Role of Corridors in Plant Dispersal: an Example with the Endangered *Ranunculus nodiflorus*

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Abstract: *The proposition that the migration of organisms between habitat patches could be enhanced by corridors has been much discussed among conservation biologists. But the few experimental studies supporting the usefulness of corridors have all concerned animal species. We investigated the role of corridors in seed dispersal, studying population genetic and demographic structure in metapopulations of the rare, pond-dwelling, autogamous plant species* Ranunculus nodiflorus *L. in the Fontainebleau Forest (France). Differentiation on three polymorphic isozyme markers was strong among local populations (ponds) within metapopulations (sites) and moderate among metapopulations. Partial Mantel tests revealed that the connection of ponds through temporarily flooded natural corridors, facilitating seed migration, had a strong negative effect on genetic differentiation between local populations and that a pond was more likely to be colonized when connected by corridors to other occupied ponds. Thus, corridors are probably a key element of landscape structure for metapopulation dynamics in* R. nodiflorus. *From a conservation perspective, our results suggest that corridors could increase the chance of persistence of plant species living in fragmented habitats by promoting seed dispersal between habitat patches.*

El Papel de los Corredores en la Dispersión de Plantas: el Ejemplo de la Especie en Peligro *Ranunculus nodiflorus*

Resumen: *La propuesta de que la migración de organismos entre parches de hábitat puede ser incrementada por corredores ha sido muy discutida en biología de la conservación. Pero los pocos estudios experimentales que apoyan la utilidad de los corredores han sido enfocados hacia especies de animales. Investigamos el papel de los corredores en la dispersión de semillas, estudiando la genética poblacional y la estructura demográfica en metapoblaciones de la especie de planta rara, autógama, habitante de estanques* Ranunculus nodiflorus *L. en el bosque Fontainebleau (Francia). La diferenciación de tres isozimas polimórficas marcadoras fue fuerte entre las poblaciones locales (estanques) dentro de metapoblaciones (sitios) y fue moderada entre metapoblaciones. Las pruebas parciales de Mantel revelaron que la conexión de estanques a través de corredores naturales inundados facilitando la migración de semillas, tuvo un efecto negativo fuerte en la diferenciación genética entre poblaciones locales y que un estanque fue más probable de ser colonizado cuando se conectaba por corredores con otros estanques ocupados. Por lo tanto, los corredores probablemente*

son un factor clave de la estructura del paisaje para dinámicas metapoblacionales en R. nodiflorus. *Desde una perspectiva de conservación, nuestros resultados sugieren que los corredores podrían incrementar la probabilidad de persistencia de especies de plantas que viven en hábitats fragmentados al promover la dispersión de semillas entre parches de hábitats.*

Introduction

Species living in fragmented habitats have to face two major constraints: restricted habitat patch sizes that limit local populations to small sizes, and isolation of habitat patches that restricts individual and gene exchanges between populations (Andrén 1994). These constraints can bring about various threats to species persistence. Small populations have higher risks of extinction than others because they are more susceptible to environmental fluctuations and demographic stochasticity (Shaffer 1987; Lande 1993), loss of genetic variation through drift (Huenneke 1991; Lynch 1996), and inbreeding depression (Charlesworth & Charlesworth 1987; Lande 1995; Saccheri et al. 1998).

In a fragmented habitat, migration among populations can substantially alleviate these threats. On a demographic level, immigrants can reinforce small populations and preserve them from extinction through environmental or demographic stochasticity: this is the "rescue effect" (Stacey et al. 1997). Moreover, when extinctions occur, migration allows for the recolonization of empty habitat patches. On a genetic level, the arrival of genes from other populations counteracts the effects of genetic drift and may prevent inbreeding depression (Mills & Allendorf 1996). So, for a species occurring in a set of local populations, migration is thought to substantially increase the chances of regional persistence over the long term (Colas et al. in press), although over time gene flow might also prevent local adaptations in patches exhibiting different selective pressures (Storfer 1999) or lead to outbreeding depression (Waser & Price 1989; Quilichini et al. 2001).

A major problem for species living in fragmented habitats is that isolation of habitat patches may drastically reduce migration among populations. That is why biological corridors have been the subject of considerable interest to conservation biologists (Simberloff et al. 1992). Corridors—strips of land facilitating movements of individuals between habitat patches—are often seen as major landscape components that can achieve conservation goals because they may increase species persistence by enhancing among-population migration (Stacey et al. 1997).

The implementation of corridors has been recommended and carried out in many conservation plans, but few scientific studies up to now have demonstrated their role in a rigorous and unambiguous way, and supporting experimental data to justify the use of corridors for conservation purposes is lacking (Simberloff & Cox 1987;

Simberloff et al. 1992; Rosenberg et al. 1997). Some recent studies have provided convincing evidence that, in some cases, corridors can enhance migration rates among patches (Andreassen et al. 1998; Aars & Ims 1999; Haddad 1999; Mech & Hallet 2001) and fitness components (Boudjemadi et al. 1999). These studies have all concerned animal species, however, and to our knowledge there has been no investigation of the effect of corridor connection among habitat patches on plant species. It has been proposed that connection by water and corridors facilitating animal movements could promote among-population migration for plants insofar as both water and animals are often good vectors for seed dispersal, but this remains untested. However, there have been some studies, based on correlations between species distribution and seed-floating capacity, documenting the role of rivers in plant dispersal (Johansson et al. 1996).

To provide some initial insight into the role of corridors in plant dispersal, we tested for the effect of connection among populations on migration in *Ranunculus nodiflorus* L. (Renonculaceae), an endangered plant species living in a naturally fragmented habitat. In the Fontainebleau Forest (France), we studied metapopulations that consisted of a spatially and temporally variable number of local populations living in ponds. Some of these ponds were connected through natural corridors temporarily flooded after rain. We hypothesized that corridors may enhance the migration of floating seeds between ponds and influence the genetic and demographic structure of metapopulations. We addressed the following questions: (1) How does the connection pattern among ponds affect genetic differentiation among populations? (2) Does the occurrence of corridors to other ponds harboring a population affect the probability of pond occupation?

Methods

Study Species

Ranunculus nodiflorus is a rare annual plant living in damp environments in Spain, Portugal, and France. The species is on France's list of priority endangered species (Olivier et al. 1995). It has experienced a large decline during the last century as a result of the drainage of wetlands and the reduction of grazing, which has led to the disappearance of open and humid habitats (Danton & Baffray 1995).
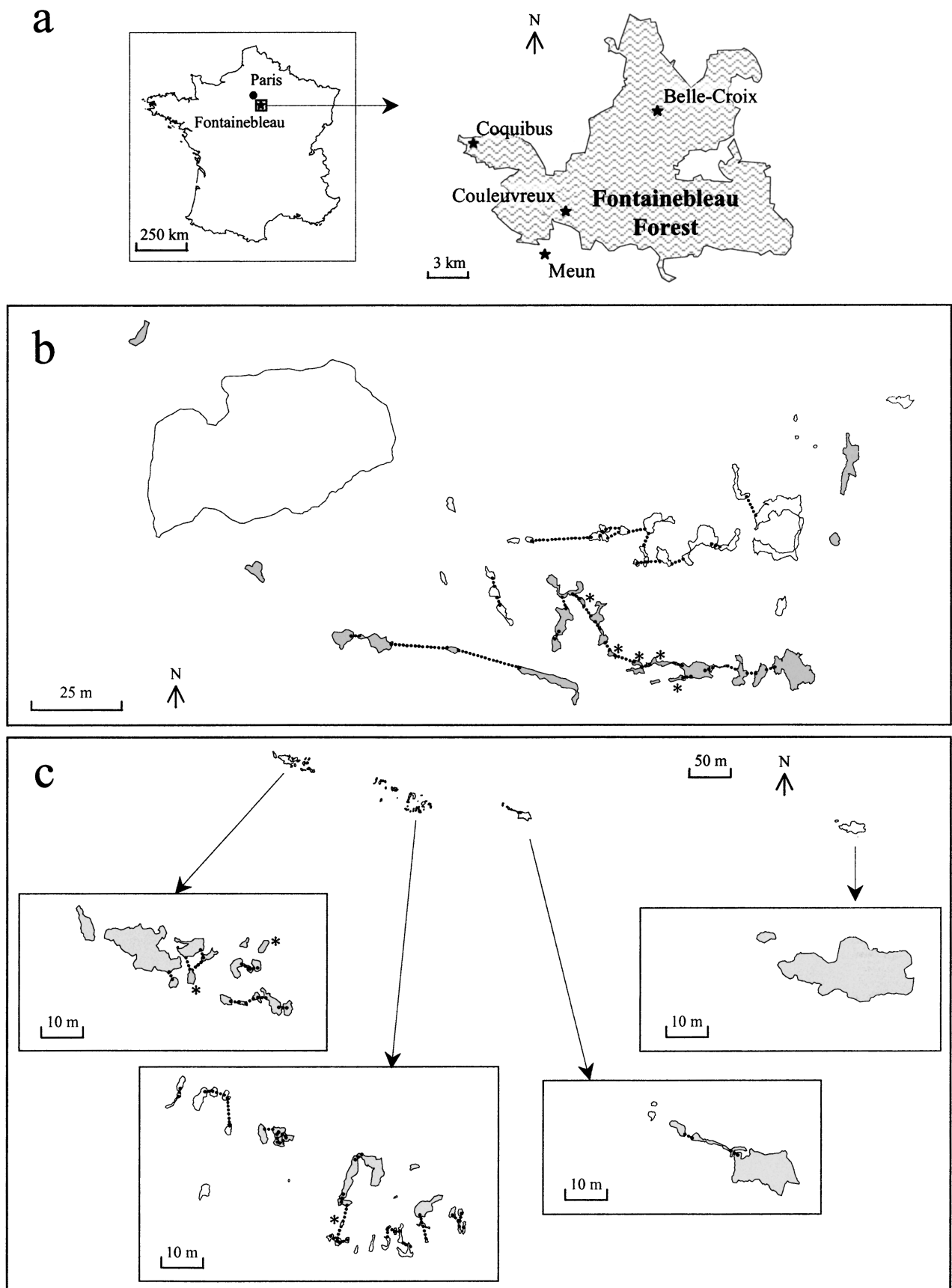
Figure 1. Geographic distribution of Ranunculus nodiflorus populations in the Fontainebleau Forest (70 km south from Paris): (a) location of the four sites studied and pond distribution for the two sites that harbored numerous ponds, (b) Coquibus and (c) Meun. Shaded ponds represent colonized habitats, unshaded ponds are empty suitable habitats, asterisks indicate ponds that were empty in 1999 and colonized in 2000, and dotted lines are natural corridors that allow for temporary connections between ponds when they become flooded after rain.

Unlike many other *Ranunculus* species, whose pollination success depends on pollinator visits (Steinbach & Gottsberger 1994), *R. nodiflorus* is thought to be predominantly autogamous. Indeed, the species presents tiny, odorless flowers with small petals (<1 mm long), and no insect foraging on the flowers was seen by any of us in the field (Fontainebleau Forest) during the 1999 and 2000 flowering seasons. To confirm the self-fertilization ability of the species, we bagged 10 individual plants before the flowers opened to investigate seed set in the absence of pollinators. Only three individuals remained bagged during the 4 weeks of the experiment, but all set seeds, showing that at least some individuals in the Fontainebleau Forest are self-compatible and capable of autonomous self-pollination. The plant produces small akenes (about 1.5 mm in size) apparently devoid of any particular structure that could facilitate their dispersal, but which are able to float (20 akenes dropped in water remained on the surface for more than 4 weeks). Seeds germinate in spring, when the water level in ponds has begun to decrease. The plants then complete their life cycle within 1 or 2 months and die during summer after the ponds have dried. As an annual, unable to reproduce vegetatively (Danton & Baffray 1995; F.K. & J.-B.F., field observations), the species depends entirely on soil seed banks for its persistence.

Study Area

Our study was carried out in the Fontainebleau Forest, which is about 70 km south of Paris, where *R. nodiflorus* is relatively abundant and occurs in numerous, well-delimited, temporary ponds found at five different sites (sandstone open areas) within the forest (Fig. 1a). Ponds were formed on the sandstone slab, lined with a thin soil layer and free of high vegetation; they were oligotrophic, acid, and generally shallow. Narrow paths of land, a few tens of centimeters wide without vegetation, could be easily identified as potential natural corridors for seed dispersal between ponds. Subject to strong variations in water level, ponds established water connections when corridors were flooded after rain (Fig. 1b & c).

The species is a poor competitor, and forest progression over the ponds has caused the extinction of populations. Recolonization is possible when fires regenerate open areas, which have become rare because of forest management, or when human-driven cleaning operations reopen ponds. In such a landscape, *R. nodiflorus* is thought to experience metapopulation dynamics with local extinctions and recolonizations.

The three hierarchical spatial scales we considered were ponds, networks, and sites. *Ponds* were suitable habitats of 1–2250 m^2 that could be colonized or not. Individuals dwelling in a pond were considered a population. Ponds could be linked in a *network* through corridors. A *site* was a set of pond networks and isolated ponds within an open area of 1 ha at most. This last geographical scale corresponds to the metapopulation scale.

We studied the populations found in four sites in the Fontainebleau Forest (the fifth site, which had a single population, was not included in the study because it was too small). In the four sites, we identified all colonized ponds and ponds in which the plant did not occur but that we considered favorable for its settlement. Identifying these potentially suitable ponds was relatively easy because of the peculiar characteristics of the species habitat. Every pond and connection was mapped by means of a global positioning system (GPS). A total of 110 ponds were mapped, of which 55 and 62 were occupied in 1999 and 2000, respectively.

Some local populations appeared to be isolated: the three populations of the Belle-Croix site and the single population of the Couleuvreux site were, respectively, 10 and 3 km away from any other population (Fig. 1a), and no empty suitable pond was located nearby. The two other sites (Coquibus and Meun) harbored a number of pond networks made of 2–16 connected ponds, with about half the ponds being colonized in 1999 and 2000 (Table 1; Fig. 1b & 1c).

Genetic Analysis

We collected young leaves in April 1999. Overall, we sampled leaves from 664 individual plants (about 2% of all individuals in 1999) from 44 populations (ponds;

Table 1.   Data from demographic surveys of *R. nodiflorus* populations in four sites[a] in the Fontainebleau Forest (France) in 1999 and 2000.

Site	No. of networks[b]	No. of favorable ponds[c]	No. of occupied ponds		No. of flowering plants	
			1999	2000	1999	2000
Coquibus	5	48	20	25	24,600	25,400
Meun	12	58	31	34	8,700	20,500
Couleuvreux	0	1	1	1	51	70
Belle-Croix	0	3	3	3	172	65
Total	17	110	55	63	33,523	46,035

[a] *Sandstone open areas in the forest.*
[b] *Networks are sets of more than one favorable pond connected by corridors.*
[c] *Favorable ponds are suitable habitat patches for the species that can be occupied or not.*

Table 2). Sample sizes ranged from 5 to 75 individuals per population.

Leaves were stored in liquid nitrogen after collection and transferred to a freezer at $-80°$ C until processing. They were ground in liquid nitrogen for enzyme extraction in a tris-HCl 0.1M grinding buffer at pH 7.5 (Soltis et al. 1983) and absorbed onto wicks that were then stored at $-80°$ C. We used two starch-gel buffer systems and assayed samples for six enzymes (modified from Soltis & Soltis 1989; recipes available from F.K. upon request). We scored 14 loci: Pgm1, Pgm2, Pgm3, Cat1, Lap1, Lap2, Mdh1, Mdh2, Mdh3, Mdh4, Mdh5, Pgi1, Pgi2, and Aat1. Three loci were polymorphic: Pgm2 and Cat1 were codominant, biallelic loci. Locus Lap1 was biallelic with a null allele.

To consider locus Lap1 in the analysis along with the two other polymorphic loci, phenotype frequencies for that locus were converted into genotype frequencies. We hypothesized that F_{IS} for locus Lap1 was equivalent to the mean bilocus F_{IS} estimate over all populations for loci Pgm2 and Cat1, and we calculated Lap1 allele frequencies for every population from the following formula:

$$X_{22}^i = (x_2^i)^2 + F_{IS} x_2^i (1 - x_2^i),$$

where x_2^i is the allele frequency of the null allele in population i and X_{22}^i is the genotype frequency of null homozygotes in that population (Hartl & Clark 1997). We then computed genotype frequencies X_{11}^i and X_{12}^i according to the hypothesized F_{IS} value. Converting phenotype frequencies into genotype frequencies made it possible to calculate multilocus estimates of gene diversity and F statistics over the three polymorphic loci. But this did not allow us to carry out population differentiation tests on data sets including locus Lap1 because allele frequencies were no longer independent among loci. Therefore, monolocus and multilocus tests were only performed for the two codominant loci.

To analyze population genetic structure, we performed a hierarchical analysis of molecular variance (AMOVA) using TFPGA (Tools for population genetics analyses 1.3: a Windows program for the analysis of allozyme and molecular population genetics data. Computer software distributed by M. P. Miller), which computes F statistic estimates according to method of Weir and Cockerham (1984).

Considering the hierarchical structure of the data set (several sites, several networks within sites, and several populations within networks), we calculated five statistics (for each polymorphic locus and over loci): F_{IS}, F_{ST} among populations within networks ($F_{ST}^{pop(net)}$), F_{ST} among networks within sites ($F_{ST}^{net(si)}$), F_{ST} among sites (F_{ST}^{si}), and F_{IT}. Mean F_{IS} over all populations was tested with GENEPOP (Raymond & Rousset 1995*a*, 1995*b*), which computes exact tests for Hardy-Weinberg equilibrium. We used TFPGA to perform Fisher exact tests (Fisher 1954) for population differentiation. Global F_{ST} among all populations and F_{ST} at the three geographical scales (sites, networks, and populations) were tested for each of the two co-dominant polymorphic loci (Pgm2 and Cat1). We used Fisher's combined probability test (Fisher 1954; Sokal & Rohlf 1995) to determine the overall significance for these two loci.

We performed a partial Mantel test to determine the relative effect of geographic distance and connection through flooded corridors on genetic distances among populations. Genetic distance was estimated as the F_{ST} calculated over the three polymorphic loci. Pairwise F_{ST}s were computed for all population pairs. Explanatory variables were the log-transformed edge-to-edge geographic distance between two populations (Rousset 1997) and three different measures of the degree of connection (C) between populations: (1) C1 = 1 if two ponds were directly connected; otherwise, C1 = 0. (2) C2 = 1 if the two ponds belonged to the same network; otherwise, C2 = 0. (3) C3 = $1/n_{ij}$, where n_{ij} is the number of corridors between two ponds i and j (C3 = 1 for ponds directly connected, 0.5 for ponds connected in a network through a third pond, and so forth, and 0 for ponds that did not belong to the same network). We tested the effect of these explanatory variables separately on Meun and Coquibus following a forward stepwise procedure (Legendre et al. 1994).

Demographic Analysis

We counted individuals of *R. nodiflorus* in 1999 and 2000 to analyze the occupancy pattern of ponds in the two study years. Only the sites that had more than three

Table 2. Number of populations sampled for genetic analyses, sample size, allele frequencies for loci Pgm2 and Cat1[a] and phenotype frequencies for locus Lap1[b] in the four sites from which *R. nodiflorus* was studied in the Fontainebleau Forest.

Site	No. of sampled ponds	Sample size	Pgm2 (allele 1)	Cat1 (allele 1)	Lap1 (dominant phenotype)
Coquibus	19	334	0.60	0.12	0.25
Meun	23	289	0.37	0.03	0.78
Couleuvreux	1	18	1	0.29	0
Belle-Croix	1	23	0	0	1
Total	44	664	0.49	0.08	0.49

[a] *Pgm2 and Cat1 were codominant, biallelic loci.*
[b] *Locus Lap1 was biallelic with a null allele.*

occupied ponds (Coquibus and Meun) were retained for the analyses.

Pond colonization may depend on the geographic distance or connection through corridors to extant populations in neighboring ponds. Thus, ponds cannot be considered statistically independent from one another. A regression analysis on local population size by isolation and the degree of connection, for example, would be done using nonindependent points, and probability results would not be clearly interpretable. So we computed a statistic D_{ij} for each pair of ponds i and $j,$ such that:

$$D_{ij} = |s_i - s_j|,$$

where s_i and s_j represent the colonization status (occupied, 1; empty, 0) of ponds i and $j.$ The statistic $D_{ij} = 0$ when the two ponds had the same status (either occupied or empty), and $D_{ij} = 1$ when one of the ponds was occupied and the other was empty. With this statistic, the occupancy pattern in Coquibus and Meun sites could be represented as a semi-matrix. We used the statistical procedure described in the previous section (a partial Mantel test with a forward selection procedure; Legendre et al. 1994) to test the correlation between the matrix of D_{ij} and four matrices of explanatory variables. This method explicitly takes into account the non-independence of neighboring ponds. Explanatory variables were the log-transformed edge-to-edge geographic distance and the three measures of connection C1, C2, and C3 defined above for the genetic analysis. The effects of these explanatory variables were tested separately on Coquibus and Meun.

Results

Genetic Analysis

Out of the four sites, two were polymorphic for the three loci (Coquibus and Meun), Couleuvreux was polymorphic only for Cat1, and Belle-Croix was monomorphic for all loci (Table 2). Genetic structure at the different spatial scales was highlighted by a hierarchical AMOVA

(Table 3). Mean F_{IS} value over the two codominant loci was high ($F_{IS} = 0.6093, p < 0.0001$). Genetic differentiation calculated over polymorphic loci was strong among populations within networks and among networks within sites ($F_{ST}^{\text{pop(net)}} = 0.7167$ and $F_{ST}^{\text{net(si)}} = 0.6838$) but much weaker among sites ($F_{ST}^{\text{si}} = 0.1335$). Differentiation among networks was significant for both codominant loci in Coquibus ($p < 0.0001$ for both loci) but for only one in Meun ($p < 0.0001$ for Pgm2 and $p = 0.2012$ for Cat1). Differentiation among populations within networks was significant ($p < 0.05$) in three networks (one in Coquibus and two in Meun) out of the seven we studied.

For the six largest ponds, plants were sampled in different points of the population (2–5 sampling points per population, 3–9.6 m apart). This allowed us to perform exact tests for differentiation within ponds for each of the two loci Pgm2 and Cat1. Differentiation among sampling points within ponds was detected in one pond for locus Pgm2 ($p = 0.004$). The 11 other tests showed no significant within-pond differentiation ($p > 0.1$).

In addition to the mean F_{IS} estimated over Pgm2 and Cat1 ($F_{IS} = 0.6093$), we used arbitrary F_{IS} values ranging from 0 to 1 to estimate genotypic frequencies in Lap1 (biallelic locus with a null allele) and then computed multilocus F_{ST}. These multilocus F-statistic estimations did not appear to be sensitive to the F_{IS} value used to estimate Lap1 genotype frequencies. Variation in F_{ST}^{si} represented 12% of the mean, whereas $F_{ST}^{\text{pop(net)}}$ and $F_{ST}^{\text{net(si)}}$ varied by <1% of the mean. Thus, F_{ST} estimations including locus Lap1 were not markedly biased by the fact that we did not have an F_{IS} estimate at this locus.

Over all population pairs, both distance and connection played a role in determining F_{ST} between populations (Fig. 2). Genetic differentiation significantly increased with geographic distance (Mantel test: $p < 0.001, b = 0.0253$), and a chi-square test showed that connected population pairs were significantly more often below than above the regression line ($\chi^2 = 45.63, p < 0.0001$). Thus, at a given distance, genetic differentiation was significantly smaller between populations connected by corridors than between unconnected populations.

For population pairs within each site in Coquibus and

Table 3.   The F statistics estimates for *R. nodiflorus* populations computed according to Weir and Cockerham (1984).[a]

Locus	F_{IT}	F_{ST}^{si}	$F_{ST}^{net(si)}$	$F_{ST}^{pop(net)}$	F_{IS}
Pgm2	0.9644	−0.0526	0.6473	0.7031	0.8801
Cat1	0.4149	0.0549	0.0506	0.1308	0.3269
Lap1	0.9559	0.3108	0.8879	0.8885	—
Pgm2 and Cat1	0.8288	−0.0261	0.5000	0.5619	0.6093
Pgm2 and Cat1 and Lap1	0.8890	0.1335	0.6838	0.7167	—
(S.D.)[b]	(0.0859)	(0.1644)	(0.1699)	(0.1453)	

[a]*Hierarchical analyses of molecular variance were carried out for each locus, over the two codominant loci Pgm2 and Cat1 and over all three loci. The frequency of the null allele of Lap1 was estimated for each population, with $F_{IS} = 0.6093$ (see text for details). Three F_{ST} were computed: $F_{ST}^{pop(net)}$, among ponds within pond networks; $F_{ST}^{net(si)}$, among pond networks within sites; F_{ST}^{si}, among sites.*
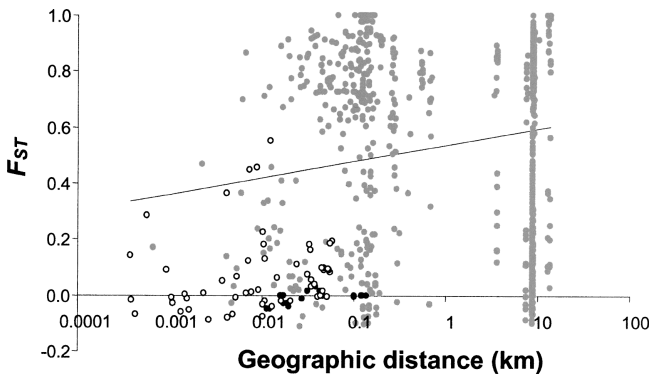[b]*Standard deviations were calculated by jacknifing over the three polymorphic loci.*

Figure 2. The pairwise F_{ST} *between* R. nodiflorus *populations as a function of geographic distance. Open circles are pairs of populations from the same pond network (i.e., populations connected via one or several corridors), and closed circles are pairs of populations from distinct networks (i.e., unconnected populations). Geographic distance has a logarithmic scale.*

Meun, distance and the three connection measures had a significant effect when tested separately on F_{ST} between populations. Distance had a positive effect (Mantel test: $p < 0.005$, $b = 0.4644$ and $p < 0.005$, $b = 0.5183$ in Coquibus and Meun, respectively) and connection had a negative effect (Mantel test: $p < 0.005$ for the three connection matrices C1, C2, and C3 in Coquibus and Meun).

When analyzing the variability in F_{ST} for population pairs with the four variables together (geographic distance, C1, C2, and C3), a stepwise procedure selected only C2 in Coquibus (partial Mantel test: $p < 0.001$) and only the log-transformed distance in Meun (partial Mantel test: $p < 0.001$; Table 4). Separate analyses for each locus gave globally concordant results (Table 4). The same analyses performed with Nei's unbiased pairwise genetic distances (Nei 1978) instead of F_{ST}s gave identical results, except for locus Lap1 in Meun. In this case, considering Nei's unbiased distances, both C2 and the log-transformed distance were selected (partial Mantel test: $p < 0.001$ and $p < 0.005$, respectively).

Demographic Analysis

Out of 110 favorable ponds, 55 were occupied in 1999 and 63 in 2000 (Table 1), and population size ranged from 1 to about 16,000 flowering individuals per pond (Fig. 3). Five ponds in Coquibus and three in Meun were empty in 1999 and occupied the following year (Table 1 and Fig. 1). The number of flowering plants was higher in 2000 than in 1999 in Meun (+136%) and Couleuvreux (+37%), lower in 2000 than in 1999 in Belle-Croix (−62%), and similar in both years in Coquibus (+3%).

When the distance and connection variables were considered separately, the analyses of pairwise dissimilarity between ponds in both Coquibus and Meun showed a significant positive effect of the log-transformed distance (Mantel test: $p < 0.01$ and $p < 0.001$ in 1999 and 2000, respectively) and a significant negative effect of the connection (Mantel test: $p < 0.01$ and $p < 0.005$ for the three connection matrices C1, C2, and C3 in 1999 and 2000, respectively).

When the four explanatory variables were tested together, only one variable was retained in each case by the stepwise procedure. In Coquibus C3 was selected in 1999 and C2 in 2000 (partial Mantel test: $p < 0.001$ in both cases). In Meun C2 was selected in 1999 and the log-transformed distance in 2000 (partial Mantel test:

Table 4. Results of the analyses of pairwise genetic differentiation (multilocus F_{ST}, F_{ST} for loci Pgm2, Cat1, and Lap1) between populations, and pairwise demographic dissimilarity (*D* for years 1999 and 2000) between favorable ponds in *R. nodiflorus*.[a]

	Site	Dependant variable	Explanatory variables selected[b]
Genetic analysis	Coquibus	multilocus F_{ST}	C2***
		F_{ST} Pgm2	C2***, Dist*
		F_{ST} Cat1	Dist*
		F_{ST} Lap1	Dist***, C2**
	Meun	multilocus F_{ST}	Dist***
		F_{ST} Pgm2	Dist***
		F_{ST} Cat1	—
		F_{ST} Lap1	Dist***
Demographic analysis	Coquibus	D_{1999}	C3***
		D_{2000}	C2***
	Meun	D_{1999}	C2***
		D_{2000}	Dist***

[a] *Mantel tests were performed separately in Coquibus and Meun sites. Significant variables were selected following a forward stepwise procedure. The demographic dissimilarity was defined as follows:* D = 0 *if two ponds had the same status (either colonized or empty); otherwise,* D = 1 *(one pond colonized and the other empty).*
[b] *The explanatory variables tested were the semi-matrix of log-transformed geographic distances (Dist) and three semi-matrices of connection (C1, C2, and C3) between ponds (see text for details). Only the variables retained in the model are presented: dash, no significant variable;* *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.*
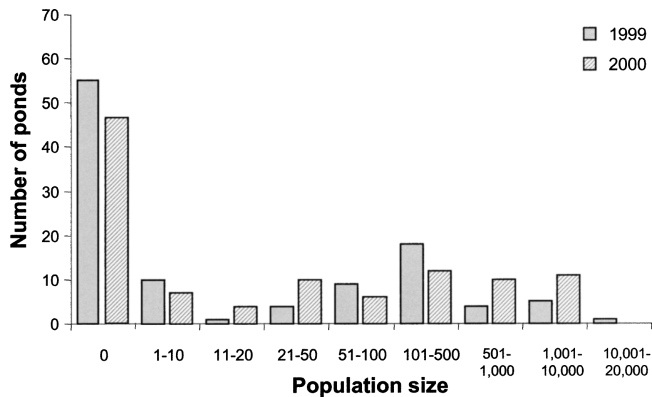
Figure 3. Distribution of population size of R. nodiflo-rus *(number of flowering plants per pond) in 1999 and 2000.*

$p < 0.001$ in both cases; Table 4). Thus, once the effect of connection on similarity was taken into account, the geographic distance between ponds appeared to have no significant effect in 1999 for either Coquibus or Meun or in 2000 for Coquibus. Globally, ponds belonging to the same network were therefore more similar than ponds belonging to separate networks. Most networks were either empty or entirely colonized, and few were partially occupied (6 and 4 networks out of 17 in 1999 and 2000, respectively; Fig. 1).

The strong correlation between distance and connection (for example, between distance and C3: $r + 0.69$, $p < 0.001$, and $r + 0.53$, $p < 0.001$, in Coquibus and Meun, respectively) may affect the stepwise selection procedure so that the first variable retained does not necessarily better explain the variability in F_{ST} or D than the other variables tested. An additional analysis was performed to take this correlation into account. When we tested with two successive Mantel tests the effect of distance on F_{ST} or D, and then the effect of connection on the residuals of the first regression, the results confirmed those presented above. Distance was significant in all cases ($p < 0.01$), and results for connection were the same as those from the stepwise analysis (Table 4), except for D_{1999} in Meun where C2 became only marginally significant ($p = 0.057$).

Discussion

Population Genetic Structure

Genetic variation was highly structured among *R. nodiflorus* populations within every site. The mean F_{ST}s among populations within networks and among networks within sites were much greater than F_{ST}s generally reported in the literature for autogamous and restricted species (Hamrick & Godt 1989, 1996). This

result is all the more striking because, unlike in most genetic studies conducted on natural populations, we estimated genetic structure on a relatively small spatial scale: the two largest sites, Coquibus and Meun, were 230 and 760 m wide, respectively.

The genetic structure of presumably neutral markers depends on the balance between random drift and migration. Given the substantial differences observed in *F* statistics among loci, selection may operate on locus Cat1. High levels of differentiation among ponds in *R. nodiflorus* suggest, however, that local drift is much stronger than migration. Strong drift within ponds may be explained by the annual life cycle of the species and the relatively small population sizes (Fig. 3), subject also to strong year-to-year fluctuations (such as those observed between 1999 and 2000 in Meun) caused by variations in water level in ponds (environmental stochasticity). Neither seed bank nor migration between ponds seemed to efficiently counterbalance the effects of genetic drift.

Recurrent founder events may also be responsible in part for the observed genetic structure among populations (Slatkin 1977; Whitlock & McCauley 1990; Pannell & Charlesworth 1999). Some ponds that were empty in 1999 were occupied in 2000, which suggests that recolonization could be frequent in *R. nodiflorus*. But seed bank might also account for these observations. One thing is clear, however: gene exchanges among ponds were restricted enough to have allowed strong divergence among populations.

This landscape structure of small, suitable habitat patches within sites had an important role in the maintenance of genetic diversity. Indeed, genetic differentiation between sites (metapopulations) was much lower than among populations within sites, but we cannot infer that gene flow was stronger between sites separated by several kilometers than between ponds a few tens of meters apart. Rather, this genetic structure can be viewed as an illustration of the theoretical prediction that, in a metapopulation harboring local populations that are more or less isolated, the random fixation of different alleles in different local populations allows the maintenance of global genetic diversity in the entire metapopulation. Thus, drift at the metapopulation level is slower than it would be in an unstructured landscape, and metapopulations are weakly differentiated from one another. On the contrary, the two sites with only one and three ponds (Couleuvreux and Belle-Croix, respectively) fixed one allele at almost all loci (Table 2).

Role of Corridors

Although our genetic study was conducted on only three polymorphic loci, it demonstrated a strong effect of corridors on gene flow among populations in *R. nodiflorus*. Genetic differentiation between two populations

strongly depended on whether or not those populations were connected ($p < 0.005$). The connection pattern best explained genetic differentiation in Coquibus (C2, $p < 0.001$; Table 4) but not in Meun, where distance was the best explanatory variable ($p < 0.001$). The difference between the two sites probably results from a lower variance in connection in Meun (pond networks in that site were numerous but smaller than in Coquibus; Fig. 1), leading to a less significant effect of corridors. Given the autogamous nature of the species and the type of corridors we considered—narrow paths of land connecting ponds when flooded—we hypothesized that corridors enhance seed migration rather than pollen migration. Our genetic analysis showed the effect of the connection pattern on genetic differentiation between ponds but did not allow us to address this hypothesis. Demographic results, on the other hand, unambiguously demonstrated that corridors provided connection between populations by promoting seed dispersal between connected ponds.

Connected-pond networks were either entirely empty or made up of ponds that were almost all colonized (Fig. 1b & 1c). The statistical analysis of pond occupancy revealed that the probability that a pond was occupied significantly increased with its degree of connection to occupied ponds (C3, in 1999 in Coquibus; Table 4) or with its belonging to a network that was at least partially colonized (C2, in 1999 in Meun and in 2000 in Coquibus). Such a pattern suggests that seed dispersal between ponds mainly occurs between ponds in the same network, whereas dispersal between different networks may be infrequent (but may occasionally occur when storms cause extensive flooding of entire sites). Distance between ponds explained the occupancy pattern better than connection in only one case out of four combinations of population and year (in 2000 in Meun; Table 4).

The existence of flood-prone corridors seems to determine the possibilities for seed exchanges between ponds, and water plays thus a predominant role in seed movements. On the scale of a pond, water probably acts to homogenize seed genotypes over the entire pond area (only 1 test out of 12 revealed a significant genetic differentiation between sampling points of the same pond), and, among ponds within a network, water allows for seed exchanges thanks to flooded corridors. Because of the absence of any preferential direction of flow through these corridors, it is likely that bidirectional seed transfers can occur between connected populations.

Our analyses of genetic structure and pond occupancy demonstrated that corridors represent a major component of *R. nodiflorus* habitat in the Fontainebleau Forest. The existence of totally empty networks and the strong genetic differentiation among networks indicated that migration among networks was weak. Seed dispersal appeared to occur mainly within networks of connected ponds, where its frequency was low but increased with the degree of corridor connection between ponds. Each of these networks might therefore be considered a small metapopulation within which seed dispersal meets a stepping-stone pattern of migration.

Implications for Conservation

Our results suggest that the existence and number of corridors determine, at least partly, the number of ponds that the species can colonize. Some of the ponds suitable for *R. nodiflorus* were probably empty solely because the plants were unable to disperse seeds to them. Corridors may therefore be important for the regional persistence of the species. In addition, corridors may alleviate the risks of local extinction through demographic stochasticity (rescue effect; see Stacey et al. 1997).

Formerly, recurrent fires had an important role in the landscape in opening ponds and corridors, but today fires in the Fontainebleau area are suppressed by forest management. Thus, from a conservation perspective it would probably be a good strategy to maintain existing corridors by removing invasive vegetation and to create new corridors so that *R. nodiflorus* could disperse to unoccupied pond networks. This could enable gradual, pond-by-pond recolonization in empty, suitable networks.

To our knowledge, ours is the first study to suggest that connection among habitat patches plays a role in the dispersal of a plant species. Given the potential conservation value of corridors, new studies need to be conducted to determine whether habitat connection can promote dispersal in other plant species. Aside from flooded corridors, which provide connection via water, fencerows and forested corridors frequented by animal vectors may enhance seed transfers in a number of species.

We did not carry out a truly experimental study, as advocated by different authors to investigate the role of corridors on migration (Simberloff & Cox 1987; Simberloff et al. 1992; Rosenberg et al. 1997). Such experimental studies are useful because several factors such as width of corridors or distance between patches can be controlled to determine the role of corridors on migration rates. But artificial corridors created and maintained by humans may not be suitable for the species under study, even if their design is based on a good knowledge of the species' biology. Instead, we investigated the role of corridors in a naturally patchy system in which the species studied has evolved over a long period of time. We used two approaches and applied them to a large set of connected and unconnected habitat patches to indirectly test the influence of natural corridors on among-population seed transfers. We suggest that other studies combining genetic and demographic methods would give more insight into the role of natural corridors in metapopulation dynamics.

Acknowledgments

Literature Cited

Aars, J., and R. A. Ims. 1999. The effect of habitat corridors on rates of transfer and interbreeding between vole demes. Ecology **80:**1648–1655.

Andreassen, H. P., K. Hertzberg, and R. A. Ims. 1998. Space-use responses to habitat fragmentation and connectivity in the root vole *Microtus oeconomus.* Ecology **79:**1223–1235.

Andrén, H. 1994. Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. Oikos **71:**355–366.

Boudjemadi, K., J. Lecomte, and J. Clobert. 1999. Influence of connectivity on demography and dispersal in two contrasting habitats: an experimental approach. Journal of Animal Ecology **68:**1207–1224.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics **18:**237–268.

Colas, B., C. D. Thomas, and I. Hanski. Evolutionary responses to landscape fragmentation. In R. Ferrière, U. Dieckman, and D. Couvet, editors. Evolutionary conservation biology. Cambridge University Press, Cambridge, United Kingdom (in press).

Danton, P., and M. Baffray. 1995. Inventaire des plantes protégées en France. Nathan, Paris.

Fisher, R. A. 1954. Statistical methods for research workers. Oliver and Boyd, Edinburgh, United Kingdom.

Haddad, N. M. 1999. Corridor and distance effects on interpatch movements: a landscape experiment with butterflies. Ecological Applications **9:**612–622.

Hamrick, J. L., and M. J. W. Godt. 1989. Allozyme diversity in plant species. Pages 43–63 in A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir, editors. Plant population genetics, breeding and genetic resources. Sinauer Associates, Sunderland, Massachusetts.

Hamrick, J. L., and M. J. W. Godt. 1996. Conservation genetics of endemic plant species. Pages 281–304 in J. C. Avise and J. L. Hamrick, editors. Conservation genetics: case histories from nature. Chapman and Hall, New York.

Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics. 3rd edition. Sinauer Associates, Sunderland, Massachusetts.

Huenneke, L. F. 1991. Ecological implications of genetic variation in plant populations. Pages 31–44 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, Oxford, United Kingdom.

Johansson, M. E., C. Nilsson, and E. Nilsson. 1996. Do rivers function as corridors for plant dispersal? Journal of Vegetation Science **7:**593–598.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. The American Naturalist **142:**911–927.

Lande, R. 1995. Mutation and conservation. Conservation Biology **9:** 782–791.

Legendre, P., F.-J. Lapointe, and P. Casgrain. 1994. Modeling brain evolution from behavior: a permutational regression approach. Evolution **48:**1487–1499.

Lynch, M. 1996. A quantitative-genetic perspective on conservation issues. Pages 471–501 in J. C. Avise and J. L. Hamrick, editors. Conservation genetics: case histories from nature. Chapman and Hall, New York.

Mech, S. G., and J. G. Hallett. 2001. Evaluating the effectiveness of corridors: a genetic approach. Conservation Biology **15:**467–474.

Mills, L. S., and F. W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology **10:** 1509–1518.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89:**583–590.

Olivier, L., J.-P. Galland, H. Maurin, and J.-P. Roux. 1995. Livre rouge de la flore menacée de France. Tome 1. Espèces prioritaires. Muséum National d'Histoire Naturelle, Paris.

Pannell, J. R., and B. Charlesworth. 1999. Neutral genetic diversity in a metapopulation with recurrent local extinction and recolonization. Evolution **53:**664–676.

Quilichini, A., M. Debussche, and J. D. Thompson. 2001. Evidence for local outbreeding depression in the Mediterranean island endemic *Anchusa crispa* Viv. (Boraginaceae). Heredity **87:**190–197.

Raymond, M., and F. Rousset. 1995*a*. An exact test for population differentiation. Evolution **49:**1280–1283.

Raymond, M., and F. Rousset. 1995*b*. GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. Journal of Heredity **86:**248–249.

Rosenberg, D. K., B. R. Noon, and E. C. Meslow. 1997. Biological corridors: form, function, and efficacy. BioScience **47:**677–687.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F* statistics under isolation by distance. Genetics **145:**1219–1228.

Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature (London) **392:**491–494.

Shaffer, M. L. 1987. Minimum viable population size: coping with uncertainty. Pages 69–86 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, New York.

Simberloff, D., and J. Cox. 1987. Consequences and costs of conservation corridors. Conservation Biology **1:**63–71.

Simberloff, D., J. A. Farr, J. Cox, and D. W. Mehlman. 1992. Movement corridors: conservation bargains or poor investments? Conservation Biology **6:**493–504.

Slatkin, M. 1977. Gene flow and genetic drift in a species subject to frequent local extinction. Theoretical Population Biology **12:**253–262.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. W.H. Freeman, New York.

Soltis, D., D. C. Hauffer, D. C. Darrow, and G. J. Gastony. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. American Fern Journal **73:**9–27.

Soltis, D., and P. Soltis. 1989. Isozymes in plant biology. Dioscorides Press, Portland, Oregon.

Stacey, P. B., V. A. Johnson, and M. L. Taper. 1997. Migration within metapopulations: the impact upon local population dynamics. Pages 267–291 in I. Hanski and M. E. Gilpin, editors. Metapopulation biology, ecology genetics and evolution. Academic Press, San Diego, California.

Steinbach, K., and G. Gottsberger. 1994. Phenology and pollination biology of five *Ranunculus* species in Giessen, central Germany. Phyton **34:**203–218.

Storfer, A. 1999. Gene flow and endangered species translocations: a topic revisited. Biological Conservation **87:**173–180.

Waser, N. M., and M. V. Price. 1989. Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. Evolution **43:**1097–1109.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F statistics for the analysis of population structure. Evolution **38:**1358–1370.

Whitlock, M. C., and D. E. McCauley. 1990. Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evolution **44:**1717–1724.