolution* **57**: 995–1007.
- Fréville, H., Colas, B., Ronfort, J., Riba, M. & Olivieri, I. 1998. Predicting endemism from population structure of a widespread species: case study in *Centaurea maculosa* Lam. (Asteraceae). *Cons. Biol.* **12**: 1269–1278.
- Fréville, H., Imbert, E., Justy, F., Vitalis, R. & Olivieri, I. 2000. Isolation and characterization of microsatellites in the endemic species *Centaurea corymbosa* Pourret (Asteraceae) and other related species. *Mol. Ecol.* **9**: 1671–1672.
- Fréville, H., Justy, F. & Olivieri, I. 2001. Comparative allozyme and microsatellite population structure in a narrow endemic plant species, *Centaurea corymbosa* Pourret (Asteraceae). *Mol. Ecol.* **10**: 879–890.
- Hardy, O.J. & Vekemans, X. 2002. SPAGED1: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol. Ecol. Notes* **2**: 618–620.
- Hedrick, P.W. 2001. Conservation genetics: where are we now? *Trends Ecol. Evol.* **16**: 629–636.
- Jamieson, A. & Taylor, S.S. 1997. Comparisons of three probability formulae for parentage exclusion. *Anim. Genet.* **28**: 397–400.
- Jones, A.G. & Ardren, W.R. 2003. Methods of parentage analysis in natural populations. *Mol. Ecol.* **12**: 2511–2523.
- Konuma, A., Tsumura, Y., Lee, C.T., Lee, S.L. & Okuda, T. 2000. Estimation of gene flow in the tropical-rainforest tree *Neobalanocarpus heimii* (Dipterocarpaceae), inferred from paternity analysis. *Mol. Ecol.* **9**: 1843–1852.
- Kwak, M.M., Velterop, O. & Andel, J. 1998. Pollen and gene flow in fragmented habitats. *Appl. Veg. Sci.* **1**: 37–54.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* **241**: 1455–1460.
- Lavigne, C., Klein, E.K., Vallée, P., Pierre, J., Godelle, B. & Renard, M. 1998. A pollen-dispersal experiment with transgenic oilseed rape. Estimation of the average pollen dispersal of an individual plant within a field. *Theor. Appl. Genet.* **98**: 886–896.
- Les, D.H., Reinartz, J.A. & Esselman, E.J. 1991. Genetic consequences of rarity in *Aster furcatus* (Asteraceae), a threatened, self-incompatible plant. *Evolution* **45**: 1641–1650.
- Levin, D.A. 1981. Dispersal versus gene flow in plants. *Ann. Missouri Bot. Gard.* **68**: 233–253.
- Levin, D.A. 1996. The evolutionary significance of pseudo-fertility. *Am. Nat.* **148**: 321–332.

- Levin, D.A. & Kerster, H.W. 1974. Gene flow in seed plants. *Evol. Biol.* **7**: 139–220.
- Lipow, S.R., Broyles, S.B. & Wyatt, R. 1999. Population differences in self-fertility in the 'self-incompatible' milkweed *Asclepias exaltata* (Asclepiadaceae). *Am. J. Bot.* **86**: 1114–1120.
- Marr, D.L., Leebens-Mack, J., Elms, L. & Pellmyr, O. 2000. Pollen dispersal in *Yucca filamentosa* (Agavaceae): the paradox of self-pollination behavior by *Tegeticula yuccasella* (Prodoxidae). *Am. J. Bot.* **87**: 670–677.
- Marshall, T.C., Slate, J., Kruuk, L.E.B. & Pemberton, J.M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**: 639–655.
- Meagher, T.R. 1986. Analysis of paternity within a natural population of *Chamaelirium luteum*. I. Identification of most-likely male parents. *Am. Nat.* **128**: 199–215.
- Miyazaki, Y. & Isagi, Y. 2000. Pollen flow and the interpopulation genetic structure of *Heloniopsis orientalis* on the forest floor as determined using microsatellite markers. *Theor. Appl. Genet.* **101**: 718–723.
- Nei, M., 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Olivieri, I., Michalakis, Y. & Gouyon, P.-H. 1995. Metapopulation genetics and the evolution of dispersal. *Am. Nat.* **146**: 202–228.
- Petit, C., Fréville, H., Mignot, A., Colas, B., Riba, M., Imbert, E., Hurtrez-Boussès, S., Virevaire, M. & Olivieri, I. 2001. Gene flow and local adaptation in two endemic plant species. *Biol. Cons.* **100**: 21–34.
- Pourret, P.A. 1788. *Chloris narbonensis*. *Mém. Acad. Sci. Toulouse* **3**: 310.
- Raymond, M. & Rousset, F. 1995. GENEPOP (ver 1.2): population genetics software for exact tests and eucumenism. *J. Hered.* **86**: 248–249.
- Reinartz, J.A. & Les, D.H. 1994. Bottleneck-induced dissolution of self-incompatibility and breeding system consequences in *Aster furcatus* (Asteraceae). *Am. J. Bot.* **81**: 446–455.
- Richards, C.M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. *Am. Nat.* **155**: 383–394.
- Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* **145**: 1219–1228.
- Schnabel, A. 1998. Parentage analysis in plants: mating systems, gene flow, and relative fertilities. In: *Advances in Molecular Ecology* (G. R. Carvalho, ed.), pp. 173–189. IOS Press, The Netherlands.
- Slatkin, M. 1973. Gene flow and selection in a cline. *Genetics* **75**: 733–756.
- Smouse, P.E., Dyer, R.J., Westfall, R.D. & Sork, V.L. 2001. Two-generation analysis of pollen flow across a landscape I Male gamete heterogeneity among females. *Evolution* **55**: 260–271.
- Sork, V.L., Davis, F.W., Smouse, P.E., Apsit, V.J., Dyer, R.J., Fernandez-M, J.F. & Kuhn, B. 2002. Pollen movement in declining populations of California Valley oak, *Quercus lobata*: where have all the fathers gone? *Mol. Ecol.* **11**: 1657–1668.
- Stacy, E.A., Hamrick, J.L., Nason, J.D., Hubbell, S.P., Foster R.B. & Condit, R. 1996. Pollen dispersal in low-density populations of three neotropical tree species. *Am. Nat.* **148**: 275–298.
- Streiff, R., Ducousso, A., Lexer, C., Steinkellner, H., Gloessl, J. & Kremer, A. 1999. Pollen dispersal inferred from paternity analysis in a mixed oak stand of *Quercus robur* L. and *Q. petraea* (Matt.) Liebl. *Mol. Ecol.* **8**: 831–841.
- Tufto, J., Engen, S. & Hindar, K. 1997. Stochastic dispersal processes in plant populations. *Theor. Pop. Biol.* **52**: 16–26.
- Wilson, G.A. & Rannala, B. 2003. Bayesian inference of recent immigration rates using multilocus genotypes. *Genetics* **163**: 1177–1191.
- Wright, S. 1943. Isolation by distance. *Genetics* **28**: 114–138.

Received 11 April 2003; revised 28 January 2004; accepted 28 January 2004