Theor Appl Genet (1997) 94:904–908

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Genetic variability in wild cherry populations in France. Effects of colonizing processes

Received: 26 November 1996 / Accepted: 20 December 1996

Abstract Isoenzymes were used to evaluate gene diversity and genetic differentiation among six populations of wild cherry (*Prunus avium* L.) in France. We contrast the genetic characteristics of a population resulting from a recent colonization with those of a much older population of the same species. No significant genetic structure was observed among populations; in this respect wild cherry does not differ from other forest trees. No founder effects could be detected in the newly colonized population. To explain the results, we discuss classic explanations for the lack of genetic differentiation among populations, including balancing selection and neutral drift/migration. In order to account for the absence of founder effects, we propose a hypothesis based on the life cycle of forest trees, namely that the length of the juvenile phase reduces the impact of small numbers of initial founders.

Key words Gene diversity · Genetic differentiation · Founder effect · Isoenzymes · *Prunus avium* L.

Communicated by J. Mackey

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Introduction

Knowledge of population structure, genetic diversity and the mating pattern are essential for the management of a species' genetic resources. Genetic variability at several spatial levels can be characterized by appropriate genetic markers; isozymes in particular provide a powerful tool for the study of basic questions in forest-tree population genetics.

Recent reviews (Hamrick et al. 1992; Kremer 1994) have shown that the distribution of genetic variability in forest-tree species follows a distinct pattern and depends on the markers used in the analysis. For isozymes, trees maintain a high level of genetic diversity within species and within populations. In comparison to herbaceous species, trees generally have low levels of genetic variation among populations. This pattern for forest trees is assumed to reflect a combination of recent historical events, life-history characteristics, and the mating system (Gregorius and Bergmann 1995). To assess the role of colonization events on the characteristics of forest-tree populations, it is useful to compare the genetic structure of young populations that have recently colonized new sites to that of older populations that have occupied the same site for many centuries. Using wild cherry (*Prunus avium* L.) as a study species, we were able to characterize the genetic variation on a regional scale, and to examine the differences between populations according to age.

P. avium L. is a strictly outcrossing forest tree (Crane and Lawrence 1931). Pollination is entomophilous, and seeds are ingested and dispersed by animals. It has a widespread distribution, extending west from eastern Europe to Brittany in France, and from southern England to northern Africa (Meusel 1978). Two previous studies (Santi 1988; Frascaria et al. 1993) based on isozymes indicated very low levels of differentiation among populations (mean Fst = 0.012 and 0.049 respectively). These values are far lower than the mean

Gst = 0.084 reported by Hamrick et al. (1992) for long-lived woody species. Since these earlier studies considered only a limited geographic range, a primary goal of the present work was to extend the analysis throughout France.

We used isozymes to examine genetic variability in six populations of *P. avium* in France. One of the populations was known to have originated from comparatively recent colonization. Our goals were: (1) to provide a more complete description of genetic variation in this species in France, and (2) to compare the characteristics of a newly founded population to those of long-established populations.

Materials and methods

Sampling and geographic distribution of the populations

Sampling was carried out under the direction of CEMAGREF (the French Institute of Agricultural and Environmental Engineering Research), which currently directs a program aimed at managing and conserving the genetic diversity of wild cherries.

Plant material (buds and young leaf tissue) was collected in wild populations in Brittany or for the Grand-Nord, Est, Franche-Comté and Poitou-Charente populations from a collection of clones maintained at Orleans, France. The total sample size for six populations was 232 individuals.

The two samples from Brittany were chosen so as to contrast a long-established population to one of more recent origin. Population no. 1 (n = 92 individuals) consisted of a sample drawn from old forests in Brittany; population no. 2 (n = 47) consisted of individuals from a more recently established population. The latter is located in a military camp (Coëtquidan) created on villager lands in 1873; since that time trees have invaded previously cultivated fields. Wild cherries are found in a forested area covering approximately 285 ha, where the dominant species are oaks [*Quercus petraea* (Mattus.) Liebl. and *Quercus robur* L.] and hornbeams (*Carpinus betulus* L.). The beginning of the colonization process thus dates from about one century.

Electrophoretic analysis

Enzymatic extractions were made using a buffer system adapted from Machon et al. (1995). The eight enzyme systems used in the analysis were aconitase (ACO, E.C. 4.2.1.3), acid phosphatase (ACP, E.C. 3.1.3.2), leucine aminopeptidase (LAP, E.C. 3.4.11.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), phosphoglucoisomerase (PGI, E.C. 5.3.1.9), phosphoglucomutase (PGM, E.C. 5.4.2.2), phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44) and shikimate dehydrogenase (SDH, E.C. 1.1.1.25). ACO, MDH and SDH were resolved using a tris electrode and a gel buffer adapted from Pasteur et al. (1987). PGI, PGM and PGD were resolved using a histidine electrode and a gel buffer adapted from Tanksley and Orton (1983). Lastly, LAP and ACP were resolved using a lithium-borate electrode and a gel buffer adapted from Soltis and Soltis (1989).

Measures of genetic variability in the populations of wild-cherry

Percentages of polymorphic loci and Nei's genetic distances (Nei 1972) were estimated by the software Biosys-1 (Swofford and Selander 1989). The diversity (or average heterozygosity) at each locus (Nei 1973), as well as the average gene diversity and the variance (Nei and Roychoudhury 1974), were calculated. The proportion of the total diversity among populations was measured by the Gst index (Nei 1973).

Comparison of populations in Brittany

Total alleles at each locus and the frequencies of genotypes were compared in both populations. For population no. 2, the genotypes were analysed relative to their geographical location to determine if identical genotypes were clustered.

Gene diversity at each locus, average gene diversities, and Gst were calculated. Average gene diversities were compared. In order to detect differences between allelic frequencies in population no. 1 and population no. 2, χ^2 tests were performed.

Results

Enzymatic markers

The eight enzymatic systems revealed 14 loci: one locus each for ACP, LAP and SDH, two loci each for ACO, MDH, PGD and PGI, and three loci for PGM. ACO-1, MDH-2, PGD-2, PGI-2, PGM-1 and PGM-3 were monomorphic over all the populations. Each of the remaining, polymorphic, loci had precisely two alleles.

Genetic variability of wild cherry in France

The mean number of alleles per locus over all French populations was 1.4 (Table 1), with no significant differences between populations. The mean percent polymorphic loci was 33.2%. The most diverse region was Poitou-Charente (42.9% of polymorphic loci) and the least diverse was Grand-Nord (21.4%). Differences between regions were not statistically significant. Mean values of Nei's gene diversity between populations (0.111; see Table 1) and estimated Gst (0.052) were both low, indicating a limited level of differentiation among the populations.

Comparison of populations in Brittany

Both samples from Brittany contained the same number of alleles. Considering genotypes, the two homozygotes and heterozygote were observed for ACO-2, ACP and SDH. For MDH-1, PGD-1 and LAP, the rarer homozygote was only present in population no. 1, but for LAP the heterozygote was only found in the younger population (no. 2). For PGI, the rarer homozygote was observed in neither sample. In population no. 2, 27 different genotypes were found (n = 47 analysed individuals).

Comparison of average gene frequencies (Table 2) indicated a slight loss of diversity, but standard statistical tests showed no significant differences between

Table 1 Genetic variability at 14 loci in five populations of wild cherry

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of polymorphic loci[b]	Average gene diversity[c]
Brittany[a]	92.0 (0.0)	1.5 (0.1)	35.7	0.118 (0.043)
Grand-Nord	15.4 (1.0)	1.3 (0.1)	21.4	0.093 (0.048)
Est	19.5 (0.0)	1.4 (0.1)	35.7	0.131 (0.052)
Franche-Comté	20.1 (1.1)	1.4 (0.1)	28.6	0.103 (0.042)
Poitou-Charente	28.8 (1.2)	1.5 (0.1)	42.9	0.109 (0.035)
Mean	35.2	1.4	33.2	0.111
Gst	0.052			

[a] For Brittany, individuals from Coëtquidan (the newly-colonized population) were not considered
[b] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95
[c] Unbiased estimate (see Nei 1978). Standard errors in parentheses

Table 2 Comparison of populations 1 and 2 in Brittany. Standard errors in parentheses

Locus	Gene diversity no. 1	Gene diversity no. 2
ACO-1	0	0
ACO-2	0.316	0.271
ACP	0.382	0.395
LAP	0.022	0.062
MDH-1	0.336	0.157
MDH-2	0	0
PGD-1	0.377	0.225
PGD-2	0	0
PGI-1	0.074	0.042
PGI-2	0	0
PGM-1	0	0
PGM-2	0	0
PGM-3	0	0
SDH	0.141	0.326
Average gene diversity	0.118 (0.043)	0.106 (0.038)
Gst	0.014	

Table 3 χ^2 tests comparing allele frequencies between population no. 2 and population no. 1

Locus	Item	Population no. 2/ population no. 1	Significance
ACO-1		NC	ns
ACO-2	*df*	1	ns
	χ^2	0.704	
	P	0.40	
ACP	*df*	1	ns
	χ^2	0.008	
	P	0.09	
LAP	*df*	1	ns
	χ^2	1.56	
	P	0.21	
MDH-1	*df*	1	*
	χ^2	7.126	
	P	0.01	
MDH-2		NC	ns
PGD-1	*df*	1	*
	χ^2	5.64	
	P	0.02	
PGD-2		NC	ns
PGI-1	*df*	1	ns
	χ^2	0.884	
	P	0.35	
PGI-2		NC	ns
PGM-1		NC	ns
PGM-2		NC	ns
PGM-3		NC	ns
SDH	*df*	1	*
	χ^2	8.431	
	P	0.04	

NC: non-calculable. ns: non-significant
* $0.01 < P < 0.05$

means. Considering the allelic frequencies and sample sizes, no significant differences were observed between the two populations for 11 of the 14 systems studied (Table 3). χ^2 tests gave significant differences for MDH-1, PGD-1 and SDH. The low value of Gst (0.014) indicates very little differentiation between the populations.

Discussion

Our results for wild cherry are consistent with those obtained via isoenzymatic markers for forest trees in general: little differentiation among populations and a comparatively high level of genetic diversity. The lack of differentiation we observed agrees with the low mean Gst (0.051) given by Hamrick et al. (1992) for trees with ingested seeds (Table 4). Nevertheless, mean Gst for

Table 4 Levels of allozyme variation within populations and among populations of long-lived woody species according to the breeding system and the means of seed dispersal (from Hamrick et al. 1992)

Categories	Hep[a]	Gst[b]
Breeding system		
Selfing	–	–
Mixed-animal	0.035	0.122
	(0.013)	(0.038)
Mixed-wind	0.110	–
	(–)	
Outcrossing-animal	0.163	0.099
	(0.011)	(0.017)
Outcrossing-wind	0.154	0.077
	(0.008)	(0.009)
Seed dispersal		
Gravity	0.141	0.131
	(0.012)	(0.022)
Gravity-attached	0.104	0.099
	(0.017)	(0.024)
Attached	0.144	0.065
	(0.027)	(0.035)
Explosive	0.072	0.092
	(0.036)	(–)
Ingested	0.208	0.051
	(0.022)	(0.010)
Wind	0.149	0.076
	(0.008)	(0.009)

[a] Hep: genetic diversity within populations calculated for monomorphic and polymorphic loci
[b] Gst: proportion of the total diversity among populations. Standard errors are in parentheses

outcrossing, animal-dispersed, trees is much greater (0.099). Our calculated values for gene diversity within populations (Hep) are far lower for trees with ingested seeds (0.208) and for trees which are outcrossing/animal dispersed (0.163). This result could, however, be due to the relatively small number of polymorphic loci we analysed.

There are two classical explanations for the lack of differentiation between populations. One is that the loci are under balancing selection (e.g. overdominance), which maintains the allelic frequencies at similar proportions in different locations. This could apply to tree populations in the case of a limited number of loci, or to trees located in comparable ecological conditions. However, the fact that the majority of markers in all species studied show the same low level of differentiation makes this hypothesis difficult to accept as a general explanation. The neutral theory of molecular evolution (Kimura 1983) suggests that even a very low rate of migration (a few individuals per generation) is sufficient to homogenize populations once the drift/migration equilibrium is attained. This explanation is currently the most prevailing one (Hamrick et al. 1992). However, its explanatory power is limited concerning the pattern seen in forest trees. According to the neutral hypothesis, there should be at least some differentiation between tree species due to varied dispersal ability. Also, the theoretical results are valid only at equilibrium, and forest-tree populations have certainly not reached equilibrium since the post-glacial re-colonization of temperate continents.

Consequently, there may be characteristics of forest trees that allow them to colonize progressively larger territories without losing genetic variation. We offer as one possible explanation the length of the juvenile stage. In most plant species, the loss of variation in newly colonized sites results from the small number of initial founders. Generally, the founders determine the genetic make up of the next generation. Subsequent migrants will not much influence the next generation if their numbers are negligible compared to the progeny of installed individuals. The length of the juvenile stage in trees, however, is such that once the first seeds have germinated, the next cohort is again a cohort of migrants. More importantly, subsequent migrants will have fully invaded the site before the initial colonists have started reproducing. The foundation of a new population by forest trees thus may involve little or no founder effect. A new population contains virtually all the diversity of the source(s) of migrants before individuals start to reproduce.

Our comparison of a newly established population to one that has occupied the same territory for centuries helps us assess this hypothesis. The same alleles were found in both populations, and genetic diversity parameters are nearly identical. The younger population has kept all the genetic characteristics of the older, there are no detectable founder effects. Although some genotypes were found in a limited area, this is probably due to the effect of vegetative reproduction in *P. avium*; Frascaria et al. (1993) showed that genetically homogeneous groups can cover about 0.5 ha. A particularly low Gst among our populations from Brittany is easily explained by their geographical proximity. Lastly, this analysis was done with allozyme markers; cytoplasmic markers would probably indicate a greater level of differentiation.

In general, the results support our hypothesis. Since *P. avium* L. is an entomophilous species, high pollen flow (which would homogenize the populations) is improbable, even if wild cherry pollen is easily located by insects (Frascaria et al. 1993).

In the present paper, we have described genetic variability in *P. avium*, and have compared the genetic characteristics of a newly colonized population with those of a long-established population. The life history of trees is one possible explanation for the genetic structure seen in forests. Moreover, it provides insights into the process by which trees have re-colonized continents. If our hypothesis is correct, the data suggest that large numbers of migrants have become established in new sites before reproduction has occurred. This should be taken into account in any scenario of re-colonization, because it will have a significant impact on the development of genetic structure in forest-tree

populations, and thus on the evolution of biodiversity following global climate change.

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