Low RAPD variation and female-biased sex ratio indicate genetic drift in small populations of the dioecious conifer *Taxus baccata* in Switzerland

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Abstract

Small populations are prone to genetic drift as a consequence of random sampling effects. We investigated whether we could detect such random sampling effects in the English yew (*Taxus baccata*), a dioecious conifer species occurring in scattered populations in Switzerland. Seven pairs of small and large populations were analyzed using random amplified polymorphic DNA (RAPD) marker bands from 20 individuals per population. Several genetic parameters (mean marker band frequency deviation, molecular variance, population differentiation) indicated that small populations experienced genetic drift. These genetic differences between small and large populations of yew were paralleled by an increased sex ratio bias towards a higher number of females in the small populations. Our findings support earlier assumptions that the Swiss occurrences of yew may be described as metapopulation dynamics, characterized by local colonization and extinction events leading to the observed genetic drift.

Introduction

The current cultural landscape in Central Europe is the result of long-lasting human activities that have strongly influenced and shaped natural habitats. These traditionally managed habitat types, often referred to as semi-natural, have been dramatically reduced or even been lost due to management changes, a trend that has culminated in the last century. As a consequence, a decline in total area and an increased fragmentation of many species' habitats have resulted. However, there are species that show naturally disjunct distribution patterns, irrespective of landscape changes, because their specific ecological requirements restrict them to patchily occurring locations. Such "old rare species" (Huenneke 1991; Holderegger 1997) are expected to be adapted to habitat isolation.

Thus, it is assumed that they experience a lower severity of detrimental effects of those population genetic processes that are thought to relate to fragmentation or isolation of populations. However, should a metapopulation network be disrupted, even small and isolated populations of an "old rare species" may experience genetic drift and loss of genetic variation, which might eventually threaten local population persistence. To date, these concepts have rarely been tested empirically (Levin 1995).

In plants, it is mainly herbaceous species in which the effects of population isolation have been studied, whereas there are fewer investigations on woody species (Ellstrand and Elam 1993; Young et al. 1996; Young and Boyle 2000; Frankham et al. 2002). Various reasons account for this bias, e.g., the often short generation cycles and easy

manipulation in field or common garden experiments of herbaceous species. Corresponding studies have shown that isolation negatively correlates with local population size and neutral genetic variation as measured by isozymes or diverse DNA markers (e.g., Lienert et al. 2002). This means that isolated populations tend to be small and contain lower neutral genetic variation in comparison with populations from a more contiguous distribution. In addition, neutral genetic variation may also correlate with plant fitness parameters such as seed set (Fischer and Matthies 1998). The particular life history characteristics of tree species, e.g. long individual life spans, might result in patterns of genetic variation that differ substantially from those found in herbaceous plant taxa. As shown by Hamrick and Godt (1996), such differences are found, for instance, in the partitioning of neutral genetic variation within and among populations. Being long-lived, predominantly outcrossed, and often wind-pollinated and -dispersed, temperate tree species have a large portion of their total neutral genetic variation within populations, whereas differentiation among populations tends to be lower than in herbaceous species. However, this trend is mainly based on studies in widespread tree species. Studies dealing with the partitioning of neutral genetic variation in scattered tree species are still scarce (Young and Boyle 2000) with the majority of the investigated species growing in tropical, as opposed to temperate, areas (but see Oddou-Muratorio et al. 2001).

We selected the English yew (*Taxus baccata*) as a model species to study the genetic effects of population isolation and size in a temperate tree species. In contrast to the situation in many other European countries (Thomas and Polwart 2003), this species is still widely distributed in Switzerland (Barengo et al. 2001), but its populations are often small and naturally isolated from each other because of the species-specific habitat preferences. Forestry practice has negatively affected population number, connectivity, and size during the last two centuries (Paule et al. 1993; Rajewski et al. 2000). Habitat quality has also changed towards reduced light availability in the understory because tree species competing with yew mainly for light grow higher and denser under current forest management (Svenning and Magård 1999). This limits successful natural regeneration

of yew, which is further reduced by browsing (Svenning and Magård 1999; Thomas and Polwart 2003).

A study on the pattern of genetic variation and population differentiation in Switzerland, using random amplified polymorphic DNA (RAPD), has shown that regional differentiation is, at best, marginal, but that populations within regions are highly differentiated (Hilfiker et al. 2004). A similar structure was found in *T. canadensis* by Senneville et al. (2001). Based on the substantial among-region migration rates inferred for *T. baccata* in northern Switzerland, Hilfiker et al. (2004) interpreted the pattern of genetic variation as the result of a regional metapopulation network, maintained by occasional long-distance seed dispersal (cf. Godoy and Jordano 2001; Allnutt et al. 2003), even though explicit reports on extinction or colonization events were not available. Despite this, the high degree of pairwise population differentiation within regions, attributed to the species' peculiar life history traits, led to the conclusion that conservation efforts are necessary to prevent the interruption of metapopulation dynamics and increased isolation in *T. baccata*.

We focused our study on the following questions: (1) Do small populations show effects of genetic drift when compared with large populations? (2) If so, is such an effect expressed as reduced neutral molecular variance in small populations? We used the RAPD data set of Hilfiker et al. (2004) to answer these two questions. In addition, we explored whether the sex ratio may serve as an easily scorable genetic proxy for possible drift effects in *T. baccata*. We therefore asked: (3) Does the sex ratio in yew populations have a greater deviation from an expected 1:1 ratio (Delph 1999) in small than in large populations?

Methods

Species

The natural range of the gymnosperm *Taxus baccata* L. extends from southern Scandinavia to Greece, and from Portugal to the Baltic states (Thomas and Polwart 2003). The taxonomic status

of yew occurring as far east as the Himalayans is disputed (Zoller 1991). In Switzerland, *T. baccata* is widespread in the colline and montane zones. Though most Swiss populations are scattered (Barengo et al. 2001), the species is more abundant in Switzerland than in other parts of its natural range in Europe. Natural gene pools in Switzerland are thus considered important for species conservation from a European perspective (Barengo et al. 2001).

Taxus baccata is a subordinate, patchily distributed species and is often found in the understorey of beech forests (Ellenberg 1988) as a relatively shade-tolerant species (but see Zoller 1991). It is noteworthy, however, that regeneration by seed is restricted underneath dense canopies because seedlings and saplings are light-demanding (Svenning and Magård 1999). This might also hinder persistence of established, undisturbed populations of the evergreen *T. baccata*. In addition, particularly young individuals are susceptible to browsing (Svenning and Magård 1999). The species is strictly outcrossing, given the separation of the sexes among individuals. Its seeds are endozoochorous, with birds as the predominant vectors, whereas pollen is anemophilous

(Zoller 1991). Clonal spread is rare, but polycormic growth after tree felling is possible (Thomas and Polwart 2003).

Sampling

Our sampling scheme adhered to a regional delimitation of the species within Switzerland but excluded the area south of the Alps (Barengo et al. 2001). We selected four regions (Jura, Albis/Zürich, Hörnli/Bodensee, Valais) to sample two pairs of small (≤ 150 individuals) and large populations (> 200 individuals) of *T. baccata* (Table 1). In the central-Alpine valley of Valais, we included only one pair because yew populations are rare there. However, we wanted to consider this region because several species are known to have experienced local population histories differing from those in northern Switzerland (e.g., Mátyás and Sperisen 2001). In early summer 2001, we collected leaf material from 20 individuals per population for genetic analysis. We tried to avoid the sampling of possible ramets of clones by respecting large distances between trees chosen. To assess the populations' sex ratio, all individuals were considered in the small populations. In large popula-

Table 1. Region, location, population code, population group and size, mean deviation from average RAPD band frequencies, molecular variance as taken from the analysis of molecular variance (AMOVA), and sex ratio of the 14 Swiss *Taxus baccata* populations studied

Region	Location	Code	Size	Mean band frequency deviation	Molecular variance	Sex ratio [% females]
Jura	Vorberg	J1	L (650)	0.13	4.40	54
	Montpelon	J2	S (21)	0.18	3.56	71***
	Dottenberg	J3	L (700)	0.12	5.23	55
	Einolte	J4	S (60)	0.15	5.20	62
Albis/Zürich	Uetliberg-Falätschen	A1	L (2500)	0.13	4.48	52
	Elefantenbach	A2	S (29)	0.13	4.50	59
	Scheren/Sihl	A3	L (300)	0.11	4.84	56
	Etzelwald	A4	S (47)	0.13	4.52	68**
Hörnli/Bodensee	Guegenhard	H1	L (1600)	0.14	5.10	50
	Schneitberg/Affenberg	H2	S (68)	0.17	4.13	60
	Hagebuechtobel	H3	L (550)	0.18	5.86	55
	Schoostobel	H4	S (150)	0.19	4.89	49
Valais	Unterer Darewald	W1	L (500)	0.18	5.21	55
	Blindtal	W2	S (35)	0.30	3.56	51

Population sizes (in parentheses) represent estimates of foresters for large (L) populations and census data from direct counts for small (S) populations. Significant deviations from an equal sex ratio per population (χ^2 -tests) are indicated by asterisks; ** $P < 0.01$; *** $P < 0.001$.

tions, we recorded the sex of 100 individuals along several transects across each population.

RAPD-PCR

We performed polymerase chain reactions (PCR) on all samples with four decamer primers. Technical details on the molecular analysis are given in Hilfiker et al. (2004). Repeatability of RAPD banding patterns was tested during initial primer screening and confirmed by consistent amplification even after further DNA extract purification (Wizard DNA clean-up kit; Promega). To endorse consistency in the RAPD-PCRs, we kept aliquots of a single master mix per primer for all samples, only adding *Taq* DNA polymerase and DNA extract before PCR. All PCRs were run on the same thermal cycler (MJ Research PTC-100; Bioconcept). From the 41 reliable marker bands, 7 of which were monomorphic, we obtained RAPD phenotypes for 276 of the 280 individuals collected. In the remaining four individuals, repeated PCR amplification even of purified DNA extracts was not successful. The presence or absence of marker bands from agarose gels were transformed into population and overall band frequencies.

Data analysis

To explore whether deviations from mean marker frequencies differed between small and large populations, we applied a one-sided paired *t*-test, omitting those bands that showed either low (<0.2) or high (>0.8) overall frequencies (Hartl and Clark 1997; Landergott et al. 2001). It is assumed that deviations should be more pronounced in small than in large populations (Hartl and Clark 1997). The same statistic was used to assess differences between the means of molecular variance, representing neutral genetic variation within populations. This measure is based on population sums of squares obtained from the analysis of molecular variance (AMOVA) using the software WinAmova version 1.55 (Excoffier 1993), following Fischer and Matthies (1998). Values of pairwise genetic population differentiation (Φ_{ST} -values, the analogue to F_{ST} ; Excoffier et al. 1992) from the AMOVA were grouped according to the populations involved, i.e., comparisons between small–small, small–large, or large–large populations. These groups served as treatment factor levels in a one-way

analysis of variance (ANOVA) with orthogonal linear contrasts (without assuming equal variances). We checked for deviations from equal sex ratios, assuming binomial distributions, with a χ^2 -test within populations and one-tailed, one-sample Kolmogorov–Smirnov tests in each of the two groups of populations. Subsequently, we tested for a difference between the two groups (small *versus* large populations) with a one-sided Wilcoxon test to examine potential differences in the sex ratio deviance, which we expected to be larger in small than in large populations. Data sets were checked for deviation from the standard normal distribution (Kolmogorov–Smirnov test with Lilliefors adjustment, $\alpha = 5\%$). All statistics were calculated using either SyStat vers. 10 (SPSS 2000) or SPSS vers. 11.0 (Miller and Campling 2002).

Results

Deviations from mean marker frequencies

In the majority of the 34 polymorphic RAPD marker bands used (out of the 41 scorable marker bands), we observed either the lowest or the highest population band frequencies in one or in several (with equal band frequencies) of the small populations of *Taxus baccata* (data not shown). Given this, we expected greater band frequency deviations from the mean frequencies over all populations in the small compared to the large populations. In accordance, we indeed observed that the deviations in the small populations (mean 0.18 ± 0.02 SE) significantly exceeded those found in the large populations (0.14 ± 0.01 , Table 1, Figure 1A; $t = 2.320$, $df = 6$, $P < 0.05$).

Molecular variance

The mean value of molecular variance (Table 1) detected in the small populations (4.34 ± 0.24) was significantly lower than that in the large populations (5.02 ± 0.19 , Figure 1B; $t = 2.987$, $df = 6$, $P < 0.05$). This indicates that genetic diversity is higher in populations with a larger number of individuals.

Pairwise genetic population differentiation

All but two of the 91 pairwise Φ_{ST} -values were statistically significant (Hilfiker et al. 2004). The

Figure 1. Indicators of genetic drift in small versus large populations of *Taxus baccata* in Switzerland. Means are given, vertical bars indicate $\pm$ one standard error. (A) Deviation from overall RAPD marker band frequencies. (B) Molecular variance, serving as an indicator of genetic diversity, as obtained from the sums of squares in an analysis of molecular variance (AMOVA).

ANOVA showed a significant difference among the three groups of population-pair Φ_{ST} -values ($P < 0.001$; Table 2, Figure 2). As seen from the contrasts, population differentiation was higher between those pairs comprising at least one small population as compared to pairs of only large populations ($P < 0.001$; Table 2). Hence, small populations were generally more strongly differentiated than large populations.

Deviation from equal sex ratio

The percentage of females was higher in small populations (mean = 61.8%, range = 49–71%) than in large populations (mean = 53.9%, range = 50–56%). Among all populations sampled, except for one small population, there was an excess of female trees, but only in two small pop-

Table 2. Analysis of variance (ANOVA) using values of pairwise genetic population differentiation (Φ_{ST}), based on RAPD marker bands, in *Taxus baccata*

Source of variation	df	MS	F-ratio	P-value
Groups of pairs	2	0.386	7.524	<0.001
{S-S/S-L}	1	0.211	4.129	<0.001
versus L-L				
S-S versus S-L	1	0.091	1.781	Not significant
Residuals	88	0.051		

Values were grouped according to the population sizes involved in the population pairs (S = small, L = large) and tested using orthogonal linear contrasts. df = degrees of freedom, MS = mean sums of squares.

Figure 2. Φ_{ST} -values of population pairs comprising either two small, two large, or one small and one large population of *Taxus baccata* in Switzerland. For the levels of significance among groups see Table 2.

ulations was this significant (J2, A4; Table 1). The mean percentage of females in both small and large populations significantly deviated from an expected value of 50% (Figure 3; $D_{\max} = 0.464$, $P < 0.05$ for small, $D_{\max} = 0.714$, $P < 0.001$ for large populations). Furthermore, small populations showed a significantly higher percentage of females compared to large populations (Figure 3; $Z = 1.863$, df = 6, $P < 0.05$).

Discussion

The scattered forest tree species *Taxus baccata* shows clear signs of genetic drift in small as com-

Figure 3. Percentage of females assessed in small and large populations of *Taxus baccata* in Switzerland. Means are given, vertical bars indicate $\pm$ one standard error.

pared to large populations in Switzerland. Evidence for this comes from various parameters based on molecular markers and also from observations of population sex ratios. It remains unclear, however, whether there is a direct or indirect causality to these population traits.

The indications of genetic drift observed in the small populations of *T. baccata* in Switzerland, e.g., the tendency towards fixation of marker bands or pronounced differentiation, may be assigned to various causes. Historic population processes, such as founder effects or bottlenecks, are among the reasons that genetic variation was lower in the small than the respective large populations (Figure 1), and genetic differentiation among small and between small and large populations was thus higher (Figure 2). A population bottleneck has the same effect on the total within-population genetic variation as a founder effect, but allele frequencies are expected to level out at intermediate values (Nei et al. 1975). Since the small populations in our sample often showed extreme values of RAPD marker band frequencies, with greater mean deviance than large populations (Table 1, Figure 1A), it is likely that historic founder effects, rather than population bottlenecks, account for the greater part of the lower genetic variation in small *versus* large populations. A positive correlation between population size and neutral genetic variation within populations has been shown for various species (e.g., Fischer and Matthies 1998; Lienert et al. 2002), but there are also studies showing no relation between these two parameters (Ellstrand and Elam 1993; Hoebee and

Young 2001). It has become evident that historic demographic processes may explain the within-population genetic variation better than does the actual population size (Schmidt and Jensen 2000; Landergott et al. 2001). Thus, historic information on the populations studied would help in evaluating which random sampling effect has caused the distribution of genetic variation detected in our study.

Many dioecious plant species are characterized by sex chromosomes. Usually, females are homomorphic, carrying two X-chromosomes, whereas males are heteromorphic, thus containing one X- and one Y-chromosome (Ainsworth 2000; Charlesworth 2002). The most simple sex ratio to be expected in a dioecious species is therefore 1:1, as a consequence of matings between homomorphic and heteromorphic individuals. However, exceptions are frequent in plants, and various mechanisms have been described where extrinsic, i.e., environmental, or intrinsic, e.g., developmental, influences may modify gender expression of a given genotype (Richards 1997).

We found deviations from an even gender distribution in both small and large yew populations. There was almost always a female bias, which was more pronounced in small than in large populations (Table 1, Figure 3). It is not likely that this observation is due to a systematic error during our gender assessment. In the small populations, a complete inventory of the individuals was made, which excludes the possibility of an unrepresentative sample. Our approach taken in large populations was to assess the gender of individuals along transects. Thus, even if individuals of the same gender should be clumped, e.g., if there was substantial vegetative spread, this strategy would have helped to avoid a biased sample.

In yew, the few studies which describe population sex ratios show that they vary between 44.7 and 70% (see review by Thomas and Polwart 2003). The ratio of 2.3:1, which García et al. (2000) observed in one Andalusian population, is significantly female-biased, but it is not clear whether the deviation from unity is also significant. To date, our study is the only one available that relates gender bias to population size and furthermore to genetic variation.

We did not find any information on the regulation of sex determination in yew, e.g., sex chromosomes, which could provide arguments for the

female bias. A sex-specific molecular marker could indicate the likely presence of sex chromosomes (Ainsworth 2000; Stehlik and Blattner 2004). However, we did not score a RAPD band that could be assigned to either of the genders. This would have been highly unlikely given the relatively low number of marker bands assessed. We are therefore only able to elaborate potential causes for our observations on the bias in the sex ratio of *T. baccata*. Theoretically, several factors may be responsible for an uneven population sex ratio. These may be assigned to (i) developmental processes, (ii) environmental effects, (iii) differential fitness, or (iv) population genetic processes.

It is well known that a sex bias, usually towards more females, can result from different developmental fitness. Male pollen may have less siring success than female pollen, or abortion rates might be higher in male than in female embryos. These processes can lead to a gender bias in the seed pool of a given mother plant (certation). Certation has been shown in, e.g., *Rumex acetosa* by the use of molecular markers (Korpelainen 2002). Nothing is known about the occurrence of certation in gymnosperms. Environmental effects on the sex ratio have been observed in dioecious species (Richards 1997). In Spanish *Juniperus communis* populations, a male bias was found which was attributed to variation in environmental stress at different elevations (Ortiz et al. 2002). Even more so, a change in gender may occur as a consequence of an altered environment, e.g., increased light availability after removal of surrounding competitors. Several authors report on the existence of chimerae in *T. baccata* or even a change in sex within individuals (Pridnya 1984; Thomas and Polwart 2003), and cosexual individuals were also observed in the dioecious *T. brevifolia* (DiFazio et al. 1996). In our case, without any specific information on habitat quality, one could argue that the small populations were small simply because they grew in unfavorable habitats. This could directly link environmental effects to possible selective advantages of females, e.g., higher shade tolerance, higher establishment rate of female seeds, or increased vegetative propagation. Given the increased shading in contemporary forests (Paule et al. 1993) and the consequently lower germination rate of yew under dense canopies (Thomas and Polwart 2003), such a gender-related fitness advantage could be highly relevant with respect to sex ratio.

Unfortunately, no experimental data are yet available for *T. baccata*. Thus these suggestions remain purely speculative. However, we are confident that the sex ratios we observed are not exclusively due to environmental effects because they are consistent in space (across regions). On a smaller scale and congruent with findings in *Juniperus virginiana* (Vasiliauskas and Aarssen 1992), no clumping of trees of the same sex was apparent within populations. In addition, it is predicted that, given one gender has a lower fitness, it is always the heterogametic and thus usually the male which is negatively affected (Haldane 1922). Originally deduced for crosses between races or species, Haldane's rule has been applied to within-species sex ratios as well (Richards 1997). If one assumes that females are homogametic in *T. baccata*, the data observed in our study would fit this rule.

Founder effects seem a likely explanation for the greater female bias found in small yew populations in Switzerland. This result is paralleled by those from the RAPD data, where the small populations exhibit genetic characteristics that are indicative of drift. Genetic drift is expected to be manifested in relatively recently colonized populations owing to the random sampling effects of founding individuals. Thus, when taken together, we conclude that indeed founding events have markedly imprinted the current population genetic structure. The supposed metapopulation dynamics of these populations, based on the distribution pattern of genetic variation (Hilfiker et al. 2004), coincides with the population genetic processes assumed above. Local extinction in concert with colonization events after long-distance seed dispersal (cf. Godoy and Jordano 2001; Allnutt et al. 2003) could explain this pattern of population variation as well as the population differentiation found in the present study.

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