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ORIGINAL PAPER

# Multiple introductions and no loss of genetic diversity: invasion history of Japanese Rose, *Rosa rugosa*, in Europe

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**Abstract** The shrub *Rosa rugosa* (Japanese Rose), native to East Asia, is considered one of the most troublesome invasive plant species in natural or semi-natural habitats of northern Europe and has proven very difficult to control. We aimed at disentangling the species' invasion history in Europe, including determining the number of introductions and their geographic origin, and at investigating whether populations in the introduced and native ranges differ in genetic diversity, structure and degree of differentiation. We found that introduced ($n = 16$) and native ($n = 16$) populations had similar levels of genetic diversity at seven nuclear SSR (microsatellite) loci. European populations lack isolation by distance and are less genetically differentiated than are populations in East Asia. Multiple and at least three independent colonization events, one of which was particularly successful, gave rise to current *R. rugosa* populations in Europe. The geographic distribution patterns of these three genetic clusters could not be explained by natural dispersal alone, indicating that human mediated secondary dispersal is driving the expansion in Europe. One cluster representing three of the European populations was most likely derived from NW Japan, whereas the origin of the remaining thirteen populations could not clearly be resolved. The introduction and expansion in Europe occurred with no significant loss of genetic diversity. We conclude that high propagule pressure at the primary establishment phase is the most parsimonious explanation for this pattern. A potential for long distance seed dispersal, coastal habitat connectivity and an outcrossing breeding system are factors likely to have enabled populations of *R. rugosa* to avoid detrimental effects of genetic bottlenecks and will further increase the species' range size and abundance in Europe. We recommend that human-mediated dispersal should be prevented in order to halt the continued expansion.

**Keywords** Assignment analysis · Genetic diversity · Invasion history · Microsatellite · Phylogeography · Population genetics

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## Introduction

Biological invasions—the success of non-native species in novel environments—may seem counterintuitive because such newly formed populations are

likely genetically depauperate, yet they surpass native species in fitness-related properties. Colonization by newly arrived species is expected to involve a population bottleneck because the number of founding individuals must be relatively small (Nei et al. 1975; Allendorf and Lundquist 2003). Given the relationship between genetic diversity (i.e. evolutionary potential) and population viability, this constitutes an apparent paradox in invasion biology: small population sizes and low genetic diversity on the one hand and the success and high competitive ability resulting in successful establishment and invasiveness on the other (Frankham 2005). However, in empirical studies, it is often found that genetic diversity within populations of introduced species does not differ from that in native populations (Bossdorf et al. 2005; Wilson et al. 2009; Gaudeul et al. 2011), although both lower (Dlugosch and Parker 2008) and higher levels of variation (Kolbe et al. 2004; Genton et al. 2005) have been reported. This discrepancy seems to be related to the number of introduction events and propagule sources, as genetic diversity within populations is expected to increase with the number of source populations as well as with the number of introduction events (e.g. Bossdorf et al. 2005; Lockwood et al. 2007; Ward et al. 2008; Le Roux and Wieczorek 2009; Wilson et al. 2009). It is not unusual that multiple introductions from several native sources may relieve introduced populations from negative effects associated with bottleneck events (e.g. Rosenthal et al. 2008; Alexander et al. 2009; Chun et al. 2009). Genetic data cannot only provide information on the level of genetic diversity, but also on the differentiation and structuring of invasive populations (e.g. Dlugosch and Parker 2008; Marrs et al. 2008b; Alexander et al. 2009). By comparing the genetic composition of introduced populations with those in the native range, it may be possible to find the regional sources of origin and thus reconstruct the invasion history (e.g. Besnard et al. 2007; Marrs et al. 2008a; Rosenthal et al. 2008; Fitzpatrick et al. 2012). Finding the origin of introduced population may in turn may help to determine the range of physiological and ecological tolerances of the invasive species (Miura 2007) and identifying locally adapted antagonistic organisms in the region of origin (McFadyen 1998; Le Roux and Wieczorek 2009).

The rhizomatous shrub *Rosa rugosa* (Japanese Rose or Rugosa Rose) has caused great concern as an invasive exotic species in most coastal regions of northern Europe and north-eastern America, where it primarily invades dry coastal habitats and forms dense thickets (Bruun 2005; Weidema 2006). *Rosa rugosa* is probably the worst invasive plant species occurring in natural or semi-natural habitats in northern Europe (Thiele et al. 2009), as the occurrence of *R. rugosa* has strong negative effects on native species diversity and alters the physical dynamics and soil properties of coastal ecosystems, such as sand dune and shingle beaches (Hellemaa 1998; Isermann 2008a, b; Thiele et al. 2010). Control of *R. rugosa* is extremely challenging due to its vast clonal spread, high dispersal potential and vigorous growth (Bruun 2005; Weidema 2006; Weidema et al. 2007; Kollmann et al. 2011). The introduction history is likely to be complex potentially involving several transport pathways, and secondary naturalized dispersal in the introduced range is expected to be facilitated by humans, as it's a frequently planted and popular ornamental shrub with hips of nutritional value (Bruun 2005).

In this study we aimed at investigating the population genetic patterns underlying the expansion of *Rosa rugosa* in Europe, inferred from microsatellite markers. We addressed the following questions: (1) Are the non-native European populations of *R. rugosa* genetically depauperate relative to the native East Asian populations as expected after a recent founder event? (2) Are the exotic populations genetically differentiated and if so, how are the populations geographically structured? (3) How many primary introductions have founded the European populations and can specific East Asian sources be identified?

## Methods

### Study species

The clonal shrub *Rosa rugosa* Thunb. ex Murray Rosaceae, Japanese Rose, is native to Eastern Asia where it occur in dry coastal habitats i.e. sandy dune systems, dune grassland and dune shrub. It is tolerant of extreme environmental conditions such as drought, salt and sand covering, and is generally resistant to fungal and bacterial pathogens as well as insect herbivory, particularly in the introduced range (Bruun

2006). Individuals has a high potential for clonal spread by rhizomes, which enables formation of large and dense thickets as well as long distance seed dispersal by birds, ocean currents and humans (Bruun 2005). It is outcrossing and predominantly self-incompatible (Ueda et al. 1996; Dobson et al. 1999), i.e. random mating within populations can be assumed.

The native geographical distribution of *R. rugosa* ranges from north-eastern China and the Korean peninsula to the Russian Far East regions of Primorsky Krai, Khabarovsk Krai, Sakhalin, Kamchatka and the Kurile Islands as well as central and northern Japan including the island of Hokkaido (Bruun 2005, Fig. 1b). *Rosa rugosa* may have been introduced to gardens in Europe as early as 1796 and certainly from the mid nineteenth century (Bruun 2005). A historical record of naturalization exists, albeit rather incomplete. It was observed naturalized in Denmark around 1910 and the British Isles around 1920, then a decade later in Sweden, Germany, the Baltic countries, western Russia near St Petersburg and Finland and lastly reported from southern Norway in the 1940s. Clones taken into cultivation were extensively planted as a landscape ornamental around the 1950s in Denmark (Østergaard 1953). It is now considered an invasive species in northern Europe (Weber 2003; Weidema 2006, Fig. 1a) and north-eastern North America (USDA 2009), where it colonizes dry coastal habitats (Bruun 2005; Weidema 2006). There are historical records of several transport pathways and potential areas of origin, including Japan, Island of Sakhalin and Kamchatka Peninsula in Russia and China (Bruun 2005).

## Sampling

Leaf material from *R. rugosa* was sampled from a total of 32 populations, 16 from Europe and 16 from East Asia, representing a well-balanced subset of localities in the introduced and native ranges, excluding North America (see Fig. 1; Table 1). The average sample size for European and East Asian populations were 18 and 19 individuals, respectively (range 5–30). Undamaged leaf material was collected for DNA analysis from different individuals (>12 m apart, except HO; see Table 1 for population abbreviation) in natural or naturalized populations in coastal habitats.

## DNA extraction

Complete genomic DNA was extracted from silica gel dried leaf material using a CTAB-protocol (Zhang et al. 1998) adjusted for dried samples (Jaramillo and Garcia 2006). DNA concentration was quantified using a NanoDrop ND-1000 spectrophotometer on selected samples ($n = 101$) representing populations from both Europe and East Asia, which yielded an average DNA concentration on 230 ng $\mu l^{-1} \pm 147$ SD.

## Microsatellite analysis

Twenty microsatellite primer pairs developed for other *Rosa* species were selected based on allelic polymorphism and positive cross-species amplification (Esselink et al. 2003; Yan et al. 2005; Zhang et al. 2006; Oyant et al. 2008, see Electronic Supplementary Material Table S1) as no SSR markers had been developed specifically for *R. rugosa*. These were tested using a standard PCR procedure: denaturing at 95 °C for 30 s, and extension at 72 °C for 60 s, and with a gradient annealing temperature from 56 to 50 °C for 30 s per 0.5 °C, run on a Thermo PCR PXE 0.2 Thermal Cycler. Sixteen primer pairs produced amplification products visible on a 2 % ethidium bromide agarose gel and were further analyzed for allele variation in a sample of individuals representing European and East Asian populations (Electronic Supplementary Material Table S1). PCR products were run on a 3130xl Genetic Analyzer with LIZ-500 as internal size standard and analyzed with GeneMapper® Software Version 4.0 (Applied Biosystems). Eight loci appeared to be polymorphic (>2 alleles, ID: A, B, C, I, N, Q, S and T) and were used for further analysis of the complete set of DNA samples from all populations. The proportion of missing values (alleles) in the data set was 4.6 %.

## cpDNA analysis

The chloroplast DNA *trn*L-F region known to show intraspecific haplotypic variation in several species of Rosaceae (Fér et al. 2007; Wang et al. 2009) was amplified using the universal primers c (B49317) and f (A50272) designed by Taberlet et al. (1991). PCR products were purified using standard binding and

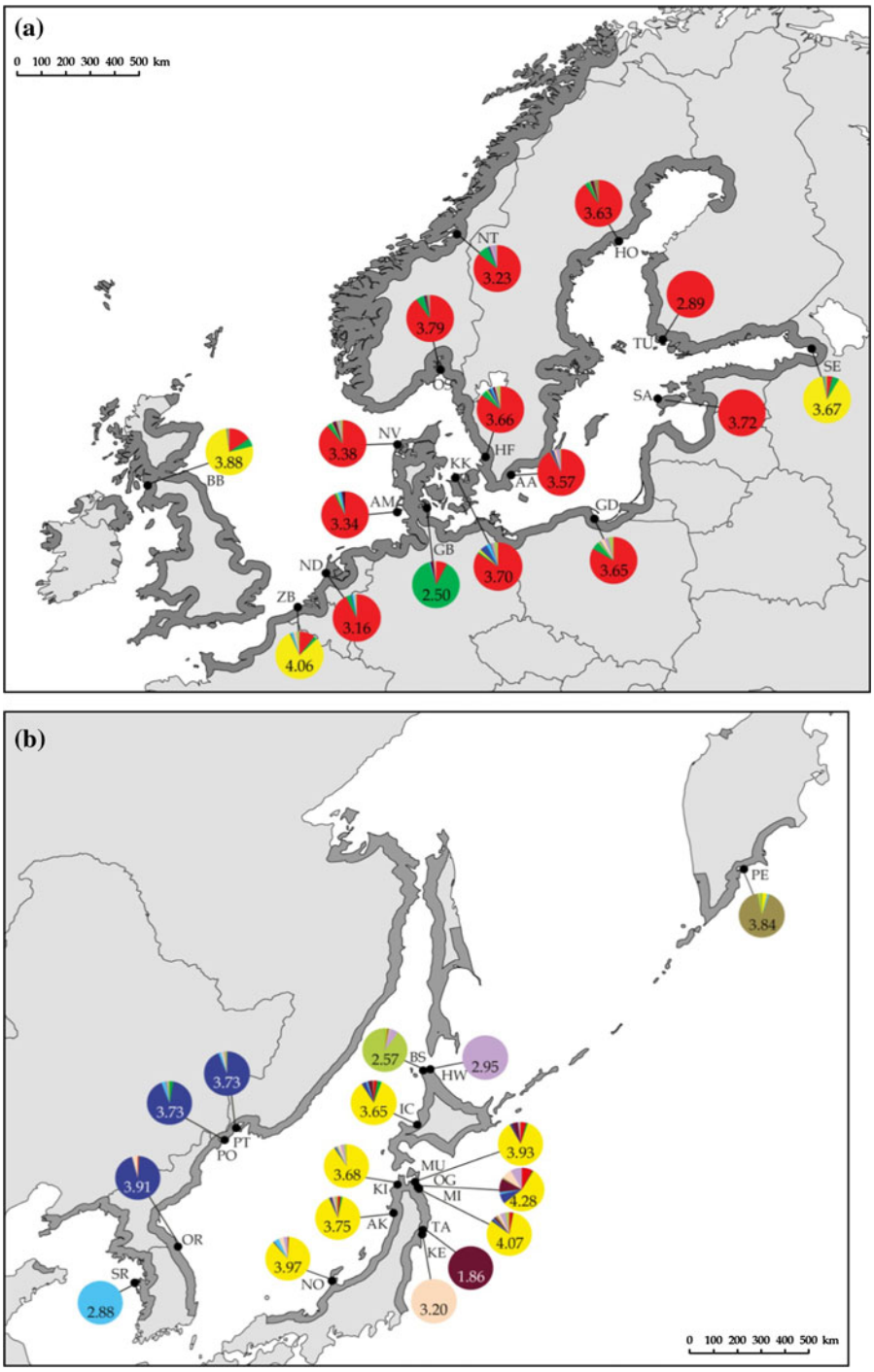


**Fig. 1** **a** European introduced and **b** East Asian native distribution of *Rosa rugosa* (hatched) showing the 32 populations sampled. *Pie charts* indicate the proportional assignment of individuals to genetic clusters for the most likely partitioning, $K = 10$ (Fig. 5). *Numbers* are the average allelic richness across loci, $A_R$ (Table 2). *Shaded areas* indicate known natural or naturalized coastal distribution of *Rosa rugosa*

elution buffers, and cycling sequencing was performed following denaturing at 96 °C for 10 s, annealing at 50 °C for 5 s and extension at 60 °C for 4 min. Sequences were scored, checked by eye and aligned using the software BioEdit version 7 (Hall 1999). No variation was found in 11 individuals representing a well-balanced subset of all populations (Europe: GD, NV, NL, SE; Asia East: IC, KE, NO, OR, PE, PT, YS; see Table 1 for population abbreviations), so this marker was not used for further analysis.

**Table 1** Samples of *Rosa rugosa*

| Country | Population | $ID_{Pop}$ | *N* | Date | Latitude | Longitude | Collector |
|---|---|---|---|---|---|---|---|
| *Introduced* | | | | | | | |
| Belgium | Zeebrugge | ZB | 20 | 01/09/2008 | N51.3255300 | E003.1653200 | Sonia Vanderhoeven & Jean-Philippe Bizoux |
| Denmark | Knarbos Klint | KK | 20 | 30/07/2008 | N55.8165167 | E011.3767333 | Hans Henrik Bruun |
| Denmark | Nørre Vorupør | NV | 30 | 24/07/2008 | N56.9496667 | E008.3576833 | Andreas Kelager |
| Estonia | Saaremaa | SA | 5 | Summer 2008 | N58.4865600 | E021.9226000 | Triin Reitalu |
| Finland | Turku | TU | 14 | 25/07/2008 | N60.4304600 | E022.1788400 | Maarten Christenhusz |
| Germany | Amrum | AM | 20 | Summer 2008 | N54.6383056 | E008.3526917 | Volker Wissemann |
| Germany | Geltinger Birk | GB | 17 | Summer 2008 | N54.7833333 | E009.9000000 | Volker Wissemann |
| Netherlands | Noordhollands Duinreservaat | ND | 21 | 20/07/2008 | N52.5243568 | E004.6337976 | Dirk-Jan ten Brink |
| Norway | Oslo | OS | 20 | 30/08/2008 | N59.4386527 | E010.6080037 | Olav Skarpaas |
| Norway | Nordtrøndelag | NT | 21 | 03/09/2008 | N63.7952700 | E011.4530700 | Liv S. Nilsen |
| Poland | Gdansk | GD | 20 | 15/10/2008 | N54.4138750 | E018.6231500 | Marcin G6rniak |
| Russia | Sestroretsk | SE | 32 | 17/09/2008 | N60.1272667 | E029.9418900 | Andreas Kelager |
| Scotland | Barassie Beach | BB | 24 | 12/10/2008 | N55.5575000 | W004.6580556 | Charles Coyle |
| Sweden | Åhus | AA | 20 | 19/08/2008 | N55.9136600 | E014.2855900 | Hans Henrik Bruun |
| Sweden | Hökafältet | HF | 20 | 19/08/2008 | N56.5298300 | E012.9460500 | Hans Henrik Bruun |
| Sweden | Hörnefors | HO | 18 | Summer 2008 | N63.5828778 | E019.8896361 | Barbara Giles & Ulla Carlsson-Granér |
| *Native* | | | | | | | |
| Japan | Akita | AK | 35 | 25/08/2008 | N39.6885000 | E140.0626500 | Hans Henrik Bruun & Andreas Kelager |
| Japan | Bakkai/Sarubetsu[a] | BS | 9 | 2004 | N45.3500000 | E141.6500000 | Teruyoshi Nagamitsu |
| Japan | Higashiura/Wakkanai[a] | HW | 12 | 2004 | N45.4000000 | E142.0333333 | Teruyoshi Nagamitsu |
| Japan | Ishikari Coast | IC | 30 | 25/07/2008 | N43.2275970 | E141.3285390 | Hajime Matsushima |
| Japan | Kesennuma | KE | 13 | 28/08/2008 | N38.8264867 | E141.5930200 | Hans Henrik Bruun & Andreas Kelager |
| Japan | Kizukuri | KI | 30 | 26/08/2008 | N40.8402100 | E140.2750217 | Hans Henrik Bruun & Andreas Kelager |
| Japan | Misawa | MI | 30 | 27/08/2008 | N40.6761233 | E141.4335367 | Hans Henrik Bruun & Andreas Kelager |
| Japan | Mutsu-wan | MU | 15 | 27/08/2008 | N40.9560567 | E141.2140033 | Hans Henrik Bruun & Andreas Kelager |

**Table 1** continued

| Country | Population | $ID_{Pop}$ | $N$ | Date | Latitude | Longitude | Collector |
|---|---|---|---|---|---|---|---|
| Japan | Noto | NO | 19 | 24/08/2008 | N36.9212400 | E136.7655700 | Hans Henrik Bruun & Andreas Kelager |
| Japan | Ogawara-ko | OG | 6 | 27/08/2008 | N40.7348033 | E141.3580750 | Hans Henrik Bruun & Andreas Kelager |
| Japan | Takata | TA | 15 | 28/08/2008 | N39.0029283 | E141.6260533 | Hans Henrik Bruun & Andreas Kelager |
| Russia | Petropavlovsk | PE | 33 | Summer 2008 | N52.9671667 | E158.8093333 | Valentina Vetrova |
| Russia | Poset | PO | 29 | Autumn 2008 | N42.6172667 | E131.0268639 | Valentin V. Yakubov |
| Russia | Pestshany | PT | 30 | Autumn 2008 | N43.1000833 | E131.6340500 | Valentin V. Yakubov |
| South Korea | Oho-ri | OR | 19 | Autumn 2008 | N38.3333883 | E128.5218050 | Jin-Seok Kim |
| South Korea | Sindu-ri | SR | 11 | Autumn 2008 | N36.8506367 | E126.1992883 | Jin-Seok Kim |

$ID_{Pop}$: population abbreviation, $N$ number of individuals; date of collection; latitudinal and longitudinal coordinates; name of collector. See also Fig. 1 for population locations

[a] Samples received as DNA aliquots

### Tests for inbreeding and linkage disequilibrium

The eight microsatellite loci analyzed were tested for deviations from Hardy–Weinberg proportions and for linkage disequilibrium (genotypic disequilibrium) using FSTAT version 2.9.3.2 (Goudet 1995, 2001) based on 4,480 and 13,440 permutations, respectively.

### Genetic diversity and bottleneck detection

Genetic diversity was analyzed for the entire dataset and separately for the regional subsets of introduced (Europe) and native (East Asia) populations. FSTAT was employed to calculate the number of alleles per locus or per population ($A$), allelic richness per population ($A_R$, based on the minimum sample size of four individuals), the average observed heterozygosity per locus ($H_O$), Nei's unbiased gene diversity (average expected within-population heterozygosity, $H_S$) per locus or per population, the global gene diversity ($H_T$) and the inbreeding coefficient ($f$ or $F_{IS}$) for each population. We tested for differences in allelic richness ($A_R$), gene diversity ($H_O$ and $H_S$) and inbreeding coefficient ($f$) between groups of populations in FSTAT based on 10,000 permutations. The within-population observed heterozygosity ($H_O$) and the number of private alleles ($A_P$) for populations as well as groups of populations were calculated using GenAlEx version 6.3 (Peakall and Smouse 2006).

Potential population bottlenecks were examined using two methods. First, we employed the program BOTTLENECK version 1.2 (Cornuet and Luikart 1996; Piry et al. 1999) to statistically detect bottlenecks from an excess of observed heterozygotes ($H_e$) compared to expected heterozygotes at equilibrium ($H_{eq}$). We used a two-phase mutation model (TPM) with default settings, 1000 iterations and a one-tailed Wilcoxon's signed rank test. Second, we calculated the $M$-ratio, a relationship between the mean number of alleles and the range of allele size (Garza and Williamson 2001a), using the software M_P_Val.exe with settings $\theta = 4.0$, $\Delta_g = 2.8$ and $P_s = 0.12$, and statistically tested by the software Critical_M.exe (Garza and Williamson 2001b) using 10,000 simulated replicates and assuming an effective population size of 40 (about double the average sample size per population). Values of $M$ and $\Delta H$/SD for introduced and native populations, respectively, were subject to an $F$-test for equal variances and a $t$ test for differences in mean values.

### Population differentiation and structure

We studied population differentiation calculating Weir and Cockerham's $\theta$ (1984) using FSTAT version

2.9.3.2 based on 10,000 permutations to estimate the genetic differentiation ($F_{ST}$) between all populations, within and between regions (introduced and native), and between genetic clusters inferred by BAPS (see methods below). Similarly, $F_{ST}$ for pairs of populations were calculated based on 9,920 permutations. As the value of $\theta$ is affected by the allelic diversity at the marker loci applied, we further calculated the standardized $G'_{ST}$ (Hedrick 2005, equation 4b), and the estimator $D_{EST}$ (Jost 2008, equation 12) as alternative quantifications of the genetic differentiation, making comparisons with studies based on other marker loci possible (Heller and Siegismund 2009).

To examine possible genetic isolation by distance within regions we employed a Mantel test implemented in FSTAT with 10,000 randomizations of the association of pairwise $F_{ST}$ values and geographic distances between populations. We further tested for difference in sampled range sizes between Europe and East Asia by comparing the mean of the geographic distances between populations using a jackknife procedure over populations to avoid pseudo-replication.

A Principal Component Analysis (PCA) implemented in the software PCA-GEN version 1.2 (Goudet 1999) was used to obtain a population based multivariate ordination of samples based on allele frequencies. Significance tests of principal component axes were based on 10,000 randomizations of multilocus genotypes. A three level hierarchical Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) was calculated using GenAlEx version 6.3 (Peakall and Smouse 2006) with 9,999 permutations to estimate the variance component of genetic distances, within and among populations as well as between the native and introduced regions.

We studied the among-population genetic pattern employing the Bayesian algorithm implemented in the software BAPS version 5.2 (Corander et al. 2003, 2008b) to cluster group of individuals (populations) based on allelic frequencies and genetic divergence. The most likely number of genetically distinct clusters ($K$) was estimated both by running 100 iterations for each fixed $K$ in the range 2–32 and by the generic learned clustering method setting the upper bound to 32. The level of admixture within populations was examined based on the group clustering mixture analysis for the most likely $K$ with a minimum of 5 individuals to define a population (population SA) using the parameter settings recommended by Corander et al. (2008a).

### Assignment of introduced individuals

European individuals were assigned probabilistically to each reference population using a Bayesian method (Rannala and Mountain 1997) as implemented in the software GeneClass version 2.0.h (Piry et al. 2004). A simulation algorithm using the Monte Carlo re-sampling method to infer migration rates (Paetkau et al. 2004) was employed with 10,000 simulated individuals and $\alpha = 0.01$. All populations were used as reference samples to obtain the proportion of self-assignment as well as likelihood values of allocation to populations from East Asia. The probability values for each individual were used for calculating the relative assignment likelihood mass for each native populations and genetic clusters according to Doorduin et al. (2010).

## Results

### Inbreeding and linkage disequilibrium

*F*-statistics for each locus are listed in the Electronic Supplementary Material Table S2. The test for HW disproportions revealed that the overall inbreeding coefficient $f$ ($F_{IS}$) was not significantly different from zero ($f = 0.009$, 95 % bootstrap C.I. 95 % [–0.017; 0.034]). Of the eight loci analyzed, only locus N had a significantly positive $f$-value ($f = 0.380$, $P = 0.001$), possibly due to null alleles, and was omitted from subsequent analyses. Tests for linkage disequilibrium revealed only a few inconsistent significant results (Electronic Supplementary Material Table S3), so all loci were kept in further analyses. The introduced population HO were found to have a significant deficit of heterozygotes likely due to some sampling of identical clones. The native population TA had a significant excess of heterozygotes ($f = -1.00$), caused by sampled individuals having identical genotypes (Table 2). We did not find significant differences in the inbreeding coefficient between the introduced and native range (Table 3).

### Genetic diversity and bottleneck detection

Native and introduced populations did not differ significantly in within-population genetic variation measured as allelic richness and gene diversity. A total of 117 alleles were detected in the entire dataset, with 86 (12 private) and 105 (31 private) alleles found in the

**Table 2** Diversity indices for populations and genetic clusters of *Rosa rugosa*, sorted after countries

| Population | $ID_{Pop}$ | $N$ | $A$ | $A_R$ | $A_P$ | $H_O$ | $H_S$ | $f$ ($F_{IS}$) | $\Delta H/SD$ | $M$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Introduced | | | | | | | | | | |
| Zeebrugge | ZB | 16 | 50 | 4.06 | 2 | 0.685 | 0.693 | 0.013 | −0.375 | 0.643 |
| Knarbos Klint | KK | 20 | 43 | 3.70 | 0 | 0.611 | 0.659 | 0.073 | −0.279 | 0.568 |
| Nørre Vorupør | NV | 25 | 42 | 3.38 | 0 | 0.594 | 0.616 | 0.034 | −0.458 | 0.610 |
| Saaremaa | SA | 5 | 28 | 3.72 | 0 | 0.743 | 0.714 | −0.040 | 0.626 | 0.457 |
| Turku | TU | 14 | 30 | 2.89 | 2 | 0.585 | 0.506 | −0.155 | −0.780 | 0.473 |
| Amrum | AM | 20 | 39 | 3.34 | 0 | 0.573 | 0.631 | 0.093 | −0.347 | 0.564 |
| Geltinger Birk | GB | 16 | 28 | 2.50 | 0 | 0.468 | 0.428 | −0.094 | −1.255 | 0.489 |
| Noordhollands Duinreservaat | ND | 21 | 36 | 3.16 | 0 | 0.557 | 0.611 | 0.087 | −0.571 | 0.572 |
| Nordtrøndelag | NT | 21 | 34 | 3.23 | 0 | 0.576 | 0.604 | 0.046 | −0.235 | 0.594 |
| Oslo | OS | 17 | 42 | 3.79 | 0 | 0.721 | 0.686 | −0.052 | −0.011 | 0.588 |
| Gdansk | GD | 20 | 46 | 3.65 | 3 | 0.536 | 0.622 | 0.138 | −1.234 | 0.569 |
| Sestroretsk | SE | 30 | 51 | 3.67 | 2 | 0.650 | 0.641 | −0.013 | −0.573 | 0.593 |
| Barassie Beach | BB | 21 | 43 | 3.80 | 0 | 0.583 | 0.688 | 0.153 | 0.339 | 0.596 |
| Åhus | AA | 20 | 42 | 3.57 | 1 | 0.625 | 0.632 | 0.011 | −0.419 | 0.593 |
| Hökafältet | HF | 19 | 43 | 3.66 | 0 | 0.625 | 0.650 | 0.039 | −0.098 | 0.586 |
| Hörnefors | HO | 18 | 37 | 3.63 | 1 | 0.541 | 0.680 | 0.205 | 0.221 | 0.546 |
| Native | | | | | | | | | | |
| Akita | AK | 30 | 58 | 3.75 | 0 | 0.570 | 0.598 | 0.047 | −0.966 | 0.691 |
| Bakkai/Sarubetsu | BS | 9 | 22 | 2.57 | 0 | 0.647 | 0.503 | −0.285 | −0.158 | 0.469 |
| Higashiura/Wakkanai | HW | 11 | 28 | 2.95 | 0 | 0.626 | 0.552 | −0.135 | −0.207 | 0.537 |
| Ishikari Coast | IC | 30 | 52 | 3.65 | 0 | 0.584 | 0.586 | 0.003 | −0.662 | 0.620 |
| Kesennuma | KE | 13 | 32 | 3.20 | 1 | 0.686 | 0.569 | −0.205 | −0.051 | 0.547 |
| Kizukuri | KI | 30 | 55 | 3.68 | 1 | 0.569 | 0.599 | 0.051 | −1.221 | 0.646 |
| Misawa | MI | 30 | 63 | 4.07 | 1 | 0.614 | 0.670 | 0.082 | −1.058 | 0.664 |
| Mutsu-wan | MU | 15 | 47 | 3.93 | 2 | 0.644 | 0.686 | 0.061 | −0.317 | 0.572 |
| Noto | NO | 19 | 53 | 3.97 | 3 | 0.612 | 0.657 | 0.068 | −0.637 | 0.597 |
| Ogawara-ko | OG | 6 | 35 | 4.28 | 0 | 0.657 | 0.707 | 0.071 | 0.576 | 0.613 |
| Takata | TA | 15 | 13 | 1.86 | 0 | 0.857 | 0.429 | −1.000 | 1.437 | 0.456 |
| Petropavlovsk | PE | 29 | 57 | 3.84 | 8 | 0.622 | 0.652 | 0.047 | −1.613 | 0.557 |
| Poset | PO | 21 | 45 | 3.73 | 1 | 0.655 | 0.623 | −0.051 | 0.205 | 0.605 |
| Pestshany | PT | 30 | 52 | 3.73 | 0 | 0.624 | 0.644 | 0.031 | 0.071 | 0.660 |
| Oho-ri | OR | 18 | 45 | 3.91 | 1 | 0.705 | 0.654 | −0.078 | 0.401 | 0.655 |
| Sindu-ri | SR | 11 | 25 | 2.88 | 0 | 0.488 | 0.480 | −0.017 | 0.453 | 0.574 |
| Genetic cluster | | | | | | | | | | |
| Cluster 1: AA, AM, GD, HF, HO, OS, KK, ND, NT, NV, SA, TU | | 220 | 73 | 4.84 | 8 | 0.597 | 0.647 | 0.077 | | |
| Cluster 2: GB | | 16 | 28 | 3.18 | 0 | 0.468 | 0.428 | −0.094 | | |
| Cluster 3: BB, SE, ZB, AK, IC, KI, MI, MU, NO, OG | | 227 | 96 | 5.88 | 15 | 0.608 | 0.659 | 0.077 | | |
| Cluster 4: OR, P O, PT | | 69 | 66 | 5.62 | 2 | 0.655 | 0.661 | 0.009 | | |
| Cluster 5: SR | | 11 | 25 | 3.41 | 0 | 0.488 | 0.480 | −0.017 | | |
| Cluster 6: TA | | 15 | 13 | 1.86 | 0 | 0.857 | 0.429 | −1.000 | | |
| Cluster 7: PE | | 29 | 57 | 5.31 | 8 | 0.622 | 0.652 | 0.047 | | |
| Cluster 8: KE | | 13 | 32 | 4.11 | 1 | 0.686 | 0.569 | −0.205 | | |
| Cluster 9: HW | | 11 | 28 | 3.68 | 0 | 0.626 | 0.552 | −0.135 | | |
| Cluster 10: BS | | 9 | 22 | 3.13 | 0 | 0.647 | 0.503 | −0.285 | | |

$ID_{Pop}$ population abbreviation; $N$, average number of genotyped individuals; $A$, total number of alleles; $A_R$, average allelic richness across loci adjusted for sample size using rarefaction (based on 4 individuals); $A_P$, total number of private alleles; $H_O$, average observed heterozygosity; $H_S$, Nei's unbiased expected within-population heterozygosity; $f$ ($F_{IS}$), Weir & Cockerham's inbreeding coefficient; $\Delta H$/SD, standardized difference for heterozygosity excess averaged over all loci under the two-phase model (95 % SMM, variance 12); $M$, ratio between allele number and range. Genetic clusters are for the most likely partitioning, $K = 10$; see Fig. 5 for colour codes of cluster groups. Underlined values were significant after sequential Bonferroni correction

**Table 3** Diversity indices for range of *Rosa rugosa* populations

| Range | $N$ | $A$ | $A_R$ | $A_P$ | $H_O$ | $H_S$ | $H_T$ | $f$ ($F_{IS}$) | $\theta$ ($F_{ST}$) | $G'_{ST}$ | $G'_{ST}$ | $D_{EST}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Introduced | 303 | 86 | 3.48 | 12 | 0.599 | 0.627 | 0.656 | 0.043 (0.030) | 0.045 (0.007) | 0.045 | 0.113 | 0.075 |
| Native | 317 | 105 | 3.50 | 31 | 0.626 | 0.610 | 0.674 | −0.026 (0.020) | 0.104 (0.012) | 0.115 | 0.277 | 0.189 |
| Overall | 620 | 117 | 3.49 | – | 0.620 | 0.615 | 0.680 | 0.009 (0.014) | 0.089 (0.003) | 0.098 | 0.253 | 0.174 |
| 95 % bootstrap interval | | | | | | | | [−0.017; 0.034] | [0.084; 0.095] | | | |
| $P$ values for group comparison | | | 0.926 | | 0.238 | 0.437 | | 0.108 | 0.042 | | | |

$N$, number of individuals; $A$, total number of alleles; $A_R$, average allelic richness adjusted for sample size using rarefaction (based on three individuals); $A_P$, number of private alleles; $H_O$, average observed heterozygosity; $H_S$, Nei's unbiased expected within-population heterozygosity; $H_T$, Nei's overall heterozygosity; $f$ ($F_{IS}$), Weir & Cockerham's inbreeding coefficient; $\theta$ ($F_{ST}$), Weir & Cockerham's genetic differentiation; $G'_{ST}$ , Nei's unbiased genetic differentiation; $G'_{ST}$, Hedrick's standardized genetic differentiation; $D_{EST}$, Jost's genetic differentiation. Values in parentheses are standard errors; underlined values are significant after sequential Bonferroni correction; squared brackets are overall 95 % bootstrap intervals. $P$ values are given for comparing estimates of the two ranges based on 10,000 permutations

introduced and native range, respectively (Table 3). Six of the seven microsatellite loci showed high polymorphism with the number of alleles ranging from 4 to 25, whereas one locus was dimorphic (Electronic Supplementary Material Table S2). The average allelic richness per population ($A_R$) across loci was 3.49 (range 1.86–5.88; Fig. 1; Tables 2 and 3), with nearly identical estimates for introduced and native populations (3.50 vs. 3.48; $P = 0.926$). The average within-population gene diversity ($H_S$) for introduced and native populations were 0.627 and 0.610, respectively, and not significantly different ($P = 0.437$). The ranges of $A_R$ and $H_S$ were almost identical for the two regions (Fig. 2). As expected, there was a very strong and significant correlation between $A_R$ and $H_S$ (introduced: $r^2 = 0.87$; native: $r^2 = 0.91$; both $P < 0.001$). The global average gene diversity for all populations ($H_T$) was 0.680, with a slight but significantly lower overall value for the introduced than the native range (0.656 vs. 0.674; jackknife over populations, $P < 0.001$; Table 3).

Two populations (one introduced and one native) were found to have a significant surplus of observed heterozygosity ($H_e$) compared to the expected equilibrium gene diversity ($H_{eq}$), indicating previous bottleneck events ($\Delta H$/SD in Table 2), while no bottleneck event could be detected in the remaining populations. Changing the mutation model parameters did not alter these results (with a TPM variance of 30 or SMM proportion of 85 and 90 %). All populations, regardless of origin, appeared to have undergone a bottleneck according to the $M$-ratio statistics, as all

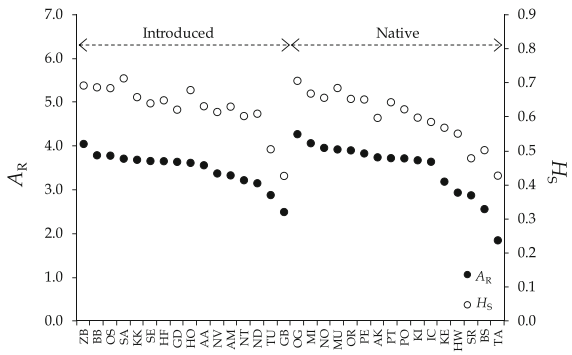


**Fig. 2** Average genetic diversity in populations of *Rosa rugosa*. $A_R$, allelic richness adjusted for sample size using rarefaction (based on four individuals); $H_S$, Nei's unbiased expected within-population heterozygosity. Data for introduced and native populations, respectively, are ranked after descending $A_R$ estimates

$M$-values were below the lowest critical $M$-value ($M_C = 0.724$; Table 2). Average values of $M$ and $\Delta H$/SD, however, did not differ significantly between introduced and native populations ($M = 0.565$ vs. 0.591; two-sided $t$ test, $P = 0.222$; $\Delta H$/SD = –0.341 vs. –0.234; $P = 0.651$).

## Population differentiation and structure

Six European populations had private alleles (between 1 and 3), and nine populations in East Asia had 1–8 private alleles (Table 2), whereas 12 and 31 private alleles were found in the introduced and native ranges, respectively (Table 3). Significant population structure was found among all populations ($\theta = 0.089$;

95 % C.I. [0.084; 0.095], Table 3) as well as within the introduced (0.045) and native (0.104) range, with significantly lower genetic differentiation between introduced populations than for populations in the native range ($P = 0.042$). Similar results were obtained with the alternative estimators of genetic differentiation $G'_{ST}$ and $D_{EST}$ (Table 3).

The pairwise population differentiation values ($\theta$, $F_{ST}$) were within the range [−0.01; 0.36] and the proportion of significantly differentiated population pairs was 27 and 73 % for the introduced and native range, respectively (Electronic Supplementary Material Table S4a). We found no significant association between genetic and geographic distance in the introduced range ($R^2 = -0.008$, $P = 0.933$; Electronic Supplementary Material Fig. S1). This was, however, significant for native populations ($R^2 = 0.0882$, $P < 0.001$), indicating genetic isolation by geographic distance. The average geographic distance between sampling sites within East Asia was significantly higher than for Europe (848 vs. 808 km; two-sided Wilcoxon test on jackknifed means, $P < 0.001$; Electronic Supplementary Material Fig. S2), but only by 40 km and so differences in spatial scales are unlikely to have influenced the results.

The Principal Component Analysis (PCA) showed a strong and significant ($P < 0.001$) first axis which explained 23 % of the genetic variance among populations and clearly separated introduced and native populations (Fig. 3), whereas there was no significant relationship for the second axis ($P = 0.156$). Accordingly, the three-level AMOVA showed that 4.2 % of the genetic variance among individuals was found between the two regions, 11.2 % among populations within regions, and 84.6 % among individuals of the same populations (Electronic Supplementary Material Table S5).

The Bayesian clustering analysis using BAPS was run 16 times independently with non-fixed $K$, giving $K = 10$ as the most likely number of genetically distinct groups (posterior probability $P = 0.999$; $K = 11$, $P = 0.01$). Similarly, using a fixed $K$ clustering, we found that the most probable partitioning was within the range of 7–13 groups with maximum log-marginal likelihood at $K = 10$ (Electronic Supplementary Material Fig. S3). This gave a clear distinction between the geographical regions, with three clusters in the introduced range and eight in the native range (Fig. 4), indicating multiple independent introduction events into Europe (see also Fig 1a). Further, a pronounced sub-regional structure was found in East Asia with one minor cluster (C4) consisting of three populations along the west coast of the Japanese Sea and one major cluster (C3) of seven Japanese populations (Fig. 1b). Low levels of admixture were found for both regions (Fig. 1). The pairwise genetic differentiation ($\theta$, $F_{ST}$) of the 10 clusters ranged between 0.02 and 0.36 and all cluster pairs except C6 and C10 were significantly differentiated from each other (Electronic Supplementary Material Table S4b).

## Assignment of introduced individuals

The average proportion of self-assignment of individuals in introduced populations was 0.75 (range 0.63–0.85; Electronic Supplementary Material Table S6a). All introduced populations had the highest relative assignment likelihood to the Japanese population Misawa (MI) with 15–25 % probability, except for the German population Geltinger Birk (GB), which was assigned to Akita (AK) with a probability of 0.18. Assignment likelihood masses for introduced clusters (C1-3) are shown in Fig. 5a. All introduced clusters were assigned to the Japanese cluster C3 with likelihood masses of 82–86 %, to cluster C4 with 10–13 % likelihood mass and to the remaining native clusters with <3 % likelihood mass (Fig. 5b). Results from the individual based assignment averaged over

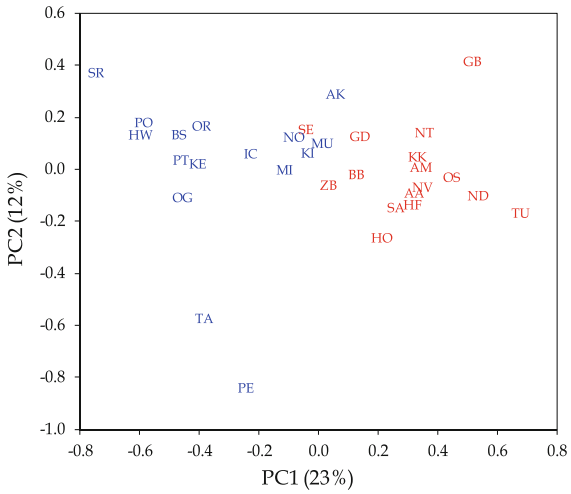


**Fig. 3** Principal component analysis of microsatellite allele frequencies of introduced (*red*) and native (*blue*) *Rosa rugosa* populations. $P$ values: PC1, $P < 0.001$; PC2, $P = 0.156$

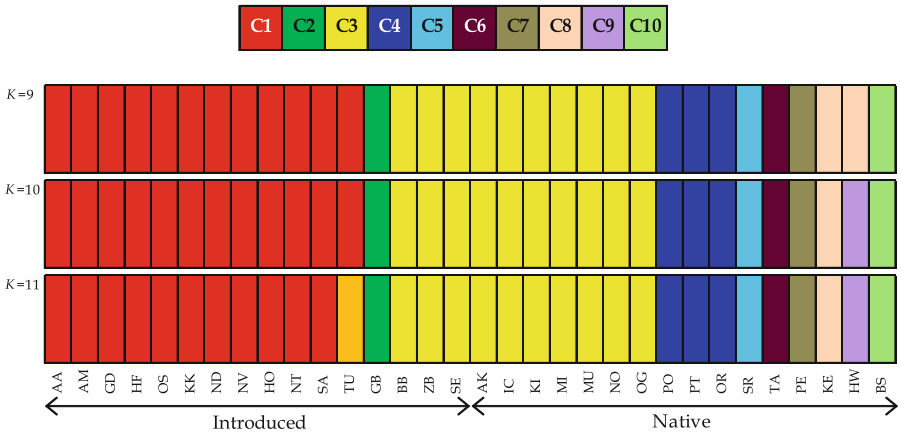


**Fig. 4** Population based clustering for all populations of *Rosa rugosa* using BAPS for $K = 9$–11, sorted after region and associated genetic cluster. *Legend* indicate cluster groups (C) and *colour code* for the most likely partitioning, $K = 10$ (see Electronic Supplementary Material Fig. S3 for log(ml) distribution for $K = 2$–32, and Fig. 1 for geographic distribution of cluster membership based on admixture results for $K = 10$ clusters)

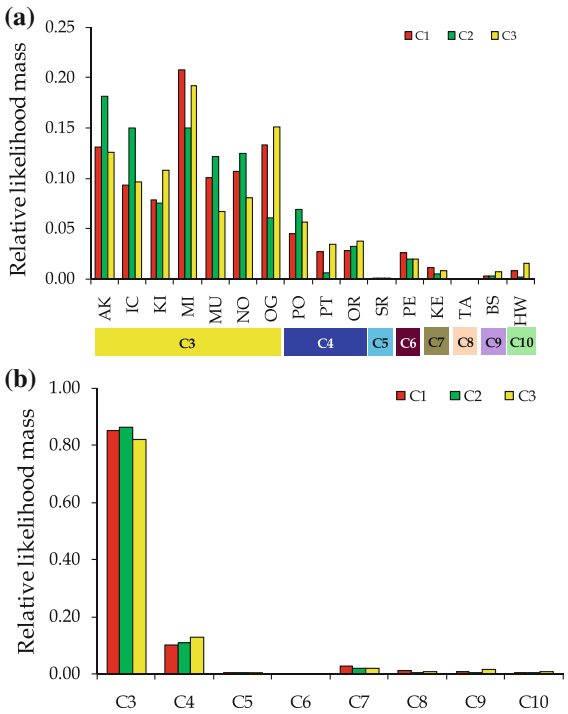


**Fig. 5** Introduced individuals of *Rosa rugosa* within genetic clusters assigned to native populations using GeneClass and noted with relative assignment likelihood mass per **a** introduced populations and **b** genetic cluster. Populations are sorted after associated genetic cluster for the most likely partitioning $K = 10$; see Fig. 5 for *colour codes* and labels **c** of cluster groups

populations are provided in Electronic Supplementary Material Table S6 and Electronic Supplementary Material Fig. S4.

## Discussion

We found that *Rosa rugosa* in the exotic range has similar within-population genetic variation as populations in the native range. The between-population differentiation is considerably smaller because the species have spread in Europe from multiple original introductions from native sources in East Asia and secondary non-admixing colonization has been facilitated by human dispersal. This adds to the growing number of reports on invasive species, particularly plants, where successful establishment in the introduced range is increased by the assistance of human transportation (e.g. Bossdorf et al. 2005; Wilson et al. 2009). Three spatially disconnected European populations were found to have a northern Japanese origin, whilst the source(s) for the remaining exotic populations were not unambiguously identified, likely due to insufficient sampling in the native range. Attention has been pointed towards designing unbiased population genetic studies on invasive species, accounting for both samples size and geographic range covered, as sampling bias can have great influence on the estimations of genetic diversity and differentiation between populations (Bossdorf et al. 2005; Ward 2006; Ward et al. 2008). In our study, we have an unbiased sampling design with equal number of populations and similar samples size per populations and the affects of the slightly higher geographic coverage in East Asia is negligible.

### Introduced populations of *Rosa rugosa* maintain high genetic diversity

Our study shows that European populations of *R. rugosa* have not lost within-population genetic diversity as both measures of genetic diversity, allelic richness ($A_R$) and expected heterozygosity ($H_S$) displayed comparable levels to populations from the native range (Fig. 2; Tables 2 and 3). During colonization, the founding population is expected to experience a reduction in size, and consequently also genetic variation within population, relative to the source population (Allendorf and Lundquist 2003). Empirical molecular studies of other invasive species have documented exactly this (e.g. Besnard et al. 2007; Dlugosch and Parker 2008; Rosenthal et al. 2008; Alexander et al. 2009; Henry et al. 2009), however, increasing evidence for similar or even higher levels of within-population genetic diversity in exotic populations has typically been explained by high propagule pressure, multiple introductions or admixture in the introduced range of individuals from different source populations (e.g. Bossdorf et al. 2005; Ward et al. 2008; Alexander et al. 2009; Le Roux and Wieczorek 2009; Wilson et al. 2009).

The two bottleneck tests gave inconclusive results (Table 3), either showing that all populations experienced past bottlenecks (*M*-ratio test) or that all but two of our populations had not gone through a bottleneck event (Bottleneck test, $\Delta H$/SD). The two bottleneck tests employed in this study detect bottleneck events at different temporal scales and severity levels. Where the *M*-ratio test (Garza and Williamson 2001a) detects old or prolonged bottleneck events, the $\Delta H$/SD-test (Cornuet and Luikart 1996) detects more recent or less severe bottlenecks (Williamson-Natesan 2005). Concern of using bottleneck tests as relative measures of founding population sizes in studies of invasive species has been raised by Fitzpatrick et al. (2012), who argues that the bottleneck test, $\Delta H$/SD (Cornuet and Luikart 1996) should not be used on invasive populations. However, we have not used the tests to detect bottleneck events in single populations, but rather compared bottleneck estimates between ranges. We did not find significantly different *M*-values or $\Delta H$/SD values between populations from Europe and East Asia, thus confirming that no significant genetic change in the populations has occurred during or after the introduction into Europe. This indicates that, in concert with the within-population genetic variation, founding populations in Europe must have been relative large or that establishment of relatively large population sizes occurred over short temporal scales.

The success of exotic *Rosa rugosa* populations in Europe appears to not be limited by genetic constraints such as inbreeding, bottleneck effects or low evolutionary potential, which is common for outbreeding as well as widely cultivated plant species (Novak and Mack 2005). *Rosa rugosa* is an outbreeding species (Dobson et al. 1999) and is thus expected to have a relatively high genetic variation at the population level (Hamrick and Godt 1996; Nybom 2004) and be less affected by bottlenecks (Novak and Mack 2005), which our results support. Similarly, estimated inbreeding coefficients for exotic and native populations were not significantly different, implying no evolutionary change away from outbreeding or other changes of the original mating system, in Europe.

### Low population differentiation and human-influenced genetic structure in Europe

We found a markedly lower genetic differentiation between introduced populations compared to differentiation between populations in the native range (Table 3) and no isolation by distance in the invasive populations ($R^2 = -0.008$, Electronic Supplementary Material Fig. S1). Twelve out of the 16 European populations clustered together, suggesting a common origin for this group of genetically similar populations (Figs. 1a, 4). Eighty-five percent of the genetic variation was found within populations, 11 % between populations and just 4 % between Europe and East Asia (Electronic Supplementary Material Table S5). Despite the low regional differentiation, the difference between exotic and native populations was significant; a pattern caused by dissimilarities in allele frequencies rather than in allelic identities (Table 3; Fig. 3). These results, together with the similar levels of genetic diversity within population and comparable patterns of bottleneck between ranges, support the hypothesis of high propagule pressure at the primary establishment and invasion phase, i.e. that many individuals from one source have co-founded the now widely distributed genetic cluster C1, which is also the most parsimonious explanation. An alternative explanation for the results observed for the genetic cluster 1 and the European populations of cluster 3 could be that

propagules may have been transported repeatedly from the same source, but this is less likely, as it would require multiple collections from the same sources across temporal scale.

The relatively low genetic differentiation in exotic *R. rugosa* populations is concordant with a pattern often detected among introduced plants. Bossdorf et al. (2005) reported lower levels of differentiation in five out of six invasive species examined, despite multiple introductions that had occurred in most of the cases. For *R. rugosa*, the limited genetic differentiation of exotic populations was mainly caused by a large number of genetically similar populations which are continuously distributed geographically (Figs. 1a, 4). We could not detect an association between genetic and geographic distances in the invasive populations (i.e. no isolation by distance), a pattern commonly found in invasive species where human-mediated dispersal has played a key role in the secondary colonization process (e.g. Okada et al. 2007; Ross et al. 2008; Gaudeul et al. 2011). This observation together with the patterns of within-populations genetic diversity, the lack of isolation by distance for the exotic populations, and the spatially disconnected distribution of cluster C3 populations (Fig. 1a), is evidence of different dispersal mechanisms acting in Europe compared to East Asia. Incidentally, the geographical range of the successful genetic cluster 1coincides with the range where *R. rugosa* has been evaluated as most problematic (NOBANIS 2008), suggesting that the ecological impact may have a genetic background.

In general multiple introductions from distinct source regions into a few recipient areas will reduce genetic differentiation because geographically structured genetic material is admixed in the exotic range. Novel establishments by secondary dispersal in the introduced range resulting in admixture will similarly lead to lower genetic differentiation, i.e. the invasion history has large effects on the patterns of genetic differentiation (Prentis et al. 2009). In our study, the low genetic structure (i.e. low genetic differentiation) in Europe was caused by the success of one group, cluster 1, consisting of genetically similar populations with high genetic diversity. Based on the low pairwise $F_{ST}$ values between C3 and C1 and higher values between C1 and C4 to 10 (Electronic Supplementary Material Table S4b) and the high assignment probability of C1 to C3 (Fig. 5b), we find it unlikely that interspecific introgression or even hybridization known to generate invasive species (e.g. Ward et al. 2008; Le Roux and Wieczorek 2009) has played a role in the shaping the invasion of *R. rugosa*, although we cannot exclude this possibility.

The Bayesian clustering analysis revealed that the entire data set was best explained by partitioning the 32 populations into 10 genetic clusters with the introduced populations allocated to three of them (Figs. 1, 4) and the Principal Component Analysis (Fig. 3) and pairwise differentiation estimates (Electronic Supplementary Material Table S4) were supportive of this genetic relationships. Genetic material from different native sources has not been mixed in Europe, as only low levels of admixture were detected. The geographical distribution of these three introduced clusters (Fig. 1a) cannot be explained by natural post-establishment migration alone. While cluster C1 seems to have a spatially continuous distribution, it lacks the genetic patterns indicative of natural dispersal, i.e. significant isolation by distance and differences in genetic diversity. The lack of isolation by distance is often found in invasive species where secondary dispersal has been facilitated by humans (e.g. Okada et al. 2007; Marrs et al. 2008a; Gaudeul et al. 2011). Cluster C3 is partitioned into two or three regions with maximum possible geographic separation (SE in western Russia, ZM in Belgium and BB in Scotland), which must be explained by at least two consecutive transfer and establishment events. The low genetic differentiation in European populations as well as comparable levels of bottleneck values and genetic diversity within populations all support that the success of the invasive populations of *R. rugosa* in Europe is driven by humans.

Both the genetic data presented here and the known history of widespread cultivation and planting suggest that human-mediated dispersal has been the main contributor to the establishment of *R. rugosa* populations in coastal habitats in Europe. However, the sparse written records on European introductions (reviewed by Bruun 2005) are not fully concordant with our results. Saint Petersburg is reported as the origin of plants introduced into several countries, including the UK, Finland, Latvia and Lithuania. In contrast, genetic evidence suggests a different origin of the SE population near St Petersburg from all the neighbouring populations in the Baltic Sea. A possible scenario is that *R. rugosa* was transferred from St

Petersburg to Scotland and Belgium (populations BB and ZB of cluster C3; Fig. 1a), by human mediated-dispersal. An explanation for this apparent discrepancy between genetic data and the historical records may be that only transfers between botanic gardens (see also Bruun 2005) are likely to be recorded, whereas commercial plant trade (and natural dispersal) remains unreported. However, it is barely surprising that dispersal facilitated by humans appears to be the main contributor to the successful colonization of Europe. The species has been frequently planted in coastal habitats since the 1950 s and, in addition, hips may be collected for cooking and thus moved around. Further, Jørgensen and Kollmann (2009) found positive correlation between the occurrence of *R. rugosa* and anthropogenic disturbance in coastal dunes. If local naturalized populations of cluster 1 originate from nearby planted individuals, as it has been suggested (Weidema 2006), it would require that the material in the nurseries all share a common ancestry. Future research should examine this hypothesis in order to determine the importance of planted individuals in relation to the invasiveness of *R. rugosa*. However, we recommend that human-mediated dispersal are limited or prevented entirely and that European and national environmental agencies should focus on communicating this recommendation to both nature managers and the broader public.

## Multiple introduction events and Japanese origin

Our results indicate multiple and a minimum of three successful primary colonization events of *R. rugosa* in Europe (Figs. 1a, 4). The amount of seed or plant material originally transferred must have been significant or, alternatively and less likely, there must have been multiple introductions from the same native sources, to account for the high genetic diversity in almost all introduced populations relative to native populations. Secondary colonization and population admixture within genetic clusters in the introduced range are expected to have contributed to the maintenance of high genetic diversity within populations as well. Such gene flow could explain the distribution of within-population genetic variation in cluster C1 (Fig. 1a). We did not find evidence for any significant admixture between different native sources, in the introduced populations. The overall genetic diversity ($H_T$) in European populations is—quite remarkably—only slightly lower than in the native range (Table 3), and this is unlikely to be explained by differences in the spatial scales studied alone (Ward 2006).

We further conclude that the European populations of cluster C3 originates from Japan, possibly from one or several populations of northern Honshu or southern Hokkaido (Figs. 3, 4, 5). The origin of the remaining thirteen European populations (clusters C1 and C2) is not easily resolved, but affiliation to northern Japan is strongly indicated by our results (Fig. 5). However, alternative sources cannot be excluded due to incomplete sampling of populations in the native range, an explanation supported by the high number of private alleles found overall in Europe (12 private alleles, Table 3) but also in the genetic cluster 1 alone (8 private alleles, Table 2). Further, Bruun (2005) mention other potential sources in East Asia including China and Sakhalin (Russia). However, given the high assignment likelihood to a set of Japanese population and very low assignment probabilities to populations outside of Japan, a non-Japanese source for the genetic clusters 1 and 2 seems unlikely. The exotic populations of cluster C1, representing 75 % of the European sites studied, are genetically similar and likely share a common origin, i.e. one source population or few genetically similar populations in the native range. This implies that *Rosa rugosa* is one among a growing number of species where detailed studies of the invasion history have revealed that a single source population was particularly successful in colonising the introduced range (e.g. Downie 2002; Hanfling et al. 2002; Miller et al. 2005; Lombaert et al. 2010; Vogel et al. 2010). Whether such success is a result of historical factors (contingency) or of increased invasive abilities in the successful lineage (evolution) is an important focus for future studies of invasive species. These results could aid in locating coevolved herbivorous arthropods and pathogenic microorganisms in the native range, likely in the northern parts of Honshu or southern Hokkaido in Japan, in future screening and development programmes using the potential biological control agents identified by Bruun (2006).

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