Influence of asymmetrical mating patterns and male reproductive success on the maintenance of sexual polymorphism in *Acer pictum* subsp. *mono* (Aceraceae)

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Abstract

Populations of *Acer* species often contain more than three sex phenotypes with complex sexual polymorphism including duodichogamy, protandry and protogyny. We identified the mechanisms that maintain sexual polymorphism in *Acer pictum* subsp. *mono*, a temperate tree from northern China, by investigating maternal mating patterns and male reproductive success. We used paternity analyses to estimate rates of outcrossing and disassortative mating, as well as male outcrossed siring success, in a population of *A. pictum* subsp. *mono* with uneven sex phenotype ratios (duodichogamous 69.1%, protandrous 19.6%, protogynous 11.3%). We used a pollen-transfer model to investigate whether the unequal ratios of sex phenotypes could be explained by the observed patterns of mating. Most progeny resulted from outcrossing, particularly disassortative among the sex phenotypes. Although the duodichogamous phenotype showed a significant amount of intraphenotypic mating, the frequency did not exceed that of disassortative mating. We detected no significant differences in male outcrossed siring success among the sex phenotypes. The pollen-transfer model demonstrated that sex phenotype ratios could be maintained by the observed mating pattern in the population. Our results indicate that disassortative mating among the sex phenotypes can maintain sexual polymorphism in *A. pictum* subsp. *mono* and that ratios biased towards duodichogamy can result from frequent intraphenotypic mating in this phenotype.

Keywords: disassortative mating, duodichogamous, heterodichogamous, intraphenotype mating, paternity analysis, sexual plasticity

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Introduction

Typically, populations of flowering plants contain a single sex phenotype that can mate with most other conspecific individuals. However, some populations are sexually polymorphic, in which individuals have restricted mating options and can be categorized into distinct mating groups that differ in morphology (e.g. heterostyly, in which two or three floral morphs occur) or phenology (e.g. heterodichogamy, in which two different morphs occur that differ in the timing of maternal and paternal sex function). Generally speaking, the maintenance of sexual polymorphism results from negative frequency-dependent selection, whereby the fitness of a sexual morph is inversely proportional to its relative frequency in the population (Clarke *et al.* 1988; Eckert *et al.* 1996; Thompson *et al.* 2003). Identifying and understanding the ecological and evolutionary factors that maintain sexual polymorphism and are responsible for variation in morph frequencies is an important challenge for plant evolutionary biologists.

In many sexually polymorphic species, especially those that are heterostylous, mating between different morphs (disassortative mating) is prevalent, and this results in morph frequencies of 1:1 or 1:1:1 (isoplethy) at equilibrium (Fisher 1941; Charlesworth & Charlesworth 1979; Barrett & Hodgins 2006). However, in sexually

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polymorphic species without heteromorphic incompatibility, morph frequencies often deviate from isoplethy (Eckert *et al.* 1996; Barrett & Harder 2005). If such populations are not in equilibrium, biased morph frequencies may result from stochastic processes, such as genetic drift and founder effects, especially when clonal propagation dominates (Morgan & Barrett 1988; Barrett 1993; Eckert & Barrett 1995). If, on the other hand, such populations are at equilibrium, biased morph frequencies may result from asymmetrical mating among morphs (Hodgins & Barrett 2008). A number of factors can cause asymmetrical mating, including morph-specific changes in compatibility relations or differences in rates of self-fertilization and assortative (intramorph) mating (Weller 1992; Barrett & Hodgins 2006).

Although heterostyly is a well-known example of sexual polymorphism, sexual polymorphism can also occur on a temporal basis, for example, as a result of two or three floral phenotypes that differ in the timing of male and female sex function. Heterodichogamy is a common form of temporal sexual polymorphism that usually involves two morphs: a protandrous morph (in which the anthers mature before the stigma) and a protogynous morph (in which the stigma is receptive before the anthers). The flowering phases of the two morphs are synchronous and reciprocal resulting in disassortative mating (Renner 2001), as has been shown directly in a few species by using molecular genetic markers (Bai *et al.* 2007; Gleiser *et al.* 2008).

In addition to heterodichogamy, some species exhibit more complex temporal sexual polymorphic strategies consisting of three or more morphs. In these populations, besides protogynous and protandrous morphs, a phenotype called duodichogamy (individuals that flower in the sequence male → female → male) is often present (de Jong 1976; Sato 2002; Luo *et al.* 2007; Kikuchi *et al.* 2009). *Acer* is such a clade and presents useful material to study the maintenance and evolution of sexual polymorphism (de Jong 1976; Renner *et al.* 2007). Sexual plasticity is well documented in *Acer*, with evidence that a low proportion of individuals transition between the sex phenotypes during their lifetime, including from protandry to duodichogamy as well as male to protandry (de Jong 1976; Sato 2002; Renner *et al.* 2007; Gleiser *et al.* 2008). This plasticity might account for the maintenance of sexual polymorphism, but there are other possible explanations, especially for its maintenance in populations that contain high percentages of protandrous or protogynous individuals. It is known from previous work on heterostylous species that mating patterns and male or female reproductive success are important factors for the maintenance of sexual polymorphism (Barrett & Harder 2005; Hodgins & Barrett 2006, 2008). As an example, in populations of

Narcissus triandrus, a tristylous species (long-, mid- and short-styled morphs) with L-morph-biased ratios, sexual polymorphism is maintained by disassortative mating (Hodgins & Barrett 2008). However, the commonly observed L-morph-biased ratios largely result from significant assortative mating in this morph (Barrett & Harder 2005; Hodgins & Barrett 2006, 2008). In comparison with heterostylous species, there have been few investigations into the factors maintaining temporal sexual polymorphic populations.

Acer pictum subsp. *mono* is a temporal sexual polymorphic taxon with no physiological incompatibility within or between sex phenotypes. It is distributed in China, Korea, Japan and eastern Russia (van Gelderen 1994). Its sexual system has been described as heterodichogamous (Kikuchi *et al.* 2009). However, this categorization ignores the second phase of male flowers in the duodichogamous individuals. According to our field observations in six populations from northern China over four consecutive years, more than 50% of individuals in the populations are duodichogamous, and thus, *A. pictum* subsp. *mono* is different from typical heterodichogamous species, which have only protogynous and protandrous individuals; *A. pictum* subsp. *mono* may have duodichogamous, protandrous, protogynous and pure male individuals.

The main goals of this study on *A. pictum* subsp. *mono* are as follows: (i) to determine the possible mechanisms that maintain sexual polymorphism and (ii) to identify the mechanisms that may account for the observed frequencies of sex phenotypes. Specifically, we began by conducting a 4-year survey on the frequency of sex phenotypes (duodichogamy, protandry, protogyny and pure male) in a selected population. We then estimated mating patterns in the population by using paternity analysis to test the prediction that sexual polymorphism in *A. pictum* subsp. *mono* is maintained by negative frequency-dependent selection resulting from disassortative mating. We also compared differences in male reproductive success and rates of assortative mating among the sex phenotypes by using paternity analysis and then investigated how the rate of assortative mating could give rise to the observed ratio of phenotypes by using a pollen-transfer model. These data were used to test the prediction that differences in mating patterns among duodichogamous, protandrous, protogynous and pure male phenotypes may explain their biased frequencies.

Materials and methods

Study species

Acer pictum subsp. *mono* is a monoecious, bee-pollinated, temperate deciduous tree. Flowers are

functionally unisexual, due to either abortion of the pistil or failure of the anthers to dehisce. Flowers are borne in corymbs before the leaves emerge in early spring. Each ovary has two locules with two ovules, but usually only one ovule develops after fertilization. The unfertilized flowers are capable of developing into parthenocarpic fruits (de Jong 1976).

Study site

The study site was located on Dongling Mountain, 114 km west of Beijing, China (39°58′N, 115°26′E; 1120 m above sea level). This region is dominated by brown mountain earth, with a temperate continental monsoon climate. The mean annual temperature is 4.8 °C (January −10.1 °C, July 18.3 °C). The average annual precipitation is 612 mm/year, of which 78% occurs in June–August (Li & Chen 1999). *Juglans mandshurica*, another heterodichogamous species (Bai *et al.* 2007), and *A. pictum* subsp. *mono* are common species in this region. The study population is located in a long, narrow valley (Fig. 1).

Sexual expression and phenotype ratios

A survey of the sex phenotypes of flowering individuals in the study population (a total of 97 trees) was conducted over a 4-year period (2008–2011). Four sex phenotypes were clearly recognizable: duodichogamy, protandry, protogyny and pure males. We evaluated sexual transitions for each flowering tree during the 4-year survey. In addition, the diameter at breast height of the 97 individuals was measured to determine whether there was a relationship between plant size and sexual expression.

Paternity analysis

The 97 individuals in the study population were mapped and genotyped as potential pollen donors. For all individuals, we collected leaf tissue in June 2008.

Three months later, 1041 seeds were collected from 58 maternal individuals (842 from 45 duodichogamous trees, 181 from 11 protogynous trees and 18 from two protandrous trees; mean = 17.9 seeds per individual). DNA from the dried leaf and embryo tissue was extracted with a plant total genomic DNA kit (Tiangen, Beijing, China). Extracted DNA was then amplified at 10 microsatellite loci that were initially developed for three other *Acer* species (Pandey *et al.* 2004; Terui *et al.* 2006; Kikuchi & Shibata 2008) (Table S1, Supporting information).

PCR was carried out with a Veriti 96-well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Amplifications were conducted in 20 μL PCR mixture consisting of the following: 20 ng of DNA template, $MgCl_2$ (1.5 mM), dNTPs (0.2 mM), two primers (0.2 μM of each) and *Taq* polymerase (0.8 U; TaKaRa, Tokyo, Japan). PCR cycling condition was as follows: 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at the annealing temperature specific for each primer (51–56 °C) and 45 s at 72 °C, followed by a final extension of 10 min at 72 °C. Amplified products were visualized on an ABI 3730 DNA Analyzer (Applied Biosystems), and allele sizes were determined using GENEMAPPER 3.7 software (Applied Biosystems).

Data analysis

We calculated the following single-locus and multi-locus measures for the 97 adult trees and 1041 seeds using FSTAT 2.9.3 software (Goudet 2001): number of alleles, observed and expected heterozygosity, paternity exclusion probability and inbreeding coefficient. The presence of null alleles was tested using the program MICRO-CHECKER 2.2 (van Oosterhout *et al.* 2004).

Paternity analysis. Paternity analysis was performed using CERVUS 3.0 (Marshall *et al.* 1998; Kalinowski *et al.* 2007), a computer program that uses a maximum likelihood (ML) method to assign parentage (Meagher 1986). There are three steps when conducting paternity
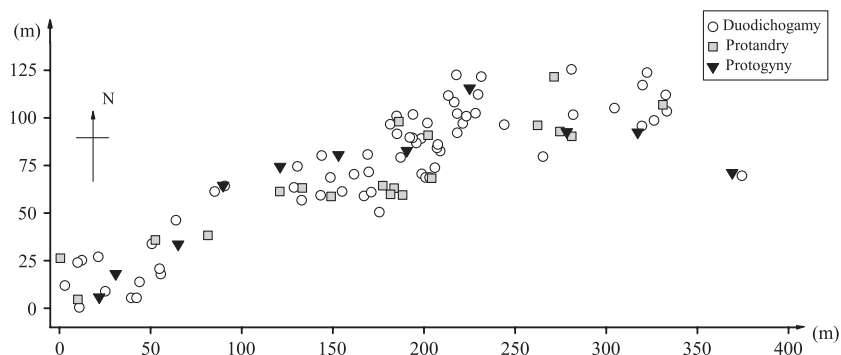

Fig. 1 Map of the study population. Circles, duodichogamous trees (*n* = 67); squares, protogynous trees (*n* = 19); triangles, protandrous trees (*n* = 11).

analysis with CERVUS: (i) allele frequency estimation for the parents; (ii) simulations of paternity analysis to determine the confidence intervals for assignment and (iii) paternity analysis. The simulation parameters were as follows: 10 000 offsprings, 203 candidate male parents, 0.48 as the proportion of candidate male parents sampled, 0.9947 as the proportion of loci typed, 0.01375 as the rate of typing error, 95% for the strict confidence level and 80% for the relaxed confidence level. Note that candidate male parents mean all adults known to be or thought to be present at the time of mating irrespective of whether or not they are sampled. The product of the number of candidate male parent and proportion of candidate male parents sampled is equal to the number of sampled male parents (97 in the study population). For the simulation of paternity analysis (step 2), the total number of candidate male parents and the proportion of male parents sampled were adjusted to yield the best match between observed and expected assignments. For the paternity analysis (step 3), we used the same rate of typing error as used in the simulations and accepted the single most likely father according to the 80% confidence interval.

Mating pattern. Mating patterns among sex phenotypes were determined by identifying seed to which a single male parent was assigned. Next, the phenotype of the most likely father for each seed was identified. Selfing was included in intraphenotypic mating. Goodness-of-fit tests (Sokal & Rohlf 1995) were used to examine the significance of the mating pattern among the phenotypes and compare the observed mating patterns by CERVUS with those that would be expected, given random mating among the sex phenotypes. Random mating was estimated by two strategies. In the first, it was estimated from the frequencies of phenotypes in the population and the number of seed from each maternal phenotype to which a male parent was assigned successfully. In the second, given that the distance between fathers and mothers can also be an important factor of mating pattern, the potential mating probability was adjusted using the distance model (Smouse et al. 1999) as:

$$p_i = \exp(\gamma_d D_i) / \sum_{97}^{i=1} \exp(\gamma_d D_i)$$

where γ_d is the estimated regression coefficient (see the section entitled 'Male reproductive success' below), D_i is the distance of the ith father from the mother and $\sum p_i = 1$ for each mother. Furthermore, the observed mating patterns among the sex phenotypes inferred from the CERVUS analysis were adjusted for frequency of sex phenotype with the equation:

$$q'_{ji} = (q_{ji}/f_j)/(q_{ii}/f_i + q_{ji}/f_j + q_{ki}/f_k)$$

where q_{ji} is the proportions of seeds produced by phenotype i and sired by pollen from phenotype j. f_j is the frequency of sex phenotype j.

Pollen-transfer model. The model of frequency-dependent selection of morph ratios in a temporally sexually polymorphic population followed previous models developed by Lloyd & Webb (1992) and Barrett et al. (2004). When applying their models, we used a transfer equation to obtain the ratios of sex phenotypes at each generation. The transfer equation is $\vec{p}_{n+1} = \vec{p}_n E$, where the vector $\vec{p}_n$ is the frequency of the sex phenotype in the nth generation, and E is the matrix of pollen-transfer probabilities calculated by CERVUS after adjusting for the frequency of the sex phenotypes (Table 1). The observed frequency of sex phenotypes (duodichogamous 0.69, protogynous 0.20 and protandrous 0.11) was regarded as an initial value for the pollen-transfer equation. We performed a repeated iterative operation with 100 generations to determine whether the observed mating pattern could maintain a stable sex phenotype ratio. For comparison, we also performed the iteration with random mating and complete disassortative mating. The model was run using the R statistical package (R Development Core Team, 2008).

Male reproductive success. Given that the distance between fathers and mothers can be an important factor for male reproductive success, we used the PatQuest 4.0 program (Meagher 2002), a log-linear regression mating model that includes distance between candidate fathers and mothers, to estimate male reproductive success as follows (Verdú et al. 2006; Gleiser et al. 2008):

$$\log(\lambda_{jk}) = \gamma_d \delta_{jk} + \beta z_k$$

where λ_{jk} is the male reproductive success of the kth father with the jth mother, δ_{jk} is the distance between the kth father and jth mother, γ_d and β are the estimated regression coefficients and z_k means the sex phenotype

Table 1 Matrix of pollen-transfer probabilities considered in the pollen-transfer model in *Acer pictum* subsp. *mono*, calculated from the mating patterns assigned by CERVUS after adjusting for sexual phenotype frequency

Donating sexual phenotype	Receiving sexual phenotype		
	Duodichogamous	Protogynous	Protandrous
Duodichogamous	0.42	0.34	0.24
Protogynous	0.62	0.38	0
Protandrous	0.44	0.56	0

group of the *k*th father. We calculated exp(*β*) to obtain the reproductive success of one sex phenotype relative to any other sex phenotype and assessed the significance of the estimated parameters by bootstrap permutations using 1000 iterations (Morgan & Conner 2001).

Results

Sexual expression and sex phenotype ratios

Of the 97 trees sampled in the first year, 67 (69.1%) were duodichogamous, 19 (19.6%) protandrous, 11 (11.3%) protogynous, and two were pure males. From 2008 to 2011, 13 trees showed a change in sexual expression; the average frequency of annual sexual transitions was 4.5%, with most switches observed between the duodichogamous and protandrous phenotypes (Table 2). The two pure males switched to protandry within the study period; hence, we considered maleness to be an 'inconstant phenotype' and assigned these individuals to the protandrous phenotype in the paternity analysis. We observed no significant difference in diameter at breast height among the sex phenotypes (duodichogamous 9.33 ± 3.43 cm, protogynous 9.84 ± 4.28 cm, protandrous 10.03 ± 4.27 cm; $P = 0.77$, ANOVA).

Categorical paternity analysis

The nuclear microsatellite loci analysed were highly polymorphic, with 12.4 alleles on average. For the parental population, the diversity parameters were as follows: the expected heterozygosity (H_E) varied from 0.35 to 0.86, whereas the observed heterozygosity (H_O) varied from 0.37 to 0.85. Inbreeding coefficients (F_{IS}) for reproductive individuals were 0.007 and not significantly different from zero (Table 3). Null alleles were not detected at any of the loci. For the progeny, diversity measures were as follows: the multi-locus expected heterozygosity (H_E) was 0.68, the observed heterozygosity (H_O) was 0.65 and the inbreeding coefficient was 0.05 (Table 4).

In the paternity analysis, we could assign a single male parent to 581 (55.8%) of the 1041 offspring analysed with 80% confidence. For 460 offspring (44.2%), we were unable to identify the most likely male parent at the 80% confidence level, which indicated that their male parents would have come from outside the population.

Mating pattern

Among the 581 offspring for which the male parent occurred within the population, the frequency of self-fertilization was 8.7% ($N = 91$; duodichogamous 10.3%, protogynous 2.2% and protandrous 0%). Significant deviations from random mating were observed among the sex phenotypes (without distance correction: $G_4 = 18.73$, $P < 0.001$; with distance correction: $G_4 = 60.93$, $P < 0.001$; Fig 2). The proportions of seeds produced by duodichogamous individuals and sired by pollen from duodichogamous, protogynous or protandrous individuals were 74.6%, 17.8% and 7.6%, respectively. However, after adjustment for the frequency of sex phenotype, the corresponding values were 40.6%, 34.3% and 25.1%, which shows mating was non-random in the population (without distance correction: $G_2 = 9.58$, $P = 0.008$; with distance correction: $G_2 = 8.35$, $P = 0.015$). The proportions of seeds produced by protogynous individuals and sired by pollen from duodichogamous, protogynous or protandrous individuals were 75.0%, 13.5% and 11.5%, respectively, but the corresponding values were 39.0%, 24.8% and 36.2% after adjustment for the frequency of sex phenotype. Mating in the protogynous phenotype was not significantly different from random without correction for distance ($G_2 = 2.49$, $P = 0.288$), but was significantly different from random with distance correction ($G_2 = 39.78$, $P < 0.001$). All seeds produced by protandrous individuals were sired by pollen from duodichogamous individuals, and the mating was non-random (without distance correction: $G_2 = 6.66$, $P = 0.036$; with distance correction: $G_2 = 12.80$, $P = 0.002$).

Pollen-transfer model

The results obtained using the pollen-transfer model showed that mating patterns can influence frequencies of sex phenotype when mating is driven by frequency-dependent selection. For the observed rate of disassorta-

Sexual phenotype in year (*n*)	Sexual phenotype in year (*n* + 1)			
	Duodichogamous	Protogynous	Protandrous	N
Duodichogamous	0.428	0.000	0.044	0.528
Protogynous	0.020	0.580	0.000	0.400
Protandrous	0.135	0.000	0.541	0.324
N	0.756	0.111	0.133	0.000

Table 2 Sexual transitions matrix obtained from a 4-year survey in *Acer pictum* subsp. *mono*. Each cell (x_{ij}) represents the fraction of the possible cases, in which a tree of the *i*th sexual phenotype in a year (*n*) flowered as the *j*th sexual phenotype in the following year (*n* + 1). N: non-flowering

Table 3 Number of observed alleles (A_o), observed and expected heterozygosity (H_o and H_e), paternity exclusion probability (EP) and inbreeding coefficient (F_{IS}) at each locus, for 97 adult individuals of *Acer pictum* subsp. *mono*

Locus	A_o	H_o	H_e	EP	F_{IS}*
Am116	8	0.598	0.596	0.334	0.015
Am118	7	0.722	0.717	0.468	−0.004
Am340	12	0.742	0.796	0.609	0.070
Am607	11	0.722	0.751	0.535	0.045
Am742	11	0.845	0.863	0.716	0.014
Am258	8	0.649	0.638	0.372	−0.017
Map09	9	0.845	0.817	0.635	−0.043
Aca22	8	0.719	0.749	0.542	0.034
Aca24	6	0.365	0.348	0.203	−0.056
Aca17	6	0.552	0.529	0.238	−0.039
Multilocus	8.6	0.676	0.680	0.999	0.007

*Significance levels were determined after 10 000 randomizations: all were non-significant($P > 0.05$).

Table 4 Number of observed alleles (A_o), observed and expected heterozygosities (H_o and H_e) and inbreeding coefficient (F_I) at each locus, for 581 progeny individuals of *Acer pictum* subsp. *mono*

Locus	A_o	H_o	H_e	F_I
Am116	17	0.613	0.617	0.006[ns]
Am118	9	0.693	0.723	0.046*
Am340	16	0.763	0.792	0.034*
Am607	15	0.693	0.747	0.076*
Am742	17	0.819	0.858	0.049*
Am258	9	0.610	0.634	0.042*
Map09	12	0.781	0.809	0.041*
Aca22	9	0.696	0.758	0.087*
Aca24	7	0.367	0.364	−0.003[ns]
Aca17	13	0.498	0.536	0.081*
Multilocus	12.4	0.653	0.683	0.048*

Significance levels were determined after 10 000 randomizations. ns, non-significant ($P > 0.05$); *$P < 0.05$.

tive mating, a stable ratio of sex phenotypes (duodichogamous 0.50, protogynous 0.38 and protandrous 0.12) can be achieved after approximately five generations. For completely disassortative mating, frequencies of sex phenotypes oscillate with decreasing amplitude to approach the equilibrium (isoplethy) after 10 generations. In contrast, random mating results in isoplethy at equilibrium in one generation (Fig. 3). This is equivalent to mating pattern in the population containing a single mating type.

Male reproductive success

The significant and negative estimate of the distance parameter with the log-linear regression model indicated that mating among neighbours occurred more frequently than among individuals that were more spatially separated ($\gamma_d = −0.96$, $P < 0.001$). More importantly, the analysis revealed no significant differences in male reproductive success among sex phenotypes ($\beta_{DUO} = −0.20$, $P = 0.87$; $\beta_{PG} = 0.10$, $P = 0.18$; $\beta_{PA} = −0.14$, $P = 0.16$), which suggested that the sex phenotypes had equal levels of male fertility.

Discussion

Our results show that mating patterns in *Acer pictum* subsp. *mono* are predominantly outcrossing and disassortative, and are primarily determined by flowering phenology and distance to mates. Theoretical analysis of a pollen-transfer model indicated that a ratio biased towards the duodichogamous phenotype would result from a greater degree of assortative mating within the duodichogamous sex phenotype than within the protogynous and protandrous sex phenotypes.

Mating patterns among sex phenotypes

Similar to *Narcissus triandrus*, a tristylous species in which equilibrium populations deviate from isoplethy (Barrett *et al.* 2004), *A. pictum* subsp. *mono* has no physiological incompatibility within and between sex phenotypes and also has biased sex phenotype ratios. Non-random mating involving disassortative mating is a requirement for the maintenance of sexual polymorphism (Hodgins & Barrett 2008). Our paternity analysis provides clear evidence of deviations from random expectations. Moreover, after correction for sex phenotype frequencies, the frequencies of assortative mating were 40.6%, 24.8% and 0% for the duodichogamous, protogynous and protandrous sex phenotypes, respectively, which were less than the frequencies of disassortative mating for each of these phenotypes. The pollen-transfer model indicated that the frequencies of sex phenotypes could be maintained by the observed mating pattern in the population. Therefore, similar to other sexually polymorphic species, in general, the conditions for maintaining sexual polymorphism were met in the *A. pictum* subsp. *mono* population.

The frequencies of assortative mating for the protogynous and protandrous phenotypes were less than that for the duodichogamous phenotypes. Reasons for this might include the fact that the protogynous and protandrous phenotypes were less frequent than the duodichogamous phenotype in the study population and that flower phenology within the protogynous and protandrous phenotypes was more synchronous than that of the duodichogamous phenotype, which resulted in a low probability of mating within a sex phenotype. Our
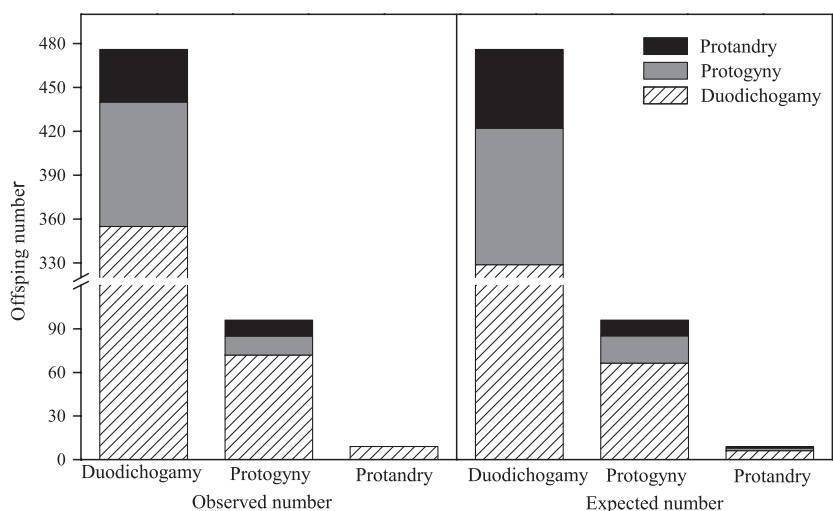
Fig. 2. Mating patterns among the sex phenotypes in the population of *Acer pictum* subsp. *mono*. The results are from paternity analyses conducted with CER-VUS 3.0 (80% confidence level). For each maternal phenotype, the observed number of progeny sired by each phenotype is shown, together with the expected number of progeny sired by each phenotype from random mating on the basis of phenotype frequencies in the population.
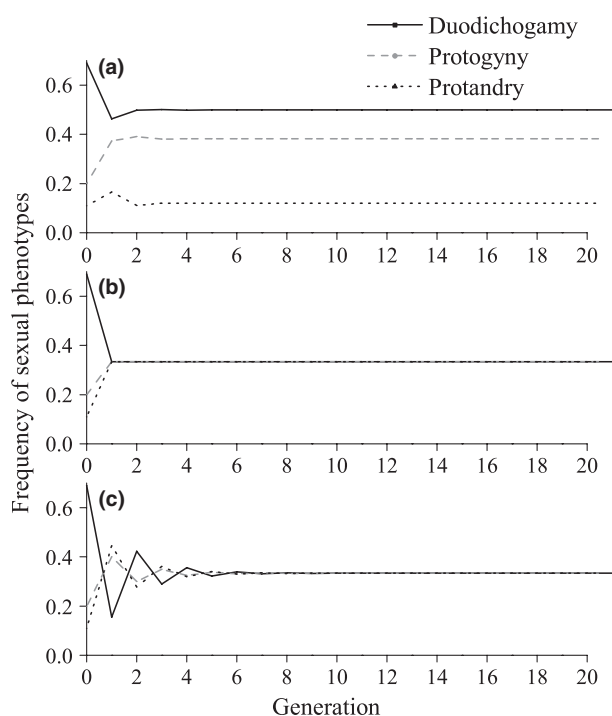

Fig. 3 Results of iteration of the pollen-transfer model with the observed sex phenotype ratio (duodichogamy 0.79, protogyny 0.20 and protandry 0.11) as the initial value. (a) Mating patterns assigned by CERVUS; (b) random mating; (c) completely disassortative mating.

estimate of protandrous mating patterns might have been biased, because fewer seeds were collected for this phenotype than for the others, due to sampling difficulties and low fruit set by protandrous individuals. Nine seeds produced by protandrous individuals were sired by duodichogamous trees, which might be due to the fact that the maternal protandrous trees were surrounded by duodichogamous individuals.

The maintenance of duodichogamous-biased sex phenotype ratios

We detected significant assortative mating by the duodichogamous phenotype of *A. pictum* subsp. *mono*. Moreover, using our estimates of the rate of assortative mating for the three sex phenotypes, we obtained duodichogamous-biased phenotype frequencies at equilibrium (duodichogamous 0.499, protogynous 0.381 and protandrous 0.120); these estimates were roughly similar to the observed phenotype frequencies (duodichogamous 0.690, protogynous 0.196 and protandrous 0.113). Consequently, significantly higher assortative mating by the duodichogamous phenotype than by the other two mating types can help to explain the duodichogamous-biased ratio that was observed in the study population.

There are several possible explanations for the higher rate of assortative mating by the duodichogamous phenotype. First, the absence of self-incompatibility or incompatibility among duodichogamous individuals affords opportunities for selfing and assortative mating. Second, duodichogamous individuals flower in the sequence male → female → male, so they pass twice through a hermaphroditic stage in the course of sexual transition, during which time selfing could occur. Thus, the opportunity for selfing is greater in duodichogamous than in protogynous and protandrous individuals (selfing rate: duodichogamous 10.3%, protogynous 2.2% and protandrous 0%), and selfing was included in our estimate of intraphenotypic mating. If selfing was excluded, the frequency of assortative mating by duodichogamous phenotype would fall to 34.0% from 40.6%. Third, flowering phenology within the duodichogamous phenotype is not as synchronous as that of the protogynous and protandrous phenotypes (H. Shang, Y. Y. Yin, J. Gao, Y. B. Luo and W. N. Bai, unpublished data),

which could result in more frequent mating between duodichogamous individuals. Lastly, given that male flowers produce both pollen and nectar, in early spring, insects would be more attracted to male flowers than to female flowers, which offer only nectar. Duodichogamous individuals have two cycles of male flowers, which might be visited more frequently than flowers of protogynous and protandrous individuals. As a result, this could increase the probability of cross-pollination among individuals of the duodichogamous phenotype.

Male reproductive success and sexual plasticity

Skewed morph ratios or the consistent loss of a morph from populations can be accounted for by differences in fitness among morphs (Charlesworth 1979; Heuch & Lie 1985; Barrett *et al.* 1989). Theoretical analysis of *Narcissus triandrus* has indicated that a bias in morph ratio can result from differences in maternal fitness among the morphs and that these effects are magnified by asymmetrical mating (Hodgins & Barrett 2006). It is unfortunate that sex phenotype-specific differences in female fertility could not be investigated in the present study because of sampling difficulties. However, no significant differences in male fertility were observed among phenotypes, which suggested that male fertility would have little effect on the evolution of the sex phenotype ratios in the study population. Thus, the skewed ratios of sex phenotypes in *A. pictum* subsp. *mono* may result from asymmetrical mating only. Nevertheless, different populations associated with specific geographical factors might show different mating patterns and reproductive success, which could lead to variation in morph ratios (Barrett *et al.* 2004). In future work, we will focus on the geographical variation in sex phenotype ratios that are associated with local phenotype-specific reproductive success, especially female reproductive success.

In *Acer*, the protandrous and duodichogamous phenotypes are often inconstant, and protandrous and duodichogamous trees are capable of switching between the two mating types (de Jong 1976). In our study, similar to the findings for *Acer japonicum* (Sato 2002), plant size did not differ significantly among the sex phenotypes, which indicated that *A. pictum* subsp. *mono* was not ontogenetically labile, but rather may adjust its sexual expression according to annual environmental conditions. Renner *et al.* (2007) argued that sexual phenotypic plasticity might be a fixed heritable sexual strategy in *Acer*, and thus might be a strategy for maintaining sexual polymorphism. On the basis of the results of our population-level study, we propose that negative frequency-dependent selection resulting from disassortative mating among the sex phenotypes largely maintains sexual polymorphism

in *Acer*. The following results support this proposal. First, sexual transitions occurred at a very low frequency (4.5% each year), which might not affect the phenotype frequencies significantly. Second, sexual transitions occurred mainly between the duodichogamous and protandrous phenotypes in the study population; thus, it is difficult to explain the existence of protogynous individuals in the population on the basis of sexual plasticity. Third, if all sex phenotypes simply represent different sexual phases of a single sex phenotype, the mating patterns would be random rather than disassortative, because there is no frequency-dependent selection in such a population. However, our results do not enable us to eliminate the hypothesis of sexual phenotypic plasticity. It might be that, in contrast to other sexually polymorphic species, a disassortative mating pattern and sexual phenotypic plasticity contribute jointly to maintaining sexual polymorphism in *Acer*.

Acknowledgements

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Data accessibility

Sample locations, sex phenotypes and microsatellite data: DRYAD entry doi: 10.5061/dryad.v5t5t5d5.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Microsatellite loci analysed, PCR conditions, motif and allele size ranges in *Acer pictum* subsp. *mono*.

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