Gene flow in *Plantago* I. Gene flow and neighbourhood size in *P. lanceolata**

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The genetic population structure and gene flow in the obligatory outbreeding plant species *Plantago lanceolata* L. were determined in the Westduinen (Wd) pasture population in the South-West of the Netherlands. Three experiments were performed: the measurement of genetic structure using allozyme variation, pollen flow in a wind-tunnel and the distribution of an allozyme marker in a garden experiment. The position and allozyme genotype of 524 plants from eight subpopulations in the Westduinen population were determined. The population exhibited local differentiation: genotype frequencies within subpopulations were mostly in Hardy–Weinberg equilibrium, and gene frequencies varied significantly between subpopulations. The wind-tunnel experiments showed that wind speed, source height and capture height of pollen influenced the pollen-mediated gene flow and therefore neighbourhood sizes. In the garden experiment a clear picture of actual pollen flow could be shown.

Estimates of neighbourhood and gene transport parameters from the data of the three experiments indicated restricted gene flow: neighbourhood size (N_e) was between 14 and 20 plants, mean gene transport per generation (M) between 0.2 and 1.4 metres. We argue that this combination of small effective number and low gene transport leads to the substructuring of the Westduinen population of *P. lanceolata*.

INTRODUCTION

Several theoretical studies (Wright, 1943; 1951; Jain and Bradshaw, 1966; Rohlf and Schnell, 1971) suggest that the effect of gene flow on local genetic differentiation is to erode the effects of contrasting selection pressures and random drift. Restricted gene flow would then stimulate the process of local adaptation. However, that this process is more complicated has recently been shown by Stam (1983). He showed that genetical divergence of flowering time, triggered by an environmental difference, could only occur in the presence of sufficient pollen flow. Restricted pollen flow retarded the divergence. Moreover many experiments (see e.g., Millicent and Thoday, 1961 and for more examples, Endler, 1977) show that quite marked differentiation may evolve quickly in spite of high levels of gene flow.

Information on the relative importance of gene flow in processes like differentiation or adaptation of local wild plant populations was collected, e.g., by Wolfenbarger (1959), Levin and Kerster (1974),

Levin (1981). Levin concludes—mainly from observations on entomophilous species—that “even if gene flow distances were twice as large as we now think, the spatial scale of gene dispersal: (1) is still small enough to allow substantial differentiation over short distances with moderate selective differentials and (2) is too small to be a major cohesive force within a species”.

The impact of gene flow on the level of local inbreeding in populations is mostly considered in Wright's neighbourhood model (1943; 1946). The neighbourhood is the area (N_a) from which the parents of central individuals may be treated as if drawn at random. The effective size of a neighbourhood (N_e) is in this model equivalent to the number of reproducing individuals in N_a . The circle of radius 2σ , twice the standard deviation of gene flow distance, would include 86.5 per cent of the parents of individuals at the centre. This linkage between the formulae describing neighbourhood size ($N_e = 4\pi d\sigma^2$ where d = density) and isolation by distance (the parent is situated within a circle of radius 2σ with a probability of $1 - 1/e^2 = 0.865$) only holds under a restricted set of conditions (Crawford, 1984a; van Dijk, 1985a, b). These

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authors further adapt the formulae for N_a and N_e (see Materials and Methods) but also question the applicability of the method for seed plants.

The measurement of the genetic neighbourhood structure of a population requires the estimation of the following parameters: population density, mating system and dispersal distances of pollen and seed. Plant populations are well-suited to study the flow of genes, e.g., because parental individuals are on the average sessile, mature progeny (seed) can be sampled along with the maternal parents and genes are mostly distributed by two distinct developmental stages: pollen and seed. Dispersal can be followed relatively easily if genetical markers are present.

This report presents the measurement of the genetic structure in a field population and estimates of gene flow in the self-incompatible, wind-pollinated, perennial, herbaceous plant species *Plantago lanceolata* L. The species shows the typical syndrome of anemophilous species (sensu Faegri and van der Pijl, 1973): reduced flowers with extending anthers, with sticky, feathery stigmas, large amounts of non-sticky pollen grains with a diameter of 20–30 μm (Hammer, 1978; Primack, 1978; Bos *et al.*, 1985). However some populations of the species are also reported to be insect pollinated (Stelleman, 1984).

Estimates of gene flow were derived from three different types of experiments: (1) The determination of the genetic structure of a field population using allozyme variation. (2) Release of pollen and seed in a wind-tunnel. (3) The distribution of an allozyme marker in an experimental garden population.

MATERIALS AND METHODS

1. Determination of population structure

In order to determine the genetic structure in part of the Westduinen (Wd) population, a dune grassland used as a meadow for more than three centuries in the South-West of the Netherlands, most of the adult plants from eight distinct subpopulations were collected and transported to the Institute Garden. Their position coordinates within the subpopulations were noted. The position of the subpopulations is given in fig. 1. The figure also provides as an example the position of the plants in plot H together with the genotype for the Pgm-2 locus. Numbers of adult plants used for electrophoresis and plants present in (and size of) these subpopulations are presented in the bottom lines of table 1. Environmental clines within the

Figure 1 (Above) Sample sites A-H in the Westduinen population. The predominant wind direction is SW. (Below) Position of Pgm-2 genotypes in the site H.

subpopulations were avoided as much as possible, although small height differences could not always be prevented.

All plants were screened for allozyme variation on eight loci with polyacrylamide-gel electrophoresis. Extract preparation, electrophoresis and staining methods were applied according to van Dijk and van Delden (1981). The eight loci were: Isocitrate dehydrogenase (Idh), Glutamate-oxaloacetate-Transaminase (Got, 2 loci), Leucine-Amino-Peptidase (Lap, 1 locus), Phospho-Glucomutase (Pgm, 2 loci), 6-Phospho-Gluconate dehydrogenase (6-Pgd, 2 loci).

To describe genetic differentiation between subpopulations Nei's (1975) genetic distance (D) and Wright's (1969) fixation indices $F_{ST} = (F_{IT} - F_{IS}) / (1 - F_{IS})$ were used. To describe within subpopulation differentiation we use F_{IS} and deviations from Hardy-Weinberg expectations as indicators. Especially the F statistics developed by Wright to analyse subdivision in hierarchical populations by describing the correlation between uniting gametes—measured relative to the original, theoretical population—is quite informative. The

Table 1 Allele frequencies in the eight subpopulations of the *Westduinen*; subpopulation size (number of adults), number of plants used for electrophoresis (n) and subpopulation area (m^2)

		A	B	C	D	E	F	G	H	Average
IDH	<i>S</i>	—	0.01	—	—	—	—	—	—	<0.01
	<i>N</i>	1.00	0.96	0.99	0.98	0.99	1.00	1.00	1.00	0.99
	<i>F</i>	—	0.03	0.01	0.02	0.01	—	—	—	0.01
GOT-1*	<i>S</i>	0.07	0.16	0.15	0.14	0.11	0.05	0.26	0.07	0.13
	<i>I</i>	0.92	0.67	0.80	0.83	0.88	0.90	0.72	0.93	0.83
	<i>F</i>	0.01	0.17	0.05	0.03	0.01	0.05	0.02	—	0.04
GOT-2*	<i>S</i>	0.72	0.78	0.61	0.76	0.83	0.83	0.77	0.66	0.75
	<i>F</i>	0.28	0.22	0.39	0.24	0.17	0.18	0.23	0.34	0.25
LAP-1*	<i>SO</i>	0.03	—	—	—	—	—	—	—	<0.01
	<i>SI</i>	0.09	0.10	0.32	0.12	0.15	0.31	0.10	0.11	0.16
	<i>I</i>	0.58	0.64	0.30	0.59	0.67	0.39	0.54	0.73	0.55
PGM-1	<i>F</i>	0.30	0.26	0.38	0.29	0.18	0.30	0.36	0.16	0.29
	<i>S</i>	0.10	0.05	0.01	0.01	0.06	0.02	—	0.02	0.04
	<i>N</i>	0.87	0.92	0.97	0.99	0.94	0.98	1.00	0.98	0.95
PGM-2*	<i>F</i>	0.03	0.03	0.01	—	—	—	—	—	0.01
	<i>S1</i>	0.06	0.02	0.02	0.06	0.02	0.12	0.02	—	0.04
	<i>S2</i>	0.20	0.14	0.19	0.13	0.21	0.06	0.12	0.36	0.18
	<i>S3</i>	—	—	0.06	—	0.01	—	—	—	0.01
	<i>I</i>	0.62	0.42	0.46	0.59	0.44	0.52	0.14	0.48	0.46
	<i>F1</i>	0.02	0.11	0.01	0.07	0.01	0.02	0.23	—	0.06
6PGD-1	<i>F2</i>	0.10	0.31	0.26	0.15	0.31	0.28	0.49	0.16	0.25
	<i>S</i>	0.01	—	—	0.01	—	0.01	—	—	<0.01
	<i>N</i>	0.99	1.00	1.00	0.99	0.99	0.99	1.00	1.00	1.00
6PGD-3	<i>F</i>	—	—	—	—	0.01	—	—	—	<0.01
	<i>N</i>	0.99	0.98	1.00	0.99	0.99	1.00	0.99	1.00	0.99
	<i>F</i>	0.01	0.02	—	0.01	0.01	—	0.01	—	0.01
Subpop. size		83	78	79	92	94	67	61	32	73.3
	<i>n</i>	78	66	70	90	72	63	57	28	65.5
Sample area		0.72	1.49	0.72	1.80	0.56	1.62	1.28	2.05	1.28

* The χ^2 -test for heterogeneity was applied for these loci and χ^2 's all appeared to be significant ($P < 0.0005$).

correlation can be partitioned into within groups (F_{IS}), between groups (F_{ST}) and total correlations (F_{IT}). For more details of the methods used in our analysis see Schaal (1975). By analogy with Wright's (1943; 1946; 1969) F -statistics van Dijk (1985a, b) suggested an F_r (fixation index varying with distance r). This index—which is independent of allele frequencies—describes the genetic similarity of alleles in individuals of the population at spatial distance r from a central individual. van Dijk shows for a completely outcrossing, windpollinated species like *P. lanceolata*, that

$$\bar{F}_{IS} = \sum_r \bar{F}_r P_r N_r \quad (1)$$

(P_r = the probability of a plant at distance r to become the male parent; N_r = the number of plants at distance r from the mother) and that, using a normal distribution function for pollen dispersal,

$$P_r = \frac{C \cdot e^{-r^2/2\sigma_p^2}}{\sum_r \psi_r N_r} \quad (2)$$

(ψ_r = pollen density at distance r ; C = a constant).

For a series of σ_p values the value of (1) can now be calculated and the particular σ_p value, which corresponds to the expected value of $\bar{F}_{IS}$ will be the best estimate for this population. So, with this method it is possible to calculate standard deviations of pollen flow (σ_p); the same holds for standard deviations of seed flow (σ_s); van Dijk (1985a, b). With these parameters neighbourhood area (N_a) and size (N_e) are calculated for *P. lanceolata* as:

$$N_a = 4\pi(\sigma_s^2 + \frac{1}{2}t\sigma_p^2) \quad (\text{Crawford, 1984a; van Dijk, 1985a, b})$$

$$\frac{1}{N_e} = \frac{(1 - \frac{1}{2}t)^2}{4\pi d\sigma_s^2} + \frac{t(1 - \frac{1}{2}t)}{4\pi d(\sigma_s^2 + \frac{1}{2}\sigma_p^2)} + \frac{\frac{1}{4}t^2}{4\pi d(\sigma_s^2 + \sigma_p^2)} \quad (\text{van Dijk, 1985a, b})$$

(where d is the density of flowering plants/ m^2 and t the outcrossing rate). In addition a more straightforward measure of isolation by distance, "the mean gene transport in n generations" (van Dijk,

1985a, b) was used:

$$M_n = \sqrt{\frac{1}{2}\pi n(\sigma_s^2 + \frac{1}{2}t\sigma_p^2)} \quad n = \text{number of generations}.$$

2. Wind-tunnel experiments

In a large wind-tunnel (Knottnerus, 1979) situated indoors with a closed air circuit the dispersal of pollen (coloured with a IIK-solution) and seed was studied. The dimensions of the tunnel were $20 \times 0.70 \times 0.70$ m. A schematic diagram of the experimental procedure is given in fig. 2. Pollen

Figure 2 Schematic diagram of the experimental procedure in the wind-tunnel experiments. → direction of the wind; □ sticky ribbon; ψ artificial vegetation. Dimensions of the tunnel: length 20 m, width 0.70 m, height 0.70 m.

was released from an artificial scape: a pipecleaner, on top of a buoyant bar. To release the pollen, the "scape" was dipped in pollen, tightened and set free with a string and then stopped by a rigid bar, transverse in the tunnel. In this way the scape was acting as an instantaneous point source. In each experiment 3–5 puffs were released in an identical way. Two source-heights were used: 0.15 m and 0.40 m above floor level. Every experiment was duplicated. Pollen was collected at variable distances from the source (0.15, 0.50, 1, 2, 4, 7, 11 and 15 metre) and on three heights (0.15, 0.10 and 0.05 m with source-height at 0.15 m; 0.40, 0.25 and 0.10 m with source at 0.40 m) on sticky ribbons and counted under a microscope. One low and one high wind speed were used: 1.5 and 6.0 m/sec (Beaufort ± 1 and 4). The floor of the tunnel was covered with an artificial vegetation of about 0.10 m height.

Seed was released from an artificial source and caught on glued paper on the floor of the tunnel. The source was a small open platform.

Assuming a normal distribution of pollen-dispersal along the linear axis of the tunnel σ_p could be determined as the distance over which 68.3 per cent of the pollen at a certain height in

the tunnel was caught. This was acceptable for source height, but also used for the other receptor heights, although we realise that these σ_p 's by doing so become slightly overestimates. The distances were determined in the graphs of fig. 3, which were constructed from the pollen counts on the sticky ribbons (see above).

With the above conditions two field situations were simulated in a simplified way: (A) The Westduinen, meadow situation: low δ source close to the top of the vegetation with windspeed 1.5 or 6 m/sec and collectors below source height. (B) A contrasting situation ("hay-field") in early spring: high δ source 0.3 m above the vegetation, situation otherwise as in A.

3. Pollen-mediated gene flow in a garden experiment

750 male-sterile (MS1; van Damme and van Delden, 1982) plants were regularly planted (spacing 0.5 metre) within a circle with a radius of 8 metre (fig. 4). The circle was surrounded by a mixed non-*Plantago* vegetation of about the same height to decrease border effects. The experiment was done in June and July 1982 in the Botanical Garden, University of Groningen. Dispersal of pollen from two groups of four hermaphrodite individuals (*H*) into the circular field with *MS* plants was followed. The *H* plants in the South corner were all homozygous *FF* at the *Idh* locus and *FF* at the *To* (tetrazolium oxidase) locus, the West group was *Idh* – *FF*, *To* – *NN* except one plant which appeared to be *To* – *NF*. Seeds were collected from the *MS* mothers (all *Idh* – *NN*, *To* – *NN*), seedlings grown up and 10 young plants per mother used for electrophoresis. The origin of the male gametes could be traced in most of the progeny plants and σ_p calculated (see results) assuming a two dimensional normal distribution with $\mu = 0$.

RESULTS

1. Genetic structure of the Westduinen-population (*Wd*)

Allele frequencies within the eight *Wd*-subpopulations are given in table 1. With these data, the exact location and genotype of individual plants within the subpopulations, some methods of measuring the genetic structure of our populations were employed. The χ^2 -test for heterogeneity was applied to four loci: *Got*-1, *Got*-2, *Lap*-2 and *Pgm*-2 (Workman and Niswander, 1970). For all loci

Figure 3 Relative proportion of pollen, caught at various distances from the source. A. Source height = 0.15 m; receptors at 0.15 m (●---●), 0.1 m (■---■) and 0.05 m (▲---▲). B. Source height = 0.4 m; receptors at 0.4 m (●), 0.25 m (■) and 0.1 m (▲). 1. Windspeed = 1.5 m/sec., 2. Windspeed = 6 m/sec.

the allelic frequencies between subpopulations did deviate more than would be expected by chance alone ($P < 0.0005$). This means that a genetic structure was present in the Wd population (see table 1).

A χ^2 -test for heterogeneity was also applied for the same loci to all possible combinations of subpopulations in pairs (28); all subpopulations except (A-D) and (E-H) differed significantly from each other ($P < 0.05$).

The genetic distances (D) between subpopulations over all loci and the spatial distances are presented in table 2. Correlation between genetic

and spatial distance was non-significant for seven loci and significant only for PGM-1 ($r^2 = 0.53$). The correlation combined for all loci was non-significant.

Within the subpopulations deviation from Hardy-Weinberg expectation was determined where possible. Deviation was found to be significant (table 3) in 4 out of 30 possible comparisons: Got-1 in subpopulation C, Got-2 in D, Pgm-2 in E and G; in all four cases there was a shortage of heterozygotes ($P < 0.01$). When all subpopulations were taken together and

Table 2 Genetic distances (beneath the diagonal) and spatial distances (above the diagonal; meters) between the eight subpopulations (A-H) in the Westduinen population

	A	B	C	D	E	F	G	H
A	—	13.7	38.9	48.4	52.1	56.3	65.1	65.0
B	0.0181	—	28.8	34.5	35.6	43.0	51.2	51.3
C	0.0236	0.0274	—	12.1	20.0	18.9	28.6	29.0
D	0.0045	0.0089	0.0198	—	9.2	8.8	18.9	18.9
E	0.0118	0.0078	0.0287	0.0079	—	12.5	17.3	17.3
F	0.0161	0.0187	0.0136	0.0110	0.0136	—	11.0	11.8
G	0.0475	0.0163	0.0354	0.0325	0.0245	0.0337	—	1.6
H	0.0100	0.0190	0.0304	0.0114	0.0092	0.0281	0.0423	—

Figure 4 (Above) Actual gene flow from *Idh* - *FF* pollen sources in a circular-field with *Idh* - *NN* male sterile plants. The proportion of progeny with the *F*-allele in each spike is shown by the shaded fraction of each circle. ■ *FF*-source. (Below left) Relative frequencies of wind directions per 30 degrees in July, 1982.

considered as one population three out of four loci showed a significant deviation from H.W.-expectation (table 3).

As a third method to measure the population structure *F*-statistics were employed. The different *F*-values are presented in table 3. F_{IS} values and F_{IS} (averaged over subpopulations) values showed large differences from zero. However F_{IS} averaged over all loci and subpopulations, is almost zero (0.0041), indicating a very slight deviation from panmixia within the subpopulations. In contrast the F_{IT} value deviated far more from zero which meant that on population level there was a shortage of heterozygotes. As a result the F_{ST} values indicated differentiation between subpopulations. The pollen flow parameters estimated from the genetic structure were: $0.13 \text{ m} < \sigma_p < 0.28 \text{ m}$ (table 4). For the PGM-2 locus an estimation of both parameters could be made: $\sigma_p = 0.23 \text{ m}$ and $\sigma_s = 0.08 \text{ m}$, which resulted in $N_a = 0.40 \text{ m}^2$, $N_e = 13.7$ plants and $M = 0.20 \text{ m}$.

2. Wind-tunnel experiments

Fig. 3 shows that in all treatments at source height 80–100 per cent of the pollen grains were collected within 0.5 m distance from the source. These distributions resembled a normal distribution. Graphs made from collections at lower than source height showed peaks away from the source and are obviously non-normal (with the Kolmogorov-Smirnov test: six out of eight distributions).

Table 3 Fixation indices F_{IS} , F_{IT} and F_{ST} . The body of the table gives F_{IS} values

Locus	IDH	GOT-1	GOT-2	LAP-1	PGM-1	PGM-2	6-PGD-1	6-PGD-3	Mean	S.D.
Subpop.										
A	—	-0.0759	-0.0764	-0.0409	-0.0144	-0.1007	-0.0748	-0.0748	-0.0654	0.0284
B	-0.0294	0.0772	-0.0154	-0.1798	-0.0723	0.0165	—	-0.0114	-0.0307	0.0800
C	-0.0276	0.3331†	0.1317	-0.1402	-0.0423	-0.0290	—	—	0.0376	0.1690
D	-0.0328	0.1686	0.2772†	-0.0216	-0.0213	-0.0382	-0.1167	-0.1167	0.0123	0.1387
E	0.0009	-0.0016	0.0016	0.0997	-0.0509	0.1194†	0.0009	0.0009	0.0214	0.0575
F	—	0.1991	-0.0994	0.1304	-0.0082	-0.1174	-0.0001	—	0.0174	0.1253
G	—	0.0667	0.1030	-0.0242	—	0.2689†	—	0.0165	0.0862	0.1130
H	—	-0.0829	0.2817	-0.1715	-0.0102	-0.1628	—	—	-0.0029	0.1857
F_{IS}	-0.0222	0.0855	0.0755	-0.0435	-0.0314	0.0054	-0.0477	-0.0371	0.0041	
S.D.	0.0156	0.1339	0.1390	0.1095	0.0225	0.1320	0.0581	0.0564		
F_{IT}	-0.0196	0.1531†	0.1152†	0.0206	-0.0019	0.0676†	-0.0380	-0.0184	0.0348	
F_{ST}	0.0025	0.0739*	0.0429*	0.0614*	0.0286	0.0726*	0.0093	0.0181	0.0387	

* Significant deviation from expectation with χ^2 -test for heterogeneity ($P < 0.0005$).

† Significant deviation from Hardy-Weinberg expectation ($P < 0.01$).

S.D. = standard deviation.

Table 4 Standard deviations of pollen dispersal (σ_p) in cm. d = number of adult plants/m²

Locus Subpop.	d	Got-1	Got-2	Pgm-2	Total
B	44.4	—	—	—	27.5
C	97.2	—	—	—	22.5
E	116.6	27.5	22.5	—	—
F	40.4	12.5	—	—	17.5
Total	63.6	12.5	—	22.5	12.5

Comparing wind speeds within each source height experiment, it is clear that with increasing wind speed concentration peaks shifted away from the source and showed tails to different extents. Comparing source heights at the same wind speed we noticed that peaks again shift away from the source and become wider. Another way of showing this trend is presented in table 5: the increase of dispersal with wind speed and with the lowering of the level of pollen collection. Standard deviations (σ_p) were derived from the surfaces of the graphs in fig. 3. The points, at which 68.3 per cent of the grains were deported, were determined. The distance from this point in the tunnel to the source is the σ_p on a certain height. Estimations from distances moved are (e.g. in situation A, table 5) close to these values and are 0.67, 0.85, 0.32 and 1.46 m respectively.

Table 5 also gives the neighbourhood area (N_a) and mean gene transport (M) calculated from the σ_p and the σ_s values. It is clear, that reception of pollen on a level below source height will increase N_a as well as M , especially with increasing source height and windspeed.

Table 6 shows the increase of σ_s with increasing source height and wind speed.

Table 6 The dependence of the standard deviation of seed flow on source height (h) and wind speed (V)

h (m)	V (m/sec)	σ_s (m)
0.20	1.35	0.06
0.20	2.50	0.09
0.20	5.25	0.35
0.40	1.35	0.09
0.40	2.50	0.26
0.40	5.25	1.28

Under the Westduinen (A) conditions genes flow from 0.3–1.4 metres per generation (M). In “hayfield” (B) conditions gene flow was higher.

3. Pollen-mediated gene flow in a garden experiment

During pollination mean flowering height of the *MS* mothers was 31.6 ± 0.3 cm ($\bar{x} \pm S.E.$; when measured, spikes were female flowering), for *H* plants the corresponding figure was 57.9 ± 1.3 cm (spikes were male flowering). *MS* spikes were initially protected against pollination with paper bags and then exposed for 1 day, 7 days or 14 days to pollination. Since the weather conditions—percentage sun, mean temperature, wind speed and direction—were very similar during the three pollination periods and since there was no lapse of gene movement with pollination time (test against lapse in k samples: $P = 0.37$; De Jonge, 1963) the results of the periods will be treated together (table 7). The direction of wind varied considerably during the total period—even within the 1 day pollination experiment from *SE* to *N*—which can be

Table 5 Replicates (σ_1 and σ_2) and average σ -pollen ($\bar{\sigma}$), neighbourhood area (N_a) and mean gene migration (M) from wind-tunnel experiments (A1, A2, B1 and B2 are referring to situations in fig. 3)

Situation	Source height (m)	Wind speed (m/sec)	Receptor height (m)	σ -pollen (m)			N_a (m ²)		M (m)	
				σ_1	σ_2	$\bar{\sigma}$	(1)*	(2)	(1)	(2)
A1	15	1.5	15	0.44	0.34	0.39	1.04	1.56	0.36	0.44
			10	0.71	0.81	0.76	3.71	4.13	0.68	0.72
A2	15	6.0	15	0.29	0.43	0.36	0.89	1.32	0.33	0.41
			10	1.00	2.10	1.60	16.17	16.59	1.42	1.44
B1	40	1.5	40	0.24	0.23	0.24	0.44	0.87	0.24	0.33
			25	1.90	1.10	1.50	14.22	14.64	1.33	1.35
			10	3.60	2.80	3.20	64.42	64.84	2.84	2.85
B2	40	6.0	40	1.10	1.50	1.30	10.70	11.12	1.16	1.18
			25	2.70	9.30	6.00	226.28	226.70	5.32	5.32
			10	6.70	11.60	9.20	531.89	532.31	8.15	8.16

* (1) calculation with $\sigma_s = 0.08$ m from Population structure-experiment, (2) calculation with $\sigma_s = 0.20$ m from the reasoning that $\sigma_s = \frac{1}{2} \times \text{scapellength}$ (see Discussion 2) in situation B.

Figure 5 Number of *F* and *N* alleles found in the seeds at distances from the *Idh* - *FF* pollen sources. Data are from the experimental field shown in fig. 4.

inferred from the relative frequencies of wind direction during July (fig. 4). Fig. 4 (above) also shows that there was a rapid increase in proportion of spikes without any seeds with the *F* allele from the *Idh* - *FF* sources, as distance from the source increased. The standard deviation of the axial normal distribution of pollen flow (Crawford, 1984a) was calculated as $\sigma_{p,axial} = \sqrt{\sum x_i^2 / 2N}$ ($N = 57$; x_i is the distance of the i th *F*-pollen grain) and became 3.8 metre (table 7). This dispersal was

obtained with pollen source at about 0.58 m and reception at about 0.32 m height (comparable with situation B, reception 0.3 m below source height; table 5). With the data from the wind-tunnel we can adjust σ_p to the Wd field situation (comparable with situation A, reception 0.05 m below source height). In the Wd population σ_p decreases then with a factor 4 or 6 and becomes approximately 0.8 m.

DISCUSSION

1. Experiments

1.1. Determination of population structure The results show that the genotype frequencies within the subpopulations deviate slightly from Hardy-Weinberg equilibrium (χ^2 -tests and F_{IS} : almost zero, table 3) and that there are significant genetical differences between the subpopulations of the Westduinen population (significant χ^2 -tests for heterogeneity: table 1 and high F_{ST} for four out of eight loci: table 3). The genetic distances between the subpopulations (D) are different from zero, but no correlation is present between genetic and spatial distances (table 2).

In a comparison of seven populations of *P. lanceolata* in the Netherlands (max. distance 150 km) van Dijk *et al.* (1985) find the same variance in allele frequencies among populations, measured by F_{ST} , as we find among subpopulations: $F_{ST} = 0.0369$ and 0.0387 respectively.

The standard deviation of pollen flow, estimated from the genetic structure within the different subpopulations, is: $0.13 \text{ m} < \sigma_p < 0.28 \text{ m}$ (table 4). For the Pgm-2 locus with some alleles at intermediate frequencies a value $\sigma_s = 0.08 \text{ m}$ is found. These estimates are estimates after establishment and indicate low gene flow. van Dijk *et*

Table 7 Weather conditions and pollen flow in the garden experiment. Weather conditions are from the nearest-by weather station (at 10 km) Eelde. Deviations are standard errors

Pollinations	(1)	(2)	(3)	Total:
Length (days)	1	7	14	
Period	15-16 July	27 July-3 Aug.	23 June-14 July	
Mean temp. (°C)	21.7; 17.4	21.4 ± 1.0	17.2 ± 0.6	
% sun	52; 63	76.1 ± 4.2	46.8 ± 5.8	
Wind speed (m/sec)†	3.5; 4.5	4.0 ± 0.3	4.0 ± 0.3	
Wind speed (m/sec)*	0.4-1.4	1.0 ± 0.1	no data	
Pollen flow (m)	4.35 ± 1.0	2.59 ± 0.6	4.21 ± 1.3	3.61 ± 0.5
$\sigma_{p,axial}$	4.3	2.6	4.5	3.8
Number of <i>F</i> -pollen	20	23	14	57
(from total scored)	(289)	(251)	(337)	(877)

* In the experimental field at 0.25 m height.

† Average of 6 hour periods at 10 m height (weather station Eelde).

al. (1985) find neutrality of the allozyme variation in the *P. lanceolata* Wd population (no significant differences between variances in allele frequencies among populations as measured by their F_{ST} values—Lewontin and Krakauer, 1973—and no significant differences between allozyme loci within populations, as measured by the homogeneity of the F values among loci). If we assume neutrality in our Wd population, composed of eight small (table 1) distinct subpopulations, the genetic microdifferentiation of this population can be obtained as the combined result of genetic drift and restricted gene flow, even within the subpopulations.

1.2. Wind-tunnel experiments In the Wd population plants are small and strongly exposed to wind. From the wind-tunnel experiments an estimation of the dependence of gene flow by wind on gene source height and wind speed can be obtained. The overall conclusion is not unexpected: an increase of flow distance with height and speed (table 5). We are not reporting here the increase obtained by removing the artificial vegetation. Calculations of pollen flow distances assume a two-dimensional distribution of the bell-like form one obtains by rotating a normal distribution while the realistic distribution on a height below source height is like the form of a turban cake. As a result pollen flow is overestimated. Under Wd (A) conditions in the wind-tunnel σ_p is between 0.36 and 1.60 m (table 5) and σ_s is between 0.06 and 0.35 m (table 6). We emphasise that situation B with low reception height is unrealistic in field situations.

1.3. Pollen-mediated gene flow in a garden experiment The measurement of pollen flow in this experiment is done probably under optimal weather conditions for a temperate climate (table 7). We therefore may consider the observed disper-

sal an upper limit. Another reason to suppose the values to be an overestimate is the fact that we used male sterile individuals as female plants, which implies no local sources of pollen. $\sigma_p = 0.8$ m.

2. General discussion

From our experiments we learn that neighbourhood estimates change with wind speed, source and reception height and estimation method. To formulate generalisations is therefore a delicate exercise. Only the combination with other information, like the position and number of genotypes in the population and gene frequencies can help to give some broad conclusions about the situation in a population like the Westduinen (Wd). Crawford (1984a) points out that neighbourhood parameters should be estimated accurately within the limitations of the methods involved and shows for some published examples the implications of taking different methods. In table 8 we show the differences in the calculation of N_e with van Dijk (1985a, b) or with Wright's formulae expanded by Levin and Kerster (1974)— $N_e = 2\pi(\sigma_p^2 + \sigma_s^2)d$. It is clear, that N_e (van Dijk) is insensitive to changes in σ_p if σ_s is small, while N_e (Levin and Kerster) is clearly not. Since Crawford (1984a) and van Dijk (1985a) use good arguments for adapting formulae, we used estimations made with their formulae for our hypothesis modelling. Since in the more complex situation of seed plants like *P. lanceolata* the elegance of the coincidence of the local effective population size and the number of reproducing individuals within the area N_a is lost and the relation $N_e = N_a d$ does not exist any more, van Dijk proposes a more straightforward measure of isolation by distance M , the mean gene transport per generation. To calculate M we also

Table 8 Summary of estimates of pollen flow (σ_p) in the Westduinen (Wd) population, obtained in three different ways: from the genetic structure of the Wd population, from the dispersal in the wind-tunnel and from the pollen flow in the garden experiment. Effective population size (N_e ; van Dijk, 1985 or Levin and Kerster, 1974), neighbourhood area (N_a ; van Dijk, 1985 and Crawford, 1984a) and migration per generation (M ; van Dijk, 1985) are calculated with $\sigma_s = 0.08$ m, $d = 63.6$ plants/m² and $t = 1$

	σ_p (m)	N_e van Dijk	N_e Levin and Kerster	N_a (m ²)	M (m)
Estimate from:					
Population structure	0.23	13.7	23.7	0.4	0.2
Wind-tunnel					
min.	0.36	16.6	54.4	0.9	0.3
max.	1.60	20.2	1025.0	16.2	1.4
Garden experiment	0.80	19.5	258.3	4.1	0.7

need estimations of σ_p and σ_s . The summary of σ_p estimates obtained in our three experiments (table 8) for the Wd population range from 0.23 to 1.60 m, roughly similar to Tonsor (in press).

For σ_s we have found in experiment 1, 0.08 m; in experiment 2, 0.06–0.35 m. From the observation that most of the seed falls at the circumference of a circle with a radius (r) of scape length around the plant and that mean scape length ($\pm$ S.E.) in the Wd population is 0.07 ± 0.005 m, we can argue that $2\sigma_s$ is about r . So $\sigma_s = 0.035$ m. Some seed flow by wind from the ripe spike (van Damme, personal communication) could perhaps increase σ_s to the value from experiment 1. The distribution of *MS* genes and cytoplasmic types in the Wd (van Damme, in preparation) from *MS* plants occurs only by seeds. From this we also have (circumstantial) evidence of low seed flow. Calculations show that changes of σ_s up to 0.20 m do not change N_a or M substantially (table 5).

With the above values of σ_p and $\sigma_s = 0.08$ m a survey of neighbourhood parameters from the Wd population (table 8) shows clearly that gene flow in this population with small plants, 0.07 m height, is restricted: $0.2 < M < 1.4$ m. The sample area of the eight subpopulations lies between 0.6 and 2.1 m^2 (table 1), which corresponds—dependent on the value of N_a chosen—with 1.5 and 5.3 (for $N_a = 0.4 \text{ m}^2$) or 0.04 and 0.1 (for $N_a = 16.2 \text{ m}^2$) neighbourhood areas. Since within the subpopulations gene frequencies are not always in Hardy-Weinberg equilibrium we may assume that the actual size of the sample areas is somewhat larger than one neighbourhood area in the sense of Wright (1943; 1946). So, on the average a neighbourhood in the Wd population is closer to our smaller estimations than to the largest value.

The centres of neighbouring subpopulations are separated on the average by 14 metres (fig. 1). With $M = 1.4$ m we can argue that it takes a gene from a central plant $(14:1.4)^2 = 100$ generations to migrate to the centre of the next subpopulation. With a half life of two years for *P. lanceolata* (van Dijk *et al.*, 1985 after van der Toorn *et al.*; van Damme, 1983) this becomes 200 years. This long duration can explain the significant genetic heterogeneity and the genetic distances (tables 2 and 3) of the subpopulations. The size of this micro differentiation is comparable with the differentiation found by van Dijk *et al.* (1985) on macro scale. These calculations are applicable in a continuous population and less in the situation of scattered islands with incidental individual plants in between. Therefore the figure of 100 generations is an underestimate. The overestimates of gene flow

noted in the discussion of the experiments also increase the time a gene needs to travel a given distance.

In the Wd we studied the situation of eight subpopulations (islands) and found no relation between geographic and genetic distances (table 2). This and the random incidental long distance dispersal of seed (e.g., by grazing cattle and walking scientists) make the situation comparable with the Island model, although gene flow within the subpopulations is dependent on distance.

From Wright (1969)'s island model we know that if m (number of migrants/individual/generation) is small, then:

$$F_{ST} = \frac{1}{4Nm + 1} \quad (N = \text{mean number of adult plants/subpopulation}).$$

Solving the equation with $F_{ST} = 0.04$ (table 3) and $N = 73.3$ (table 1) gives $m = 0.08$, which tells that in an average subpopulation with 73.3 reproducing individuals every generation $Nm = 73.3 \times 0.08 = 6$ individuals enter the population. This apparently explains that although the subpopulations are genetically heterogeneous and some N_a 's apart, they are only moderately different ($F_{ST} = 0.04$). This figure is close to the Nm value 10 for *P. lanceolata* in van Dijk *et al.* (1985). We realise that the Wd situation with scattered subpopulations (fig. 1) and incidental plants in between, is not a pure "Island" situation and therefore that our figure is a rough approximation.

The major conclusion that may be drawn from the above analysis is, that we find genetic differentiation on micro-scale in the Wd population and that this is explicable especially in the situation of the Wd with small N_a and N_e , scattered subpopulations and restricted gene flow within and between the subpopulations.

Table 9 presents N_a and N_e values for seven wild herbaceous plant species. We also present the methods (expression I to IV after Crawford, 1984a) used for calculation. The N_a estimates are small and rather similar, despite the large variability in breeding and pollination mechanisms. We realise that most of the estimates are based upon potential gene flow rates (movement of pollinators, pollen or seed) and therefore tend to be too small (Levin, 1981) and also that estimations are based upon assumptions which rarely are achieved in nature (Crawford, 1984b). But even if the real values were five-fold the values presented, groups of plants which are conventionally called populations—pastures, hill sides, road sides, hay field, stands—contain many breeding units or evolutionary units.

Table 9 Dispersal estimates in wild plant populations. N_a = neighbourhood area, N_e = neighbourhood size, I = insect pollination, W = wind pollination (I-IV see text)

Paper	Species		N_a (m ²)	N_e	Breeding system	Pollination
1: Kerster and Levin, 1968	<i>Lithospermum caroliense</i>	IV	5-21	2.2-5.4	36% selfing	I
2: Levin and Kerster, 1968	<i>Phlox pilosa</i>	I	11-21	75-282	selfincomp.	I
3: Levin and Kerster, 1969	<i>Liatris aspersa</i>	II	17-30	30-191	outcrossing	$I-W$
4: Antlfinger, 1982	<i>Borrichia frutescens</i>	IV	0.87	20-30	100% outcross	I
5: Rai and Jain, 1982	<i>Avena barbata</i>	II	1-8	40-400	selfing	W
6: Schaal, 1980	<i>Lupinus texensis</i>	IV	2.8-6.3	42.2-95.4	obl.	I
					outcrossing	
7: This study	<i>Plantago lanceolata</i>	IV	0.4-16.2	13.7-20.2	selfincomp.	W

We therefore think that our results obtained with three methods of estimation and the agreement of these results with gene flow estimates in other herbaceous wild plants, justify the generalisation that gene flow in *Plantago lanceolata* populations will be restricted. As is shown, this can lead to population substructuring not only in plant species that are insect pollinated (Schaal, 1975), but also in wind pollinated species with low seed dispersal.

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