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RESEARCH ARTICLE

Strong genetic exchange among populations of a specialist bee, *Andrena vaga* (Hymenoptera: Andrenidae)

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Abstract Habitat fragmentation is believed to be a key threat to biodiversity, with habitat specialists being stronger affected than generalists. However, pioneer species might be less affected by fragmentation, as their high colonization potential should increase gene flow. Here, we present an analysis of the genetic structure of populations of the solitary bee *Andrena vaga*, which naturally occurs in sandy habitats and is specialized on willow (*Salix*) pollen as larval food and sandy soils as nesting sites. While the species is widespread in the young sandy landscapes of our main study area (Emsland, northwestern Germany), it occurs less frequently in the Lower Rhine valley. Our analyses of six polymorphic microsatellites show that the populations are only slightly differentiated, suggesting a relatively strong gene flow. No genetic structure corresponding to the geographic origin was found as the variability within populations accounted for the major proportion of variation. F_{ST} values were higher and allelic richness was lower in the Lower Rhine valley, supporting the hypothesis that habitat availability affects the degree of genetic exchange between populations. Inbreeding coefficients were generally high and nearly all populations had a heterozygote deficiency, which could be explained by the breeding strategy of *A. vaga*, which nests in aggregations.

Keywords Fragmentation · Specialization · Connectivity · Isolation by distance · Inbreeding

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Introduction

Habitat loss and fragmentation are major problems for the maintenance of biodiversity in modern cultural landscapes (Fahrig 2003; Henle et al. 2004; Hanski 2005). The persistence of plant and animal species within habitat fragments depends on several parameters, such as size, age, spatial isolation and the structure of the surrounding area (Tscharntke et al. 2002). Due to the disruption of the remaining suitable habitats, populations become increasingly isolated and small. Hence, they often have a reduced genetic diversity due to the reduced gene flow (Ellis et al. 2006). This reduction in genetic diversity is considered to enhance inbreeding depression and decrease the adaptability to environmental changes (Darvill et al. 2006), which often results in negative effects on the survival of populations. While the influence of inbreeding on population persistence is discussed controversially in the recent literature (reviewed in Hedrick and Kalinowski 2000), it is generally acknowledged that the survival of small populations is negatively affected by the loss of genetic diversity (Frankham et al. 2002).

Habitat specialization is generally believed to be an important trait affecting the vulnerability of species (Primack 2002) and is thus a fundamental concept explaining their extinction risk (McKinney 1997). Since the availability of suitable habitat is usually lower for specialists than for generalists, the effects of fragmentation are thought to be stronger in the former group (e.g., Kitahara and Fujii 1994; Kelley et al. 2000; Bonte et al. 2004; Polus et al. 2006). Many species of wild bees (Hymenoptera, Apoidea) are specialized on pollen resources and nesting habitats (Westrich 1989; Kratochwil 2003). Hence, the distribution of such oligolectic bees is limited by the availability of their specific floral hosts (Packer et al. 2005).

The recent decline of many species of wild bees and particularly of specialized species has been attributed to habitat fragmentation (Steffan-Dewenter 2003) and the resulting ecological and economic consequences ("pollination crisis") are currently strongly debated (Cane and Tepedino 2001; Goulson 2003; Biesmeijer et al. 2006; Butler et al. 2007). While the degree of specialization is probably an important factor influencing the genetic population structure of a species, the life history strategy might substantially influence the consequences of fragmentation. Pioneer species with a high dispersal capability and comparatively large population sizes might be less prone to effects of fragmentation as they should be adapted to dynamic processes in their natural habitats. Hence, it could be proposed that populations of pioneer species might be strongly connected despite a strong degree of specialization. Moreover, the availability of habitats is probably more important than the degree of specialization. Thus, it is reasonable to suggest that highly specialized species are able to persist as long as the habitat availability remains high.

Here we test the hypothesis that populations of pioneer species are strongly connected. We analyzed the genetic differentiation among populations of a widespread specialist bee species (*Andrena vaga* Panzer, 1799) using six polymorphic microsatellite loci. *A. vaga* is a floodplain pioneer with a high dispersal capability, which is specialized on willow pollen (*Salix*) and sandy habitats with sparse vegetation. It is widespread in northern Germany due to the predominance of sandy soils and a high availability of suitable nesting habitats but more restricted in the Lower Rhine valley, where the landscape structure is strongly shaped by intensive agriculture and urbanization.

Materials and methods

Study area and sampling

The main study region is located in the Emsland area in northwestern Germany (Lower Saxony, Fig. 1), which is mainly characterized by alluvial soils and utilized as arable farmland and pine plantations. Due to the dominance of Pleistocene sands in northern Germany, the availability of nesting habitats for *A. vaga* in the Emsland is relatively high. A total of 32 nest aggregations of *Andrena vaga* were located in the floodplains of the rivers Ems and Hase, eleven of which were large enough to be selected for further analyses. While the watercourse of the Ems is characterized by a high amount of adjacent forests and arable fields, the floodplain of the Hase is dominated by open habitats due to restoration measures, which were carried out between 1998 and 2001. These measures included the removal of dikes, the restoration of inland dunes and the reconnection of oxbows.

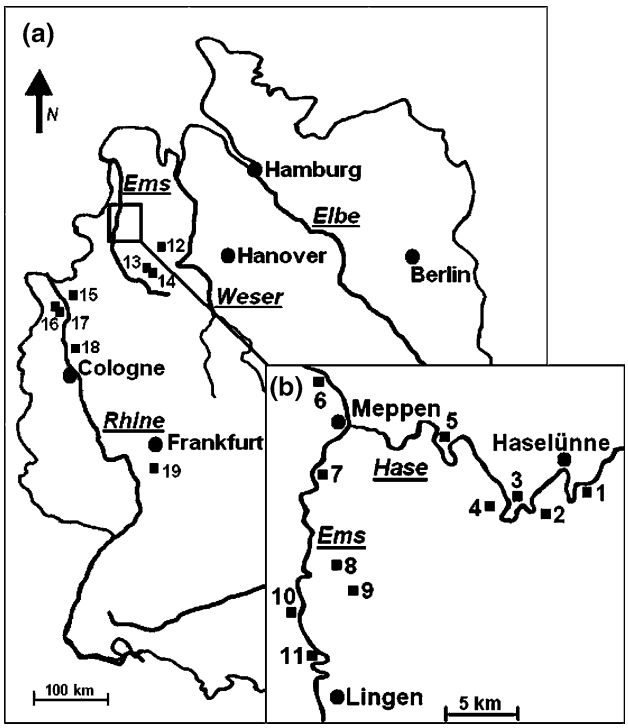

Fig. 1 Map of the locations of the 19 *A. vaga* populations studied within Germany. (**a**) Map of the complete study area in Germany, (**b**) Main study site in the Emsland region of Lower Saxony (note change of scale between **a** and **b**)

Samples of *Andrena vaga* were collected from 2002 to 2006. We analyzed a total of 201 females from eleven nest aggregations (12–25 individuals per aggregation). The distance of nests within aggregations (ca. 10 cm, max. 1 m) was considerably smaller than the distance between aggregations (>750 m). The individuals were sampled as they were haphazardly encountered. To study the influence of geographical distance on the genetic differentiation, we included eight nest aggregations (156 individuals) with increasing distance to the study area. Three of them were situated in the surroundings of Osnabrück (Lower Saxony), further four from the Lower Rhine valley (North Rhine-Westphalia) and another one near Darmstadt (Hesse; see Table 1 and Fig. 1). The Lower Rhine valley is characterized by intensive agriculture and urbanization (van Dijk et al. 2006). While in the Emsland many sandy paths exist which are frequently colonized by *A. vaga*, paved tracks predominate in the Lower Rhine valley. Hence, the availability of nesting habitats for *A. vaga* is rather low compared to the Emsland. The minimum distance between nest aggregations was 750 m, the maximum distance 330 km. The number of nests per aggregation varied from 100 to more than 5,000 nests.

Table 1 Characteristics of the analyzed *Andrena vaga* populations (N = sample size; A = mean number of alleles per locus; R = mean allelic richness; F_{IS} = inbreeding coefficient)

Region	Population	N	A	R	F_{IS}
Emsland-Hase	1	21	7.17	6.11	0.26
	2	23	8.67	7.30	0.26
	3	11	7.50	7.50	0.42
	4	19	7.67	6.82	0.33
	5	22	8.33	6.85	0.29
Emsland-Ems	6	12	7.17	6.95	0.30
	7	17	6.17	5.68	0.36
	8	19	6.67	5.94	0.26
	9	18	8.33	7.53	0.36
	10	20	8.17	7.21	0.28
	11	19	7.17	6.39	0.34
Osnabrück	12	18	6.83	6.06	0.40
	13	18	7.50	6.50	0.48
	14	17	6.67	5.94	0.35
Rhineland	15	25	7.00	6.10	0.51
	16	22	5.83	5.41	0.57
	17	21	7.33	6.18	0.43
	18	15	5.00	4.90	0.52
Darmstadt	19	20	8.17	7.03	0.45

DNA extraction and amplification

Genomic DNA was extracted from abdominal or thoracic tissue using the DNeasy Tissue Kit™ (Qiagen), following the manufacturer's protocol. Each sample was typed at six microsatellite loci (vaga01, vaga02, vaga05, vaga08, vaga09 and vaga13) developed by Mohra et al. (2000). The loci were amplified separately using the HotMasterMix™ (Eppendorf). The 5'-end of each forward primer set was labelled with a fluorescent marker, either 5-FAM, JOE or TAMRA. The products were genotyped on an ABI PRISM 377 automated DNA sequencer (Applied Biosystems Inc.). Fragment lengths were determined using GENESCAN 3.1 and GENOTYPER 2.5 (Applied Biosystems Inc.).

Statistical analysis

GENEPOP 3.4 (Raymond and Rousset 1995) was used to calculate genotypic linkage disequilibrium, using Fisher's exact test and the Markov-chain method. With the same program a global test for departure from Hardy-Weinberg equilibrium (HWE) was performed with the null hypothesis of random mating and an alternative hypothesis of heterozygote deficiency. The significance of departure from HWE was estimated using the Markov-chain method (1,000 iterations). Since the data departed significantly from HWE, they were inspected for the presence of null alleles using MicroChecker 2.2.3 (Van Oosterhout et al. 2004).

FSTAT 2.9.3 (Goudet 1995) was used to estimate the mean number of alleles, the allelic richness R and the inbreeding coefficients F_{IS} for each nest aggregation. The measure of allelic richness R (El Mousadik and Petit 1996) was used as it is independent of sample size. The expected (H_E) and observed (H_O) heterozygosity for each locus and each nest aggregation was determined using GenAlEx 6.0 (Peakall and Smouse 2006), which performs a χ^2-test to asses the significance of a departure from HWE. The same program was also used to inspect the occurrence of private alleles (alleles which can only be found in one population) for each nest aggregation.

To examine the genetic structure within and between nest aggregations an analysis of molecular variance (AMOVA) was performed in GenAlEx 6.0 (Peakall and Smouse 2006) based upon Wright's F-statistics (Wright 1951). F_{ST} values are based on the variance in allele frequencies among the nest aggregations. For this purpose, aggregations belonging to the main study region (Emsland) were assigned to two groups corresponding to their origin from the floodplains of the rivers Hase and Ems. The genetic structure was tested at three different levels: floodplains (groups of nest aggregations), nest aggregations within floodplains and individuals within nest aggregations. The same analysis was performed for the complete data set, in which nest aggregations were grouped according to their geographic origin (Emsland, Osnabrück and Rhineland together with Darmstadt).

To examine the pairwise population differentiation within each floodplain (Ems, Hase) we performed a log-likelihood based exact test (G-test), which tests the distribution of genotypes between each pair of aggregations (Goudet et al. 1996) as implemented in FSTAT (Goudet 1995). The significance of these tests was adjusted using standard Bonferroni corrections. This method seems to be particularly efficient for non-random mating populations (Goudet et al. 1996) and a low overall population structure (Petit et al. 2001).

To test for isolation-by-distance, pairwise genetic distances (F_{ST} calculated in GenAlEx 6.0 as described above) and geographical distance matrices were checked for a correlation assuming that F_{ST} is linearly related to the geographical distance between populations. We employed a Mantel-test of matrix correlations and used a reduced major axis (RMA) regression to estimate the intercept and slope of the isolation-by-distance relationship within the program IBD 1.52 (Bohonak 2002). This procedure was first applied for the eleven Emsland populations. Afterwards, the populations from further south were included to assess the influence of increasing distance on the genetic differentiation using the complete data set.

Moreover, the relationship between genetic and geographical distance was checked for spatial autocorrelation of the genetic distance estimates as implemented within GenAlEx 6.0 (Peakall and Smouse 1999). This method is based on a multivariate technique combining alleles and loci to reduce stochastic noise. The theory of spatial autocorrelation is based on the assumption that samples collected at any locality will have a greater similarity to those from locations in their vicinity. Thus, positive correlation coefficients should occur between populations from neighbouring areas whereas a negative correlation coefficient is expected for populations separated over a greater spatial scale. No spatial autocorrelation, indicated by values close to zero, gives evidence for a random pattern of genetic distance over the studied spatial scale.

Since the availability of habitat differs among the studied areas (more potential habitats in the Emsland than in the Rhineland), we tested our data for effects of the sampled regions. We performed ANOVAs to test for differences in allelic richness, expected (H_E) and observed heterozygosity (H_O) and inbreeding coefficients (F_{IS}) using "region" as the explanatory variable and the average values across all loci as response variables. In case of significance, we conducted pairwise *t*-tests with Bonferroni correction. These analyses were carried out with the program "R 2.5.1" (R Development Core Team 2007).

Results

The complete data set contained 19 populations with a total of 357 females of *Andrena vaga* (Table 1). A global test of genotypic linkage disequilibrium across all populations revealed no significant departure for any combination of microsatellite loci. A global test for departure from Hardy-Weinberg equilibrium revealed a significant deviation from random mating ($P < 0.001$) with an excess of homozygotes. Null alleles were detected in nearly all populations in at least one locus, but all loci did amplify in all individuals (and also in 12 males, which were not included in the statistical analyses).

Genetic diversity

In all populations the observed heterozygosity was lower than the expected heterozygosity with 66 of 114 tests for each locus showing a significant departure from HWE. This means that each of the 19 populations had a heterozygote deficiency for at least one locus (Table 2). Inbreeding coefficients (F_{IS}) within populations were high, ranging from 0.26 to 0.57 (mean 0.38 ± 0.095 SE; Table 1). Nevertheless, we detected a high genetic variability: The average number of alleles per population (7.23 ± 0.93 SE) and the resulting allelic richness (6.44 ± 0.73 SE) was high for all populations, with the lowest values observed in a population within the Rhineland (population 18) and the highest number of alleles detected within the Emsland area. The average gene diversity was also high ranging from 0.74 to 0.86 (0.79 ± 0.04 SE). Private alleles occurred in a low number and frequency in eleven populations.

Genetic differentiation

Assigning nest aggregations to the river catchments revealed no genetic structure corresponding to their origin from the floodplains of the rivers Hase and Ems. The highest genetic variance detected by the AMOVA was measured among individuals within aggregations (95%, variance component 2.4, $P = 0.01$). Pairwise estimates of population differentiation revealed a stronger differentiation among aggregations located at the Ems than at the Hase (Table 3). Although the average F_{ST} values within these regional groups were generally low (Hase: $F_{ST} = 0.03 \pm 0.01$ SE; Ems: $F_{ST} = 0.07 \pm 0.02$ SE), the values were significantly higher for nest aggregations belonging to the Ems group (ANOVA, $F_{1,23} = 26.71$; $P < 0.001$). The highest F_{ST} values were detected in the Rhineland (0.09 ± 0.02).

Analyzing the combined dataset of all nest aggregations revealed also no genetic structuring corresponding to the spatial arrangement of populations. The results of the AMOVA showed that 92% of the molecular variance was explained by variation within nest aggregations (variance component 2.4; $P = 0.01$). The remaining variance was partitioned among aggregations within regions (6%, variance component 0.17, $P = 0.01$) and variation among regions (2%, variance component 0.05, $P = 0.01$). Pairwise population differentiation was significant for many populations and global F_{ST} was 0.07 (± 0.03) across all loci.

Geographical effects

Within the main study region (Emsland) there was no significant correlation between the genetic distance (pairwise F_{ST}), and the pairwise geographical distance (Mantel-test, $r = 0.17$, $P = 0.11$; intercept $= 0.01 \pm 0.007$, slope $= 0.004 \pm 0.001$). The Mantel-test for the entire study area revealed an *r*-value of 0.34 ($P = 0.02$; intercept $= 0.05 \pm 0.003$, slope $= 0.0003 \pm 0.00002$; Fig. 2), indicating a weak but significant isolation-by-distance. Further analysis of spatial autocorrelation revealed no linear relation to geographic distance (Fig. 3). Significant

Table 2 Hardy–Weinberg equilibrium and private alleles (Pop. = population; H_O = observed heterozygosity; H_E = expected heterozygosity; P = significance of a χ^2-test of Hardy-Weinberg equilibrium, pA = number of private alleles)

Region		Emsland-Hase					Emsland-Ems						Osnabrück			Rhineland				Da
Locus/Pop		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
N		21	23	11	19	22	12	17	19	18	20	19	18	18	17	25	22	21	15	20
vaga01	H_O	0.52	0.87	0.64	0.47	0.59	0.25	0.41	0.79	0.50	0.70	0.32	0.22	0.22	0.35	0.25	0.23	0.33	0.27	0.50
	H_E	0.77	0.75	0.77	0.77	0.78	0.63	0.80	0.77	0.81	0.79	0.75	0.68	0.66	0.67	0.54	0.62	0.57	0.62	0.78
	P	0.16	0.01	0.56	0.01	0.01	0.08	0.00	0.12	0.01	0.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.05
	pA																			
vaga02	H_O	0.43	0.48	0.55	0.47	0.45	0.50	0.53	0.37	0.56	0.65	0.63	0.61	0.28	0.41	0.60	0.36	0.43	0.40	0.60
	H_E	0.83	0.84	0.88	0.82	0.84	0.88	0.74	0.82	0.80	0.87	0.77	0.70	0.74	0.75	0.85	0.84	0.78	0.79	0.83
	P	0.00	0.00	0.11	0.00	0.01	0.02	0.04	0.00	0.00	0.09	0.25	0.45	0.00	0.03	0.01	0.00	0.00	0.00	0.05
	pA			1										2				1		
vaga05	H_O	0.75	0.65	0.45	0.58	0.62	0.42	0.59	0.74	0.41	0.60	0.53	0.39	0.50	0.65	0.32	0.38	0.42	0.67	0.63
	H_E	0.78	0.81	0.79	0.83	0.73	0.77	0.84	0.75	0.82	0.84	0.77	0.81	0.72	0.78	0.77	0.76	0.75	0.76	0.85
	P	0.38	0.23	0.33	0.00	0.00	0.12	0.02	0.15	0.00	0.02	0.01	0.00	0.37	0.25	0.00	0.00	0.27	0.23	0.00
	pA										1									1
vaga08	H_O	0.62	0.57	0.45	0.53	0.45	0.75	0.53	0.47	0.44	0.50	0.37	0.56	0.56	0.59	0.36	0.36	0.50	0.47	0.50
	H_E	0.77	0.85	0.82	0.83	0.66	0.69	0.69	0.56	0.87	0.84	0.75	0.77	0.86	0.84	0.79	0.80	0.81	0.72	0.74
	P	0.00	0.03	0.11	0.02	0.01	0.98	0.22	0.22	0.01	0.00	0.07	0.11	0.05	0.05	0.00	0.00	0.09	0.06	0.00
	pA								1			1		1	1		1			1
vaga09	H_O	0.62	0.57	0.45	0.68	0.73	0.67	0.35	0.84	0.56	0.45	0.68	0.44	0.56	0.41	0.36	0.23	0.57	0.13	0.10
	H_E	0.76	0.88	0.85	0.85	0.84	0.82	0.72	0.86	0.87	0.72	0.80	0.69	0.78	0.78	0.72	0.75	0.84	0.58	0.81
	P	0.75	0.02	0.02	0.52	0.10	0.21	0.00	0.43	0.00	0.00	0.61	0.05	0.00	0.04	0.00	0.00	0.05	0.00	0.00
	pA																			2
vaga13	H_O	0.62	0.61	0.45	0.63	0.50	0.75	0.59	0.11	0.83	0.70	0.58	0.56	0.33	0.65	0.36	0.36	0.38	0.20	0.40
	H_E	0.75	0.75	0.69	0.73	0.72	0.70	0.74	0.59	0.81	0.78	0.69	0.78	0.74	0.72	0.77	0.51	0.73	0.72	0.79
	P	0.48	0.84	0.29	0.34	0.38	0.62	0.14	0.00	0.94	0.83	0.21	0.00	0.03	0.44	0.00	0.18	0.00	0.00	0.00
	pA			1						2			2							

Table 3 Pairwise F_{ST} for populations located at the river Hase (1–5) and the river Ems (6–11) below diagonal, significance of pair-wise population differentiation above diagonal

		Hase					Ems					
		1	2	3	4	5	6	7	8	9	10	11
Hase	1	/	NS	**	*	***						
	2	0.026	/	NS	NS	NS						
	3	0.052	0.012	/	NS	NS						
	4	0.038	0.016	0.020	/	NS						
	5	0.052	0.027	0.026	0.035	/						
Ems	6						/	**	***	*	**	**
	7						0.092	/	**	**	**	**
	8						0.083	0.052	/	**	**	**
	9						0.059	0.050	0.069	/	NS	**
	10						0.059	0.087	0.097	0.033	/	**
	11						0.066	0.104	0.109	0.065	0.050	/

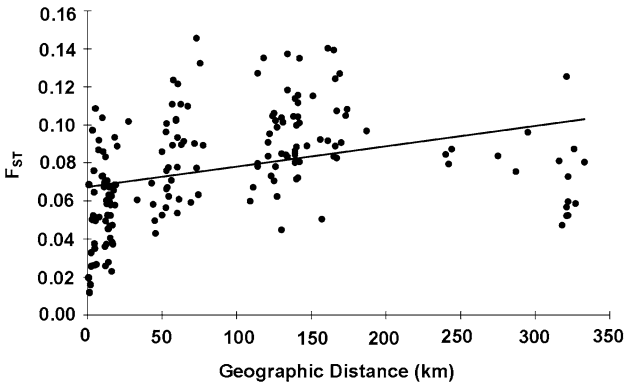

Fig. 2 Isolation-by-distance-plot for the entire study area. Note that the variability of F_{ST} values is high across the complete range of distances ($R^2 = 0.12$; $P = 0.02$)

positive correlation coefficients were only detected within a range of 20 km (10 km $r = 0.15$, $P = 0.002$; 20 km $r = 0.11$, $P = 0.001$). With increasing distance, correlation coefficients differed not significantly from zero.

Analyzing the genetic diversity and inbreeding coefficients of aggregations according to their geographic origin revealed significant differences in allelic richness ($F_{2,15} = 5.3$; $P = 0.018$), H_E ($F_{2,15} = 5.54$; $P = 0.016$; Fig. 4), H_O ($F_{2,15} = 27.45$; $P < 0.001$; Fig. 4) and F_{IS} ($F_{2,15} = 20.86$; $P < 0.001$). The aggregations from the Rhineland had the lowest allelic richness (pairwise *t*-test with Bonferroni correction, $P = 0.02$), H_E ($P = 0.02$) and H_O ($P < 0.001$), as well as the highest F_{IS} ($P < 0.001$).

Discussion

Our data show that the nest aggregations of *A. vaga* are only slightly differentiated, although the species is strongly

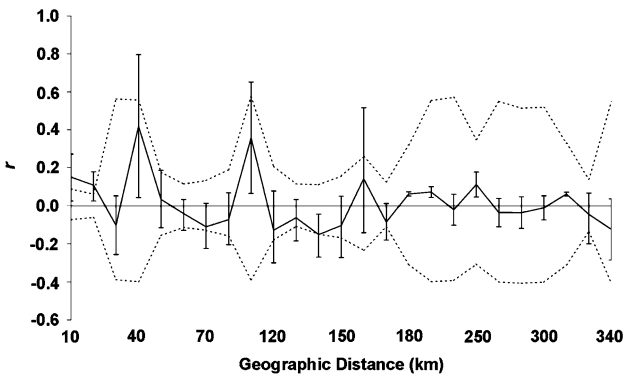

Fig. 3 Genetic autocorrelation (*r*) as a function of distance, with a null hypothesis of a random pattern of genotypes (error bars are ±SE for *r* determined by bootstrapping). The 95% confidence interval (CI) is depicted by a dotted line (note the increase in CI with larger distances due to fewer replicates)

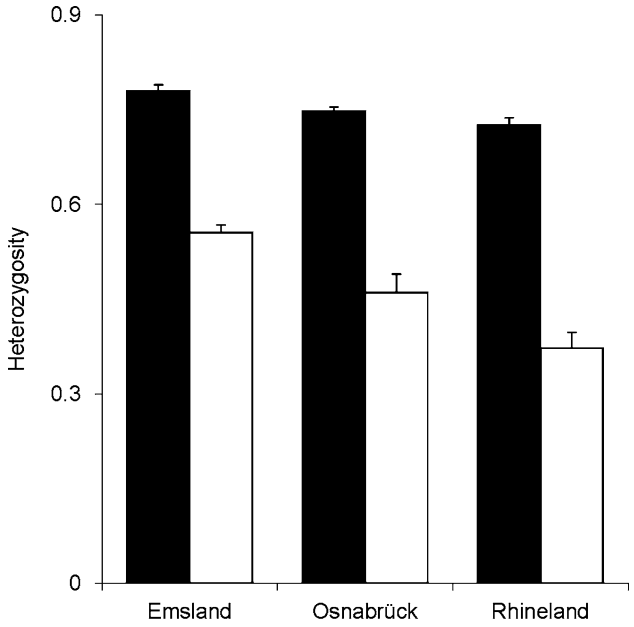

Fig. 4 Mean expected heterozygosity (H_E, black bars) and observed heterozygosity (H_O, white bars) in the three sampled regions (Emsland: $n = 11$, Osnabrück: $n = 3$, Rhineland: $n = 4$). Note that both, H_E and H_O are significantly higher in the Emsland than in the Rhineland (error bars are ±SE)

specialized on willow (*Salix*) pollen as larval food and sandy soils as nesting habitats. These results support the hypothesis that pioneer species are able to maintain a high level of gene flow despite a strong degree of specialization. Due to its high dispersal capability and large population sizes, *A. vaga* is well adapted to the natural floodplain dynamics. Similar low levels of population differentiation were found in highly specialized, but dispersive fig wasps (Zavodna et al. 2005) and bark beetles (Sallé et al. 2007). Nevertheless, we found a stronger degree of genetic differentiation in regions with stronger anthropogenic impacts, indicating that a reduced habitat availability increases the fragmentation of populations even in this highly dispersive species.

The AMOVA indicated that the highest variance occurred among individuals within aggregations and was only slightly influenced by the affiliation to or spatial arrangement of nest aggregations. Even if the Rhine populations were included, only a weak influence of the geographical distance on the genetic differentiation was found. Within a small spatial scale (<20 km), there was a significant positive spatial autocorrelation, indicating that nest aggregations within this distance are of greater genetic similarity (Haper et al. 2003). However, our results show no linear decline in the correlation coefficient suggesting a random pattern of differentiation.

The low F_{ST} values at the river Hase (average F_{ST}: 0.03) compared to the Ems (average F_{ST}: 0.07) and Lower Rhine

valley (average F_{ST}: 0.09) might be caused by the differing landscape structure of these areas. The restoration measures at the river Hase created new habitats (Stroh et al. 2005), which might serve as stepping stones between populations resulting in genetic homogenization. The Ems area is stronger fragmented by pine forests (*Pinus sylvestris*), which might increase the separation of populations of *A. vaga*. These populations might also be of higher age than at the Hase, leading to a stronger degree of differentiation (Le Corre and Kremer 1998). Compared to the Emsland, the Lower Rhine valley is characterized by intensive agriculture and urbanization (van Dijk et al. 2006). Hence, the availability of habitats for *A. vaga* is rather low and the existing populations are stronger fragmented than in the Emsland, which has a much (four times) lower human population density. Moreover, the availability of habitats for *A. vaga* is positively influenced by the Pleistocene sands which dominate northern Germany. The degree of differentiation in the Rhine valley is comparable to the pattern found in a rare Bumblebee (*Bombus muscorum*) in Great Britain, which seems to be particularly affected by habitat loss and isolation (Darvill et al. 2006). This is also reflected by the fact that *A. vaga* is red listed in Westphalia (Kuhlmann 1999), while it is widespread in Lower Saxony (Theunert 2002). These conclusions are supported by the reduced genetic diversity (allelic richness and H_E) and higher levels of inbreeding in the Rhineland populations compared to the Emsland (Fig. 4).

The effects of habitat fragmentation are often believed to be stronger in more specialized bee species (Packer et al. 2005; Zayed et al. 2005), such as *A. vaga*. However, some authors (e.g., Sallé et al. 2007) suggest that strong food specialization favours individuals with high dispersal capabilities. It is reasonable to suggest that specialization does not represent a threat *per se* as long as the availability of nesting habitats and pollen resources is high (Peterson and Denno 1998). This might be particularly true for pioneer species, which should be able to react rapidly on changes in their dynamic habitat. Flooding events are known to have dramatic consequences for populations of *A. vaga* as the brood cells are not water resistant (Fellendorf et al. 2004). Hence, a high rate of dispersal might compensate for local extinctions. This hypothesis is supported by the fact that about 50% of the emerging females of *Andrena vaga* are known to emigrate (Bischoff 2003).

Despite the comparatively weak genetic differentiation of *A. vaga* populations compared to other insects (Darvill et al. 2006; Repaci et al. 2006), most populations had rather high inbreeding coefficients and a deficiency of heterozygotes for most microsatellite loci. In the Emsland populations heterozygote deficiency occurred only in single loci, but nevertheless inbreeding coefficients were positive, ranging from 0.26 to 0.42 (Rhineland: 0.43 to 0.57). Deviations from HWE can generally be caused by a variety of factors including non-random mating, population subdivision and the presence of null alleles (Callen et al. 1993). Although Microchecker detected null alleles, all loci were successfully amplified in all individuals, suggesting little importance of null alleles in our analysis (see also Stahlhut and Cowan 2004 for discussion). Homozygote excess has also been reported from other Hymenoptera (Paxton et al. 1996; Danforth et al. 2003; Zayed et al. 2005; Stow et al. 2007). It is usually ascribed to inbreeding events within populations and seems to be strongly related to the life history strategies of the species involved (Paxton et al. 1996; Zavodna et al. 2005; Stahlhut and Cowan 2004). One explanation for this general excess of homozygotes in Hymenoptera might be found in the lower effective population sizes of haplo-diploid organisms (Packer and Owen 2001). However, it is likely that the deviation from HWE in *A. vaga* is also influenced by its breeding system and nesting behaviour. The high nest densities of *A. vaga* increase the possibility of inbreeding as males wait at nest aggregations for females, whereas in solitary nesting species males patrol flowers or landmarks seeking for mates (Paxton 2005). The latter is suggested to support random mating (Stahlhut and Cowan 2004). In fact, we found lower degrees of inbreeding in a solitary nesting species, *Andrena fuscipes* (unpublished data), while Paxton et al. (1996) documented even higher degrees of inbreeding in the communal nesting species *Andrena carantonica* Pérez, 1902. In this species, 70% of the females mate with nestmates before emerging from their natal nest (Paxton and Tengö 1996).

Although the populations of *A. vaga* seem to be highly inbred, they are characterized by a high allelic richness (4.9–7.5) compared to other studies on bees (Darvill et al. 2006; Francisco et al. 2006). This pattern might be influenced by the wide distribution and the high mobility of this species. A high gene diversity and low degree of differentiation has also been recorded for other widespread insects (Vandewoestijne et al. 1999; Schmitt and Hewitt 2004). Solitary bees have a rather small foraging range, therefore local habitat structure is believed to be of particular importance for the maintenance of viable populations (Williams and Kremen 2007). Detailed studies on the dispersal ability of wild bees are still needed, as the existing studies mainly concern bumblebees (Osborne et al. 1999; Chapman et al. 2003; Knight et al. 2005), while our knowledge of the dispersal ability of solitary wild bees is sparse. The foraging range of pollinators is considered to be positively correlated with body size (Gathmann and Tscharntke 2002). Hence, a limited foraging range makes small species stay in one habitat (Tscharntke and Brandl 2004). However, Zayed et al. (2005) point out that the distance wild bees cover during their foraging trips are not

necessarily related to the dispersal ability and can not be used to predict gene flow. Hence, the dispersal capability of wild bees might often be underestimated substantially.

Conclusions

Our results show that populations of the specialist bee *A. vaga* exhibit a high genetic diversity associated with a low overall population differentiation. Thus, we cannot support current theories describing a generally higher threat of specialized wild bee species (Packer et al. 2005; Zayed et al. 2005). The population genetic structure seems to be dependent on the availability of the floral host and nesting habitats. Although the populations of *A. vaga* seem to be still connected in large parts of the Emsland, our results show that highly specialized species might become threatened when habitat availability is decreasing. During the last century the natural habitat of *A. vaga* has been degraded by canalisation of rivers, drainage of wetlands, and cultivation of dry grasslands resulting in decreasing availability of the floral host *Salix sp.* and nesting sites (Winfree et al. 2007). This process has probably affected the populations in the Lower Rhine valley, but not the populations in the Emsland, as the availability of suitable habitats is still high.

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