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# Can the spread of agriculture in Europe be followed by tracing the spread of the weed *Silene latifolia*. A RAPD study

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**Abstract** On the basis of gene frequency data of three flavone glycosylating genes, populations of the agricultural weed *Silene latifolia* (Caryophyllaceae) in Europe can be divided into two chemical races: an eastern and a western race. Morphological data also show a clear east-west division. When the two datasets are combined at least nine different geographical races can be distinguished using cluster analysis. Because these observations are hard to explain by selection, it has been proposed that these different races probably originated as a consequence of migration during the spread of agriculture over Europe in the past. To discriminate between selection and genetic drift many more selectively neutral easy-to-score characters are needed. In order to test whether random amplified polymorphic DNAs (RAPDs) might be suitable for this purpose, we performed a small-scale RAPD analysis on 16 geographical different populations. Using Jaccard's coefficient of similarity, we calculated genetic distances by pair-wise comparisons of both unique and shared amplification products, and a dendrogram was subsequently constructed using an unweighted pair-group method with arithmetical averages (UPGMA). On the basis of the dendrogram two clusters were discerned that clearly coincide with the aforementioned east-west division in populations. As there has been little or no artificial selection on this weed, its migration routes may be a good reflection of the different geographical routes agriculture has taken. We propose that a phylogenetic analysis of RAPD data of many more populations may provide additional information on the spread of agriculture over Europe.

**Key words** *Silene latifolia* · RAPD · Agriculture · Genetic drift · DNA polymorphism

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## Introduction

On the basis of the frequency distribution of different alleles of three flavone glycosylating genes, populations of the weed *Silene latifolia* Poiret (= *Silene pratensis* (Rafn.) Godron & Gren. = *Silene alba* (Mill.) E. H. L. Krause) (Caryophyllaceae) in Europe can be divided into two main chemical races: a western and an eastern race. The east-west dividing line coincides with the January 0 °C isotherm, which suggest that there is selection on different alleles under different climatological conditions (Nigtevecht and Brederode 1972). However, using pattern recognition techniques and testing many more populations Mastenbroek et al. (1983b) and Mastenbroek and Brederode (1986) were able to further divide *S. latifolia* populations into seven different smaller chemical races that no longer show a correlation with climatological data. In order to establish whether these differences are the result of selection for different gene combinations in different areas or a reflection of a migration from different gene pools, Prentice et al. (1983), Mastenbroek et al. (1983b) and Prentice (1986) investigated the distribution of other independent characters, seed, capule and pollen morphology and isozyme characters. The distribution of the morphological characters showed the same east-west pattern as was apparent in the frequency distribution of the three flavone glycosylating genes. The expression of the isozymes, however, was strongly influenced by the environment and therefore inappropriate for use in this type of study (Mastenbroek et al. 1984). If a large number of unrelated characters show the same distribution, it becomes very hard to explain these differences in terms of selection, although the effect of hitchhiking cannot be excluded. What is much more likely then is that the distribution is the result of a process such as migration or gene flow.

When the morphological and gene frequency data were combined, a total of nine different geographical races could be distinguished using pattern recognition

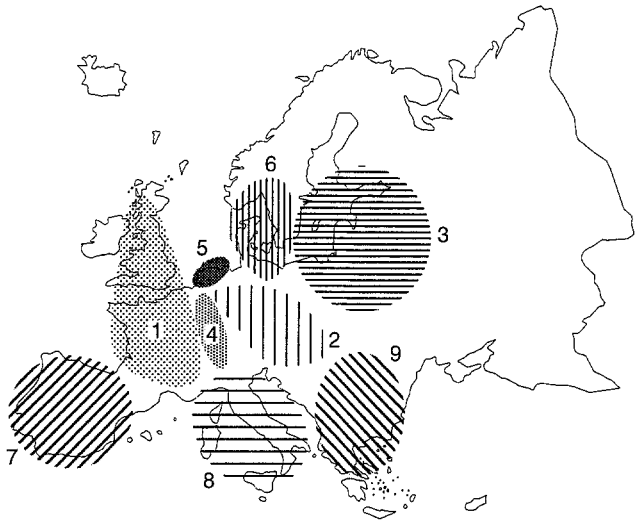


**Fig. 1** The nine geographical of *S. latifolia* in Europe according to Mastenbroek (1986). In Northern Europe the three main chemical races are those numbered *1*, *2* and *3*. In Southern Europe the races numbered *7*, *8* and *9* are the main races. The races numbered *4*, *5* and *6* are zones with intermediate properties and possible the result of hybridization between the main races

techniques (Fig. 1). The distribution of those races also showed no correlation with climatic or soil data.

On the basis of these results, it was concluded that the present-day distribution of the populations of *S. latifolia* in Europe is not the result of selection.

Mastenbroek et al. (1983a, 1986) proposed that the distribution of the different races of *S. latifolia* is the result of the last ice age. When the ice expanded over the northern part of Europe, *S. latifolia* retreated to Northern Africa. There, several genetically different populations survived during the glacial period. After the ice retreated to the north, the species (re)estiblished itself in Southern Europe, where genetically distinct groups evolved and adapted to more open habitats, such as open woodlands or montane limestone screes (where the species exists today in Southern Europe in contrast with Northern Europe where it is especially found as a weed on arable land). From these genetically distinct groups weedy forms of *S. latifolia* evolved, which followed the spread of agriculture to Northern and Central Europe via several main routes (i.e. from the coast of the Mediterranean via Spain to Northern Europe; from the Balkans to Central and Northern Europe; from Southern Russia to Northern Europe). At places where the genetically different forms of *S. latifolia* came into contact with each other, zones with intermediate types arose. The present-day distribution of the gene frequencies and the morphological characters are thus a reflection of those events.

Although in his study Mastenbroek sampled many populations (over 300) with each sampling population being more than 100 individuals, the *S. latifolia* populations have been divided into nine geographical races on the basis of relatively few informative characters (57). To determine more accurately if migration is indeed the cause of the observed distribution, we need many more independent and selectively neutral characters. An attractive possibility is the use of random amplified polymorphic DNAs (RAPDs), which allow the examination of genetic variation without prior knowledge of DNA sequences (Caetano-Anolles et al. 1991; Welsh and McClelland 1990; Williams et al. 1990). RAPDs are a class of dominant and independent genetic markers, that are easy to score and not influenced by environmental conditions, unlike morphological or isozyme characters, and as such offer a potentially powerful tool by which to discriminate between selection and genetic drift in population genetic studies. An advantage of RAPDs above all other characters used, morphological as well as biochemical, is the large number of characters they can provide. Hence, it may be possible to sample fewer populations and fewer individuals per population than with morphological or biochemical characters. RAPDs have already been used in a number of studies ranging from the individuals up to the species level (Adams and Demeke 1993; Bardacki and Sibinski 1994; Chalmers et al. 1993; Hadrys et al. 1992; Heusden and Bachmann 1992; Jain et al. 1994; Stiles et al. 1993; Vierling and Nguyen 1992; Wilde et al. 1992).

In the study presented here, we tested whether RAPDs can be used for tracing the processes of migration. For this purpose, a pilot study was performed on 16 populations of *S. latifolia*, which provide a sample from the different geographical races and the border region between the main races (east-west division), which was sampled more extensively. This pilot study may provide a basis for sampling RAPD characters of many more *S. latifolia* populations and the application of a phylogenetic analysis to the data in order to unravel the routes by which *S. latifolia* spread over Europe. In this way additional information may be obtained on the spread of agriculture in Europe.

## Materials and methods

### Plant materials

Plants of *Silene latifolia* from 16 different geographical locations in Europe were grown in the field. Total DNA was isolated from the leaves of three individuals per population.

### DNA extraction

Approximately 3 *g* of leaves of each individual were ground in liquid nitrogen. Subsequently, 10 ml of prewarmed (55 °C) extraction buffer (2% CTAB, 1.4 *M* NaCl, 0.1 *M* TRIS-HCl pH 8.0, 20 m*M* EDTA and 1% ß-mercaptoetahanol) was added, and the mixture was incubated for 20 min at 55 °C. Samples were then extracted with equal amounts of chloroform-isoamylalcohol (24:1) for 10 min and centrifugated at 12, 000 *g* for 5 min. A 1/10 volume of 10% CTAB, 0.7 *M* NaCl was added to the supernatant, followed by a chloroform-isoamylalcohol extraction as mentioned above. The DNA was precipitated by adding a 1/10 volume of 3 *M* sodiumacetate (pH 4.8) and a 7/10 volume of isopropanol (−20 °C) to the supernatant. After 10 min of centrifugation at 12, 000 *g*, the DNA pellet was washed with 76% ethanol/10 m*M* ammoniumacetate, and vacuum dried. The DNA pellet was then

resuspended in 200 ul of TE buffer (10 m*M* TRIS-HCl, 1 m*M* EDTA, pH 8.0). DNA concentration was assayed using a DNA fluorometer (Hoefer, model TKO 100).

### DNA amplification

Polymerase chain reactions (PCR) were carried out according to Yu and Pauls (1992) with some modifications. Bulked genomic DNA samples were prepared by mixing the DNA of the three sampled individuals per population in equal amounts. A total of 27 primers (random 10-mers, kits A, C, F, G and W; Operon Technologies, Alameda, Calif.) was tested. PCR buffer and Super-*Taq* DNA polymerase were purchased from Sphaero Q (Leiden, The Netherlands). The PCR was carried out in 50 ul and contained 50 ng of template DNA, 200 u*M* each of dATP, dCTP, dGTP and dTTP (Pharm), 15 ng primer, 0.5 U of *Taq* DNA polymerase and 5 ul 5 × PCR buffer. The reaction was carried out in a Cyclotherm Programmable Heat Cycler (N. B. S. Koch-Light) and consisted of 35 cycles of 5 s at 95 °C, 30 s at 37 °C and 1 min at 72 °C. Before the first cycle the samples were heated for 7 min at 95 °C; after the last cycle the samples were incubated for an additional 10 min at 72 °C. The amplification products were analyzed after electrophoresis on a 1.4% agarose gel stained with ethidium bromide.

### Interpretation and analysis of RAPD patterns

The RAPD patterns on a gel were subsequently analyzed and converted to a matrix in which fragments in a pattern were scored on the basis of their presence (1) or absence (0). Fragments of the same size in 2 samples on the same gel were assumed to be homologous. Due to the sometimes considerable variation in intensity of the same fragment between different samples, it was not always clear if a fragment was present or absent in a pattern; in this case it was scored as a missing value (9). The resulting data matrix could be used directly in a phylogenetic analysis by PAUP (phylogenetic analysis using parsimony version 3.1), but for an unweighed pair group analysis using arithmetical averages (UPGMA) the matrix had to be transformed into a genetic distance matrix. This was done by pair-wise comparisons of both unique and shared fragments using Jaccard's coefficient of similarity (Jaccard 1908): a/(a + m), in which a is the number of matches (i.e. a fragment is present in both populations) and m is the number of mismatches (i.e. a fragment is present in one but absent in the other population). The genetic distance (GD) was calculation as GD = 1 − JC (in which JC is Jaccard's coefficient of similarity).

## Results and discussion

Sixteen *S. latifolia* populations (Table 1) were tested with 27 different primers. The populations represent both the different geographical races and the intermediate zone (i.e. Germany, Switzerland, Austria) between the eastern and western races. The latter was sampled more extensively. Thirteen primers (a1, c2, c6, c11, f3, g2, g6, g11, v17, w2, w19, x14, x18; Operon Technologies) gave clearly resolved patterns on a gel with less than 20 fragments per lane, and these were used for the analysis. From Table 2 it is clear that the different primers differ in the amount of variation they can reveal: the percentage polymorphism detected varied from 71% to 100%. Due to the presence of unique fragments though, the percentage of informative fragments varies from 41% to 79%.

There are many reports that small alterations in PCR conditions and in the quality or concentration of the target DNA can radically alter the resulting patterns (Devos and Gale 1992; Williams et al. 1993). However, we observed that even a tenfold dilution (i.e. 3 ng instead of 30 ng) of the template resulted in a reproducible pattern. Also, amplification of genomic DNA from different isolations of the same material produced reproducible fragment patterns. However, the intensity of the fragments differed considerably between the different dilutions and, consequently, these intensity differences were not considered in the analysis.

In order to perform an analysis of the populations on the basis of their RAPD patterns, the amount of variation between individuals in a population had to be determined first.

As *S. latifolia* populations are heterogenous and consist of cross-breeding individuals, a large amount of variation is to be expected. When the intrapopulation variation in RAPD patterns is as large as the interpopulation variation, no distinction between populations can be made. To establish the amount of intrapopula-

**Table 1** Origin of the *S. latifolia* populations

| Accession number | Country, nearest town | Geographic position | Geographical race [a] | Cluster number [b] |
|---|---|---|---|---|
| 3100 | Poland, Poznan | 52.25N 16.53E | 3 | II |
| 3159 | Hungary, Budapest | 47.30N 19.03E | 2 | II |
| 3169 | Russia, Novosibirsk | 55.04N 83.05E | – | II |
| 3196 | Germany, Strausberg | 52.34N 13.53E | 2 | II |
| 3204 | France, Cluny | 46.25N 4.39E | 1 | I |
| 3234 | Belgium, Vilvoorde | 50.56N 4.25E | 4 | I |
| 3240 | Ukraine, Yalta | 44.30N 34.09E | – | – |
| 3288 | Denmark, Risskov | 56.11N 10.15E | 6 | II |
| 3292 | Finland, Turku | 60.27N 22.15E | 3 | II |
| 3297 | Sweden, Djuprik | 60.37N 15.00E | 6 | II |
| 3302 | Germany, Münster | 51.58N 7.37E | 4 | I |
| 3304 | Portugal, Lisbon | 38.44N 9.08W | 7 | – |
| 3312 | Austria, Vienna | 48.13N 16.22E | 2 | II |
| 3318 | Switzerland, Geneva | 46.13N 6.09E | 4 | I |
| 3337 | Luxemburg, Luxemburg | 49.37N 6.08E | 4 | I |
| 3351 | Germany, Berlin | 52.32N 13.25E | 2 | II |

[a] According to Mastenbroek (see also Fig. 1)
[b] Cluster number of the population in an UPGMA analysis of the RAPD data based on Jaccard's coefficient of similarity (see also Fig. 2)

**Table 2** RAPD primers used to test the 16 *S. latifolia* populations

| Operon primers | Total fragments (no. polymorphic) | Informative fragments | Percentage of polymorphic fragments | Percentage of informative fragments |
|---|---|---|---|---|
| A01 | 36(35) | 23 | 97 | 64 |
| C02 | 26(24) | 13 | 92 | 50 |
| C06 | 28(25) | 20 | 97 | 71 |
| C11 | 24(24) | 17 | 100 | 71 |
| F03 | 28(26) | 20 | 93 | 71 |
| G02 | 38(34) | 16 | 89 | 42 |
| G06 | 22(22) | 16 | 86 | 73 |
| G11 | 25(23) | 15 | 92 | 60 |
| V17 | 23(19) | 15 | 83 | 65 |
| W02 | 38(37) | 24 | 97 | 63 |
| W19 | 17(15) | 7 | 88 | 41 |
| X14 | 14(13) | 11 | 93 | 79 |
| X18 | 7(5) | 4 | 71 | 57 |

tion variation, three separate individuals per population were tested with a subset of the 13 primers (a1, c2, c6, c11, f3, g2, w2). In the dendrogram of an UPGMA analysis of the resulting fragment patterns, the individuals clearly clustered per population (data not shown). The only exceptions were the closely situated populations from Switzerland (3318) and Luxemburg (3337). The individuals of these populations always clustered together in the dendrograms and behaved as if they belonged to the same population. Jaccard's coefficient between individuals in a population ranges from 0.50 to 0.82 as opposed to the coefficient between populations, which ranged from 0.28 to 0.61 (Table 3). However, the similarity coefficient for the populations from Switzerland (3318) and Luxemburg (3337) is higher (0.71). This is not surprising because the individuals of both populations cluster together in a dendrogram. From these results it can be concluded that individual variation in a population is smaller than variation between populations, although there is some overlap. The RAPD analysis was made more efficient by using bulked genomic DNA samples (Yu and Pauls 1993) of three individuals per population to establish the interpopulation variation, and these were tested with all 13 primers. The fragment pattern in the bulked genomic samples was seen to be a superposition of the patterns of the three separate individuals, although sometimes faint fragments observed in one of the individuals were absent in the bulked sample (data not shown). This may be due to the higher dilution of the template sequence in the bulked sample hampering effective amplification (Michelmore et al. 1991; Yu and Pauls 1993). Analysis of the fragment patterns of the bulked genomic samples resulted in a total of 326 characters, of which 275 showed polymorphism. Of the polymorphic fragments 74 were unique fragments (i.e. they occured in only one population), and 201 were informative in a phylogenetic analysis. On average, each population had 3–4 unique fragments, but a clear exception was the population from Yalta (3240), which had 22 unique fragments. Jaccard's coefficient of similarity between populations ranged from 0.28 to 0.61 (Table 3).

In Fig. 2 we present an UPGMA analysis of the bulked genomic samples. Two main clusters can be distinguished (I and II, see Table 1): cluster I coincides with the western populations (i.e. geographical races 1, 4, 5, 7 and 8; Figs 1, 2; Mastenbroek et al. 1986); cluster II with the eastern populations (i.e. geographical races 2, 6, 3 and 9; Figs 1, 2). An exception is the population from Portugal (3304), which is excluded from clusters, but

**Table 3** Similarity matrix of the *S. latifolia* populations used in this study for Jaccard's coefficient of similarity

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3100 | | | | | | | | | | | | | | | | |
| 3159 | 0.587 | | | | | | | | | | | | | | | |
| 3169 | 0.526 | 0.524 | | | | | | | | | | | | | | |
| 3196 | 0.464 | 0.487 | 0.570 | | | | | | | | | | | | | |
| 3204 | 0.433 | 0.452 | 0.508 | 0.465 | | | | | | | | | | | | |
| 3234 | 0.409 | 0.460 | 0.440 | 0.444 | 0.507 | | | | | | | | | | | |
| 3240 | 0.295 | 0.303 | 0.296 | 0.292 | 0.312 | 0.314 | | | | | | | | | | |
| 3288 | 0.525 | 0.524 | 0.538 | 0.519 | 0.442 | 0.422 | 0.306 | | | | | | | | | |
| 3292 | 0.485 | 0.483 | 0.481 | 0.529 | 0.469 | 0.456 | 0.360 | 0.517 | | | | | | | | |
| 3297 | 0.520 | 0.485 | 0.524 | 0.535 | 0.433 | 0.481 | 0.345 | 0.614 | 0.581 | | | | | | | |
| 3302 | 0.346 | 0.438 | 0.340 | 0.352 | 0.398 | 0.510 | 0.315 | 0.392 | 0.402 | 0.426 | | | | | | |
| 3304 | 0.403 | 0.379 | 0.424 | 0.426 | 0.406 | 0.485 | 0.292 | 0.417 | 0.459 | 0.418 | 0.433 | | | | | |
| 3312 | 0.559 | 0.552 | 0.525 | 0.514 | 0.441 | 0.464 | 0.319 | 0.551 | 0.545 | 0.589 | 0.440 | 0.480 | | | | |
| 3318 | 0.408 | 0.490 | 0.457 | 0.426 | 0.521 | 0.507 | 0.327 | 0.478 | 0.445 | 0.479 | 0.510 | 0.410 | 0.504 | | | |
| 3337 | 0.418 | 0.458 | 0.436 | 0.468 | 0.472 | 0.552 | 0.346 | 0.438 | 0.455 | 0.503 | 0.514 | 0.410 | 0.471 | 0.71 | | |
| 3351 | 0.521 | 0.496 | 0.445 | 0.442 | 0.454 | 0.387 | 0.280 | 0.478 | 0.481 | 0.510 | 0.364 | 0.384 | 0.520 | 0.471 | 0.481 | |

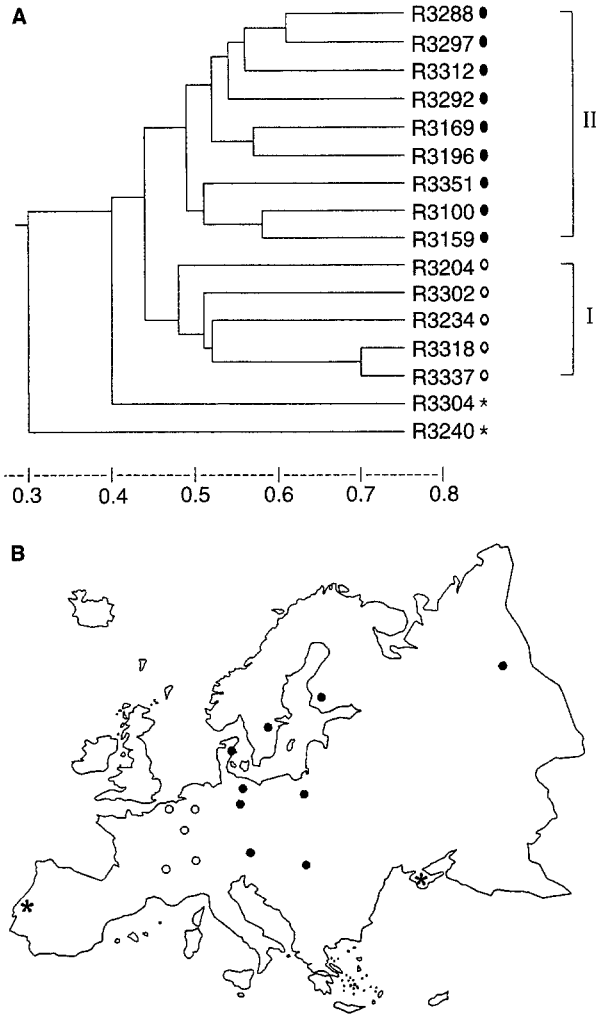


**Fig. 2** A An UPGMA dendrogram of the 16 *S. latifolia* populations based on variation in RAPD patterns obtained with 13 RAPD primers and using Jaccard's similarity coefficients. Two main clusters are clearly distinguishable: a western cluster (*I*) and an eastern cluster (*II*). **B** Map of Europe showing the geographic location of these 16 populations and the cluster they belong to: ○cluster I; ● cluster II. Populations 3304 (Portugal) and 3240 (Yalta) (both indicated by*) fall outside the main clusters

which was expected to fall in the western cluster. Due to the small size of the population sample analyzed, the main two clusters could not be further divided into smaller groups; only the larger clusters are thus appparent (although the populations from geographical race 4 clearly cluster together).

Using a phylogenetic analysis with PAUP we found the same east-west division between the populations, but in this case the populations from Portugal (3304) and Yalta (3240) were now included in the western cluster. With respect to the population from Yalta (3240) this may be explained by the many unique fragments it possesses. Therfore, the Yalta population was excluded from the two main clusters in the UPGMA analysis, while in a phylogenetic analysis these unique fragments are uninformative and thus excluded from the analysis. In a bootstrap analysis with PAUP the different branches of the resulting tree showed very low bootstrap values. When the populations at the border regions of the geographical races (i.e. 3196, 3302, 3312 and 3351) were excluded, however, the resulting bootstrap values were much higher (data not shown). The reason for this may be that a population at the border between two of the races is a hybrid between the different races and so interferes with a phylogenetic analysis, resulting in a less resolved tree.

Although detailed comparisons between the different datasets (gene frequency, morphological and RAPD data) were not possible, since the surveys were based on different population samples, generalizations can be made. Our RAPD analysis clearly supports the results obtained by Mastenbroek et al. (1982, 1983a,b): the same east-west distribution as the gene frequency and the morphological data. Geographical race 4 can also be identified in our analysis. Though the number of both populations and individuals per population sampled in our study is much smaller than in earlier analyses by Mastenbroek, the number of characters obtained with RAPDs is much larger. It can be concluded therefore, that the application of RAPDs is much more efficient than, for example, the analysis of gene frequency data, in which 358 populations with 120 individuals per population were analyzed (Mastenbroek et al. 1983b).

The distribution of the RAPD characters provide support for the hypothesis that the different geographical races of *S. latifolia* originated not as the result of a process of selection for different gene combinations in different geographical areas, but as the result of a past migration. This migration can be explained as put forward by Mastenbroek et al. (1986) – that the species adapted as a weed in Southern Europe and then spread over Europe parallel to the spread of agriculture. As there has been little or no artificial selection on this weed, a phylogenetic analysis of RAPD character distribution of *S. latifolia* populations in Europe using, for example, the closely related species *S. dioica* as an outgroup, may provide additional information on the different routes agriculture has taken.

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