Biologia, Bratislava, **59**/4: 493—500, 2004

Mutual links of demographic and genetic processes in a wild cherry population during the colonization

Dušan Gömöry

Technical University in Zvolen, Faculty of Forestry, T.G. Masaryka 24, SK–96053 Zvolen, Slovakia; tel.: ++421-45-5206 226, fax +421-45-5332 654, e-mail:gomory@vsld.tuzvo.sk

Gömöry, D., Mutual links of demographic and genetic processes in a wild cherry population during the colonization. Biologia, Bratislava, **59:** 493—500, 2004; ISSN 0006-3088.

The development of allelic and genotypic structures during the colonization was investigated in a wild cherry (*Prunus avium* L.) population, which spread over abandoned pastures and meadows at the locality Kráľová in Central Slovakia, using isozyme markers. During the population expansion, allelic richness increased. The observed and expected heterozygosities overcame a considerable fluctuation, although the difference between the initial and final stages is quite small. Significant changes of allelic frequencies were observed at one allozyme locus (*Pgm-A*). The individuals forming clonal groups exhibited significantly different allelic frequencies and lower levels of genetic diversity as compared with non-cloning ones. Relationships between the demographic expansion and the development of genetic structures are discussed.

Key words: secondary succession, genetic structure, cloning, *Prunus avium* (L.) L.

Introduction

Most pastures and meadows in the colline and montane belts of Central Europe are secondary communities, which arose after the original forests had been removed. Like other artificial ecosystems, they need to be maintained through an input of energy. Large areas of pastures and meadows have been abandoned in Central Europe due to changes in agricultural systems in the past 50 years. When mowing and grazing cease, and young trees and shrubs are no more destroyed or limited in their growth, the communities begin to change spontaneously towards the climax. Pastures and meadows are rarely a homogeneous grassland. Most frequently, they are crossed by balks, creeks, or they contain places inaccessible for agricultural use because they are rocky, steep etc. These places are mostly covered by woody vegetation rich in species, which, together with individual solitary older trees and neighbouring forest stands, may serve as a starting point of the later colonization.

Secondary succession, as a sequence of colonization of the same space by different species, is not only an ecological process, but it has a genetic component as well (Austerlitz et al., 2000; Gray, 1987). Population size and genetic structure of plant species change considerably among succession stages. In particular the gene pools of tree species, which often survive several generations of different phases of succession, evolve during the colonization. In case of rare woody species or those, which appear after long-distance colonization events, genetic structures are influenced by founder effects (Haase, 1993; Demesure et al., 2000; Ledig, 2000), accompanied first of all by allele loss (Cornuet & Luikart, 1997). Subsequently, during the population expansion allelic

richness increases again due to gene flow.

Wild cherry (*Prunus avium*) is one of the woody species, which can participate in such colonization. In Central Europe, wild cherry is a species occurring at lower altitudes, mainly in oak and beech forests. It is an entomophilous self-incompatible species. Seeds are ingested and dispersed by animals, mainly birds, and sometimes they can be transported over large distances. The lack of differentiation at cytoplasmic maternally inherited markers indicates that seed dispersal plays a much more important role in gene flow as compared to pollen dispersal than in widespread broadleaved forest tree species (MOHANTY et al., 2001). Wild cherry occurs in several succession stages. Owing to its considerable capacity of a rapid vegetative propagation through root suckers, it behaves as a colonizing species of initial succession stages. In Slovakia, it frequently occupies abandoned pastures, balks and rocky sites in the agricultural land, often forming large groups or even discontinuous stands. On the other hand, it is a component of climax or close-to-climax mesophilous forest ecosystems (DUCCI & PROIETTI, 1997). In this case, however, it grows mostly scattered or in small groups. This study focuses on the first type of wild cherry population. Its aim is to illustrate how allelic frequencies, allelic richness and diversity of allozyme genes change during the colonization process.

Material and methods

A population of wild cherry at the locality Kráľová in central Slovakia was investigated. At this site, pastures and meadows, formerly used for cattle grazing and/or mowed, were partially abandoned, and partially they are utilized as ski pistes (Fig. 1). They are crossed by several rocky balks, formed of stones removed from meadows to allow mowing. Wild cherry predominates mainly on these balks, forming here almost parallel lines. However, it occurs also on a stony ridge in the central part, although here other tree species predominate (Turkey oak, common beech, Norway spruce, silver fir). This central part has probably never been utilized for agriculture (maybe for sheep and goat grazing). Neither wild cherry nor the other tree species have ever been exploited for timber, since the individuals have rather an appearance of solitary trees than trees in a forest stand so that they are phenotypically inferior (large crowns with thick branches, short crooked trunks). No stumps indicating a former logging have been found on the area and even dead or broken trees have not been removed.

In this population, all fruit-bearing individuals (153 trees) were sampled. During the winter season 1998/1999, twigs with dormant buds were taken and the buds were used for analysis. At the same time, breast-height diameters (DBH) of the sampled trees were measured and their spatial position was determined from an aerial photograph.

Green tissue from buds was homogenized in 0.1 M Tris-HCl extraction buffer pH 7.3 with PVP 40, EDTA II, Tween 80, PEG 200, 2-mercaptoethanol (1% each), 0.025% DTT and 0.5% Na-ascorbate (LONGAUER, 1996). For enzyme separation, 12% w/v starch gels were used, employing three buffer systems (0.028 M Li-borate – 0.051 M Tris citrate pH 8.1, 0.05 M Na-borate pH 8.0–0.075 M Tris-citrate pH 8.7, and 0.05 M Tris-histidine – 0.125 M Tris-citrate pH 7.0). Staining followed CHELIAK & PITEL (1984). Twelve enzyme systems were used. They are controlled by 19 loci, but only 7 of them were polymorphic. Mendelian inheritance of the loci used was verified by KAURISCH et al. (1989) and SANTI & LEMOINE (1990).

It was not possible to determine the age of all individuals from drill cores. Therefore, we reconstructed the course of colonization on the basis of the breast-height diameter distribution. Following MEIER (1984) and SPIECKER (1994), the diameter growth of wild cherry up to 80 years is almost linear. We took drill cores from 16 trees of various heights and diameters. Based on these drill cores, we developed a diameter-increment curve, which was used to determine the age of individuals from the DBH.

The diameter-class distribution of flowering trees is presented in Fig. 2. Around 40 cm, the distribution becomes flat, and even a minor peak at 51 cm class is visible. This indicates the presence of different generations. Therefore, we considered all trees with diameter of 40 cm and more to represent the founder generation. The course of colonization was simulated by subsequent addition of 1 cm diameter classes to the population. Allelic frequencies of each developmental stage of the population were calculated based on diploid genotypes. Genetic variation was characterized by the mean number of alleles per locus, observed and expected heterozygosities.

Although the population was sampled exhaustively, the presence of further individuals at any developmental stage can not be excluded. Therefore, we treated every developmental stage as a sample, not as a population. Heterogeneity of allelic frequencies among developmental stages and/or genotype sets was tested using exact binomial tests following RAYMOND & ROUSSET (1995).

Results

Demographic development of the wild cherry population

As can be seen in Fig. 1, the parts of the area, which are used as ski pistes, are still maintained, they are regularly mowed, and trees and shrubs, which may disturb skiers, are removed. However, the other areas are abandoned and a gradual succession to forest has started here. Climax forest at sites of this type is dominated by European beech

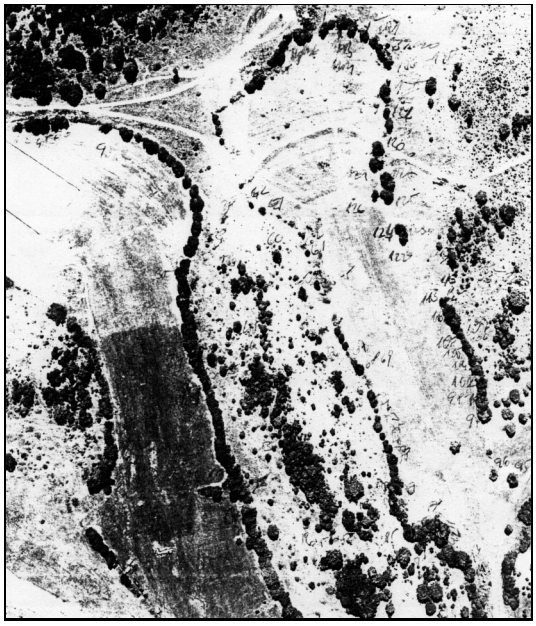

Fig. 1. Aerial photograph of the investigated area.

and sessile oak. Nevertheless, these areas are at an early stage of succession. Grasses predominate in the herbaceous layer. Junipers (*Juniperus communis*) spread rapidly in the eastern part, whereas the central part becomes covered by broadleaved shrubs and young trees (*Crataegus* sp., *Rosa* sp., *Quercus cerris* etc.), although junipers occur also here.

The gradual expansion of the investigated wild cherry population is presented in Figs 3a-d. At the initial stage, the cherry trees are distributed on more or less continuous line (Fig. 3a), which is formed of a stony balk on the western edge, and some trees are dispersed in the central part (a ridge covered by a grove with predominance of oak, beech and other broadleaves). Balks bordering the central ridge became colonized much later (Figs 3c-d). At the most recent stages cherry expands also over the area, out of lines (Figs 3d and 4).

Fig. 4 displays the distribution of putative clones. A total of 27 repeatedly occurring genotypes were identified. Some clonal groups are quite large extending over tens of meters. However, it must be emphasized that clones were identified on the basis of only 8 polymorphic loci, out of which 6 loci have only 2 alleles. The discrimination power of such a set of genes is surely quite low; some of the supposed clones probably consist of two or more genotypes. Clonal groups are clearly unevenaged; some individuals may represent secondary or tertiary suckers. Two clones were found also in the founder population.

To assess how the clonal structure developed during the colonization, we evaluated the ratio of number of different genotypes to the census number of population at each stage. This proportion decreases steadily without important fluctuations from 82 to 56%. No stage with an abrupt expansion of clonal groups and/or seedlings was identified; apparently, both ways of regeneration occurred during the colonization.

Development of the genetic structures

As shown in Table 1, there are no dramatic changes in the representation of alleles between the initial stage (founder population) and the present state at most loci. However, a unidirectional development can be identified in case of two biallelic loci: *6pdg-A* and *Pgm-A* (Fig. 5). In *6pgd-A*, the frequency of the *100* allele increased by almost 15%, but the differences between the developmental stages are non-significant (although approaching the conventional statistical significance

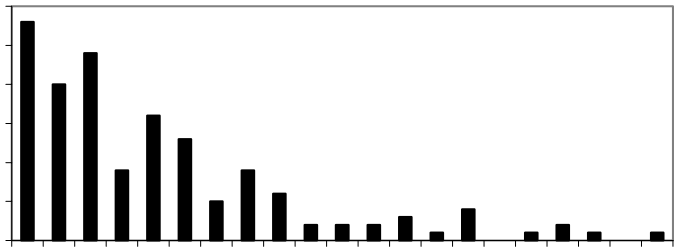

Fig. 2. Diameter class distribution in the investigated wild cherry population.

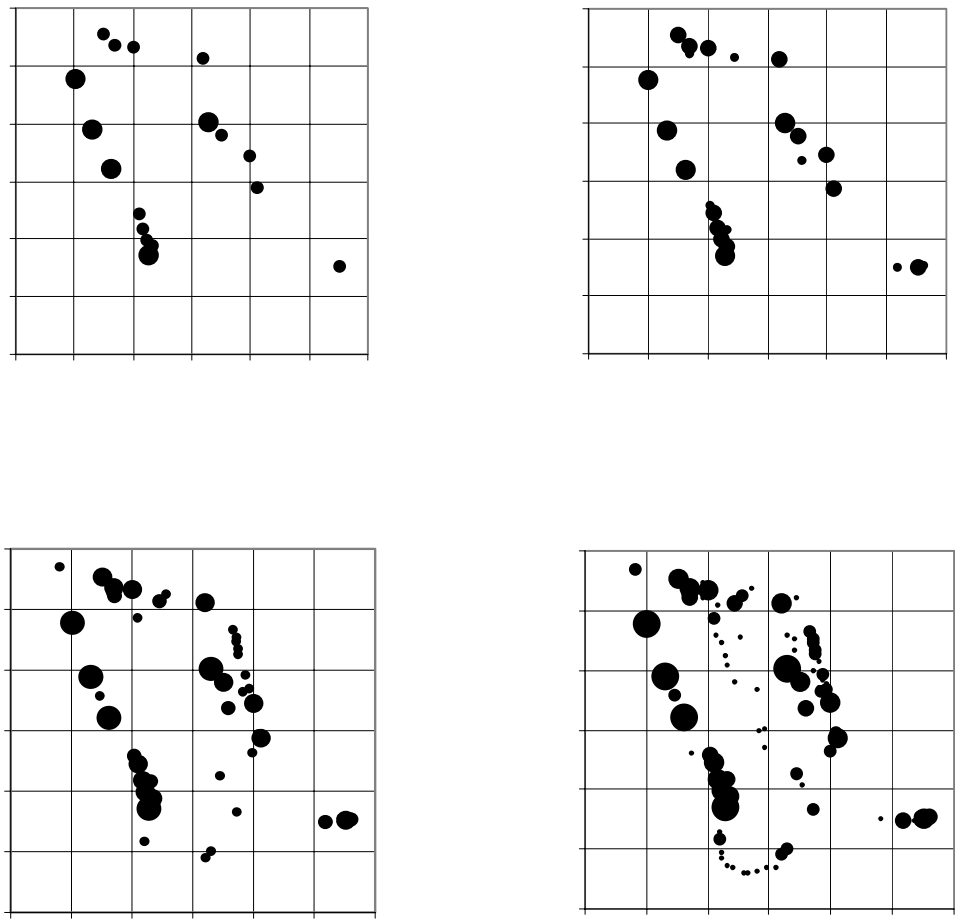

Fig. 3. Distribution of trees during the development of the population: (a) founder population, (b) at −40 years, (c) at −30 years, (d) at −20 years. Circle sizes correspond to diameter.

limit of 0.05). A clearer trend can be seen in *Pgm-A*. The frequency of the *100* allele increased from 0.250 to 0.592, i.e., more than twice. The allelic structure at this locus in the present stage differs significantly not only from the founder population, but from later stages as well.

As expected, allelic richness increases along with increasing population size (Fig. 6). Three rare alleles (*Idh-B/73, Pgi-B/86* and *Pgi-B/72*), which are absent in initial stages, appear later during the colonization. All these alleles were found exclusively in genets, not in ramets, indicating that they had not been present in the population at early stages and were subsequently imported by seeds from outside sources. However, as stated before, the presence of further individuals, which aborted or were removed from the population can not be excluded, therefore, the observed increasing trend of the allelic richness must be interpreted with caution.

There is quite a small difference in the observed heterozygosity between the founder population and the present stage, but there is an interesting development during the colonization process. The observed heterozygosity decreases in the initial phases of the population expansion, reaching the minimum at −38 years. Subsequently, it increased rapidly within the next 3 years. This rise coincides with the first appearance of two new alleles. After that, increase of the observed heterozygosity is very slow. The development of the expected heterozygosity is similar, although the am-

Table 1. Allelic frequencies at polymorphic loci in the founder population and the present population, and in the sets of individuals forming clonal groups and non-cloning ones.

Locus	Allele	Population		Genotype set		
		founder	present	cloning	non-cloning	$P^{1)}$
Idh-B	*87*	.382	.412	.463	.288	0.023
	80	.618	.565	.537	.653	
	73	.000	.024	.000	.059	
Pgm-A	*100*	.250	.592	.519	.491	0.865
	85	.750	.408	.481	.509	
Aco-B	*85*	.367	.441	.500	.321	0.067
	73	.633	.559	.500	.679	
6pgd-A	*100*	.588	.714	.722	.627	0.295
	80	.412	.286	.278	.373	
Fest-A	*153*	.059	.095	.037	.186	0.008
	100	.941	.905	.963	.814	
Got-C	*40*	.353	.316	.315	.297	0.856
	26	.647	.684	.685	.703	
Pgi-B	*128*	.000	.003	.000	.008	0.073
	114	.088	.119	.074	.203	
	100	.912	.874	.926	.780	
	86	.000	.003	.000	.008	
Mdh-A	*100*	.941	.976	.981	.958	0.668
	87	.059	.024	.019	.042	

1) Exact probability test of difference in allelic frequencies (Rousset & Raymond, 1995)

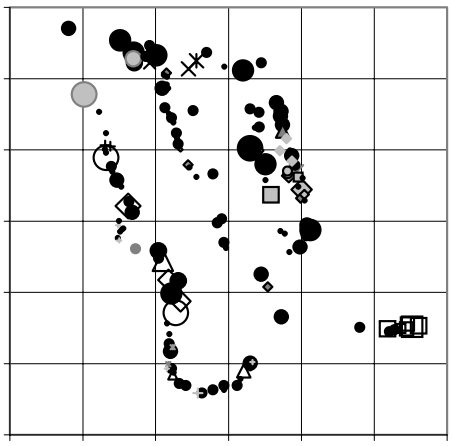

Fig. 4. The present distribution of individuals and clonal groups among fruit-bearing trees in the investigated wild cherry population. Full black circles – unique genotypes, other symbols – putative clones. Symbol sizes correlate with DBH.

plitude is much less pronounced. The minimum is achieved earlier, at −44 years, and after the maximum at −36 to −30 years, the level of the expected heterozygosity becomes more or less stabilized. There does not seem to be any association of this development with the formation of clonal groups.

Since the ability of vegetative propagation is an important feature of wild cherry, we looked at differences in the allelic and genotypic structures between sets of individuals with unique genotypes (apparently genets) and those occurring in the putative clonal groups. Obviously, some individuals of the first group may be able of suckering, but they have not proven this capability. There are considerable differences between both groups. Despite small sample sizes, significant differences in allelic frequencies were found at two out of eight polymorphic loci (Tab. 1), whereby at two further loci, the probability is less than 0.10. Putative ramets posses less alleles (mean number of alleles is 1.42 in contrast to 1.58 in the set of genets), and are genetically much less diverse (expected heterozygosity of 0.138 vs. 0.162 in genets).

Discussion

The designation of the wild cherry population at the initial stage as "founder population" is not exact. Very probably, wild cherry had been present in this locality already when the area was used for agriculture, since it occurs in neighbouring for-

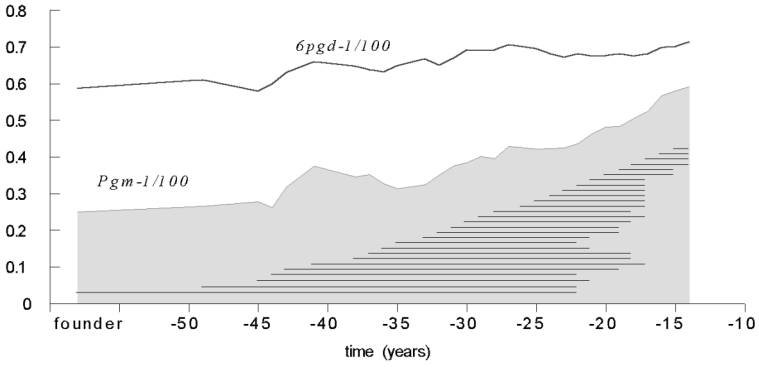

Fig. 5. Development of allelic frequencies at *Pgm-A* and *6pgd-A* loci during the colonization. Horizontal bars indicate non-significant differences of allelic frequencies at *Pgm-A* among developmental stages.

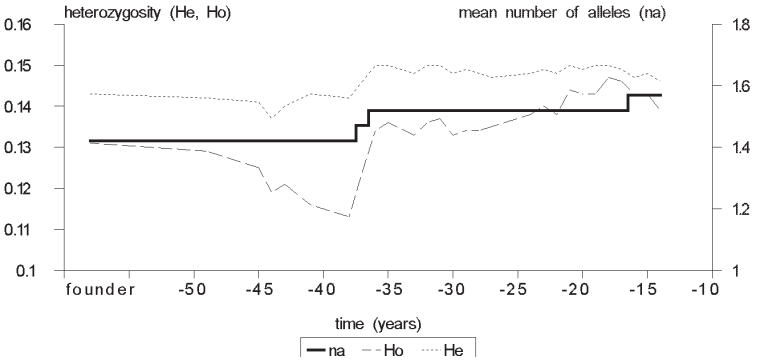

Fig. 6. Development of heterozygosity and allelic richness in the wild cherry population during the colonization.

est stands and cherry cultivars are grown in a near village. Until the mid 1950's, the whole area was intensively used by private farmers. Following collectivisation, the original small-scale structure of fields, meadows and pastures disappeared. Between 1960 and 1970, larger meadows and pastures arose, from which stones were removed to allow mowing by machinery. Most stones were piled to balks which bordered the agricultural land and which could have potentially provided niches for individual wild cherry trees. Mowing and cattle and sheep grazing did not allow to extend regeneration outside of these balks. In the eighties, agricultural use of this area was abandoned. On the meadows, two ski lifts were built, and all young trees and shrubs (including cherry seedlings and suckers) were removed. The other areas, however, have nor been managed since this time. This permitted a rapid spread of shrubs and trees, including cherry.

The ability of natural vegetative regeneration by suckering is surely one of the factors, allowing such a substantial increase of population census number within a short time period of one generation. Wild cherry forms very large clonal clusters extending over tens of meters, covering an area up to 0.5 ha, and containing from two to several tens of ramets. When cherry occurs in mixed high forests, clonal groups are mostly continuous, in pure stands; they are frequently overlapping (Frascaria et al., 1993; Ducci & Santi, 1997). In our case, the length of the largest group of individuals with a single genotype was approx. 240 m. The putative clones overlapped spatially. On one hand, this can be explained by different reproduction strategy as compared with a mixed high forest. In beech or oak stands, which are generally quite dense, there is rather little space for light-demanding wild cherry, so individual trees, growing from animal-transported seeds are widely dispersed. However, when these trees start to propagate, suckers, which can use the root systems of the parent tree at initial life stage, have a higher survival probability than seedlings, so that small continuous clonal groups are formed. Colonization of a forest-free area represents a different situation. There are more genets at start, and even if there is a root competition, sucker groups may grow one into another and acquire various forms. On the other hand, it is questionable if the number of the analysed loci and their polymorphism are sufficient for a reliable identification of clones.

FRASCARIA et al. (1993) did not include all individuals of the same genotype into a clonal group, if they had extended over unrealistic distances or if they had been separated by too many individuals of a different genotype.

Seed dispersal through animal ingestion also contributes to the capability of a rapid colonization of appropriate sites. Despite the fact that most seeds fall below the crown, birds and small mammals transport ingested seeds to places with favourable conditions, sometimes over large distances, so that even if the share of cherry in the neighbouring forests is very low, there is enough parental individuals available.

Genetic structures changed considerably during the colonization process. The greatest change in allelic frequencies was observed at the *Pgm-A* locus. We assume that in the founder population, allelic frequencies at this locus were deformed by a restricted population size, and that they developed towards the mean frequency distribution in the surrounding metapopulation. Unfortunately, we analysed only this single population, and phospohoglucomutase has not been generally used in the surveys of genetic variation in wild cherry, so that we cannot compare with other studies. The observed heterozygosity fluctuated between 0.113 and 0.147. There is an apparent decrease in the initial stages of colonization. The most plausible explanation is the expansion of highly homozygous clonal groups. However, empirical data do not support this hypothesis. Clonal groups are clearly uneven-aged, and there is no concordance between the curves of heterozygosity and the proportion of clones in the population. Another possibility is the expansion of half-sib families with a higher level of inbreeding. Considerable differences in the proportion of mating between relatives among families were found in this population (GÖMÖRY & PAULE, 2001).

A slight increase of the allelic richness was observed. Three new alleles appeared in the population during the colonization. However, these alleles are rare (frequency below 5%), so that we cannot exclude that they had been present also in the founder population. Allelic richness and genetic diversity of the present population seems to be comparable with that of the other European wild cherry populations (FRASCARIA et al., 1993; DUCCI & PROIETTI, 1997). MARIETTE et al. (1997) found that a newly colonized adult population possessed the same levels of genetic multiplicity and diversity as an old established one. Our results also indicate that within one generation, a recently expanded population is able to achieve allelic richness levels common for this species thanks to an efficient gene flow through seed dispersal and pollen transport from the surrounding stands and gardens in the near village.

Acknowledgements

This study was supported by research grants No. 1/7056/20 and 1/0126/03 from the Slovak Grant Agency for Science. The use of aerial photograph was kindly permitted by the Institute of Forest Management, Zvolen. The help of Dr. Ján ĎURSKÝ and Mrs. Anna MIKOVÁ, Department of Forest Management of the Technical University in Zvolen, with the evaluation and measurement of core drills is also appreciated.

References

AUSTERLITZ, F., MARIETTE, S., MACHON, N., GOUYON, P.-H. & GODELLE, B. 2000. Effects of colonization processes on genetic diversity: Differences between annual plants and tree species. Genetics **154:** 1309–1321.

CHELIAK, W. M. & PITEL, J. A. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Information Report PI-X-42, Petawawa National Forest Institute, Chalk River.

CORNUET, J. M. & LUIKART, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics **144:** 2001–2014.

DEMESURE, B., LA GUERROUÉ, B., LUCCHI, G., PRAT, D. & PETIT, R.-J. 2000. Genetic variability of a scattered forest tree: *Sorbus torminalis* L. (CRANTZ.). Annales des Sciences Forestières **57:** 63–71.

DUCCI, F. & PROIETTI, R. 1997. Variabilitá alloenzimatica nel ciliegio selvatico (*Prunus avium* L.) in Italia. Annali del Istituto Sperimentale della Selvicoltura **25-26:** 81–104.

DUCCI, F. & SANTI, F. 1997. The distribution of clones in managed and unmanaged populations of wild cherry (*Prunus avium* L.). Can. J. Forest Res. **27:** 1998–2004.

FRASCARIA, N., SANTI, F. & GOUYON, P. H. 1993. Genetic differentiation within and among populations of chestnut (*Castanea sativa* MILL.) and wild cherry (*Prunus avium* L.). Heredity **70:** 634–641.

GÖMÖRY, D. & PAULE, L. 2001. Spatial structure and mating system in a wild cherry (*Prunus avium* [L.] MOENCH.) population. Biologia, Bratislava, **56:** 117–123.

GRAY, A. J. 1987. Genetic change during succession in plants, pp. 274 293. In: GRAY, A. J, CRAWLEY, M. J. & EDWARDS, P. J. (eds), Colonization, succession and stability. Blackwell Scientific Publications, Oxford.

HAASE, P. 1993. Genetic variation, gene flow, and the "founder effect" in pioneer populations of *Nothofagus menziesii* (Fagaceae), South Island, New Zealand. Journal of Biogeography **20:** 79–85.

Kaurisch, P., Hackenberg, E. M., Gruppe, W. & Köhler, W. 1989. Enzympolymorphismen bei Kirscharten (*Prunus* spp.) der Sektion *Eucerasus*, *Pseudocerasus* und *Mahaleb*. Angewandte Botanik **63:** 533–542.

Ledig, T. 2000. Founder effect and the genetic structure of Coulter pine. Journal of Heredity **91:** 307–315.

Longauer, R. 1996. Genetic diversity of silver fir (*Abies alba* Mill.). Ph.D. thesis, Technical University in Zvolen, Zvolen.

Mariette, S., Lefranc, M., Legrand, P., Taneyhill, D., Frascaria-Lacoste, N. & Machon, N. 1997. Genetic variability in wild cherry populations in France. Effects of colonizing processes. Theoretical and Applied Genetics **94:** 904–908.

Meier, S. 1984. Über einen Vogelkirschenreinbestand in Schleswig-Holstein. Forst- und Holzwirt **39:** 233–235.

Mohanty, A., Martin, J. P. & Aguinagalde, I. 2001. A population genetic analysis of chloroplast DNA in wild populations of *Prunus avium* L. in Europe. Heredity **87:** 421–427.

Raymond, M. & Rousset, F. 1995. An exact test of population differentiation. Evolution **49:** 1280–1283.

Santi, F. & Lemoine, M. 1990. Genetic markers for *Prunus avium* L.: inheritance and linkage of isozyme loci. Annales des Sciences Forestières **47:** 131–139.

Spiecker, M. 1994. Wachstum und Erziehung wertvoller Waldkirschen. Mitteilungen der Forstlichen Versuchs- und Forschungsanstalt Baden-Württemberg, Freiburg i.B., Heft 181.

Received May 15, 2003
Accepted April 27, 2004