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RESEARCH ARTICLE

Genetic diversity and spatial genetic structure support the specialist-generalist variation hypothesis in two sympatric woodpecker species

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Abstract
Species are often arranged along a continuum from "specialists" to "generalists". Specialists typically use fewer resources, occur in more patchily distributed habitats and have overall smaller population sizes than generalists. Accordingly, the specialist-generalist variation hypothesis (SGVH) proposes that populations of habitat specialists have lower genetic diversity and are genetically more differentiated due to reduced gene flow compared to populations of generalists. Here, expectations of the SGVH were tested by examining genetic diversity, spatial genetic structure and contemporary gene flow in two sympatric woodpecker species differing in habitat specialization. Compared to the generalist great spotted woodpecker (*Dendrocopos major*), lower genetic diversity was found in the specialist middle spotted woodpecker (*Dendrocoptes medius*). Evidence for recent bottlenecks was revealed in some populations of the middle spotted woodpecker, but in none of the great spotted woodpecker. Substantial spatial genetic structure and a significant correlation between genetic and geographic distances were found in the middle spotted woodpecker, but only weak spatial genetic structure and no significant correlation between genetic and geographic distances in the great spotted woodpecker. Finally, estimated levels of contemporary gene flow did not differ between the two species. Results are consistent with all but one expectations of the SGVH. This study adds to the relatively few investigations addressing the SGVH in terrestrial vertebrates.

Keywords Genetic diversity · Specialist-generalist variation hypothesis · Microsatellites · Piciformes

Introduction

Species are often arranged along a continuum between "generalists" and "specialists" (e.g. Devictor et al. 2008). Generalists typically use a broad range of resources, while specialists are restricted to a narrow set of resources. Owing to their resource specialization, habitats suitable for specialists may be spatially more restricted and more patchily distributed than for generalists, and populations of specialists may hence be less connected and more subdivided than those of generalists. In addition, because of the (often) smaller population sizes in specialist than generalist species, stochastic extinctions of local populations are more likely to occur in habitat specialists than generalists (Henle et al. 2004). Thus, populations of habitat specialists are expected to have lower genetic diversity and to be genetically more differentiated due to reduced gene flow compared to populations of habitat generalists, as posited by the specialist-generalist variation hypothesis (SGVH) (Li et al. 2014). Consistent with the SGVH, generalists tend to have increased genetic diversity and lower population differentiation compared to related specialists (Li et al. 2014 and studies cited therein; Janecka et al. 2016; Khimoun et al. 2016; Matthee et al. 2018). Yet, how genetic diversity and differentiation differ between (related) species varying in specialization has received surprisingly little attention, particularly in vertebrates.

Genetic diversity among populations likely depends on the mobility of the involved taxa and the spatial distribution of suitable habitats. It is often assumed that genetic differentiation among populations of highly mobile animals such as birds is low, because their flight capability allows them to easily cross hostile habitats, thereby maintaining gene flow even across fragmented landscapes. In fact, lack

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of genetic differentiation over varying spatial scales and degrees of landscape fragmentation has been documented in a number of bird species (e.g. Alcaide et al. 2009; Mayer et al. 2009; Amos et al. 2014). In turn, genetic differentiation and relatively low levels of gene flow have been observed as well (e.g. Alcaide et al. 2009; Kozakiewicz et al. 2018), both among nearby populations of non-migratory (Kormann et al. 2012; Szulkin et al. 2016; van Rees et al. 2018) and even migratory species (Barr et al. 2008; Lindsay et al. 2008).

Woodpeckers are mostly resident species varying in habitat specialization, and their dispersal abilities are generally considered to be weak (del Hoyo et al. 2018). This assumption particularly applies to the middle spotted woodpecker (*Dendrocoptes medius*), a resident species restricted to old deciduous forests with many rough-barked trees (Pasinelli 2003). For this species, occurrence and colonization probabilities appear to be positively associated to the size and quality of old oak forests (Pettersson 1985; Robles and Ciudad 2012) and to drop drastically if distances between oak forests exceed a few kilometers (Müller 1982; Richter 1997). Further, the species seems to exhibit strong natal and breeding philopatry (Pasinelli 2003), and during postfledging movements juveniles select the same oak-dominated habitats as breeding adults (Ciudad et al. 2009). Patterns of occurrence and colonization as well as strong philopatry thus suggest low demographic and genetic connectivity, and isolation of populations has been invoked as one potentially important driver of population declines and extinction (Pettersson 1985; Bühlmann and Pasinelli 2012; but see Robles and Ciudad 2017). In contrast to this philopatric habitat specialist, the generalist great spotted woodpecker (*Dendrocopos major*) thrives in many forested habitats as well as in urbanized areas with parks and small woods (Michalek and Miettinen 2003). In addition, juvenile dispersal appears to occur over several hundred kilometres, and eruptive movements are well-documented for this species (del Hoyo et al. 2018), suggesting strong dispersal abilities and high genetic connectivity of populations. Middle and great spotted woodpeckers are not closely related and have been suggested to belong to different clades (Fuchs & Pons 2015; Shakya et al 2017), and no evidence of interbreeding has been reported.

Here, expectations of the SGVH outlined above were examined in relation to genetic diversity, spatial genetic structure and patterns of gene flow of these two woodpecker species differing in habitat specialization. More specifically, I asked whether or not sympatric local populations of middle and great spotted woodpeckers (1) differed in terms of genetic diversity, (2) showed evidence for recent bottlenecks, (3) were genetically differentiated (intraspecifically), (4) exhibited correlations between genetic and geographic distances and (5) varied in gene flow. Genetic diversity was expected to be lower in the specialist middle spotted woodpecker than in the generalist great spotted woodpecker (expectation 1). Even though the middle spotted woodpecker has recently shown strong population increases and range expansions in Switzerland (Knaus et al. 2018; Schuck et al. 2018), evidence for recent population bottlenecks was expected to be found due to the long history of population declines in many parts of central Europe (expectation 2a). Conversely, the great spotted woodpecker was an abundant and widespread species at least since the 1950ies (Knaus et al. 2011), so no recent bottlenecks were expected for this species (expectation 2b). Owing to the differences in habitat specialization and dispersal of the two species, significant genetic differentiation and spatial structure among local populations of the middle spotted woodpecker (expectation 3a) were expected, but less so or not at all among local populations of the great spotted woodpecker (expectation 3b). A positive correlation between genetic and geographic distances was expected to be present in the middle spotted woodpecker (expectation 4a), but less so or not at all in the great spotted woodpecker (expectation 4b). Finally, gene flow among local populations was expected to be lower in the specialist middle spotted woodpecker than in the generalist great spotted woodpecker (expectation 5).

Material and methods

Study populations

This study was conducted with samples of both middle and great spotted woodpeckers collected in Switzerland and Germany (Fig. S1). In Switzerland, samples were obtained from cantons known to host strongholds of the middle spotted woodpecker according to the Swiss species recovery plan (Pasinelli et al. 2008). These were the cantons Aargau (hereafter AG), Basel-Landschaft (BL), Neuenburg (NE), Schaffhausen (SH), Thurgau (TG) and Zürich (ZH). In each of these six cantons, one local population known to host at least 30 territories of each species was studied (Table 1), allowing to sample potentially 20 individuals per local population and canton, which was considered a representative subset of individuals to genetically characterize a local population (Lukas Keller, pers. comm.). In Germany, samples from one local population in Bad Homburg, Hessen (hereafter HE), were obtained to enlarge the spatial extent of the study. This local population is part of the very extensive Taunus Mountains population (Hennes 2012). Population densities of both species ranged from 1 to 2 breeding pairs per 10 ha in Switzerland (own unpublished data) and Germany (Hennes 2012). Distances among the Swiss local populations ranged from 8 to 144 km, and the Swiss populations were from 284 to 379 km apart from the German population.

Table 1 Genetic diversity in the two sympatric woodpecker species among seven local populations

Species, population	N	A	A_r[a]	H_o	H_e	HW	F_{IS}
Middle spotted wp							
AG (7.8172/47.5416)	14	4.643 (0.561)	4.626 (0.558)	0.617 (0.080)	0.641 (0.072)	0.164	0.036
BL (7.6435/47.5389)	19	5.214 (0.735)	4.879 (0.666)	0.594 (0.078)	0.628 (0.077)	**0.005**	0.053
HE (8.6182/50.2268)	16	5.429 (0.618)	5.228 (0.577)	0.635 (0.067)	0.662 (0.064)	0.265	0.034
NE (6.9413/47.0070)	18	5.071 (0.667)	4.814 (0.604)	0.595 (0.077)	0.629 (0.077)	0.013	0.053
SH (8.5699/47.6736)	18	5.357 (0.768)	5.057 (0.696)	0.639 (0.078)	0.647 (0.076)	0.516	0.012
TG (9.1062/47.6427)	15	4.643 (0.520)	4.535 (0.500)	0.586 (0.078)	0.602 (0.066)	0.050	0.027
ZH (8.6224/47.6128)	16	4.714 (0.683)	4.605 (0.656)	0.585 (0.081)	0.604 (0.075)	0.202	0.032
Great spotted wp							
AG (7.8152/47.5377)	24	8.917 (1.145)	8.415 (1.048)	0.720 (0.062)	0.746 (0.050)	0.021	0.035
BL (7.6429/47.5346)	21	7.333 (1.054)	7.175 (1.018)	0.670 (0.073)	0.654 (0.071)	0.754	−0.025
HE (8.6182/50.2268)	21	9.000 (1.211)	8.798 (1.105)	0.612 (0.054)	0.702 (0.067)	0.019	0.128
NE (6.9395/47.0056)	21	7.000 (1.066)	6.796 (1.011)	0.639 (0.079)	0.634 (0.075)	0.516	−0.008
SH (8.5598/47.6734)	20	8.250 (1.053)	8.141 (1.032)	0.660 (0.064)	0.698 (0.062)	0.039	0.054
TG (9.1197/47.6400)	21	7.917 (0.957)	7.678 (0.919)	0.663 (0.059)	0.687 (0.060)	0.610	0.035
ZH (8.6210/47.6140)	19	7.417 (0.783)	7.417 (0.783)	0.658 (0.062)	0.676 (0.056)	0.114	0.027
P (Wilcoxon)		0.016	0.016	0.047	0.016		1.000

Given are means (SE) based on 14 polymorphic markers for the specialized middle spotted woodpecker and 12 polymorphic markers for the generalist great spotted woodpecker. Population abbreviations are *AG* Aargau, *BL* Basel-Landschaft, *HE* Hessen (Germany), *NE* Neuenburg, *SH* Schaffhausen, *TG* Thurgau, *ZH* Zürich, followed in brackets by longitude and latitude calculated per species as means of nest tree coordinates. *N* number of individuals genetically analyzed, *A* number of alleles, A_r allelic richness, H_o observed heterozygosity, H_e expected heterozygosity, *HW* p-values for Hardy–Weinberg probability tests (bold = significant after sequential Bonferroni correction), F_{IS} mean values per population, *SE* standard error. *P (Wilcoxon)* p-values of Wilcoxon signed rank tests comparing values of Msw vs. Gsw

[a]Calculations based on 13 individuals in middle spotted and 19 individuals in great spotted woodpeckers

Data sampling

From the Swiss local populations, blood samples were collected from 2009 to 2011 (2009: TG, ZH; 2010: NE, SH; 2011: AG, BL). Territories of the middle spotted woodpecker were censused with playbacks of the rattle and qweek calls in March and April (Pasinelli 2003; Müller et al. 2011) and searched for active breeding cavities from April to May. Breeding cavities were detected by observing excavating birds, fresh wood chips at the base and/or on branches of the cavity tree and, from early May onwards, by listening for begging calls of nestlings. For the great spotted woodpecker, no playback census was conducted, as active breeding cavities are easy to find owing to the much louder nestling begging calls compared to the middle spotted woodpecker. Active breeding cavities of the great spotted woodpecker were recorded while searching for middle spotted woodpecker nests.

Cavity trees were climbed by professional tree climbers. Nestlings were removed from the breeding cavity with a noose (Jackson 1982), a technique successfully applied in both middle and great spotted woodpeckers in Austria (Michalek and Winkler 2001) and in endangered red-cockaded woodpeckers *Picoides borealis* in the USA (Walters et al. 1988). From each nest, two nestlings were removed and sampled for blood by puncturing the brachial vein and collecting a drop of blood with a not heparinized capillary. After ringing with one aluminum ring and color rings, the nestlings were put back into the nest. The blood was put into APS-buffer (Arctander 1988) and later on deep-frozen.

For the German population, feather samples of individuals were obtained in 2011 and 2012 from captures at a feeder located at the forest edge (permit to Rolf Hennes from Staatliche Vogelschutzwarte für Hessen, Rheinland-Pfalz und Saarland). Individuals were marked with one aluminum ring and color rings as part of an ongoing study on population dynamics (Hennes 2012), which made sure that individuals were sampled only once. From each individual, two small body feathers were taken and DNA later on extracted from skin cells at the base of the rachis (see below).

DNA extraction, microsatellite markers and genotyping

Details on DNA extraction, microsatellite markers and genotyping are given in text Appendix 1 and Table S1. In summary, 15 markers were typed in the middle

spotted woodpecker and 16 in the great spotted woodpecker (Table S2); from these, five were typed in both species.

Data analysis

In all population genetic analyses, DNA of only one randomly chosen nestling per nest was used.

Null alleles, Hardy–Weinberg expectations and linkage equilibrium

MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004, 2004) was used to examine the presence of null alleles for each locus-population combination out of HWE due to heterozygote deficiency.

Departures from Hardy–Weinberg expectations of panmixia and from linkage equilibrium between all loci pairs were tested with probability tests using GENEPOP on the web, available at http://genepop.curtin.edu.au/ (Raymond and Rousset 1995). These tests were conducted using a Markov chain with 300 batches each iterated 3000 times and a dememorization number of 3000 (Raymond and Rousset 1995). To avoid Type I statistical errors in multiple comparisons, a sequential Bonferroni correction was applied (Rice 1989).

Genetic diversity

For each species, genetic diversity was estimated using allele frequency data, from which the number of alleles per locus (A), observed and expected heterozygosities (H_{obs} and H_{exp}, Nei 1987) and F_{IS} for each local population were calculated with GENEALEX 6.501 (Peakall and Smouse 2012). For each local population, rarefied allelic richness (A_R) averaged over loci were calculated with FSTAT 2.93 (Goudet 2001).

Differences in genetic diversity between woodpecker species were assessed with Wilcoxon signed rank tests (with local populations of each species being paired). The tests were done both with all markers typed per species (see general marker-specific results below for details) and with only those five markers developed in the middle spotted woodpecker and typed in both woodpecker species.

Detailed marker-specific results on null alleles, HWE and linkage disequilibrium tests are given in Table S3 and text Appendix 2. Based on those results, all the further analyses considered 14 polymorphic markers typed in 116 middle spotted woodpeckers and 12 polymorphic markers typed in 147 great spotted woodpeckers.

Bottlenecks

Evidence for recent population bottlenecks was examined with the program BOTTLENECK 1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999) for each local population of either species. Recently bottlenecked populations (i.e., populations bottlenecked within the past few dozen generations, Luikart et al. 1998) show a faster reduction in allele number than in heterozygosity. For such populations, the heterozygosity (H_e) is thus higher than the expected equilibrium heterozygosity (H_{eq}) as calculated for a population of constant size (Piry et al. 1999), and BOTTLENECK tests for such an excess of heterozygosity (H_e). To detect recent bottlenecks, the Wilcoxon test with both the stepwise mutation model (SMM, Ohta and Kimura 1973) and the two phase model of mutation (TPM, Di Rienzo et al. 1994) was used. For the TPM, two analyses were run, with values for the proportion of single-step mutations being 0.95 (Piry et al. 1999) and 0.78 (Peery et al. 2012), respectively, and a variance in the mean size of multi-repeat mutations of 12 in both analyses (Piry et al. 1999; Peery et al. 2012). Additionally, the qualitative graphical mode-shift method (Luikart et al. 1998) was used.

Genetic differentiation and population structure

Genetic differentiation among all populations per species was assessed with F_{ST} and D_{EST} in GENEALEX 6.501 (Peakall and Smouse 2012). D_{EST} was calculated following Eq. 2 in Meirmans and Hedrick (2011). The corrections for small population size and for inbreeding were applied in the calculations of H_T (heterozygosity of the pooled subpopulations, Jost 2008) and H_S (mean heterozygosity of the individual subpopulations, Jost 2008). F_{ST}- and D_{EST}-values were tested for departure from 0 by permuting genotypes among samples (9999 permutations) in GENEALEX 6.501. To avoid Type I statistical errors in multiple comparisons, a sequential Bonferroni correction was applied to P values of both F_{ST}- and D_{EST}-analyses (Rice 1989).

Genetic population structure

STRUCTURE 2.3.4 was used to examine genetic population structure (Pritchard et al. 2000) and run with the following parameter settings: admixture model, correlated allele frequencies among populations, a burn-in period of 100,000 steps, a chain length of 100,000 and alternatively excluding or including prior information on sampling locations (LOCPRIOR models option). Following Wang (2017), STRUCTURE was run several times for each approach (i.e., excluding or including the LOCPRIOR option) by changing the settings for the prior and alpha values, using either the uniform prior (default) or the alternative prior (gamma) setting, an initial value of 1 (default) for alpha or a value of 0.143 (1 divided by 7, i.e. the local populations sampled), and allowing alpha to be either the same for all populations or to differ among populations. For each woodpecker

species, eight STRUCTURE analyses were thus done (Table S4). Finally, for each K ranging from 1 to 7, 20 runs were performed.

To infer the most likely K, information from previously used approaches ($ln\ P(X|K)$, $P(K=k)$ and ΔK) and new ones proposed by Puechmaille (2016) were combined. A Markov chain Monte Carlo (MCMC) procedure was conducted to estimate $ln\ P(X|K)$ (denoted as LnK in the following), the mean log probability of the data given K (Eq. 12 in Pritchard et al. 2000) for each value of K, averaged across runs for each K. From LnK, the posterior probability of K, $P(K=k)$ (PPK in the following), was calculated following Pritchard et al. (2010, chapter 5.1). Because it is one of the most often used methods to estimate the number of clusters, the ΔK statistics (Evanno et al. 2005) was also calculated. Puechmaille (2016) defined clusters as "spurious" if they never achieved a mean Q greater than a user-defined threshold value in any of the subpopulations of the data set. Here, threshold values increasing in 0.1 steps from 0.5 to 0.8 were used. Spurious clusters were removed in a supervised correction applied to the estimated number of clusters given by LnK, PPK and ΔK, resulting in "corrected" $LnKCor$, $PPKCor$ and and $\Delta KCor$ (for details see Puechmaille 2016).

The number of clusters was further inferred with additional four supervised methods named 'MedMeaK' (median of means), 'MaxMeaK' (maximum of means), 'MedMedK' (median of medians) and 'MaxMedK' (maximum of medians) developed by Puechmaille (2016). These four supervised methods do not depend on LnK, PPK and ΔK and were found to outperform existing methods (Puechmaille 2016). Briefly, these new methods are based on counts of the number of clusters which at least one of the user-defined subpopulations, here individuals grouped by sampling locations (i.e. local populations), belongs to (Puechmaille 2016). A subpopulation is considered as belonging to a cluster if the mean (or median) Q of its individuals is above a user-defined threshold. Here, thresholds increasing in 0.1 steps from 0.5 to 0.8 were used as suggested by Puechmaille (2016).

All calculations were done in R with a script provided by Puechmaille (2016), yielding 31 parameters for each of the eight STRUCTURE analyses per species (see Tables S6 and S7 for detailed results). For each such analysis, the number of times each K was supported was then summed across the 31 parameters and this sum used to infer the most likely K. To further examine and visualize results, STRUCTURE HARVESTER (Earl and vonHoldt 2012) and CLUMPAK (Kopelman et al. 2015) were used.

Aside from STRUCTURE, discriminant analysis of principal components (DAPC, Jombart et al. 2010) was used to identify and describe groups of genetically similar individuals. DAPC was run with the ADEGENET package in R in two ways. First, the function find.clusters was applied to identify the number of genetic clusters K based on k-means clustering and BIC values for each K. The number of clusters K with the lowest BIC and the largest decrease in BIC was then used in the subsequent DAPC (Jombart et al. 2010). No prior information on sampling locations was used. Second, DAPC was run based on groups being the local populations where sampling had occurred (that is, $K=7$ local populations, sampling location used as prior information).

The DAPCs were run with 50 PCs in the middle spotted woodpecker and 45 PCs in the great spotted woodpecker. These numbers of PCs were determined prior to the DAPCs separately for each species (for details see text Appendix 3). DAPC results were interpreted based on scatter plots of discriminant functions and on the posterior group membership probabilities calculated from the discriminant functions. Individuals were assigned to the population or cluster for which their posterior group membership probability was >0.5.

Correlations between genetic and geographic distances

To assess whether geographical distance between populations explained genetic differentiation, correlations between genetic and geographic distances were examined using a Mantel test (100,000 permutations) in GENEPOP. Pairwise genetic distance defined as $F_{ST}/(1\text{-}F_{ST})$ was regressed on the logarithm of geographic distance in km. This regression is considered linear in a two-dimensional model.

Contemporary gene flow

The first-generation migrant test implemented in GENECLASS 2.0 (Piry et al. 2004) was used to detect offspring of migrant individuals based on their multi-locus genotype data (Rannala and Mountain 1997; Paetkau et al. 2004). Because not all possible source populations had been sampled, L_h was used as the test statistic for the likelihood estimation (Paetkau et al. 2004). The test was run with the Bayesian method of Rannala and Mountain (1997) and a probability threshold value α of 0.05 based on 10′000 repetitions of the Paetkau et al. (2004) Monte Carlo re-sampling algorithm. The null hypothesis that an individual was an offspring from parents born in the local population in which the offspring was sampled was rejected when α was ≤ 0.05.

Results

Genetic diversity (expectation 1)

In line with expectation 1, measures of genetic diversity were consistently larger in the great spotted woodpecker than in the middle spotted woodpecker (Table 1). When considering all markers, the difference in favor of the great

spotted woodpecker ranged from 38 to 92% for the number of alleles A (average across populations: 59%, Wilcoxon test $p < 0.016$), 41% to 81% for allelic richness A_r (57%, $p < 0.016$) and 1% to 16% for expected heterozygosity H_e (9%, $p < 0.016$). F_{is} did not significantly differ between the species (Table 1). Results were similar when only the five markers developed in the middle spotted woodpecker and typed in both species were considered: number of alleles A (range difference, average across populations, Wilcoxon test result; A: 32–100%, 62%, $p < 0.016$), allelic richness A_r (39–92%, 56%, $p < 0.023$) and expected heterozygosity H_e (2–17%, 9%, $p < 0.016$) were higher in the great spotted woodpecker than in the middle spotted woodpecker (Fig. S2).

Bottlenecks (expectation 2)

There was evidence for recent bottlenecks in the middle spotted, but not in the great spotted woodpecker. For the middle spotted woodpecker, evidence for bottlenecks based on heterozygosity excess tests was revealed in all local populations, except HE and TG (Table S5). A characteristic mode-shift distortion in the distribution of allele frequencies indicative of population bottlenecks was detected for the populations AG and NE (Fig. S3). Expectation 2a was hence partly supported. Local populations of the great spotted woodpecker showed no evidence for recent bottlenecks, neither based on heterozygosity excess tests (Table S5) nor on the shape of the allelic frequency distributions (Fig. S3). Expectation 2b thus seemed to be supported.

Genetic differentiation, spatial genetic structure and correlations between genetic and geographic distances (expectations 3 and 4)

Pairwise genotypic population differentiation based on FST and DEST

Overall F_{ST} and D_{EST} values across populations and loci were 0.048 (SE: 0.004) and 0.044 (0.016) for the middle spotted woodpecker, and 0.035 (0.002) and 0.034 (0.011) for the great spotted woodpecker, respectively. Pairwise F_{ST} values were mostly smaller than the corresponding D_{EST} values, but both measures of genetic differentiation yielded the same results (Table 2). Local populations of the middle spotted woodpecker were moderately, but often significantly differentiated from each other. The westernmost local population NE was significantly differentiated from all the other local populations and showed the highest F_{ST} and D_{EST} values (Table 2). The German local population HE was somewhat less strongly differentiated from the Swiss local populations SH, TG and ZH than the local population NE, which was unexpected, because the local population HE is clearly farthest away from these local populations. Local populations close to each other were hardly differentiated (e.g. AG vs. BL, SH vs. ZH, Table 2).

As expected, differentiation among great spotted woodpecker local populations was overall lower than among middle spotted woodpecker local populations. However, two results were surprising. First, the local population AG was significantly differentiated from all Swiss local populations, except the local population SH. Second, the German local population HE was not significantly differentiated from any of the Swiss local populations (Table 2).

Genetic structure

Analyses without a priori information on sampling locations suggested weak genetic structure in the two woodpecker species, both when using STRUCTURE (Fig. 1) and ADEGENET. Detailed results can be found in text Appendix 4.

Middle spotted woodpecker—When prior information on sampling locations was used, STRUCTURE indicated the presence of either three or four genetic clusters in the middle spotted woodpecker (Fig. 1). In three of the four analyses, the solution with $K = 3$ was most often supported, but the solution with $K = 4$ also received considerable support. In the fourth analyses however, $K = 4$ was most often supported, but $K = 3$ also received good support. Even though the Q-plots could not resolve the question of whether $K = 3$ or $K = 4$ was most adequate, the grouping of local populations was consistent within a given cluster solution (i.e. within $K = 3$ or $K = 4$; Fig. 2). With $K = 3$, the local populations SH, TG and ZH in the east of Switzerland formed one genetic cluster, whereas the local populations HE and NE each represented separate clusters (Fig. 2). The local populations AG and BL were not assigned to any cluster, as individuals had mixed ancestry to similar extents from cluster 1 (SH, TG, ZH) and 2 (NE). With $K = 4$, the genetic clusters were formed by the same populations as in $K = 3$, except that the easternmost local population TG appeared to represent a fourth cluster (Fig. 2).

The first two PCs of the DAPC separated the local populations HE and NE both from each other and from all the other local populations (Fig. 3). The second PC additionally separated the local populations AG and BL from SH, TG and ZH. The third PC confirmed the separate positions of HE and NE and further suggested TG to slightly differ from its neighboring local populations SH and ZH in eastern Switzerland (Fig. 3). DAPC with 50 PCs thus suggested four clusters (Table S8), these consisting of individuals from AG and BL (cluster 1), HE (cluster 2), NE (cluster 3) and SH, TG and ZH (cluster 4). Reassignment of individuals based on their posterior group membership probabilities supported the presence of these four clusters (Table S8). The proportions of reassignment of individuals to these clusters were

Table 2 Pairwise genotypic differentiation among local populations of middle spotted woodpeckers (Msw) and great spotted woodpeckers (Gsw)

Species[a]	Population[b]	AG	BL	HE	NE	SH	TG	ZH
Msw	AG (14)	–	13.1	304.3	89.0	58.4	97.6	61.1
	BL (19)	−0.003	–	307.3	79.4	71.2	110.6	74.1
		0.016						
	HE (16)	**0.060**	**0.058**	–	378.6	283.9	289.5	290.7
		0.034	**0.031**					
	NE (18)	**0.044**	**0.037**	**0.075**	–	143.6	178.2	143.8
		0.030	**0.025**	**0.036**				
	SH (18)	0.021	0.021	0.036	**0.041**	–	40.5	7.8
		0.022	0.020	0.025	**0.026**			
	TG (15)	0.046	**0.044**	**0.072**	**0.084**	**0.043**	–	36.5
		0.032	**0.029**	**0.038**	**0.042**	**0.029**		
	ZH (16)	0.017	0.026	**0.052**	**0.086**	0.013	**0.045**	–
		0.023	0.023	**0.031**	**0.042**	0.019	**0.032**	
Gsw	AG (24)	–	13.0	304.7	88.8	58.0	98.8	61.2
	BL (21)	**0.045**	–	307.7	79.3	70.6	111.7	74.1
		0.021						
	HE (21)	0.031	0.028	–	378.8	284.0	290.0	290.5
		0.018	0.020					
	NE (21)	**0.053**	0.026	0.032	–	143.2	179.2	144.0
		0.023	0.019	0.021				
	SH (20)	0.017	0.019	0.013	0.028	–	42.2	8.1
		0.016	0.017	0.017	0.020			
	TG (21)	**0.055**	0.032	0.016	**0.056**	0.026	–	37.6
		0.022	0.020	0.017	**0.026**	0.019		
	ZH (19)	**0.049**	**0.043**	0.032	**0.046**	0.040	0.037	–
		0.022	**0.023**	0.021	**0.025**	0.023	0.022	

Numbers above the diagonal are distances (km) between local populations, numbers below the diagonal are D_{EST}- (upper cell value) and F_{ST}-values (lower cell value). Bold: significant after sequential Bonferroni correction. *AG* Aargau, *BL* Basel, *HE* Hesse (Germany), *NE* Neuchâtel, *SH* Schaffhausen, *TG* Thurgau, *ZH* Zürich. Note that distances differ slightly between the two woodpeckers because distances were calculated based on centroids of breeding cavity locations per species

[a]Msw: 14 autosomal loci; Gsw: 12 autosomal loci

[b]Numbers in parentheses give individuals genetically analyzed

0.929 (AG) and 0.842 (BL), respectively, for cluster 1, 0.875 for cluster 2 (HE), 1.0 for cluster 3 (NE), and 0.889 (SH), 0.993 (TG) and 0.875 (ZH), respectively, for cluster 4.

Great spotted woodpecker—For the great spotted woodpecker, K = 2 was the best supported solution in all four STRUCTURE analyses with a priori location information, even though in one analysis K = 1 was equally well supported (Fig. 1). Despite the overall good support for K = 2, Q-plots did not provide clear evidence for the two groups (Fig. 2).

DAPC supported the presence of three clusters. Plotting the first two PCs suggested two genetic clusters, one being the local population NE and the other the local population AG (Fig. 3). The third PC additionally separated ZH from the other local populations as a third cluster (Fig. 3). Reassignment of individuals based on their posterior group membership probabilities supported the presence of these three clusters (Table S8). The proportion of reassignment of individuals from AG to cluster 1 was 0.792, from NE to cluster 2 0.762 and from ZH to cluster 3 0.790, respectively. Individuals from the other four local populations were neither reassigned particularly successfully to their original local populations (TG: 0.524, HE: 0.381, BL: 0.000, SH: 0.150) nor did they collectively represent another cluster (Table S8). In summary, STRUCTURE and DAPC suggested local population NE to be one cluster, with AG and possibly ZH representing two additional clusters.

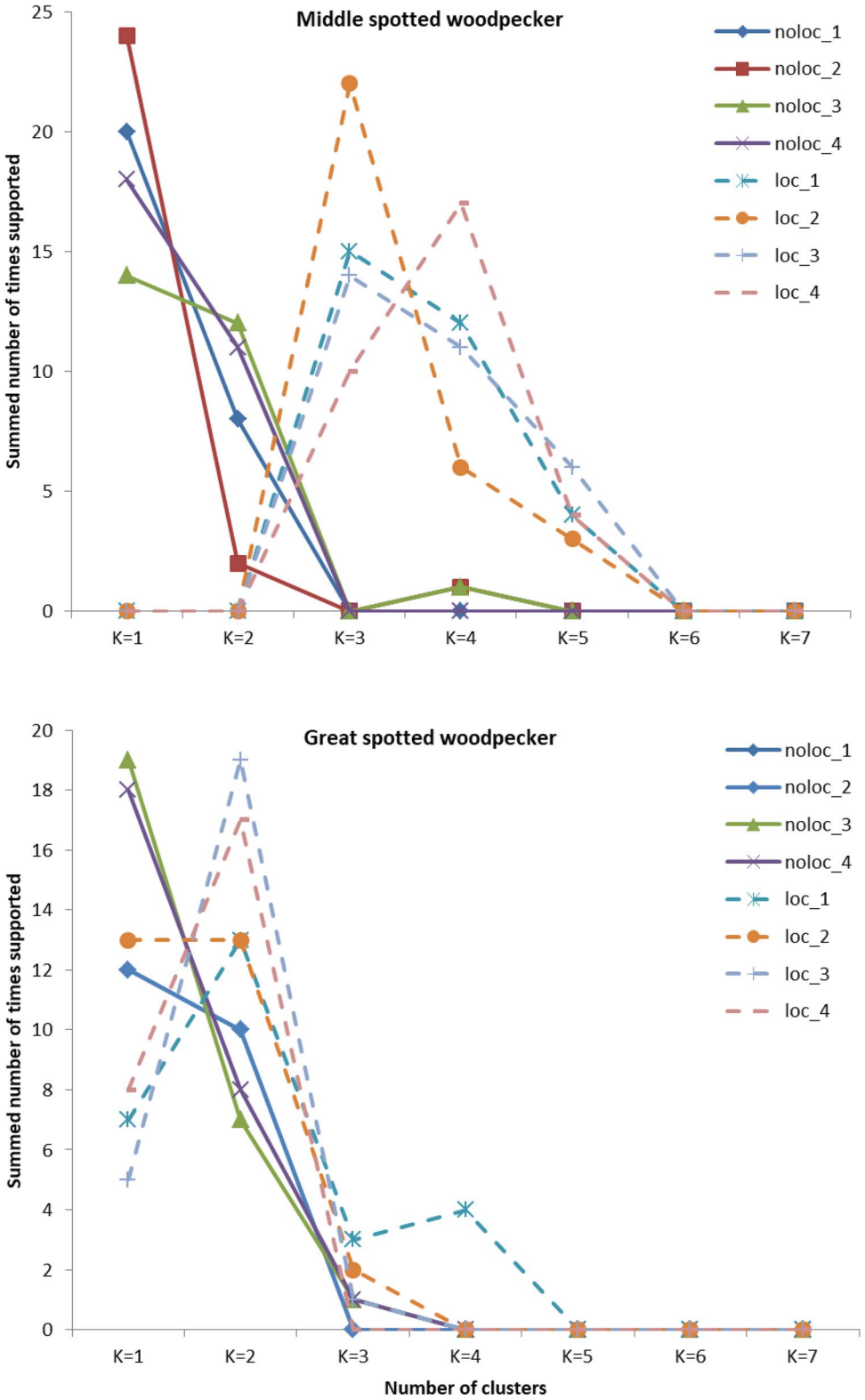

Fig. 1 Summed number of times, a particular cluster solution (K) was supported in the eight STRUCTURE analyses of the middle spotted woodpecker and great spotted woodpecker. Noloc and loc refer to analyses without and with a priori location information used by STRUCTURE, respectively. Explanations of the settings of the eight analyses per species are given in Table S4 and detailed results per species for each of the 31 parameters used to infer K are found in Tables S6 and S7. Note that for the great spotted woodpecker, noloc_1 and noloc_2 yielded exactly the same results, as indicated by their identical line and marker

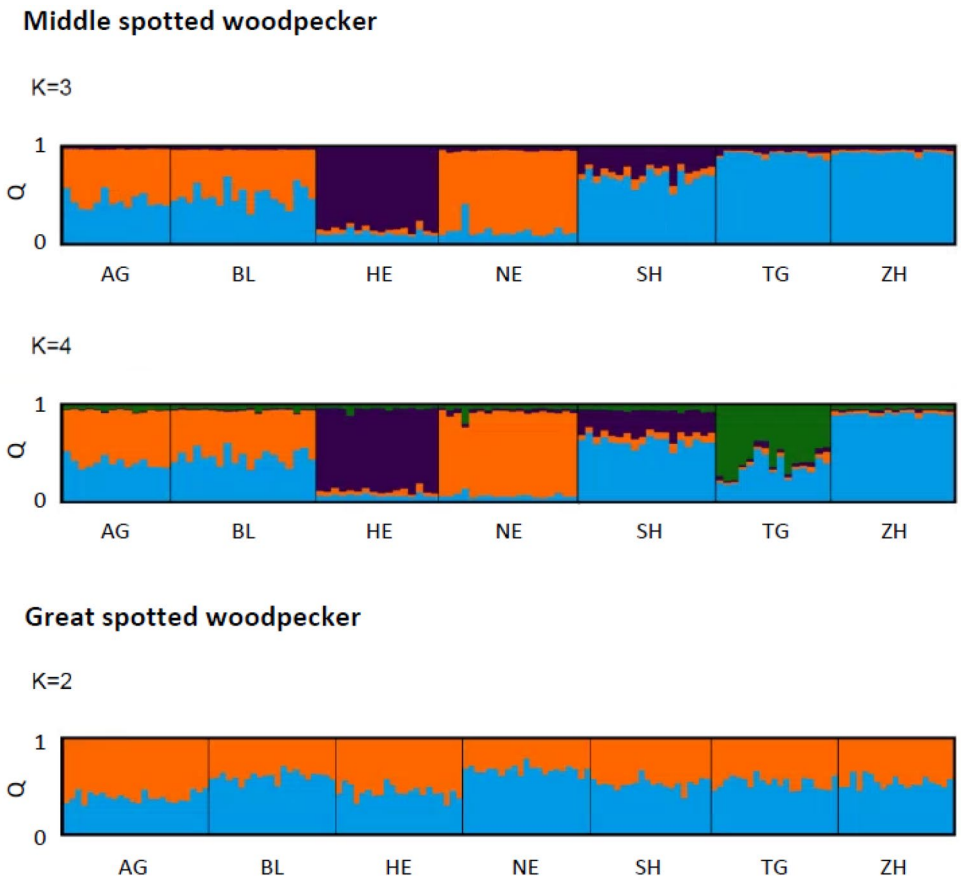

Fig. 2 Q-membership plots for STRUCTURE analyses using prior information on sampling locations. Each vertical bar corresponds to one individual. K = number of clusters. Plots were generated with CLUMPAK based on loc_1 analysis output (see Table S4; results for loc_2-loc_4 look virtually the same). Q = membership coefficients. For abbreviations of population names see Table 1

Correlations between genetic and geographic distances

The middle spotted woodpecker showed a significant correlation between genetic and geographic distances at the population level ($R^2 = 0.52$, Mantel test, $p = 0.001$, Fig. 4). Exclusion of the distant local population HE did not change the overall result ($R^2 = 0.48$, $p = 0.008$).

In contrast, the great spotted woodpecker neither showed significant correlations between genetic and geographic distances across all local populations ($R^2 = 0.14$, $p = 0.803$, Fig. 4) nor when excluding the local population HE ($R^2 = 0.03$, $p = 0.080$).

In summary, the different analyses support expectations 3a and 4a of the SGVH, i.e. significant genetic differentiation, spatial structure and correlation between genetic and geographic distances among local populations of the specialist middle spotted woodpecker. In the generalist great spotted woodpecker, some genetic differentiation and spatial structure was found, albeit overall weaker and less clear than in the specialist middle spotted woodpecker, which is in line with expectation 3b. Also, a significant correlation between genetic and geographic distances was not found in the generalist species, consistent with expectation 4b.

Contemporary gene flow (expectation 5)

Across local populations, the first-generation migrant tests in GENECLASS2 detected 12 recent migration events among the 116 middle spotted woodpeckers (10.1%) and 16 recent migration events among the 147 great spotted woodpeckers (10.9%, Table 3). The fraction of migrants did not differ between the two species (two sample t-test, $t = 0.157$, $p = 0.876$). The median migration distances among local populations were 76.8 km (25–75% quartiles: 30.7–180.1, range: 7.8–307.3, $n = 12$) in the middle spotted woodpecker and 98.8 km (56.5–180.5, 8.1–304.7, $n = 16$) in the great spotted woodpecker (Fig. S4). The migration distances did not significantly differ between the species (U-test, $p = 0.763$). Expectation 5 was thus not supported.

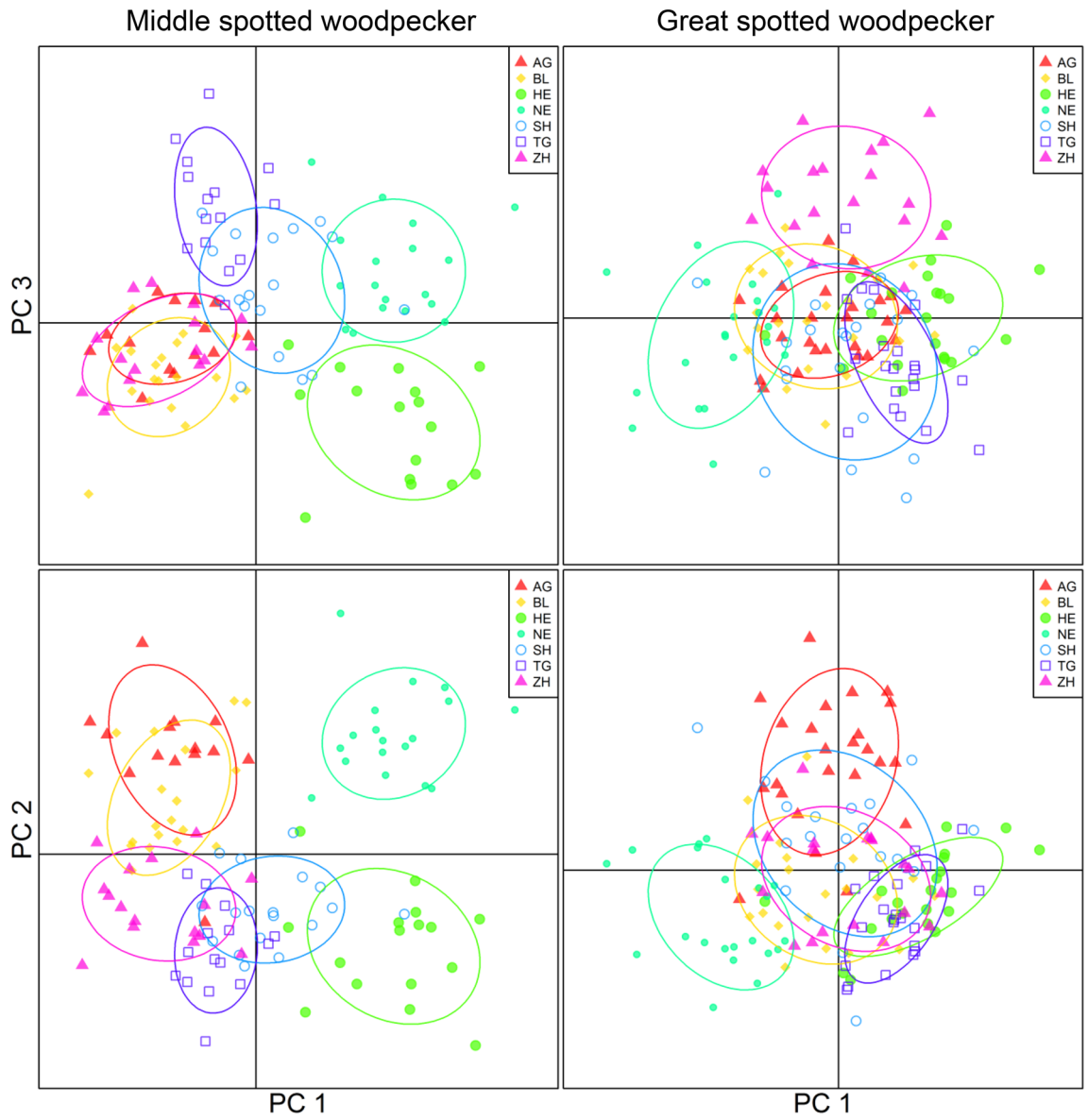

Fig. 3 Genetic structure of seven local populations of middle spotted woodpeckers (based on 50 PCs) and great spotted woodpeckers (45 PCs) according to discriminant analysis of principal component (DAPC). For abbreviations of population names see Table 1

Discussion

This study tested predictions of the SGVH by addressing genetic diversity, spatial genetic structure and contemporary gene flow in two sympatric bird species differing in habitat specialization. The results support all but one expectations of the SGVH. Compared to the generalist great spotted woodpecker, genetic diversity was lowered in the specialist middle spotted woodpecker (expectation 1 supported). Evidence for recent bottlenecks was found in some populations of the middle spotted woodpecker, but in none of the great spotted woodpecker (expectations 2a partly and 2b fully supported). Significant spatial genetic structure and correlations between genetic and geographic distances were found in the middle spotted woodpecker (expectations 3a and 4a supported), but only weak spatial genetic structure and no significant correlation between genetic and geographic distances in the great spotted woodpecker (expectations 3b and 4b supported). Finally, estimates of contemporary gene flow did not differ between the two species (expectation 5 not supported).

Genetic diversity and bottlenecks

Between-species comparison of genetic diversity based on microsatellite markers may be problematic if the

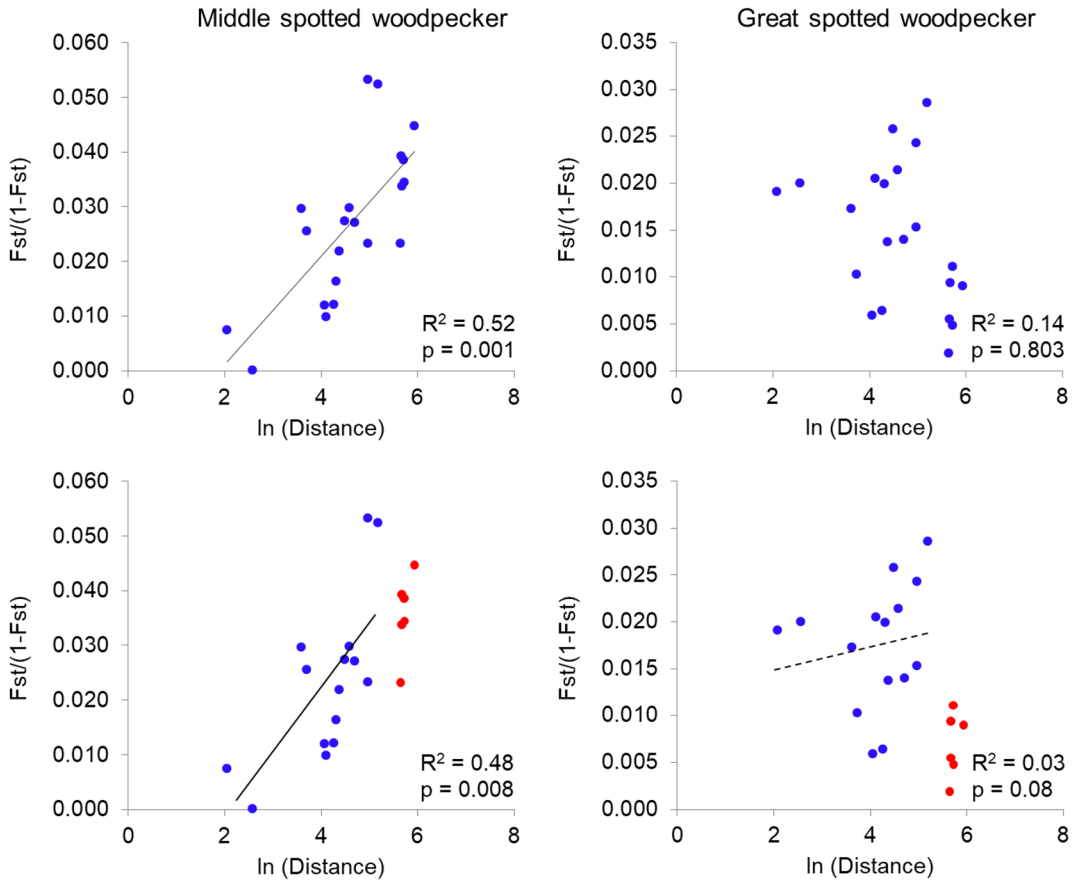

Fig. 4 Correlations between genetic distance [$F_{ST}/(1-F_{ST})$] and geographic distance (ln kilometers) in middle spotted woodpeckers and great spotted woodpeckers. Upper panels include all population pairs, lower panels only Swiss population pairs, with the excluded German-Swiss pairs shown as red points for information

Table 3 Results of first-generation migrant tests conducted in GENECLASS 2

Species	Sampled in	Assigned to								
		AG	BL	HE	NE	SH	TG	ZH	Nr. mig	% mig
MSW	AG (n = 14)	–	0	0	0	0	0	0	0	0.0
	BL (n = 19)	2	–	0	1	0	0	0	3	15.8
	HE (n = 16)	0	1	–	1	0	1	0	3	18.8
	NE (n = 18)	1	0	0	–	1	0	0	2	11.1
	SH (n = 18)	0	1	0	0	–	0	0	1	5.6
	TG (n = 15)	0	0	0	0	0	–	1	1	6.7
	ZH (n = 16)	0	1	0	0	1	0	–	2	12.5
GSW	AG (n = 24)	–	0	0	0	0	1	0	1	4.2
	BL (n = 21)	1	–	0	0	0	0	0	1	4.8
	HE (n = 21)	2	0	–	0	0	0	0	2	9.5
	NE (n = 21)	0	0	0	–	1	0	1	2	9.5
	SH (n = 20)	0	1	0	0	–	1	0	2	10.0
	TG (n = 21)	1	1	2	0	0	–	0	4	19.0
	ZH (n = 19)	2	0	0	0	1	1	–	4	21.1

Numbers indicate first-generation migrant events among the studied local populations of middle spotted woodpeckers (MSW) and great spotted woodpeckers (GSW), respectively, at a probability level of $p \leq 0.05$. For each studied population, % mig. gives the percentage of sampled individuals with recent ancestry from elsewhere than the sampling population

markers used differ between species (Frankham et al. 2010; Ellegren and Galtier 2016). In this study, only nine markers used to assess genetic diversity had been typed in both woodpecker species (Table S3). Nevertheless, the overall lower genetic diversity found in the middle spotted woodpecker compared to the great spotted woodpecker is thought to be real for two reasons. First, 12 of the 14 markers used in the middle spotted woodpecker, but only 7 of the 12 markers used in the great spotted woodpecker were species-specific markers (i.e. had been developed for the particular species). Since marker variability decreases with increasing phylogenetic distance (Primmer et al. 1996; Galbusera et al. 2000; Dawson et al. 2013), middle spotted woodpeckers should have shown at least as high genetic diversity as great spotted woodpeckers, for which fewer species-specific markers had been used. Second, genetic diversity was also lower in the middle than the great spotted woodpecker, when the same five markers developed in the middle spotted woodpecker and typed in both species were used.

Specialist species typically have lower genetic diversity than generalist species (Li et al. 2014; Janecka et al. 2016; Khimoun et al. 2016; Matthee et al. 2018). The consistently lower genetic diversity in the specialist compared to the generalist woodpecker species reported here mirrors these patterns and thus lends support to the SGVH. Moreover, the low genetic structure (see below) does not support the view that the generalist great spotted woodpecker is made up of locally adapted specialists.

Genetic diversity is generally reduced in small populations compared to large populations, owing to both stronger genetic drift and the effects of potential inbreeding (Frankham et al. 2010). Population sizes are considerably smaller in the range-restricted specialist middle spotted woodpecker (Germany: 27,000–48,000 territories, Gedeon et al. 2014; Switzerland: 1,700–2,100 pairs, Knaus et al. 2018) than in the wide-spread generalist great spotted woodpecker (Germany: 680,000–900,000 territories, Gedeon et al. 2014; Switzerland: 70,000–90,000 pairs, Knaus et al. 2018;), and this also applies to local populations of the species (Michalek and Miettinen 2003; Pasinelli 2003). These disparities in population sizes likely explain much of the difference in genetic variation observed between the two woodpecker species in this study. In addition, connectivity among populations is stronger in the great spotted woodpecker than in the middle spotted woodpecker (see below), which counteracts the loss of genetic diversity due to drift via sustained genetic exchange.

Another reason for low genetic diversity may be past bottlenecks (e.g. Keller et al. 2001). Evidence for such bottlenecks was found in five of the seven local populations of the middle spotted woodpecker, but no evidence at all in great spotted woodpecker local populations. The middle spotted woodpecker had declined in many parts of Europe (Pasinelli 2003) and in Switzerland at least since the 1950ies (Knaus et al. 2011). The declines were primarily caused by the harvesting of old forests rich in oaks and the destruction of riverine forests. These processes led to increasingly small and fragmented areas of suitable habitat allowing to sustain small local populations only. As an example, local populations of the middle spotted woodpecker in Switzerland hardly ever surpassed 50 territories between the 1970ies and early 2000s (e.g. Bühlmann et al. 2003; Mulhauser and Junod 2003). The two populations showing no evidence of a recent bottleneck were those in Hesse (Germany) and Thurgau (Switzerland). In Hesse, the middle spotted woodpecker numbers 5,000 to 9,000 territories (Stübing et al. 2010). Samples for this study were obtained from the edge of the very extensive Taunus Mountains population (Hennes 2012) and were thus part of an overall considerably larger local population than those from Switzerland. As for the canton Thurgau, it is possible that the local population sampled had been relatively small but stable for a long time, as the conversion of oak coppice-with-standards forest to oak high forest had taken place earlier than for example in the nearby canton Zürich (Bühlmann et al. 2007). Loss of genetic diversity due to population declines might have been relatively low and not resulted in a recent bottleneck.

Bottleneck tests assume random mating (no population structure) and population closure (no gene flow) (Funk et al. 2010). Non-random mating can produce genealogies resembling bottlenecks, while gene flow is generally predicted to resemble recent expansion by introducing rare alleles (Cornuet and Luikart 1996; Busch et al. 2007). Thus, the bottlenecks detected in the middle spotted woodpecker may be artefacts of non-random mating (i.e., population structure), gene flow and/or recent population expansion. Random mating within local populations (i.e., lack of genetic substructure) was generally supported by agreement with HW proportions, while evidence for some gene flow among local populations was found. In addition, middle spotted woodpeckers have recently increased in many parts of Europe, including Switzerland and the local populations studied here (Knaus et al. 2018). However, despite gene flow and the general population increases, the signature of bottlenecks in five populations and the lack thereof in two populations was relatively consistent across the analyses applied. In contrast, great spotted woodpeckers also showed gene flow among the local populations studied (with HW proportions supported) and population increases in Switzerland over the past 20 years (Knaus et al. 2018), but no evidence for bottlenecks whatsoever. Thus, the bottlenecks detected in middle spotted woodpecker appear to be real and not artefacts arising from gene flow.

Genetic differentiation and spatial genetic structure

Middle spotted woodpecker—Combining the results of the different analyses (F_{ST}, D_{EST}, STRUCTURE, DAPC) revealed significant spatial genetic structure in the middle spotted woodpecker (expectation 3a supported). Local populations HE and NE were consistently considered to be genetically different both from each other and from all the other local populations. This is not surprising for HE, given its location far from the other local populations and the comparatively limited dispersal of the species. However, that NE genetically differed from the other Swiss local populations examined was surprising, but may be explained by topography. NE is geographically separated from the other local populations by the Jura Mountain range (Fig. S1), which accounts for much of the geographic distance to the closest sampled populations AG and BL and, to a lesser extent, also to the local populations in eastern Switzerland. The slopes of these mountains (up to 1000 m high between the locations in question) are mostly covered by managed beech-conifer forests, which might compromise dispersal of this oak habitat specialist. Juvenile middle spotted woodpeckers prefer old oak forest during post-fledging dispersal (Ciudad et al. 2009) and thus similar habitats as adults for breeding (Pasinelli 2003).

Great spotted woodpecker—Phylogeographic studies using mitochondrial markers have revealed low genetic structuring across large geographic areas in this species (Zink et al. 2002; Perktas and Quintero 2013). Therefore, the expectation was to find no spatial genetic structure in this study conducted on a comparatively small spatial scale. Nevertheless, results of the different analyses (F_{ST}, D_{EST}, STRUCTURE, DAPC) based on 12 microsatellite markers revealed spatial genetic structure in this woodpecker species, but overall weak and less clear than in the middle spotted woodpecker (expectation 3b supported). Local populations AG, NE and ZH were found to be genetically different from each other in all analyses (exception: ZH not different in STRUCTURE analysis), even though they were only 61 km (AG-ZH), 89 km (AG-NE) and 144 km (NE-ZH) apart. In contrast to the middle spotted woodpecker, the Jura Mountain range extending between NE and the other two local populations (Fig. S1) is colonized entirely by the great spotted woodpecker (Knaus et al. 2018) and thus unlikely to impede dispersal and to result in the genetic dissimilarity observed. In addition, the other sampled local populations were not consistently (TG) or not at all (BL, HE, SH) genetically separated from the NE local population lying beyond the Jura mountain range. Alternatively, the local populations NE and ZH might differ genetically from AG because of differences in habitat composition. AG birds were sampled in a forest with a relatively high share of beech (*Fagus sylvatica*), while the NE and ZH populations reside in almost pure oak forests (as the other local populations examined here). It is not known whether breeding habitat selection in great spotted woodpeckers is affected by the natal environment experienced (e.g. Davis and Stamps 2004; Selonen et al. 2007) nor whether natural selection might act against immigrants from other natal environments. The sampling scheme was not designed to address a potential habitat-dependent genetic structuring in the two study species, but the pattern found in the great spotted woodpecker is reminiscent to isolation-by-environment reported for other taxa (Wang and Bradburd 2014).

Correlations between genetic and geographic distances and contemporary gene flow

As expected, middle spotted woodpeckers exhibited a significant correlation between genetic and geographic distances (expectation 4a supported), even when excluding the distant HE local population. The findings on spatial genetic structure (above) and from field studies on marked individuals suggest dispersal limitation to underlie the correlation between genetic and geographic distances found. Natal dispersal distances (from the site of birth to first breeding) based on color-ringed birds were below 3.5 km in continuous habitat and between 0 and 10 km in fragmented habitat (Kossenko 2002). Radio-tracked juveniles did not venture further than 7 km during summer dispersal in fragmented habitat (Robles et al. in prep.). Re-sightings of birds color-ringed as nestlings in our study revealed movements of 13 km (found in March of the post-ringing year), 17 km (July of ringing year) and 43 km (6 years post-ringing), while in another study, movements of ringed fledglings up to 55 km were documented (Ruge and Görze 2001). Breeding dispersal is considered to occur over even shorter distances than natal dispersal (Pasinelli 2003). Furthermore, occurrence and colonization probabilities are reduced beyond distances of around 10 km among suitable habitats (Müller 1982; Pettersson 1985; Richter 1997; Bühlmann and Pasinelli 2012), suggesting a reluctance of middle spotted woodpeckers to moving over longer distances.

On the other hand, contemporary gene flow estimated in this study suggested that dispersal in the middle spotted woodpecker can occur over much wider distances than previously thought based on ringing and radio-tracking. The median distance of recent migrant events was estimated at 76.8 km and considerably longer movements were also revealed (up to 307 km). It is clear that these estimates are affected by the distances among the local populations sampled. Nevertheless, eight of the twelve recent migrant events found were within 100 km, suggesting that dispersal mostly occurred between neighboring local populations, which complies with the correlation between genetic and geographic distances found.

In the great spotted woodpecker, no evidence for a significant correlation between genetic and geographic distances was found (expectation 4b supported). Two possible explanations for the lack of such a correlation observed are suggested. First, natal dispersal appears to often occur over distances beyond 100 km, with the maximum recorded juvenile dispersal distance being almost 600 km (Winkler et al. 2018). These numbers are in line with our estimates of distances of contemporary gene flow, which averaged 100 km and ranged up to 307 km. Moreover, neither Zink et al. (2002) nor Perktas and Quintero (2013) found a phylogeographic structure across Eurasia based on mtDNA markers. It thus seems likely that the scale of our study was insufficient to detect a significant correlation between genetic and geographic distances. Second, eruptive movements regularly occur in the great spotted woodpecker when winter food sources (coniferous cones) are scarce in the north (Winkler et al. 2018). Some invading birds might stay in their wintering areas and subsequently breed, thereby homogenizing gene pools at least across the distances relevant in this study.

That the results on spatial genetic differentiation and contemporary gene flow were not in line with each other may be explained by the different temporal time scales over which genetic signals are captured by these analyses (Manel et al. 2005). Analyses using F_{ST}, D_{EST}, genetic clustering, etc. summarize genetic patterns over longer temporal scales (i.e., over multiple generations) while analyses on contemporary gene flow try to estimate recent migration events (i.e. over few generations). It is possible that these latter analyses captured the population expansions of the middle spotted woodpecker, which has been occurring in many parts of its range since the early 2000s, while these expansions may not yet have substantially affected the other analyses considering longer time scales.

Conclusions

This study on two sympatric woodpecker species differing in habitat specialization lends some support to the SGVH. Consistent with the expectations of the SGVH, the generalist species showed higher genetic diversity, which may also in part be explained by its larger population size (see above), and overall lower genetic differentiation than the specialist species. The SGVH has received some attention in invertebrate and fish species (Li et al. 2014 and studies cited therein; Matthee et al. 2018). This study adds to the very few investigations addressing the SGVH in terrestrial vertebrates (two mammals species: Janecka et al. 2016; eight tropical bird species: Khimoun et al. 2016). By examining two non-tropical, non-passerine forest bird species, the study extends the assessment of the SGVH in terms of both latitude and phylogeny.

Spatial genetic structure of local middle spotted woodpecker populations seemed to be best described by the hierarchical island model (Jombart et al. 2010), with some local populations constituting separate clusters and exchanging more migrants among each other (i.e. within clusters) than with other local populations (among clusters). In this species, local populations thus seem to be organized as metapopulations, which is in line with extinction-colonization dynamics observed previously (Robles and Ciudad 2012). In contrast, spatial genetic structure of local great spotted woodpecker populations appeared to follow the island model, characterized by high migration among local populations (Jombart et al. 2010). Great spotted woodpecker local populations may thus best fit a patchy population (Harrison 1991).

Finally, this study revealed reduced genetic diversity in the specialist middle spotted woodpecker compared to the generalist great spotted woodpecker. Levels of genetic diversity in the specialist species were similar to those found in other threatened taxa (Spielman et al. 2004; Frankham et al. 2010), including woodpecker species (Ellegren et al. 1999; Vila et al. 2008). Whether the current spread of the species leads to the theoretically expected increase of genetic diversity in local populations requires further study, but may be critical for the long-term survival of the bottlenecked populations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10592-022-01451-9.

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Declarations

Competing interest The author declares no competing financial interest.

Consent to participate Not applicable.

Consent for publication Not applicable.

Ethical approval Capture of nestlings, blood sampling and ringing were done with permits of the cantonal veterinary offices (AG: Permit

No. 75640; BL: Permit No. 418; NE: Permit No. 01/2010; SH: Permit No. 10.01.04.1 TVSH; TG: Permit No. VoWa 01/09; ZH: Permit No. 66/2009) and the federal office for environment FOEN.

Availability of data and material *Dendrocopos major* microsatellite sequences are available on GenBank, accession JX196852-JX196858; microsatellite genotypes are available on Zenodo vogelwarte.ch Open Repository and Archive at https://doi.org/10.5281/zenodo.6798002.

Code availability Not applicable.

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