Adaptive strategy of a spreading gynodioecious plant species (*Origanum vulgare*, Labiatae) in a riparian corridor

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Backgrounds and aims – *Origanum vulgare* L. is a successfully recovering gynodioecious species in the ecological restoration project along the Common Meuse (Belgium). Strong contrasts exist between spreading – mostly newly established – populations on the one hand, and remnant, declining populations on the other hand. The flowering strategy of the species was assumed to be related to these differences in population status. Two alternative hypotheses for small, isolated populations were postulated: either they contain more sterile individuals due to their presence in marginal conditions, or they show more hermaphrodites for reproductive assurance. Additionally, we aimed at relating neutral genetic variation of the populations with their status and sex ratio.

Methods – An analysis at two levels was performed to get a consistent image of the differences in flowering strategy among and within populations. For the population-level survey, 25 populations were sampled for flowering strategy and for genetic analysis. For the within-population analysis, the survey focused on the expanding populations. Five expanding populations on the riverbanks were fully surveyed for the sex ratio of all individuals. Statistical analysis focussed on relationships between the populations' sex ratios and population genetics, size and dynamics, and stand conditions related to management intensity and isolation.

Key results – Both hypotheses were supported, but at a different level. At the population level, the smaller populations showed more sterility. But within the expanding populations, isolated parts showed less sterility. For the observed sexual expression at population level, no significant correlation was present between sexual expression and population dynamics, isolation or management intensity. For the within-population differentiation, a relationship with reproductive assurance in expanding populations was shown. For the observed differentiation in sex expression, indications are present for the flower strategy being a developmental plasticity.

Conclusion – For *O. vulgare*, the gynodioecy proves a successful plastic strategy for expanding and new establishing populations to cope with pollen limitation and inbreeding depression.

Key words – riparian corridor, colonisation, reproductive assurance, adaptation, plasticity.

INTRODUCTION

River landscapes offer opportunities for the study of population dynamics in relation to the processes of fragmentation and colonization. For some species, reproductive and flowering strategies are well adapted to benefit from the frequent habitat disturbances, and thus are the key to survival. One such species that is extremely well recovering along the River Meuse is *Origanum vulgare* L. This species is currently taking advantage of the newly established natural management of the floodplain in the framework of a large-scale river restoration programme. The observation of the contrast between strongly spreading populations of this species on the one hand, and remnant declining populations on the other hand, was the starting point for this study on the genetic and population structure of the species along the river reach. We aimed at understanding the mechanisms that were responsible for the successful recovery of the species in a restoration context, at the landscape level. Because *O. vulgare* is a gynodioecious species, the flowering strategy may be responsible for the observed contrast in population dynamics between the different populations.

It is known that the breeding system of a plant species plays an important role in its genetic response to spatial and temporal habitat dynamics (Lewis & Crowe 1955, Honnay & Jacquemyn 2007). Less is known, however, regarding the mediating role of more complex breeding systems such as gynodioecy in the response to spatial and temporal landscape dynamics (but see Gouyon & Vernet 1982, Laporte et al. 2001). Gynodioecy has been reported for many higher plant species, particularly in the Labiatae (Lewis & Crowe 1955). Gynodioecious plants have a mixture of hermaphroditic and functionally female individuals (Frank 1989). The genetic control of gynodioecy in *O. vulgare*, being cytoplasmically determined (Lewis 1941), allows for an enduring equilibrium between the different sex phenotypes in the populations (Lloyd 1974, Charlesworth & Charlesworth 1978, Charlesworth & Ganders 1979). When the sex phenotypes of gynodioecious populations are determined by cytoplasmic inheritance, females need only a slight advantage over hermaphrodites in survival, ovule production or outbreeding to persist at equilibrium (Lloyd 1975). Since this genetic control of inheritance is well understood (Dufay & Pannell 2010), gynodioecy is often seen as a selection-based genotypic adaptive strategy. Selection is thought to favour hermaphrodites during population establishment because this sex should be less prone to pollen limitation, especially if self-fertilization is possible (Baker's law, Baker 1967, McCauley & Taylor 1997). However, inbreeding depression could limit this advantage. Yet, in their study of the gynodioecious *Silene vulgaris* (Moench) Garcke, Taylor et al. (1999) found the negative effects of pollen limitation on females far outweighing the negative effects of inbreeding depression following selfing in hermaphrodites. Therefore, in isolated, small populations, more hermaphroditic plants may be expected.

Relating male sterility of *Origanum vulgare* to stand conditions, on the other hand, brought several authors to the conclusion that small and isolated populations exhibit more sterility, due to the presence of marginal stand conditions or intensive management (Elena-Rosello et al. 1976, Ietswaart et al. 1984). The ecological rationale behind this observation might be that female flowers are 'cheaper' to produce and that they promote cross-fertilization (Lewis & Crowe 1955). Furthermore, for the Labiatae it was found that female flowers produce more vigorous (Kheyr-Pour 1975) and more numerous progeny (Assouad et al. 1978).

To summarize, two alternative hypotheses for small, establishing populations can be formulated:

(1) small, isolated populations in unfavourable conditions show more sterility (Ietswaart et al. 1984, Elena-Rosello et al. 1976);

(2) small, isolated populations contain more hermaphrodites (Baker's law) for reproductive assurance.

This opposition could partly be due to scale effects of observation, as well as a consequence of a potential developmental (phenotypical) plasticity of the species, which would overrule the assumption of selection being the cause for the observed patterns. If the male sterility is a developmental plasticity instead of a genetically inherited adaptation of the species, it allows the individual plants to choose their flower strategy based on the environment they experience.

To discriminate between these hypotheses, we studied the flower strategy of the species' populations over the whole river valley with emphasis on the colonization patterns. As the two formulated hypotheses might be affected by scale effects – the first hypothesis being driven by longer term selective forces than the second hypothesis that just refers to pollen limitation, with potentially more local effects – our survey was executed at two scale levels; at population level for all the populations of the Meuse valley and at the within population level for a subset of expanding populations. The expanding populations are structured, with small, isolated patches at the edge of the population, showing processes similar to those occurring in small, isolated populations. In this way, it is justified to make the same observations at both levels. This analysis was done back to back with a study of the genetics of the populations of the Meuse valley (Van Looy et al. 2009) and the relationship between genetic diversity and population dynamics is also part of the explanation of the stated hypotheses. The potential for adaptive responses to changing environmental conditions might be influenced by genetic effects of inbreeding and genetic drift, caused by fragmentation. The sex expression of the populations as a response to environmental conditions might therefore be related to the genetics of the populations (rates of inbreeding/genetic diversity and differentiation).

MATERIAL AND METHODS

Study area

The Common Meuse between Belgium and The Netherlands is the non-impounded, non-navigable reach of the river Meuse, where the river descends from the Ardennes and enters the lowlands. The dynamic gravel-bed river character allows many species from upstream regions to settle in this river stretch (Van Looy et al. 2006). The rain-fed discharge regime knows high peak flows and extreme low flows in dryer periods with a baseflow of less than 10 m³ s⁻¹ and the extreme peak discharges being thousand times bigger. In the mid nineties two extreme peak flows occurred in 1993 and 1995 with the highest ever recorded discharges of over 3200 m³ s⁻¹. These floods revitalised the river's morphodynamic character with impressive bank retreats, scouring of flood channels and overbank sedimentation of gravel and sand. Together with this morphological revival, an impressive influx and recolonisation of species was recorded, enhancing further restoration measures (Pedroli et al. 2002).

Studied species

Origanum vulgare is a tall, tufted, aromatic herb of relatively dry and infertile, calcareous soils. It produces numerous small seeds (1.0 × 0.7 mm, mean weight 0.1 mg), which germinate in vegetation gaps during spring (Cresswell 1982). *O. vulgare* is a long-lived, perennial herb that also forms very large buried seed banks (Grime 1978). Both functionally hermaphroditic and male-sterile individuals exist in the same populations, along with the incidence of intersexes or intermediates, individuals with both hermaphroditic and male-sterile flowers. Female flowers are smaller than the bisexual ones. The sex ratio of natural populations has been observed to vary

from exclusively hermaphrodite to as much as 62% male sterility (Kheyr-Pour 1980).

For the observed populations (fig. 1), cross-fertilization is further promoted by protogynous flowering, although in literature protandry is mentioned for the species (Kühn & Klotz 2002). In the Meuse valley, *O. vulgare* is a typical river corridor species of the more elevated floodplain grasslands. With the intensification of agricultural practices mainly, it became a threatened species of borders and more extensively managed floodplain meadows (Van Looy & Meire 2009). A decade ago a restoration programme was initiated for the river and its valley, starting with the installation of a natural grazing management on several locations. *O. vulgare* was one of the species to benefit from this development and is a strongly expanding species now over the restored sites of the valley. So, it is a species that went through a period of strong fragmentation and now is spreading again along the river.

Survey and sampling

We studied the flower strategy of the species' populations with emphasis on the colonization patterns; at population level for all the populations of the Meuse valley and at the within population level for a subset of expanding populations.

Population-level survey – In the summer of 2005, all 25 populations present in the floodplain of the Common Meuse reach were studied (fig. 1). For each population, leaves of twenty randomly chosen individuals were sampled for genotyping and flowers were described for male sterility characteristics. Four populations contained only very few individuals, and these were omitted, leaving 21 populations for further genetic analysis.

Of each individual plant the flower structure of one flower per stem (up to fifteen stems) was described for male sterility characteristics. Thus male sterility was measured for flowers, and combined for individual plants (proportion of male sterile flowers). There was a strong variation in male sterility observed, within populations but also within individual plants. Furthermore, partial reduction of stamens was described, although this does not necessarily result in sterility. The staminal reduction showed different forms; flowers with total absence of stamens, or with partial reduction up to little buds of two or all four stamens, or with a petal-like form of stamens. This variety was measured as diversity of flower types within a population. The observations were assembled in a measure for flower diversity (number of flower types per individual, averaged over the sampled plants for the population) and male sterility (percentage of male-sterile individuals per population).

Because all sampled populations were monitored since 1992, we could exactly determine the time of establishment (fig. 1, table 1) and distinguished the following categories: 1: 1–4 years; 2: 5–9 years; 3: 10–15 years; 4: > 15 years. Isolation was measured as the distance to the nearest population. A further distinction was made in spreading and remnant populations. Spreading populations were either new colonisations or expanding populations. The spreading populations were compared to remnant populations with respect to flower strategy and genetic diversity and differentiation. The population expansion rate of the spreading populations was measured as

$(\log N)/t$, with N being the current population size and t the time since colonization. The population expansion rate was analysed for a relationship with genetic diversity within the populations, and with the male sterility by Pearson correlation testing in Statistica 9 (StatSoft 2009).

For each population the management was documented, and categorised according to the intensity. Four management classes were distinguished as 1: no management, 2: extensive, natural grazing (0.3 animal units ha^{-1} y^{-1}), 3: intensive grazing pastures, and 4: intensive hayfield. Kruskall-Wallis and median testing was performed to test for effects of the different age categories, the isolation, the spreading-remnant character, and the management class. In case this first explorative analysis of the data identified significant relationships, further statistical regression testing could measure the responses.

Genetic sampling, AFLP protocol and analysis – The analysis of flower strategy was done parallel to a study of the genetics of the populations of the Meuse valley (Van Looy
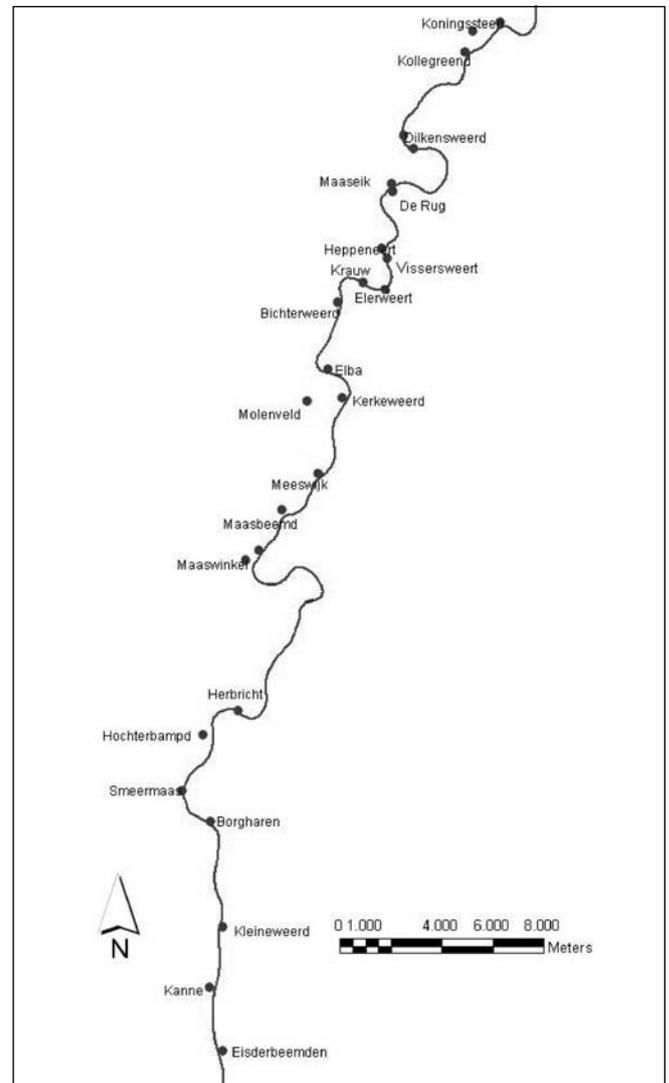

Figure 1 – Sampled *Origanum vulgare* populations along the River Meuse; named according to population names in table 1.

Table 1 – Sampled populations along the River Meuse with their characteristics.

	population	river km	isolation	age	size (N)	population expansion rate	status (expanding /remnant)	man-agement	proportion Polymorphic Loci	Nei's gene diversity Hj	Band richness Br	% male sterility	% staminal reduction	flower diversity
1	Eisder-beemden	7	2000	14	>100	1.06	E	2	87.5	0.3041	1.6	16	23	1.3
2	Kanne	9	2500	12	25–50	0.44	E	3	68.8	0.2486	1.56	43	44	1.5
3	Kleine Weerd	12	3500	>15	50–100	0.58	E	2	79.7	0.2874	1.63	40	40	1
4	Borgharen	16	2500	>15	25–50	-	R	4	-	-	-	22.5	25	1.4
5	Smeermaas	17	2250	>15	<25	-	R	4	60.9	0.2605	1.61	40	40	1.5
6	Hochter-bampd	20.5	2500	14	>1000	2.46	E	2	62.5	0.2314	1.47	14	25.5	1.6
7	Herbricht	22	3550	>15	<25	-	R	4	78.1	0.3001	1.59	23.5	33	1.7
8	Maaswinkel	32	3500	>15	50–100	-	R	1	82.8	0.2878	1.63	21	27	1.8
9	Maasbeemdoever	34	1500	14	25–50	-	R	3	60.9	0.2216	1.47	61	64	1.6
10	Maasbeemdgreend	35	2000	3	50–100	3.51	E	2	75	0.2663	1.56	27	34	1.5
11	Meeswijk	38	2500	2	<25	0.87	E	3	-	-	-	50	50	1
12	Kerkeweerd	40	1250	8	>100	1.37	E	2	79.7	0.2656	1.64	32	43.6	1.7
13	Molenveld	40.5	1250	12	25–50	-	R	4	71.9	0.2594	1.64	55	55	1.5
14	Elba	42.5	2000	>15	>1000	3.47	E	2	78.1	0.2796	1.57	38	34.5	1.6
15	Bichterweerd	44.5	2000	3	25–50	2.11	E	2	79.7	0.2844	1.68	35	52	2.6
16	Elerweert	46.5	1250	>15	50–100	-	R	1	62.5	0.2312	1.54	18.6	24	1.7
17	De Krauw	47	1250	>15	>100	0.75	E	3	76.6	0.3018	1.64	26.5	26.5	1.5
18	Vissersweert	49	1500	>15	25–50	-	R	3	-	-	-	42.5	45	1.4
19	Heppeneert	50	2000	>15	>100	-	R	1	67.2	0.2409	1.54	44	59	1.4
20	Maaseikbrug	53	1550	>15	25–50	-	R	4	71.9	0.2557	1.52	57.5	62.5	1.6
21	De Rug-Roosteren	53	1550	14	<25	0.41	E	2	82.8	0.3304	1.67	30	41	1.5
22	Dilkensweerd dijk	56	1750	14	25–50	0.49	E	4	73.4	0.2798	1.58	16	30	1.7
23	Dilkensweerd	56.5	2250	10	<25	0.47	E	2	76.6	0.2896	1.57	56.5	80.4	2.4
24	Kollegreend	60.5	510	>15	25–50	-	R	1	-	-	-	46	48	2.4
25	Koningssteen	61.5	1000	12	<25	0.32	E	2	68.8	0.2367	1.56	63	63	1.4

et al. 2009). The potential for adaptive responses to changing environmental conditions might be influenced by effects of genetic drift, caused by fragmentation. The sex expression of the populations as a response to environmental conditions might therefore be related to the genetics of the populations (genetic diversity and differentiation).

A total of 398 individuals were sampled from 21 populations located along 55 km of the river (fig. 1). This sampling and analysis is fully described in Van Looy et al. (2009). Individuals were sampled from the entire area occupied by the population in order to avoid the effects of population substructure. AFLP analysis was carried out according to Vos et al. (1995). Three measures of within-population genetic diversity were estimated: the proportion of polymorphic loci (PPL), Nei's gene diversity (H_j) and band richness (Br). PPL and H_j were estimated using AFLPsurv 1.0 (Vekemans et al. 2002). Band richness was calculated for a standardized sample size of 5 individuals according to the rarefaction method of Petit & Thompson (1998).

Within-population analysis of expanding populations – For the within-population analysis, the survey focused on the expanding populations, as the formulated hypotheses at this level can only be analysed in this group. For this analysis, the aspects studied at population level are held constant; age, management and soil conditions are identical for the selected populations. Five expanding populations on the riverbanks were fully recorded for the sex ratio of all individuals. For a total of 2090 individuals the sex ratio was recorded.

Within the expanding parts – occurring linearly along the riverbanks –, the individuals and smaller subgroups were clustered in groups to distinguish the peripheral expansion at considerable distance of the core population (two times the cross section of the core population as boundary, fig. 2).

Both the vegetation structure and soil conditions are the same for the core and the newly colonized areas. All individuals of the new colonisation and the core population were recorded. The analysis of individual plant's responses was not feasible in an unambiguous way; as different responses in relation to both the size of the core population and the presence of subpopulations interfered. Therefore, the effect of this distance to the core on male sterility was compared for the different groups with Friedman testing in Statistica 9. Further testing of male sterility in relation to size and distances for the expanding subgroups was done with Spearman rank tests in Statistica 9.
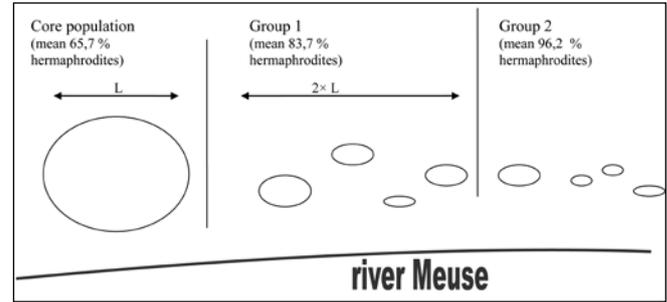

Figure 2 – Presentation of within-population survey of expanding populations, with division in (sub-)populations and observed flowering strategy (% hermaphrodites).

RESULTS

Flowering differentiation between populations

The size of the 25 populations studied ranged from eight to more than 1,000 individuals (table 1). For the male sterility a mean of 23% females is present over the populations (minimum 14%, maximum 63%). Staminal reduction had a mean of 41.2% over the populations, ranging from populations with 23%, to populations with up to 80.4% of the flowers showing staminal reduction. The diversity of flower types over the populations had a mean of 1.55, with a population minimum of 1 and a maximum of 2.6.

Small populations (< 50 individuals) showed significantly higher male sterility ($N = 25$, H = 5.2, $p = 0.02$) than larger populations. No relationship was present between the size and the rate of expansion of the populations on the one hand and the isolation – measured as least distance between neighbouring populations. There was no significant relationship between management intensity over the four classes and the male sterility, although increasing values with management intensity were observed (fig. 3A). The diversity of flower types showed highest values and a higher range for the naturally managed (extensive grazing) grasslands (fig. 3B), but no significant overall differences for flower diversity are present between the four management classes. The spreading populations show on average a higher flower diversity (1.68, SD 0.51) with a larger variation over the populations than the remnant populations (1.58, SD 0.13). This relationship with higher diversity of flower types for spreading populations parallels the fact that these are mostly present in recently established natural areas under extensive grazing (fig. 3A).

Neither for the segregation between spreading and remnant populations nor for the age categories, significant differ-

Table 2 – Size and flowering strategy of subpopulations of within-population survey for expanding populations.

	core population		group 1		group 2	
	size	% hermaphrodites	size	% hermaphrodites	size	% hermaphrodites
Hocht	975	77	26	89	39	97,5
Mazenhoven	123	58	29	79	65	98
Kerkeweerd	278	62	86	83	76	93
Bichterweerd	27	79	107	93	-	-
Krauw	226	52	33	91	-	-
Mean +- SD	326 +- 375,5	65,5 +- 11,9	56 +- 37,6	87,0 +- 5,8	60 +- 19	96,2 +- 2,7

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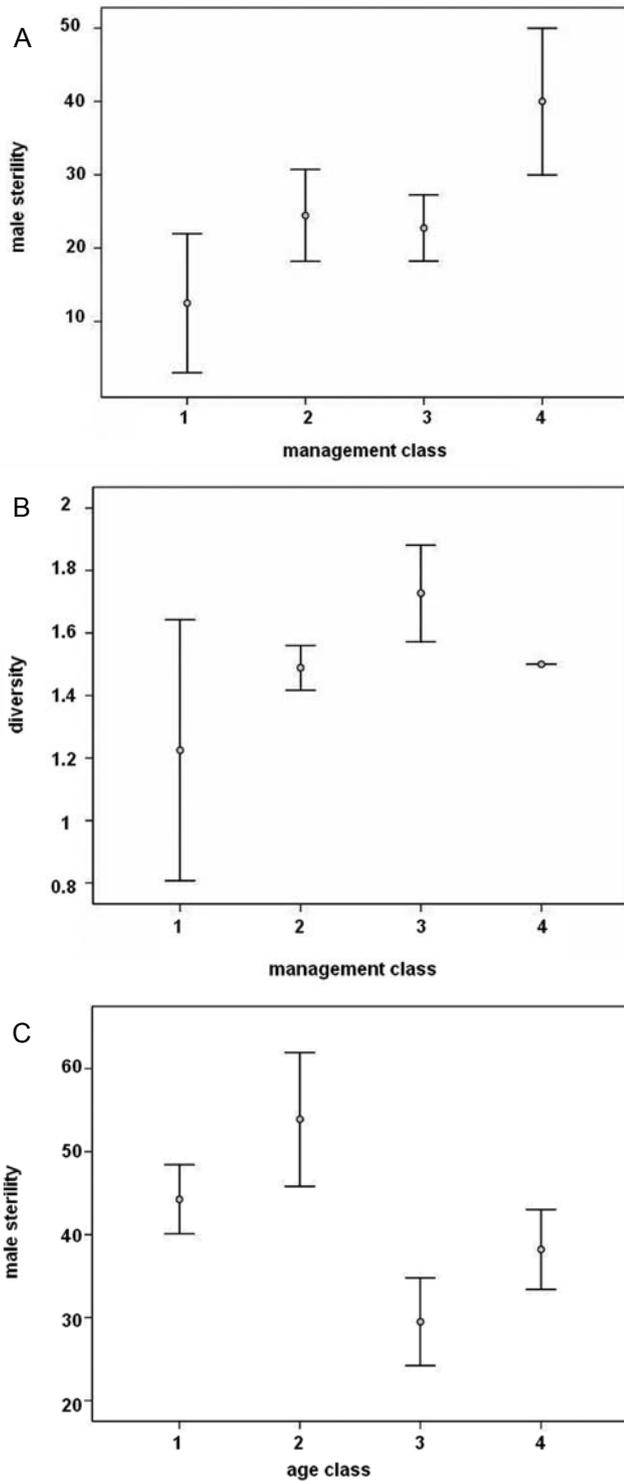
Figure 3 – The mean (+- 1 SE) male sterility (A), and flower diversity (B) for the different management classes (1: no management, 2: extensive grazing, 3: grazing pasture, 4: hayfields), and male sterility for the different age classes (C) of the populations.

▶

Figure 4 – Population expansion rate of the expanding populations in relation to measures of genetic diversity; A, % polymorphic loci P; B, Nei's gene diversity Hj; C, Band richness (Br) and flowering strategy; D, % male sterility.
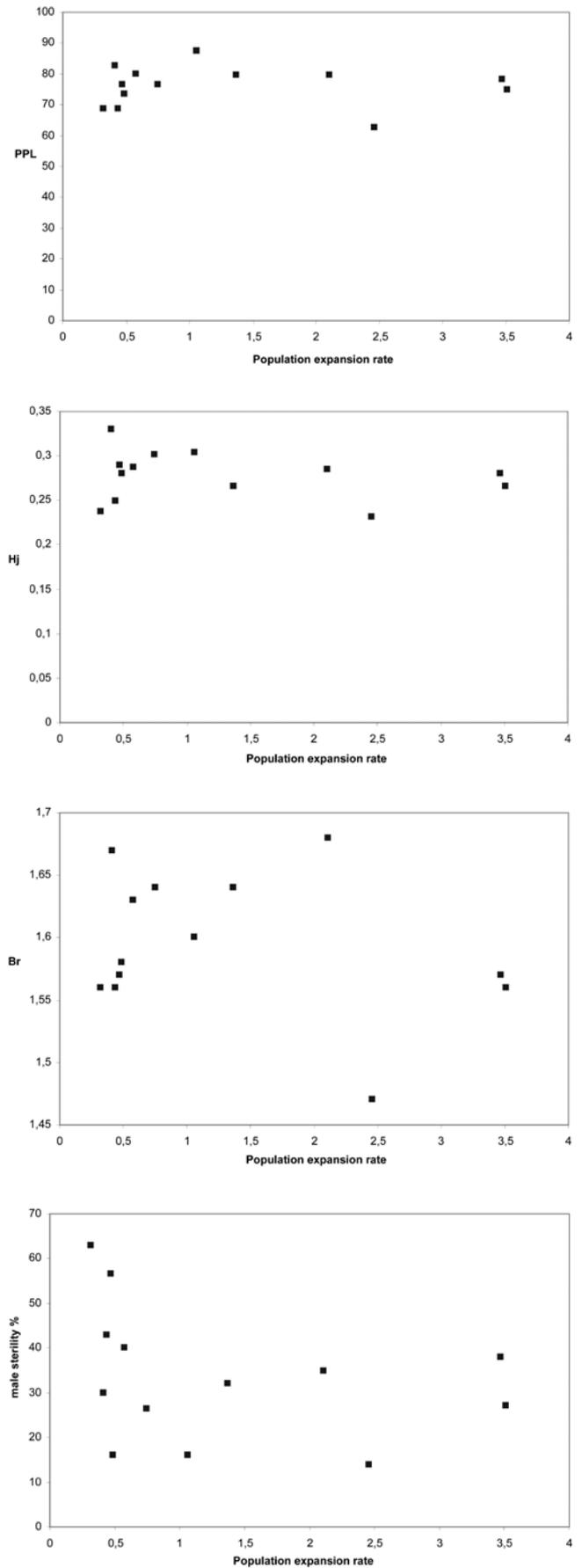
ences in male sterility were observed at population level. The younger populations show on average higher sterility (fig. 3C), with the highest values for the second age category. But also here, the spread was high and no significant distinction can be made in the set of 25 populations.

Genetic diversity and structure

No significant relationships were present between the genetic diversity measures and the staminal reduction, male sterility and flower diversity. Neither for population size, nor for the difference between expanding and remnant populations, nor for the population expansion rate, was there a relationship with the measures of genetic diversity (fig. 4).

The genetic differentiation showed no relationship with the expanding character, nor with flowering strategy. The genetic differentiation was largely explained by a colonisation pattern with extreme flood events (Van Looy et al. 2009). We can conclude that although male sterility varied strongly, no relationship between observed male sterility of the populations was present with genetic differentiation or diversity.

Within-population differentiation

Peripheral newly colonising 'subpopulations' or groups of the expanding populations showed a significantly higher percentage of hermaphrodites, with an extremely high mean of 96% hermaphrodites for the most peripheral groups (fig. 2, table 2). The higher the distance to the core of the population, the more pronounced this tendency, as was shown by the significant difference in male sterility between the distance groups ($\chi^2 = 6$, p : 0.049). The size (number of individuals) of the spreading (sub-)groups showed no relationship with the male sterility.

DISCUSSION

Genetics, population dynamics and reproductive strategy

Many studies have documented clear differences in genetic diversity and differentiation between management regimes (Kleijn & Steinger 2002, Reisch & Scheitler 2009, Reisch & Poschlod 2009) and disturbance levels (DiBattista 2008), and for the flooding influence in the riparian zone of the Meuse River valley even at short time scales (Honnay et al. 2009) and over short distances (Jacquemyn et al. 2006, 2009). For the *O. vulgare* populations no effects of disturbance or fragmentation on genetic diversity were present (Van Looy et al. 2009), as we detected no difference in genetic diversity between small or large, spreading or remnant, intensively or extensively managed populations. Neither was there a relationship with the flowering strategy. This is an important observation as these disturbance (fragmentation and management) processes can cause decreased fitness, decreased tolerance to environmental stress and, most importantly, significantly impede adaptive responses to changing and stressful environmental conditions (Bijlsma et al. 2000).

Male sterility in isolated, spreading populations

We found evidence for both of the formulated hypotheses,

though at different scale levels. At the population level, more sterility was observed in small populations. The lowest sterility is present in the large stable populations under conditions of no or extensive management. Our observations seem to correspond partially to other *O. vulgare* studies. Ietswaart et al. (1984) observed for a large set of populations over different stand conditions that small isolated populations showed more male sterility, especially those under intensive management. Elena-Rosello et al. (1976) also describe more sterility in suboptimal, marginal stands. Yet, she related marginal conditions to rough vegetation structure and soil conditions, which opposes to Ietswaart's conclusions regarding the absence of a relationship with soil conditions and the intensively managed stands offering marginal conditions. So, for the aspect of marginal stand conditions no clear indication is present to whether they are management or soil related, not in our data and contradictory in literature. Still the general hypothesis for populations showing more male sterility in small populations under marginal conditions conforms to our observations and has an obvious ecological rationale; the marginal conditions are somewhat offset by producing cheaper flowers with more progeny.

Conformity to Baker's law was observed in the within-population analysis of spreading subgroups in the edge of populations showing an almost absence of male sterility. For the isolation and colonisation aspects in the hypotheses, the scale of observation is of the strongest importance.

Overall, the gynodioecy seems a useful strategy as reaction to stress conditions; in intensively managed stands of hayfields or grazed meadows, the less investment in flowers results in an earlier seed set. The populations with the least stress are the best developed populations in the area, showing a maximum of hermaphrodites. Mixed flowers (male sterile and hermaphroditic flowers in same individual) are most abundant in intermediate management intensity levels. Also, flower diversity is highest in nature reserves with extensive grazing. In these terrains the high variety in vegetation structure and grazing intensity can explain the mixture in stress responses and strategy combinations.

Reproductive assurance in colonizing species

Especially the significant relationship within the expanding populations illustrates the importance of the gynodioecy as an adaptive strategy. Self-compatibility is generally favoured as a mechanism of reproductive assurance (i.e. Baker's law 1967) especially in colonizing new sites. Taylor et al. (1999) found support for Baker's law in the gynodioecious species *Silene vulgaris* (Moench) Garcke, suggesting that selection favours hermaphrodites during the colonization process. However, the advantage that hermaphrodites might enjoy during colonization was offset somewhat in their study by reduced genetic variation and greater inbreeding depression that occurs within the populations founded by hermaphrodites.

Baker's law holds at the individual plant level for the colonization, yet at the level of the populations, the second hypothesis holds, that there's more sterility in the small, isolated, recently colonized populations, compared to older, larger populations with high numbers of hermaphrodites. So the

species exhibits a perfect strategy by eliminating the risk of pollen limitation with hermaphroditic colonizing plants and new settlements, yet young establishing populations (3–10 years) overcome the inbreeding risks with an increased sterility. These young populations have double profit from the gynodioecy, as the females prevent inbreeding depression and at the same time produce more viable seeds (the less-investment in pollen is invested in progeny). This makes the young populations vital and very successful in their expansion over the newly installed nature reserves. It can explain the extreme ability of the species to colonize the newly realised nature restoration sites and naturally managed terrains.

Mechanism of gynodioecy

From these observations of the adaptive character of this flowering strategy, we can question whether selective processes or developmental plasticity is involved. No difference was observed in genetic structure and diversity between expanding or remnant populations. Nor was there a relationship with within-population genetic diversity with the gynodioecious strategy. The absence of a relationship between population size and age, genetic differentiation and diversity and the flowering strategy can be an indication that for *O. vulgare* the gynodioecy is a developmental plasticity (Lesica & Allendorf 1999, Rice & Mack 1991).

Especially our observations of the colonizing behaviour brings support for the flowering strategy potentially being a developmental (within-generation phenotypic) plasticity. The results of the within-population analysis indicate that the process of directional selection is not the driving force behind the observed adaptive, phenotypic differentiation. At the level of individual plants within expanding populations, higher rates of hermaphrodites were observed at the extremities of the expanding range. Taylor et al. (1999) also observed this pattern at distances of 20–160 m for a gynodioecious species. The significant difference in sex expression over such short distances and time interval for this long-lived species overrules a selective force being at play.

So, our observations indicate that a developmental plasticity governs this species strategy for colonisation and survival under marginal conditions. Further experiments in common garden or with translocation might prove this phenotypic plasticity in the species.

Plasticity was also considered the mechanism behind observed variation of reproductive strategy in *Ranunculus ficaria* L. in a riverine landscape, operating over short temporal and spatial scales (significant differences over 25 m transects) (Jung et al. 2008). A same observation was made for the phenotypic plasticity of *Ranunculus reptans* L. along a small-scale flooding gradient (Lenssen et al. 2004) and for phenological separation of *Ranunculus adoneus* A.Gray along a steep gradient, where gene flow over the micro-environments was supposed to impede adaptation (Stanton et al. 1997). For a gynodioecious wild strawberry, plastic changes in sex expression were observed in response to herbivory (Ashman et al. 2004). Non-random associations between the environment experienced by the individuals over generations might lead to genetic assimilation of developmental plasticity and local adaptation at a small spatial scale (Uller 2008).

CONCLUSION

The analysis at two scale levels proved necessary to get a sufficiently consistent image of the differences in flowering strategy among and within populations. As we anticipated that the contradiction in the hypotheses might be due to the survey scale, we can now refer to the different aspects of these hypotheses. Both hypotheses were supported, but at a different scale level. At the population level, the smaller populations showed more sterility, but within the expanding populations, isolated parts showed less sterility. For the within-population differentiation, the relationship with colonisation behaviour is evidenced. For a long-lived species like *O. vulgare*, the significant variation in sex expression is an indication of this strategy being a developmental plasticity. Its ease to colonize over short as well as long distances with flooding (Van Looy et al. 2009), together with its gynodioecious breeding strategy that allows small isolated populations as well as small new settlements to cope with pollen limitation and survive the threats of genetic drift and inbreeding depression, explains its remarkable recovery in the studied river reach.

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