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論 文 題 目 Quantitative conservation genetics of a small population of Sakhalin spruce（*Picea glehnii* Mast.）

（マツ科針葉樹アカエゾマツ（*Picea glehnii* Mast.）の小集団を対象とした定量的保全遺伝学）

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1. Introduction

Genetic aspects had been considered in conservation biology in the last decades. Small populations of trees are under threats of degradation and extinction due to genetic consequences. Stochastic loss of genes (also called "bottleneck effect") leads to the loss of adaptive potential to adapt environmental changes. At the same time, increased inbreeding results in the decline of population fitness through the accumulation of inbreeding. Molecular markers and several population genetics indicators provide primary quantitative measures to assess the genetic threat of a small population. Nevertheless, we know little about the relationship between genetic indicators and population integrity. Few quantitative guidelines for genetic conservation and management were available yet.

On the contrary, non-genetic conservation biology has addressed the quantitative assessment of population integrity. The methodological framework of population viability analysis (PVA) has been developed and applied particularly in wildlife management. PVA is not a general framework; rather it is an *ad hoc* simulation of population dynamics, which based on the observation of demography such as birth, reproduction and mortality in a focal population. Therefore, it allows realistic prediction of the viability of the focal population, and under the multiple management scenarios. PVA could include some genetic processes such as inbreeding depression, although few studies addressed genetic PVA.

In this thesis, I developed a genetic PVA for a small population of tree. Trees are long living organism: observation of entire demographic stages, which is often crucial in demographic PVA, is extremely difficult. Instead, inferring key ecological processes from available limited data is plausible challenge. Using the statistical models, I estimated two processes that shape the future genetic variability: mating and the selection against inbred offspring. Then, I conducted the simulation of future genetic variability and fitness based on the results from the statistical models.

I conducted the study in a small population of Sakhalin spruce (*Picea glehnii* Mast., Pinaceae) in Mt. Hayachine, northern Japan. Sakhalin spruce is a monoecious, wind-pollinated conifer, and distributes in the boreal region of East Asia (northern Japan, Kuril Islands and Sakhalin). Study population is located at the southern end of the species range, and it is only population known in Honshu Island, northern Japan. The population consists of 59 reproductive adult trees, and 3,186 saplings (Fig. 1).

2. Simultaneous estimation of the magnitude of early inbreeding and inbreeding depression

There is a growing interest in the inbreeding and inbreeding depression as indicators of population viability in conservation genetics. Inbreeding and inbreeding depression should have different consequences to population viability, depends on a situation. The interpretation may require intensive analysis of contemporary situations: the magnitude of inbreeding and inbreeding depression should be quantified simultaneously, in a focal population, and coupled with the related ecological factors. In this chapter, I discussed ecological and conservation aspects of the inbreeding and inbreeding depression from pollination to seed maturity.

The estimation was based on the parent-offspring genotype obtained from 59 adult trees and 1,420 mature seeds collected from 15 mother trees. The pattern of pollen dispersal, variation of individual fertility and inbreeding depression to embryo survival were estimated simultaneously by a hierarchical Bayes model. The result indicated that the population was extremely pollen-limited: selfing rates estimated at pollination and mature seeds were 0.772 and 0.443 respectively (Fig. 2). Despite of strong inbreeding, the population probably has a potential to avoid inbreeding and maintain genetic variability by purging inbred offspring. The magnitude of inbreeding depression to embryo survival, which measured as the decline in the fitness of selfed offspring relative to outcrossed offspring, was 0.721 (Fig. 3). Rather, the population would lose the genetic variability in future by strong bottleneck effect. The effective number of mating individuals was much lower than actual number: the variance of fecundity, which was measured as the ratio of observed to effective pollen donor density (d_{obs}/d_{ep}), was 54.5 (Fig. 4b), whereas the actual number of adults was 59.

3. Effect of post dispersal processes to the effective population size

Trees are characterized as the highest mortality at juvenile stages. Subsequent selection and drift should occur during the survival of juvenile, and thus it affected the future genetic variability. Inbreeding depression to the juvenile mortality may increase the future genetic variability, and lowers the inbreeding coefficient. On the other hand, joint effect of microenvironmental heterogeneity and limited seed dispersal would enlarge the unequal reproductive success of parent, and thus might have negative impact to future genetic variability. In this chapter, I discussed the effect of the processes in juvenile survival to the future genetic variability.

The estimation was based on the genetic and demographic data sets obtained from 58 adult trees and 1,938 saplings that have more than one candidate parent pairs. I estimated the patterns of pollen and seed dispersal, variance of fecundity, inbreeding depression to the survival and growth of juveniles by a hierarchical Bayes model. I detected the strong inbreeding depression: the cumulative effect of the inbreeding depression to juvenile survival was 0.730 (Fig. 5). Selfing rate at the sapling stage was 0.166. I observed apparent pattern of microenvironmental heterogeneity, which was measured as the spatial variation of juvenile sizes.

The N_{ep} of the recruits was estimated at 22.17 under the assumption of the constant population size ($n = 59$, Fig. 6c). The number of individuals in the next generation had strong impact to N_{ep}: decline in N_{ep} was occurred in the population with less than approximately 130 adult trees, or when the less than 30 % of present distribution was retained (Fig. 7a). The inbreeding depression was not affected the effective population sizes. Long-distance seed dispersal (mean: 617.1 m) might weaken spatial aggregation of siblings, and increase the N_{ep} per area.

4. Predicting future genetic variability and adaptive potential

Long-living trees may be subjected to large environmental changes in few generations. Potential to adapt rapid environmental changes were determined by the intrinsic interaction among existing genetic variability, selection and drift. Quantitative prediction of the adaptability and fitness in the future population should be crucial to risk assessment and conservation of the population. In this chapter, I developed the framework of the population viability analysis of the small population of trees.

The period of simulation was set to one generation because the evolutionary response of trees is usually slower than environmental changes. In a single generation, I simulated the processes from mating to survival of saplings based on the results obtained from the previous chapters. I assumed that each individual has a single adaptive trait that was normally distributed, and the phenotype of offspring was given by averaging that of parents. The fitness was measured by the exponentially declining function. I conducted the simulation under the several population sizes and levels of environmental change.

The quantitative trait diversity decreased in the population with the number of offspring less than 20 (Fig. 8). Neither of the offspring numbers nor the magnitude of environmental changes affected the median of population fitness. Variances of fitness

were increased in smaller populations (Fig. 9). It indicated that smaller populations were more likely to drop below the threshold level of extinction.

5. Conclusion

The results indicated that the small population of Sakhalin spruce was in not the immediate threat of degrade or extinction due to genetic factors. Despite of strong initial inbreeding and low effective number of mating individuals, selection and other processes during the survival of juvenile should recovered the potential of maintaining genetic variability. Demography has larger impact than genetics to the population viability. Threshold values of the number of individuals to maintain genetic variability ($N \simeq 30$) was smaller than the number of adult trees at present ($N = 59$). Therefore, the maintaining the number of adult trees and/or the area of the population should be sufficient to maintain the future genetic variability.

In addition, the results showed smaller threshold values for the number of individuals or N_{ep} than genetically minimum viable population sizes that were proposed previously: $N_{ep} > 50$ or $N > 500$ to avoid immediate deleterious effect of inbreeding, and $N_{ep} > 500$ or $N > 5,000$ to maintain the evolutionary potential. Many of genetic PVAs targeted to animals, which addressed multiple generations in the simulations. Whereas, trees are long-living; maintaining genetic variability within few generations could be sufficient in plausible periods for conservation, and thus resulted in smaller threshold values.

The assessment of the processes that affect genetic variability in the entire life stages was crucial in PVA of trees. While mating system analysis showed the high level of inbreeding and low N_{ep}, selections and drifts that occurred in the later of life stages had a substantial impact to those values. On the other hands, genetic PVA of trees could be simpler than my thesis. In my result, microenvironmental heterogeneity, which requires much data for estimation, did not affected N_{ep}, and thus not to future genetic variability. Realistic estimation of N_{ep} that reflected the effect of selection and drift during the entire life stages is crucial for genetic PVAs.
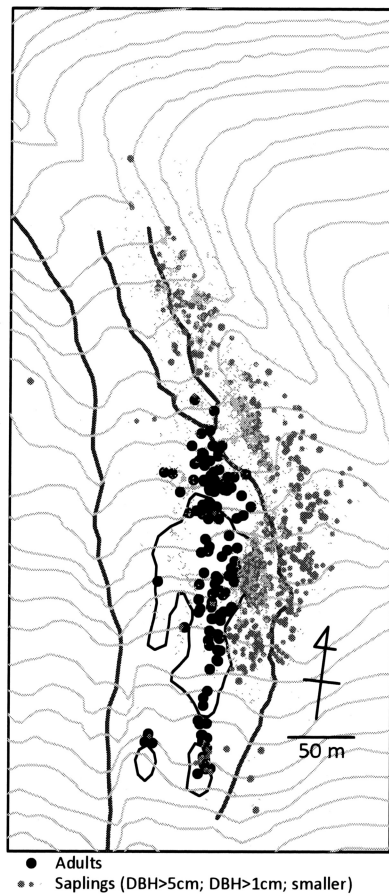
Fig. 1 Map of the study population. Contour lines were drawn in each 10 m.
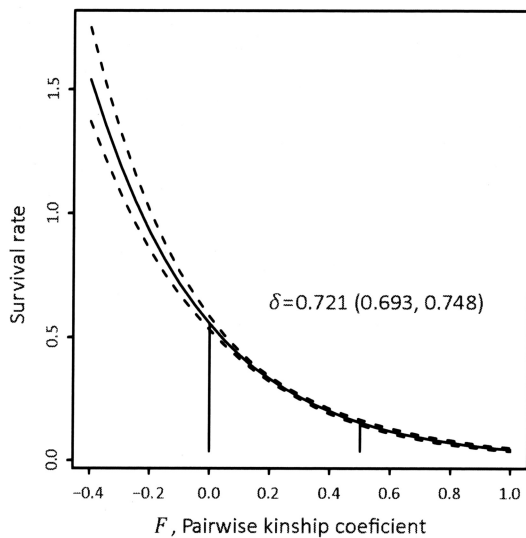
Adults
Saplings (DBH>5cm; DBH>1cm; smaller)

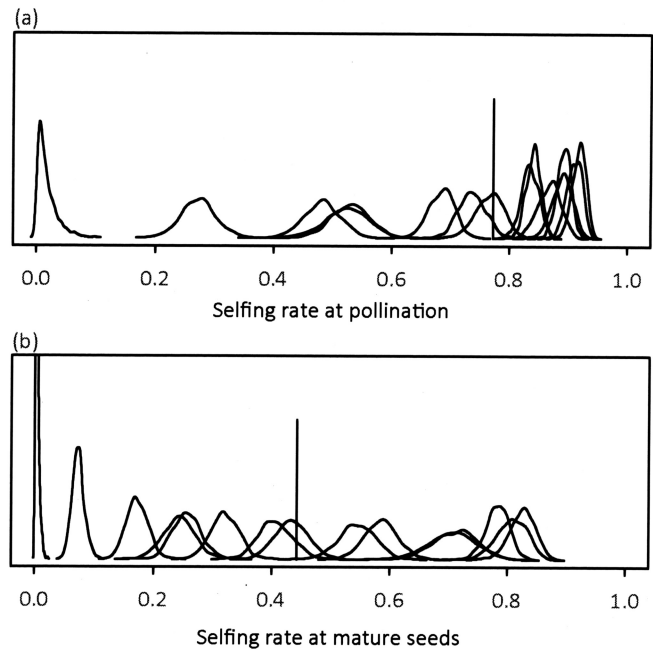Selfing rate at pollination

Selfing rate at mature seeds

Fig. 2 Selfing rates of 15 adult trees at (a) pollination and (b) mature seeds. Each peak indicates the posterior distribution of selfing rates, and the vertical lines indicate medians.
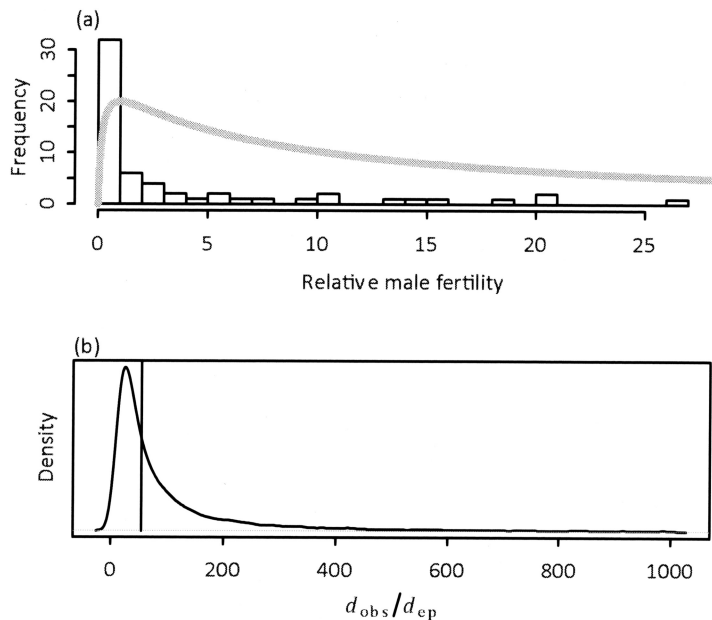

δ=0.721 (0.693, 0.748)

F, Pairwise kinship coeficient

Fig. 3 Expected survival rate of embryos. Solid line indicates the medians, and dashed lines indicas 95 % creadible intervals. Selfing (F=0.5) and outcrossing (F=0) are shown in vertical lines.

Relative male fertility

d_{obs}/d_{ep}

Fig. 4 (a) Frequency distribution of relative male fertility. The posterior medians of invidivual fertility was shown in open bars, and the variance was shown in gray lines. (b) Posterior distribution of the ratio of actural to effective density of pollen donors.
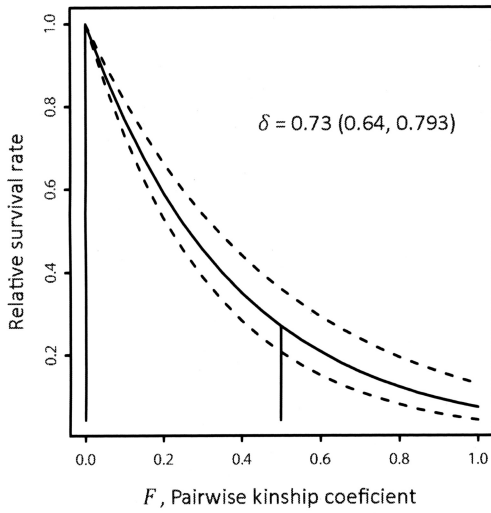
$\delta = 0.73\ (0.64, 0.793)$

Fig. 5 (top) Expected relative survival rate of saplings. Solid line indicates the medians, and dashed lines indicas 95 % creadible intervals. Selfing (F=0.5) and outcrossing (F=0) are shown in vertical lines.
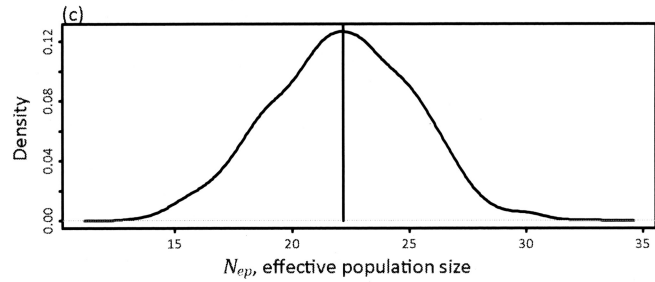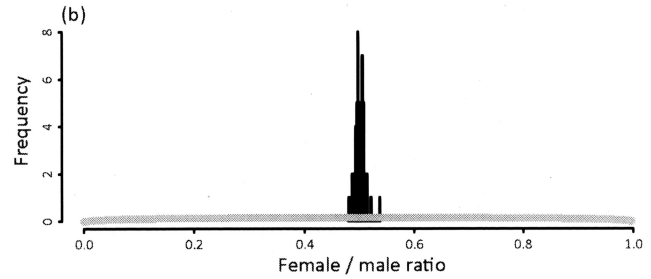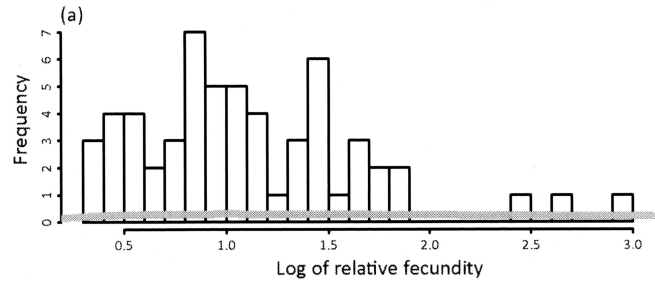
Fig. 6 (right) (a-b) Frequency distribution of relative fertility and sex ratio. The posterior medians of invidivual fertility was shown in open bars, and the variance was shown in gray lines. (c) Posterior distribution of the effective population size at constant population size (N=59).
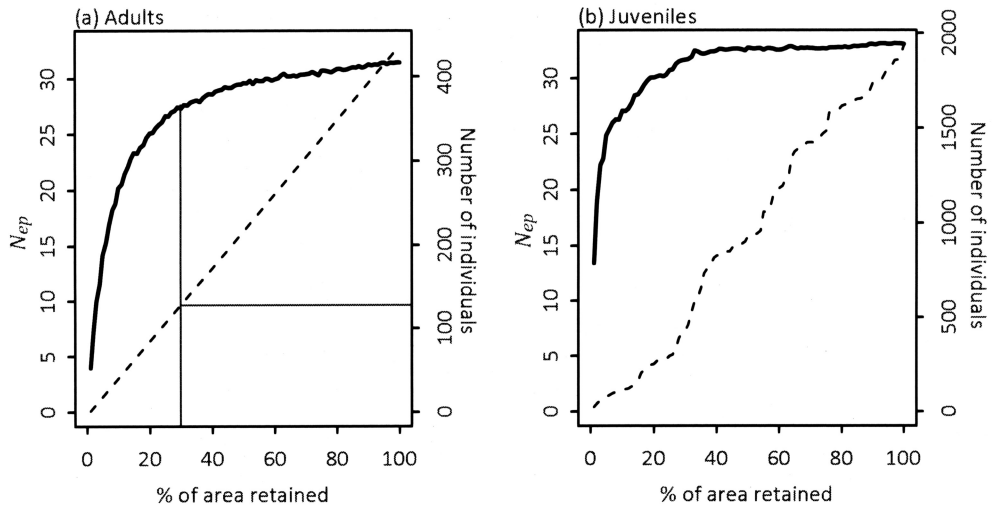
Fig. 7 Effective population sizes at various proportion of area retained (soild lines). Dashed lines are number of individuals that included in the retained area.
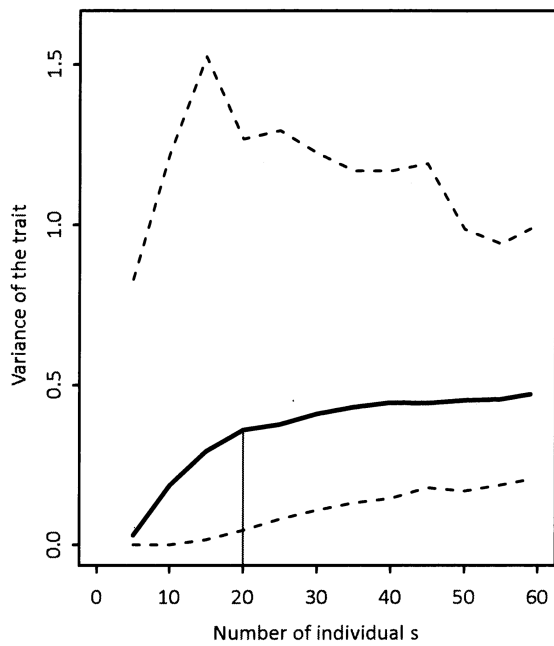
Fig. 8 (left) Simulated quantitative trait diversity under various populatoin sizes. Soild line indicates median, and dashed lines indicate 95 % condidence intervals.
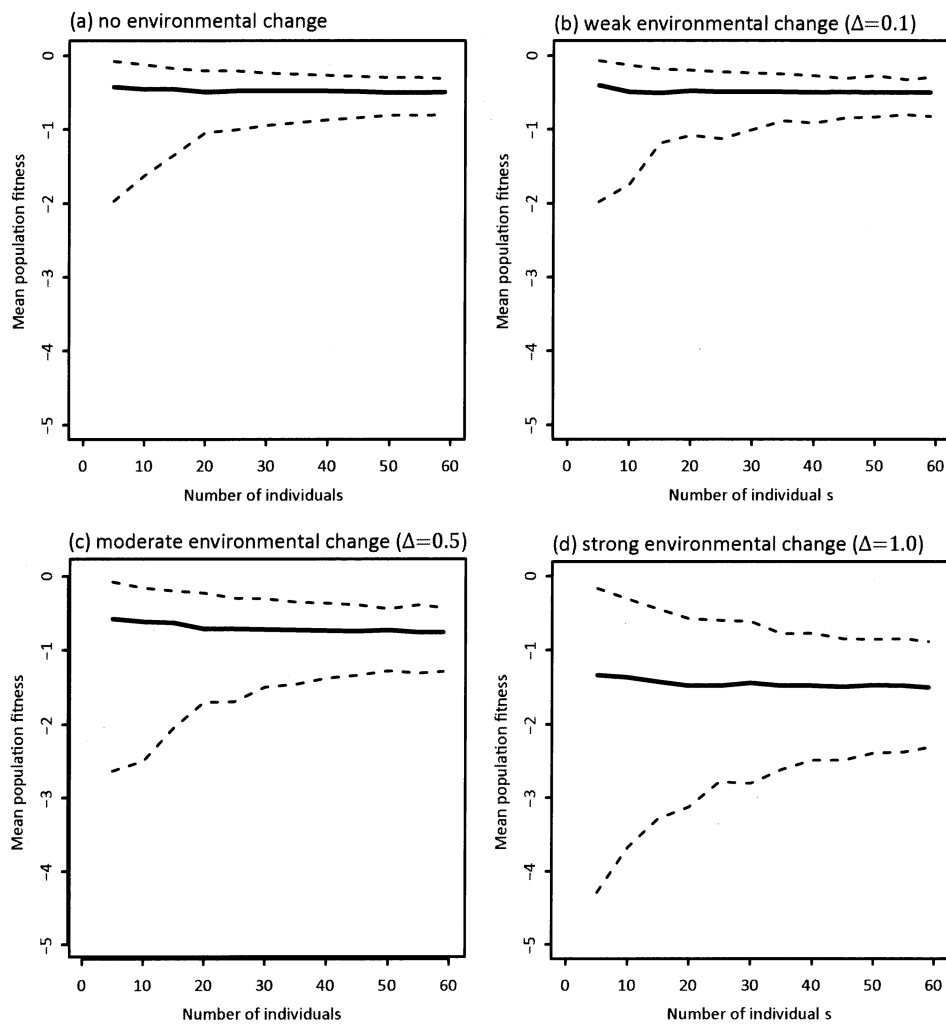

Fig. 9 Simulated mean population fitnesses under various degrees of environmental change and populatoin sizes. Soild line indicates median, and dashed lines indicate 95 % condidence intervals.

論文審査結果要旨

　生物の孤立小集団における主な絶滅要因には、個体群統計学的な要因のほかに、遺伝的要因として遺伝的多様性の喪失や近親交配・近交弱勢による遺伝的劣化などが影響すると考えられる。保全遺伝学的な視点からは、このような孤立小集団の絶滅リスクに関する定量的な評価・予測が望まれるが、そのために必要な全生活史の動態データ等を直接観測するのは、長寿命の多年生植物では極めて困難である。そこでこの論文では、限られた実測データから階層ベイズモデルを用いて必要な生態的・遺伝的要素を推定し、将来の遺伝的多様性に影響する要因を評価した。また、得られたデータを用いたシミュレーションによって、将来の遺伝的多様性・適応ポテンシャルを維持するために必要な個体数を推定した。

　材料として用いたのは、岩手県の早池峰山に本州で唯一の孤立集団として成木約 60 個体のみが分布する、マツ科針葉樹のアカエゾマツ（*Picea glehnii* Mast.）である。この集団の全ての成木だけでなく、全分布域約 6.6ha をくまなく探索して発見した全ての稚樹 3186 個体、さらに 15 個体の成木から採取した約 1500 種子を材料とした。成木および稚樹については個体サイズおよび位置情報を取得し、種子については充実種子率も算出した。これらを 8 遺伝子座のマイクロサテライトマーカーによる遺伝子型情報とともに階層ベイズモデルによって解析し、自殖率・近交弱勢の強さ・繁殖努力・有効な集団サイズなどを推定した。明らかになった主な結果は次のとおりである。

　受粉段階における自殖率の平均は 77.2％という高い値に推定されたが、強い近交弱勢（δ＝0.721）によって成熟種子段階では 44.3％まで低下した。さらに稚樹段階に至るまでに強い近交弱勢（δ＝0.731）が作用し、この段階での自殖率は 15.3％であった。繁殖に有効な集団サイズは、実際の個体数よりも小さく推定され、この値には稚樹の生育する微環境や近交弱勢の強さなどは影響しないことが示された。また、シミュレーションによって予測した将来の遺伝的多様性・適応ポテンシャルを維持するために必要な成木数は 10〜20 個体以上で、現在の集団サイズよりも小さいと推定された。

　本研究の成果は、天然記念物である早池峰山のアカエゾマツ集団の保全に必要な知見として直接的な貢献があるだけでなく、樹木集団における遺伝的多様性の将来予測として、新たな定量的リスク評価法を示した点で、保全遺伝学的分野での貢献が認められる。よって、審査員一同は本論文提出者に対して博士（農学）の学位を授与するに値するものと判定した。