

Effective population size and the viability of the Siberian jay population of Suupohja, Finland

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Tiivistelmä – Referat – Abstract <p>Genetic variation is vital for both contemporary and long-term wellbeing of populations. Whereas heterozygosity (H_o) and allelic richness (A) are commonly used to measure the level of genetic diversity in a population, effective population size (N_e) describes the speed of loss of genetic variation. Various effective population sizes are proposed as standards for safe retention of genetic variation in a Minimum Viable Population (MVP).</p> <p>Since the 1940s, several types of effective population size estimators have been developed. Earlier estimators relied on demographic parameters, whereas genetic estimators are based on the analysis of either one or two genetic samples from a population. All N_e estimators have their unique sensitivities and limiting assumptions, which complicate the choice of estimator, comparison of results of different studies and the assessment of the reliability of the results.</p> <p>N_e estimators have recently been used e.g. in the monitoring of many aquatic populations, but their reliability and comparability has not often been tested with extensive ecological and genetic data, and it is not well established how much added value they bring to the conservation of easily observable species. I tested this with an extensive dataset on the Siberian jays (<i>Perisoreus infaustus</i>) living in Suupohja, Finland (62°22'N, 21°30'E). The Suupohja Siberian jays form one of the few isolates of Siberian jays in Southern Finland. I utilised three demographic and three genetic N_e estimators to estimate the N_e and the N_e/N ratio in the Suupohja Siberian jays, and compared the findings to the H_o and A estimates calculated with the same data, and to various suggested MVP standards.</p> <p>The results showed that the ratio of effective and census population sizes (N_e/N) is close to 0.6 in the Suupohja Siberian jays. Uneven survival of offspring and population size fluctuations are the main factors in the formation of this ratio. The average genetic N_e estimate would, then, suggest a census population size of 44 % higher than the average N in the Suupohja study area. This result is probably connected to the high proportion of breeding immigrants in the data, which would cause the N_e estimates to reflect a larger genetic neighbourhood than the study area. The genetic N_e estimates also suggest that the Suupohja Siberian jays might not be able to maintain their genetic diversity in the long term if gene flow would cease due to further isolation, especially if isolation would also cause a faster demographic decline. Conservation attempts should aim at ensuring gene flow to the remaining Siberian jay isolates in Southern Finland, in order to protect them from increasing genetic uniformity and inbreeding. It is possible that while the average dispersal distances in the Siberian jay are short, occasional long-distance dispersal events have an important role in the prevention of genetic structuring in a Siberian jay population.</p> <p>N_e estimation based on demographic data was laborious in the case of the Suupohja Siberian jays, whereas the genetic N_e estimates showed large variation depending on year and estimation method used. Reliable estimation of N_e with genetic methods would have required information on the large-scale genetic structure of the population. In any case, N_e estimates gave a clearer picture on the genetic viability of the Suupohja Siberian jays than the H_o and A estimates, which did not indicate any decrease of genetic diversity during the study period.</p>			
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Tiivistelmä – Referat – Abstract <p>Geneettinen vaihtelu on elintärkeää populaatioiden hyvinvoinnille. Siinä missä heterotsygotiaa (H_o) ja alleelirik-kautta (A) käytetään yleisesti populaation geneettisen vaihtelun mittareina, efektiivinen populaatiokoko (N_e) mit-taa populaation geneettisen vaihtelun häviämisenopeutta. Useita efektiivisiä populaatiokokoja on ehdotettu ge-neettisen vaihtelun turvallisen säilyttämisen standardiksi pienimmässä elinkelpoisessa populaatiokoossa (MVP).</p> <p>Efektiivisen populaatiokoon mittareita on kehitetty 1940-luvulta lähtien. Varhaisemmat mittarit ovat perustuneet populaation demografisille ominaisuuksille. Geneettiset mittarit perustuvat yhden tai kahden geneettisen näyte-erän analyysille. Kaikilla efektiivisen populaatiokoon mittareilla on ominaisia herkkyksiään ja käyttörajoit-uksiaan, mikä vaikeuttaa mittarin valintaa, tulosten vertailukelpoisuutta ja tulosten luotettavuuden arviointia.</p> <p>Efektiivisen populaatiokoon mittausta on käytetty monien akvaattisten populaatioiden seurannassa, mutta mitta-reiden luotettavuutta ja vertailukelpoisuutta on vain harvoin voitu testata kattavalla demografisella ja geneetti-sellä aineistolla, eikä tarjolla ole selkeää näkemystä siitä, kuinka paljon lisähyötyä ne tuovat muutoinkin helposti seurattavien lajien suojeluun. Testasin tätä Suomen Suupohjassa, 62°22'N, 21°30'E, eläviä kuukkeleita (<i>Periso-reus infaustus</i>) käsittelevällä aineistolla. Suupohjan kuukkelit muodostavat yhden Etelä-Suomen harvoista kuuk-kelialueista. Käytin kolmea demografista ja kolmea geneettistä efektiivisen populaatiokoon mittaria määrit-tääkseni populaation efektiivisen koon sekä efektiivisen ja todellisen koon suhteen (N_e/N), ja vertailin tuloksia heterotsygotia- ja alleelirikkausarvoihin sekä keskusteluun pienimmästä elinkelpoisesta populaatiokoosta.</p> <p>Tulosten mukaan efektiivisen ja todellisen populaatiokoon suhde on kuukkelilla noin 0.6. Suhteen muodostumi-seen vaikuttivat lisääntymismenestyksen vaihtelu ja populaatiokoon heilahtelut. Geneettisillä mittareilla mitatun efektiivisen populaatiokoon mukaan kuukkelien todellinen populaatiokoko olisi 44 % korkeampi kuin tutki-musalueen keskimääräinen populaatiokoko. Tulos lienee sidoksissa yllättävän korkeaan immigraation osuuteen lisääntyvistä yksilöistä, mistä johtuen geneettinen aineisto heijastelisi tutkimusaluetta laajempaa geneettistä naa-purustoa. Tulokset osoittavat myös, etteivät Suupohjan kuukkelit todennäköisesti kykenisi säilyttämään geneet-tistä vaihteluaan ilman immigraatiota, etenkin jos immigraation lakkaaminen johtaisi myös populaatiokoon las-kuun. Suojeluyritysten tulisi tähdätä geenivirran varmistamiseen Etelä-Suomen jäljellä oleville kuukkelialueille geneettisen yhdenmukaistumisen estämiseksi. Koska keskimääräiset dispersaalimatkat ovat kuukkelilla lyhyitä, on mahdollista, että harvinaisemmilla pitkän matkan dispersaalitapahtumilla on keskeinen rooli geneettisen eriytymisen estämisessä.</p> <p>Efektiivisen populaatiokoon arviointi demografisen aineiston perusteella oli työlästä, kun taas geneettiset arviot ilmensivät suurta vaihtelua vuodesta ja mittarista riippuen. Luotettava efektiivisen populaatiokoon arviointi olisi edellyttänyt populaation geneettisen rakenteen parempaa etukäteistuntemusta. Efektiivisen populaatiokoon mittaus antoi kuitenkin selkeämmän kuvan Suupohjan kuukkeleiden geneettisestä hyvinvoinnista kuin mitatut heterotsygotia- ja alleelirikkausarvot, jotka eivät osoittaneet minkäänlaista geneettisen vaihtelun laskua tutkimus-ajanjaksolla.</p>			
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1 INTRODUCTION

1.1 Population size as a factor in population viability and survival

1.1.1 Demographic and genetic stochasticity in small populations

It is widely accepted among conservation biologists that a population's chances of long-term survival are not solely dependent on deterministic factors but also on population size. Wild populations are subject to several sources of natural stochasticity, and the relative impact of these stochastic factors on the fate of populations is considered to increase with decreasing population size (Shaffer 1981, 1987, Lynch *et al.* 1995). As a consequence, small populations are at an elevated risk of extinction even if no environmental cause is driving them towards a further decline.

Stochastic processes affect both the demography and genetics of populations, although they do so in different manners. Populations are subject to **demographic stochasticity** since the annual growth rate of any population depends on the annual numbers of births and deaths, both of which require realisations of probabilistic chance events on the individual level. The total numbers of realised birth and death events tend to average out in a large population, but in a small population, each individual chance event has a relatively larger impact to the total population (Lande 1993, Traill *et al.* 2010). This is considered to cause unpredicted annual population size fluctuations in small populations. Additional uncertainty to the demography of wild populations may be caused by environmental stochasticity, for instance fluctuations of climatic conditions, and natural catastrophes (Shaffer 1981, 1987). Susceptibility to stochastic demographic changes increases the extinction risk of small populations; the smaller the population, the larger the likelihood that a row of unfavourable stochastic events may drop the population size below recovery threshold.

Genetic stochasticity arises from the fact that each offspring inherits a random combination of its parents' alleles, and that chance plays a part in which individuals eventually survive and reproduce (Allendorf & Luikart 2007). This stochastic factor in the inheritance of genes may cause slight allele frequency shifts between generations. The resulting **genetic drift** (Wright 1931) may act as a counterforce to selection, since it may change the genotype composition of the population, and even cause the disappearance of certain alleles in spite of the resulting negative effects on fitness. Genetic drift is not expected to drive clearly beneficial alleles to extinction in healthy populations (Wright 1948), yet currently neutral or nearly neutral alleles are at stake. As small, isolated populations are expected to experience stronger genetic drift and develop less new variation via mutation than larger populations, they are more likely to reach a state where the overall level of genetic uniformity is increasing (Wright 1931, Soulé 1980a). The result may compromise the

population's ability to adjust to changing conditions and therefore decrease its long-term average fitness.

Although demographic and genetic stochasticity affect the population in different ways, their consequences may be intertwined. First, stochastic population size fluctuations may lead to temporary population size **bottlenecks** where genetic variation is lost (Nei *et al.* 1975). Second, unpredicted population size fluctuations and the fitness costs caused by declining genetic variation are both considered to be capable of driving the populations below recovery threshold, i.e. to a stage where the processes vital to the survival of the population are compromised. Several studies reveal decreasing fitness associated with very small population sizes or low population densities, for instance, in the form of decreased cooperation by conspecifics or difficulties in finding a mate (**Allee effects**; Allee 1931, Soulé 1980a, Stephens *et al.* 1999, Chen & Hui 2009). Additionally, if population size becomes so small that the choice of mates is limited, sexual selection is disturbed. This may lead to **inbreeding depression** (Lynch *et al.* 1995, Frankham 2005, Traill *et al.* 2010) or chance fixation of detrimental alleles, creating **genetic load** (Kimura *et al.* 1963, Willi *et al.* 2005). With these processes in mind, Giplin and Soulé (1986) argued that an insidious mutual reinforcement may occur with declining population size, creating an **extinction vortex**, where a series of events will eventually — almost deterministically — drive a population to extinction.

1.1.2 Estimation of Minimum Viable Populations

Several conservation biologists have engaged in creating probabilistic models that simulate the stochastic environments that populations are facing in the wild (Caughley 1994, Traill *et al.* 2010). Based on these models, time-dependent survival likelihoods have been estimated for various population types and sizes as a part of **Population Viability Analysis** (PVA; Caughley 1994). For instance, 99 % probability of survival in 100 years and 95 % probability of survival in 1000 years are suggested as alternative criteria for species conservation (Shaffer 1981, Traill *et al.* 2010). In addition to deterministic factors, the meeting of such time-dependent survival likelihood criteria is considered to be dependent on population size. Because of this, PVA has contributed in attempts to define a **Minimum Viable Population** (MVP) — a population size that is associated with an acceptable extinction risk level from conservation point of view (Franklin 1980, Shaffer 1981, Lande 1993, Traill *et al.* 2010).

A consensus regarding the relative importance of demographic and genetic risk factors for the survival probability of small populations has yet to be reached (Palstra & Ruzzante 2008). While some researchers stress that demographic stochasticity and local inbreeding pose more immediate threats to populations than genetic drift (Whitlock & Barton 1997, Tufto & Hindarb 2003), others point out that although risks related to genetic drift take more time to materialise, gradual genetic impoverishment due to genetic drift may take place with

population sizes that are larger than those causing apparent demographic problems (Lynch *et al.* 1995, Traill *et al.* 2010). Lynch *et al.* (1995) argue that even populations of 1000 individuals may experience small but persistent decreases in genetic diversity, which within a dozen generations may drive them towards **mutational meltdown** (Lande 1995, Frankham 2005, Traill *et al.* 2010; but see Palstra & Ruzzante 2008).

The concept of a MVP is politically sensitive, as it easily leads to conclusions regarding acceptable standards of nature conservation. Thus, a broad discussion has arisen around the topic. Some criticise that viable population sizes cannot be safely estimated with simulations, and therefore PVA may give misleading information as a basis of conservation decisions (Beissinger & Westphal 1998). In addition, there is no consensus on how to best utilise information regarding population viability: while some support conservation at any cost, others argue that it might not be sensible to focus conservation resources to already threatened populations (e.g. Game *et al.* 2008, Joseph *et al.* 2009, reviewed in Traill *et al.* 2010). Nevertheless, even a general understanding of different populations' relative chances of survival provides crucial information for conservation planning. As long as the debate concerning the risks associated with demographic and genetic factors continues, both demographic and genetic viability of populations should be monitored in order to get an overview of population viability.

1.1.3 The effect of population structure on the viability of small populations

Most of the theory concerning stochasticity in small populations is simplified by the assumption of habitat uniformity and population isolation (Reed *et al.* 2003). However, the population dynamics of real-life populations are often more complex. This complexity may alter the role of the stochastic processes in a population, affecting the population's extinction risk, and thus the population sizes needed for the safe retention of genetic variation. For instance, even small-scale immigration may have a major effect on the genetic viability of a small population, since it may be able to override the effects of genetic drift. Conservation biology knows this phenomenon as **one migrant per generation rule** (Wang 2004). On the other hand, areal variations in habitat quality may result in a **source-sink system** (Dias 1996, Nystrand *et al.* 2010), where areas of higher reproductive success and lower mortality produce a demographic surplus that complements the demographic deficits of lower-quality areas. This influences the genetic and demographic stochastic processes of such populations. **Metapopulation dynamics** are considered to be so complex that the classical theories on genetic stochasticity do not apply to them as such (Hanski 1998).

1.2 Is effective population size estimation useful for estimating viability?

1.2.1 The challenge of measuring genetic variation

Whereas the demographic stability of most populations is relatively easy to monitor by using, for instance, capture-recapture rates (Lebreton *et al.* 1992, Ferriere *et al.* 1996), the genetic viability of a population is challenging to assess for several reasons. First, the extent of genetic variation in the eukaryote genome typically varies greatly depending on the genomic location, functional importance and selective forces acting upon the locus (e.g. Fay & Wu 2000, Nielsen 2001). Second, it is difficult to estimate which variation is important to the viability and adaptability of the species. The genomes of most species are still poorly known. Third, there are differences between species (and populations) in how much inbreeding and homozygosity they can tolerate. For instance, selfing species and species with a history of severe bottlenecks may have experienced **purgings**, i.e. the exposure of detrimental recessive alleles to selection, which may reduce the population's susceptibility to inbreeding depression (Hedrick 1994, Palstra & Ruzzante 2008).

Genetic diversity is commonly estimated using average observed heterozygosity (H_o) or average allelic richness (A) in neutral genes as proxies of genome-wide genetic variation. To tackle the challenge of having varying levels of genetic diversity across the genome, it is generally recommended that H_o and A be estimated over putative neutral loci (Allendorf & Luikart 2007:110–111), such as microsatellite loci in presumably non-functional sections (but see Selkoe & Toonen 2006) of the genome. Temporal changes in H_o or A are then used to estimate whether genetic diversity is decreasing due to factors other than selection. On a population level, the heterozygosity estimate is expected to be quite reliable even with a rather low number of loci, whereas allelic richness is considered to be more sensitive to genetic bottlenecks than heterozygosity. Therefore, these estimates are expected to complement each other in many respects (Allendorf & Luikart 2007:127). However, these measures are not comparable across species, and as such, do not provide information on what kind of population sizes would be adequate for the safe retention of the current levels of genetic variation in the population.

1.2.2 Effective population size as a measure of loss of genetic variation

The basis of comparing the loss of genetic diversity among populations was laid by Wright (1931) and Fisher (1930) with their concept of an **ideal population**, which refers to a theoretical population with standardised demographic characteristics. The diploid Wright–Fisher population is free of selection, mutation, migration and overlapping generations. The

population size is stable, breeding is random, self-fertilisation is possible and the offspring numbers follow Poisson distribution. Based on these conditions, the development of genetic variation in the population can be mathematically predicted. It is known, for instance, that inbreeding increases in an ideal population at a rate of $1/(2N)$ per generation when population size is N (Wright 1931). The population genetic processes in the ideal population can therefore function as a standard against which the same processes in true populations can be compared. The size in which the ideal population would experience similar development as the studied natural population is said to be the **effective population size** (N_e) of the studied population.

The concept of effective population size can refer to two or more distinct parameters: **Variance effective population size** (N_{eV}) measures the extent of genetic drift in the population, causing random changes in allele frequencies, and **inbreeding effective population size** (N_{eI}) measures the rate of increase in relatedness in the population. The more rarely mentioned eigenvalue effective size and coalescence effective size find applications especially in more recent studies regarding effective population size (Whitlock & Barton 1997, Sjödin *et al.* 2005, Lehmman & Perrin 2006). Variance effective population size and inbreeding effective population size are considered the most important parameterisations of effective population size (Leberg 2005, Waples 2005). The values may differ, for instance, when a population is either growing or declining, or recovering from a population bottleneck (Leberg 2005, Allendorf & Luikart 2007:159, Luikart *et al.* 2010), but as in a stable population or with a longer follow-up period they are expected to approach the same value (Wang & Caballero 1999, Pollak 2002). Hence, scientific literature usually simplifies the matter by speaking about effective population size and its measurement in general (Wang 2005).

The concept of effective population size provides several benefits for estimating the genetic viability of populations. As it does not estimate the current level of genetic diversity in the populations, but the rate in which diversity is lost, it helps in deducing whether populations are capable of maintaining their current levels of genetic variation with their current size. On the other hand, using a standardised population as a common basis provides a mechanism of making comparisons between several real-life populations.

1.2.3 Different types of effective population size estimators

Population geneticists have developed various methods of estimating effective population sizes for natural populations. Various **demographic effective population size estimators** were developed in the late 20th century; these estimators are mathematical formulas based on species-specific ecological parameters, such as sex ratio of breeding adults, variation of breeding success, population size fluctuations, generation length and age-specific survival

and fecundity rates (Felsenstein 1971, Lande & Barrowclough 1987, Harris & Allendorf 1989, Nunney & Elam 1994). The complexity and precision of the demographic estimators varies from laborious to simplistic depending on the intended use of the estimator (Nunney & Elam 1994). The weakness often lies either in their assumptions that are not realistic for real-life populations, or in the difficulties related to retrieving reliable values for the parameters needed in the estimation (Harris & Allendorf 1989, Luikart *et al.* 2010). Luikart *et al.* (2010) also assume that demographic estimators overestimate N_e , as they may miss several factors that lead into the losses of genetic variation in real populations.

The development of **genetic effective population size estimators** accelerated after the improvement of DNA extraction and analysis methods in the latter half of the 20th century (Leberg 2005, Schmeller & Merilä 2007). The genetic estimators that measure contemporary levels of genetic variation can be classified into two groups from the practical point of view: **temporal estimators** are based on allele frequency shifts between the minimum of two temporally spaced samples (Leberg 2005), whereas **point estimators** are based on a varying set of genetic attributes, for instance linkage disequilibrium, within one genetic sample. The use of 10–20 highly polymorphic loci and sample sizes of ≥ 50 individuals are typically recommended (Leberg 2005).

To date, temporal estimators have formed the most commonly used group of genetic estimators of effective population size (Leberg 2005). The traditional temporal estimators, based on moment-based approximations of the allele frequency changes (Krimbas & Tsakas 1971, Nei & Tajima 1981), have lately been accompanied by more sophisticated methods of temporal data analysis, based on maximum likelihood analyses (Williamson & Slatkin 1999, Anderson *et al.* 2000, Berthier *et al.* 2002, Wang 2002), and Bayesian methods (Beaumont 2003, Tallmon *et al.* 2004). The latter methods are considered more precise, but their use has been restricted by their computer intensiveness and complexity (Wang 2002, 2005). Temporal estimators are considered robust, but their use has been limited by the fact that either the samples have to be temporally spaced 3–5 generations apart or demographic data on the age-specific fertility rates of the population has to be available (Jorde & Ryman 1995, Waples & Yokota 2007). The assumption of a closed population makes the temporal estimators sensitive to biases caused by migration (Leberg 2005).

Due to the fact that genetic samples with 3–5 generations between them are often not available for long-lived species, point estimators are actively developed. Among those, linkage disequilibrium based methods are most common and most actively developed (Hill 1981, Wang 2005, Waples 2005, Waples & Do 2008). The main challenges related to many point estimators have been their low precision, sensitivity to the violation of the assumed mating patterns, and sensitivity to immigration (Wang 2005, Watts *et al.* 2007).

The developers of effective population size estimators have attempted to overcome the limitations caused by the restrictive assumptions made by most estimators. These attempts

have resulted in methods for estimating effective population size for populations with age structure (Jorde & Ryman 1995, Wang 2005), open populations experiencing immigration from a known source population (Vitalis & Couvet 2001, Wang & Whitlock 2003) and metapopulations (Whitlock & Barton 1997, Wang & Caballero 1999, Tufto & Hindarb 2003). For large, continuous populations characterised by isolation by distance, **genetic neighbourhood size** estimators have been developed, with the intention of retrieving measures that are commensurable to N_{el} of non-continuous populations (Rousset 2000, Leblois *et al.* 2003, Leberg 2005). However, these specific estimators are typically developed to relax only a single factor violating the assumptions of the ideal population, while real populations might violate several assumptions at a time. Therefore, effective population size estimation would require a good up-front perspective on the population genetic structure.

1.2.4 Effective population size estimation and Minimum Viable Populations

Effective population size estimation has gained a noticeable position in the population genetics literature. Various authors have, for instance, attempted to define standard effective population sizes to qualify for a Minimum Viable Population. Franklin (1980) and Soulé (1980b) estimated that the prevention of acute inbreeding problems requires at least the $N_e = 50$ in a population, whereas at least $N_e = 500$ is required for maintaining a balance between mutation and loss of genetic diversity; i.e. to qualify for an evolutionary MVP (Traill *et al.* 2010). Other studies suggest that as much as $N_e = 5000$ is required for a population to be genetically viable in the longer term (Lande 1995, Franklin & Frankham 1998). Lynch *et al.* (1995) claim that populations with $N_e < 100$ are at risk of developing a substantial load of deleterious mutations within a few dozen generations.

Several studies have also attempted to define species-specific ratios between census population sizes (N) and effective population sizes (Frankham 1995). This N_e/N ratio is expected to form according to the ecology of the species, related to the patterns in which individuals pass their genetic diversity from one generation to the next. In fact, wide-ranging variation is observed in species-specific studies; an oyster species in the Pacific Ocean is associated with an N_e/N ratio of 10^{-6} (Frankham 1995), whereas the N_e/N ratio in humans would be as high as 0.99 (Wang & Caballero 1999). Being able to estimate N_e based on N , and vice versa, would be a useful concept for population geneticists. As a result, some studies have attempted to draft average N_e/N ratios, but thus far have failed to reach a consensus (Waples 2005). Recently, Palstra and Ruzzante (2008) reported a median N_e/N ratio of 0.14 across taxa. Nunney (in Nunney & Elam 1994) suggests an average ratio of 0.5 if the life span of adults is long, and according to Waples (2002), N_e would typically be 20 %

of the adult census size. So far, population viability analysts have utilised Frankham's (1995, Frankham *et al.* 2002:240–241) average N_e/N ratio of 0.1 as a baseline for estimating genetically viable census sizes (N), but due to a wide recorded range of N_e/N across species, this would lead to imprecise conclusions in most individual cases and thus be of little use for managers (Traill *et al.* 2010).

Through the allegedly constant species-specific N_e/N ratios, effective population sizes are used to analyze the population size fluctuations of marine fish populations (Hansen *et al.* 2006) and even historical population sizes based on museum specimens (Miller & Waits 2003). N_e estimation is also used in the analysis of the effects of population size fluctuations (Andren & Kapuscinski 2002) or supplementation programs (Eldridge & Killebrew 2008) to the genetic processes in populations.

1.2.5 Criticism of effective size estimation: much trouble for little benefit?

Despite the development of new effective population size estimators and regular discussion among conservation biologists, critics claim that effective population size estimation provides little value for real conservation purposes: The varying assumptions and sensitivities of the estimators limit the comparability of the results calculated in different studies (Wang 2005, Luikart *et al.* 2010). In addition, the census population size, N , is interpreted in several ways in effective population size estimation, varying from total number of observed individuals to the adult population (Nunney & Elam 1994), complicating the comparison of the N_e/N ratios across species and taxa. Sjödin *et al.* (2005) claimed that the concept of effective population size is not meaningful in open populations, which are far different from the original concept of the ideal population. Finally, the concept of the effective population size is relatively complex, which limits the ability of researchers and conservation biologists to select the most useful estimators for their purposes and to interpret the results meaningfully (Wang 2005).

To serve as a useful means for making conservation decisions, estimating effective population size should provide added value compared to the mere monitoring of population size, or such commonly measured population genetic quantities as observed heterozygosity (H_o), allelic richness (A) or F_{ST} (Rousset 1997). In case of threatened marine fish populations, effective population size estimation has earned its position (Palstra & Ruzzante 2008), but little data is available concerning its usefulness in the monitoring of populations that are easier to observe with conventional census methods. In addition, most evaluations of effective population size estimators are either based on computer simulated populations (reviewed in Wang 2005), or utilise only a few estimators. Therefore comparative studies based on a wide set of data are needed (Leberg 2005).

1.3 Siberian jay: a typical Scandinavian conservation case

1.3.1 A flagship species of the conservation of Scandinavian old-growth forests

The focus of this study is a species that has gained a status as a flagship species of conservation disputes, the Siberian jay (*Perisoreus infaustus*). The Siberian jay is a small corvid species with a distribution covering Northern Fennoscandia and Siberia. It is a year-round resident throughout its distribution range, surprisingly well adapted to survive winters in Northern latitudes despite its small size (Siitonen & Willamo 2003). Its lifestyle is based on an omnivorous diet, consisting of a creative variety of food sources: berries, beetles, flying insects, mushrooms, seeds and even lizards, tit nestlings as well as carcasses. Siberian jays have specially developed saliva glands that allow them to roll small bits of food into sticky and easily digestible preserves for the winter — these are then glued into beard moss (*Usnea*) or holes in tree bark where they are easily accessible throughout the winter, even during snowy periods (Pimenoff 2000, Siitonen & Willamo 2003, Koskimies 2009a).

Since the Siberian jays are dependent on their wintertime food storages, they are territorial birds, typically occupying a 1–4 km² large territory. Wintertime reserves can be found all around the territory, but especially in its core areas where the breeding pair establishes a nest and begins nesting early in March (Koskimies 2009a). 3–5 fledglings leave the nest in May (Lillandt *et al.* 2003), but hide in the branches of the territory cover until they are able to fly. In addition to the territory owners, the family group occupying the territory may include 1–3 juveniles that are either kin or non-kin to the breeding pair. In the autumn, juveniles compete over a right to stay in their natal territory, forcing the losers of this sibling rivalry to disperse to other nearby territories and to seek either a vacant territory or a membership in another family group (Ekman *et al.* 1994, 2002, Lillandt *et al.* 2001). The territory owners are nepotistic towards their retained offspring, granting them access to safer feeding locations compared to non-kin juveniles (Ekman *et al.* 2001, Nystrand 2006, 2007) and giving them better protection towards predators (Griesser 2009). However, even the immigrant juveniles benefit from membership in a family group in the form of feeding opportunities, learning the Siberian jay way of life, and gaining a possibility to queue for territory ownership in case the same-sex territory owner dies (Ekman & Skleptovytsch 1994). The juveniles typically attempt to establish their own territory at an age of 2–3 years (Koskimies 2009a).

The Siberian jay is specifically interesting to conservationists since its occurrence is linked to the availability of old-growth forests as habitat. The Siberian jay is adjusted to weave its way in a dense forest, and its flight in open terrain is a bit clumsy; thus it is an easy catch for goshawk (*Accipiter gentilis*) and other birds of prey outside of forests. Additionally, the Eurasian jay (*Garrulus glandarius*) and other corvid species pose a constant threat to nesting Siberian jays in the form of nest predation (Pimenoff 2000, Griesser & Nystrand 2009). These threats can be seen in the Siberian jay's preference to feed close to cover, its

increased vigilance when feeding in a more open terrain (Griesser & Nystrand 2009) and reduced visits to the nest if the nesting site is not well covered (Eggers *et al.* 2006, 2008). As a consequence, the Siberian jay is very selective in its choice of territory; the core of the territory is typically an old dense spruce swamp, where spruce branches (*Picea abies*) and dense vegetation patches of a varying-age forest provide structural cover (Edenius & Meyer 2002, Siitonen & Willamo 2003). The rest of the territory may include plots of varying ecosystems, such as groves and flood meadows (Siitonen & Willamo 2003, Sulkava 2009), but preferably not large clear-cut areas (Väisänen *et al.* 1998, von Haartman *et al.* 1967). Due to these preferences, an existing Siberian jay territory indicates that a forest is ecologically and structurally diverse and is thus also potentially suitable for other demanding old-growth forest species, such as the three-toed woodpecker (*Picoides tridactylus*) and the red-breasted flycatcher (*Ficedula parva*), various hawks and owls and sometimes also the Siberian flying squirrel (*Pteromys volans*). The territory core is also likely to host threatened mosses and lichens, and its preservation may be beneficial for declining grouse species (Tetraoninae; Helminen 2009, Sulkava 2009).

1.3.2 Siberian jays of Southern Finland

While the Siberian jay is still thriving within its main distribution area in the North, the species abundance has dramatically declined below the Oulu–Lieksa line and almost completely vanished during the last decades below the 62°N latitude, making it the fastest declining bird species in Southern Finland (Väisänen *et al.* 1998, Mäkelä 2006, Koskimies 2009a). Increased human activity is considered the main reason for the decline of the Siberian jay (Väisänen *et al.* 1998, Koskimies 2009a). Land clearing for field cultivation, construction of roads and the expansion of human settlements have fragmented the formerly continuous boreal forests into isolated patches (Esseen *et al.* 1997), separating Siberian jays from each other behind dispersal barriers that they appear to find difficult to cross (Väisänen *et al.* 1998, von Haartman *et al.* 1967). But first and foremost, forestry has changed the forests themselves: The preference of Scots pine (*Pinus sylvestris*) monoculture plantations over spruce has transformed the structure of forests (METLA 2007 in Bergholm 2007) towards being too open and spacious for the survival of the Siberian jay (Edenius & Meyer 2002, Griesser & Nystrand 2009). In addition, wintertime clear-cuttings in the core areas of the Siberian jay territory are fatal to the territory occupants (B.-G. Lillandt, pers. comm.), destroying their winter food storages, while forest thinnings increase visibility and therefore the risk of nest predation (Griesser *et al.* 2007). As a consequence, the formerly common sightings of the Siberian jay have ceased in Southern Finland, notwithstanding odd sightings here and there, and a few continuous areas where some Siberian jays still persist (Mäkelä 2006, Koskimies 2009a).

The supposedly largest area of Southern Finland that is still occupied by Siberian jays is in the Suupohja area in Southern Ostrobothnia, North–East of Kristinestad. The population has been monitored since the 1970s (Lillandt *et al.* 2003). The follow-up shows that the population is currently declining; during its best years, the study area contained almost 98 territories, whereas the number of territories was circa 45 in 2008, and the number of juveniles has dropped dramatically (Nousiainen 2008, Lillandt 2009b). The degree of isolation of the Suupohja Siberian jays from the continuous distribution of Siberian jays in Northern Fennoscandia is not known, but according to genetic studies (Uimaniemi *et al.* 2000, Jaari *et al.* 2008), the Suupohja Siberian jays show decreased genetic variation as compared to Siberian jays within the more continuous distribution of the species, which may be the result of genetic impoverishment due to isolation and inbreeding (see: Alho *et al.* 2009). On the other hand, immigration of Siberian jays to the Suupohja study area has been repeatedly reported (Lillandt 2000, 2004, Lillandt *et al.* 2003, Bergholm 2007). To the North–East of the Suupohja area, Siberian jays still occupy dozens of territories in the less thoroughly monitored areas of Suomenselkä and Central Finland (Sulkava & Sulkava and Nyholm in Koskimies 2009a).

Eastern Finland still hosts a relatively dense population of Siberian jays in the Savo–North Carelia area (Prättälä and Matero *et al.* in Koskimies 2009a), accompanied by a smaller population in South Carelia, Parikkala region. The Parikkala region currently hosts a few known Siberian jay territories, which were occupied by 5–8 breeding pairs and altogether 8–15 individuals in 2006–2007 (Kauppinen in Koskimies 2009a). The Siberian jays of Savo–North Carelia area mainly inhabit unprotected forests, while the Parikkala Siberian jays survive in a rare continuous spruce forest area owned and managed by a private forestry company UPM. Nature conservationists continuously monitor the Parikkala Siberian jays and the logging status of the forests in their territories, and several discussions over the fate of these Siberian jays have been raised in case their current territorial forests will be logged (Heikkinen 2009). The latest agreement has saved the Siberian jay territories for the time being. However, according to the forest owners, the final agreement may not entail the full conservation of the territorial forests, but instead, a planned maintenance of a continuum of old spruce forests in the area (Koskimies 2009b, Pohjalainen 2009).

The Siberian jay forests that have raised the heaviest disputes in Southern Finland are those located in the Virrat region. The continuous old spruce forest in this region is publicly owned and managed by a governmental forest management agency Metsähallitus. Conservationists have raised several campaigns against the loggings of these forests since 1999 (Mäkelä 2006, Luonto-Liitto 2008), and after several interrupted loggings, on-site demonstrations and conservation extensions, the discussion is still ongoing (Haapala 2009, Kauppinen 2009, Metsähallitus 2009). In 2006–2007, the Virrat region was counted to host only five breeding pairs and one single bird (Uppstu in Koskimies 2009a).

The worrisome situation of the remaining Siberian jays of Southern Finland has lately been recognised in the Forest Biodiversity Programme METSO organised by the Finnish Ministry of the Environment and three other relevant governmental institutions. Within the METSO programme, a collaboration network has been established for the conservation of the Siberian jay forests of Southern Finland, based on an initiative of The Finnish Association for Nature Conservation (FANC). The network aims at developing novel forest conservation methods and long-term conservation commitments in collaboration with several stakeholders, including nature and bird conservation associations, forestry enterprises and governmental forestry institutions (Helminen 2009, Lillandt 2009b, Ministry of the Environment 2009, Finnish Association for Nature Conservation 2010). The network aims to reach a consensus regarding areas needed for permanent conservation in the core areas for the Siberian jays of Southern Finland, and to improve the overall quality of the forests from the Siberian jay viewpoint by developing forest and nature management practices accordingly. Compensations to the economical losses are seeded out from increased tourism and funded forest restoration activities (Finnish Association for Nature Conservation 2010). The conservation attempts, even though ambitious and promising, rely mostly on demographic data. As the remaining refugia of the Southern Finnish Siberian jays are utterly small and possibly quite isolated, there is a great risk that genetic impoverishment would remain as a long-term threat for these isolated Siberian jays, even if the conservation attempts would succeed in securing the Siberian jays with a less turbulent environment and therefore less population size fluctuations in the future.

1.4 Study aims

The aim of my study was to evaluate the viability of the Suupohja Siberian jays from the genetic perspective with the help of effective population size estimation, and at the same time assess the usefulness of effective population size estimation for the genetic viability analysis of the Suupohja Siberian jays. As the Siberian jay is an example of a declining Scandinavian old-growth forest species, this study may give insight to the usefulness of this method in other similar conservation cases. The fact that the Suupohja Siberian jays have been extensively studied in other respects (e.g. Lillandt *et al.* 2003, Bergholm 2007, Angervuori 2008, Alho *et al.* 2009, Jaari *et al.* 2009, Gienapp & Merilä 2010, Li & Merilä 2010a, Li & Merilä 2010b) also provides the possibility to compare the findings of effective size estimation with other parameters of population dynamics. Additionally, the study can also complement the existing perspectives (e.g. Mönkkönen *et al.* 1999, Pimenoff 2000, Pihlajaniemi 2006) on what factors may be important in Siberian jay conservation.

I focused on the following five questions:

1. What is the average N_e/N ratio among the Suupohja Siberian jays? What biological and environmental factors have an effect on the N_e/N ratio?
2. What is the effective size of the population that the Suupohja Siberian jays are part of? Is this population large enough to be genetically viable?
3. If the immigration of Siberian jays to the study area would cease, would the study area population be large enough to be genetically viable? Therefore, what is the importance of dispersal pathways to the genetic viability of the Suupohja Siberian jays?
4. Based on the findings of this study, what kind of conservation actions would help to ensure the genetic viability of the Suupohja Siberian jays?
5. Compared to the required effort, how reliable and useful is effective size estimation in the analysis of the genetic viability of the Siberian jay? Which estimator types were most useful for the study, considering the characteristics of the species? How much of the same information could be gained with simpler estimators, such as monitoring changes in heterozygosity or allelic diversity?

The first question is both of general interest and relevant to the conservation of the Suupohja Siberian jays. An estimate of the N_e/N ratio based on the Suupohja Siberian jays might help relate effective population sizes to true population sizes and vice versa and to make comparisons to other species. I created an overall N_e/N ratio estimate with the help of several demographic N_e estimators, and utilised the mathematical differences of the methods to determine which factors are most significant in the formation of the ratio.

To answer the second question, I utilised two genetic N_e estimation methods and the N_e/N ratio estimate to make a census size estimate of the population, and compared the calculated N estimate against the known Siberian jay sightings around the study area in Suupohja. Based on these results, I assessed how reliable the genetic N_e estimates are in the estimation of the size and structure of the Siberian jay population around Suupohja. Finally, I compared the N_e estimate against the effective population sizes that have been proposed as minimum standards for maintaining genetic viability (see section 1.2.2).

The third question is a more speculative continuation of the second question. To answer it, I created an N_e estimate for an imaginary population of the size of the study area. I also discussed the extent to which current data can be used to estimate population viability in a changed environment.

Answers to the fourth and fifth questions arose from the results of the study.

2 MATERIALS AND METHODS

2.1 Study area

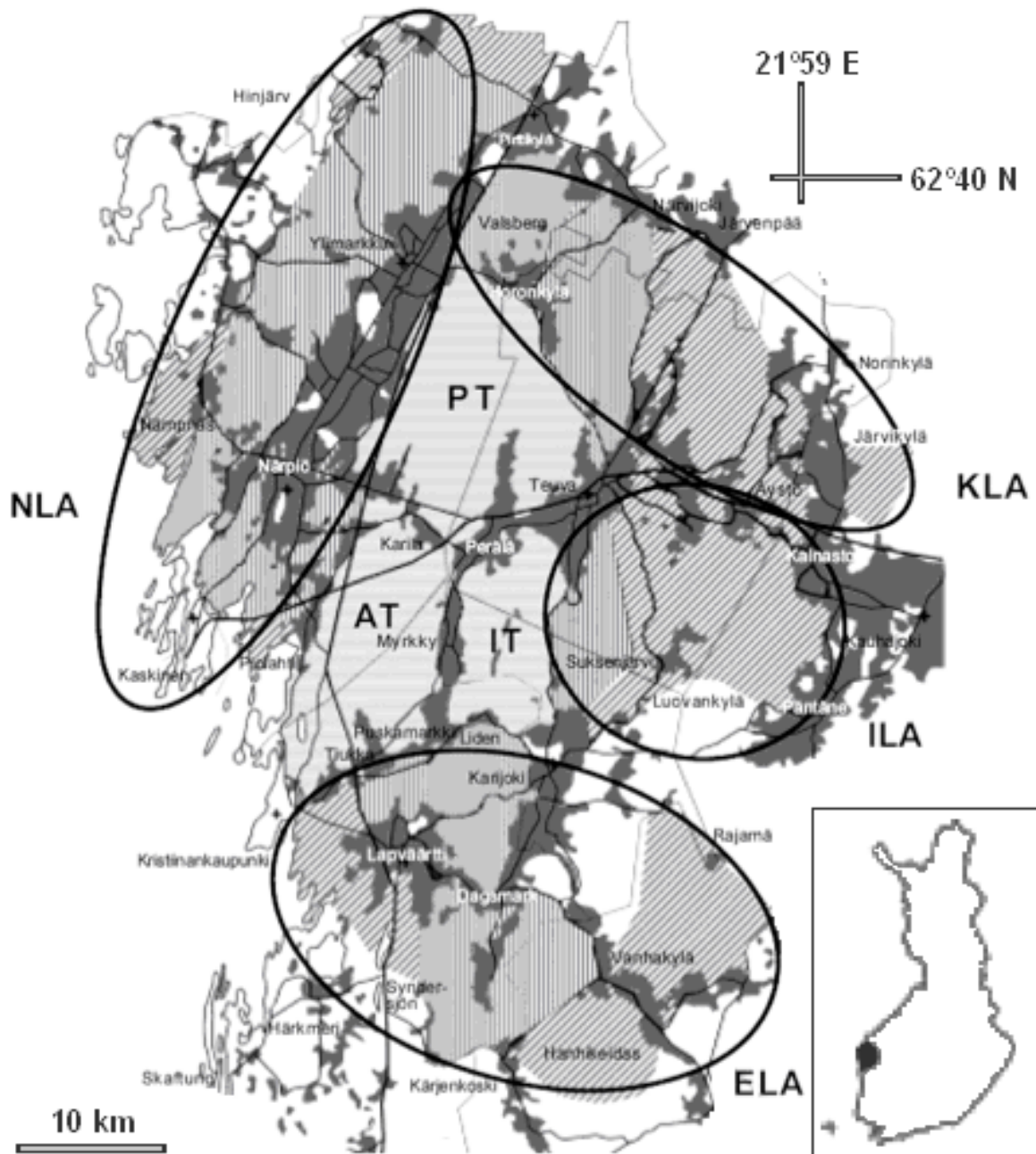


Figure 1: Map of the Siberian jay study area in Suupohja. Original study area (AT), Northern study area (PT), Eastern study area (IT), Southern additional area (ELA), Eastern additional area (ILA), North–Eastern additional area (KLA) and Närpes additional area (NLA). Map adopted from Lillandt (2002).

The Suupohja area, covering parts of seven municipalities on Western Ostrobothnia in Finland (ca. 62°22'N, 21°30'E), belongs to the Mid Boreal climatic zone. The area consists of a mosaic of agricultural fields, rural and semi-urban settlements and forest plots of various

sizes. Forests in the area are mainly privately owned, and their composition varies from spruce-dominated mixed forests to planted pine saplings. The strip-like layout of forest plots is a historical remnant from *Great Partition*, a 18th century land division process where cultivable fields were combined into larger plots and forests were divided between households based on equal access and distance (Kuusi 1914, Maanmittaushallitus 1983, Angervuori 2008). The average size of a forest strip is 0.5–2 ha (Lillandt *et al.* 2003).

Regular follow-up of Suupohja Siberian jays was originally started in a 120 km² wide study area (AT) in 1974 (Lillandt *et al.* 2003). The 155 km² wide Northern study area (PT) was included gradually during 1985–1991 and the 70 km² wide Eastern study area in 1992. The overall size of total study area grew to 1500 km² when follow-up was extended to four additional study areas (ELA, NLA, KLA and ILA) during 1998–1999 (Table 1). Then, the extensions ceased, since no Siberian jay territories could be found in the surrounding areas (B.-G. Lillandt, pers. comm.). 1000 km² of the total study area (KT) is covered by forest. MSc Bo-Göran Lillandt has been in charge of the project since 1987 and has carried out all morphologic measurements, bird ringings and sampling of DNA. During the study period, several persons have assisted in observing and catching birds (Lillandt 2000, 2002, 2003, Lillandt *et al.* 2003).

Table 1. Siberian jay study areas in Suupohja, their regional codes, areas (km²) and starting years of observation.

Study area	Regional code	Area (km ²)	Start of observation
Original study area	AT	120	1974
Northern study area	PT	155	1985 (*)
Eastern study area	IT	70	1992
Southern additional area	ELA	250	1998 (*)
Närpes additional area	NLA	250	1998 (*)
North-eastern additional area	KLA	240	1998 (*)
Eastern additional area	ILA	160	1998 (*)

(*) Follow-up was gradually expanded over the area, starting from this year; see text.

2.2 Data gathering

2.2.1 Spring and autumn follow-ups

Annual follow-ups of Suupohja Siberian jays were done each year in July–October when Siberian jay families move actively around their territory, gathering food reserves for the forthcoming winter. Territories were identified with the help of fat feeders. A fat feeder was a piece of metallic fish trap net, marked with red stripes and containing 100 g of unsalted solid

cooking fat. Feeders were placed all over the study area at 1–1.5 km intervals to cover all possible territories within the area. Feeding stations were most typically placed by an easily observable tree close to a forest track, at the height of ca. 1.5 m (Lillandt 2000, Lillandt *et al.* 2003, B.-G. Lillandt, pers. comm.).

Feeders were set out weeks prior to the commencement of yearly observations. Siberian jay families usually start to visit feeders some weeks after their introduction, although there have been cases where a feeder has stayed untouched for months before use (Lillandt 2000). Following this, feeders were visited regularly until certainty of the possible occupants of the territory was reached. Preliminary conclusions about the presence of Siberian jays were made based on the size of feeding marks in the fat and the speed of fat consumption, as only Siberian jays are known to be able to empty the feeders quickly while creating their winter storages. Other possible visitors at the feeding stations were the Eurasian jay (*Garrulus glandarius*) and flocks of various tits (*Parus* sp.; Lillandt 2000; Lillandt *et al.* 2003; B.-G. Lillandt, pers. comm.). During the actual observation period, feeders were typically observed while sitting in a car and playing Siberian jay vocalisations from a tape recorder. Siberian jays visit feeding stations even in the presence of a person observing in a car. The recording was played 1–2 times during each visit (B.-G. Lillandt, pers. comm.).

Siberian jays appearing at the feeding station were observed with a telescope. Unringed individuals were caught with a trap made of metallic fish trap net, baited with the same fat as the feeding stations, and then ringed with an ordinary aluminium ring containing an individual serial number and an individual combination of coloured rings (Lillandt *et al.* 2003). Ringed birds were identified based on their individual colour ring combinations. Each family were observed at least twice if possible. When there was certainty about the territory's occupancy status, and that all birds in the area had been observed and ringed, feeders were removed (Lillandt 2000).

In addition to autumn follow-ups, spring follow-ups were also carried out during some years. During spring follow-ups, Siberian jay nests were searched within the core areas of the territory and nestlings were ringed as explained above (Lillandt 2000).

2.2.2 Gathering and storing of DNA samples and observational data

Times and locations of observations were recorded for each individual. The behaviour of the family members was observed, since parents usually behave differently towards their own offspring than extra juveniles in the family group (Lillandt 2003, Lillandt *et al.* 2003).

During ringing, various morphological characters were measured and recorded. Age was determined and recorded based on the shape of the outermost tail feathers, which are known to be curved in juveniles (less than one-year-old birds) and straighter in adults (more than one-year-old birds; Svensson 1992). Sex was initially determined based on bird weight

and size of individuals, which is confirmed by genetic analyses to be quite a reliable method (Lillandt *et al.* 2001). Next, either one or both of the outermost tail feathers were plucked and saved in paper envelopes marked with the unique identification number of the individual, and stored at room temperature until further processing. Since 1997, a blood sample was also taken from each individual from a vein in the leg or wing (Lillandt 2000; Lillandt *et al.* 2003; B.-G. Lillandt, pers.comm.).

Blood samples were stored in a tube containing 500 µl SET buffer (0.15 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH 8.0) immediately after sampling and the tube was marked with the unique identification number of the individual. The tubes were stored either in the ambient temperature or in a cooled thermo bag until freezing within 1–12 h (average 6 h) at –20 °C. The frozen samples were transferred to the laboratory premises in a cooled thermo bag, typically within two months, except a pause in DNA sample processing during 2003–2004, during which the samples (929–1043) stayed in –20 °C for 1–2 years. In the laboratory, the samples were stored in –80 °C (with some samples stored in –20 °C for a short time period) until further processing.

2.3 Preparation of population genetic data

2.3.1 DNA extraction and genotyping of microsatellite loci

DNA was extracted using several methods depending on the period of sampling. MSc Bo-Göran Lillandt carried out DNA extractions until year sample 928 according to methods described in Lillandt *et al.* (2001). Starting from sample 929, DNA extractions were carried out within Ecological Genetics Research Unit. DNA from samples 929–1087 was extracted with High Pure PCR Template Preparation Kit (Roche) by MSc Katja-Riikka Pullinen. DNA from samples 1088–1152 was extracted with DNeasy Blood and Tissue Kit (Qiagen) by PhD Sonja Jaari (samples 1088–1111) and laboratory technician Marika Karjalainen (samples 1112–1152).

Long-term storage of DNA extracts took place in –80 °C in 1x TE buffer (samples 1–928; 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or in the elution buffer of the DNA extraction kit used (sample 929 and onwards). Since 2007, DNA extracts were stored at –20 °C. Practical dilutions of DNA extracts (1:10 dilutions in MilliQ) prepared for use until further processing were stored at +4 °C.

In this study, I used genotype data from 21 microsatellite loci listed in Table 7 (Appendix I). The first nine loci and their primers (CK1B5D-PPi1, Table 7) were developed by MSc Bo-Göran Lillandt as described in Lillandt *et al.* (2002). The remaining 12 loci (2F9–5G10, Table 7) were developed by PhD Sonja Jaari and MSc Kaisa Välimäki as described in Jaari *et al.* (2008). All individuals within the dataset were genotyped for these 21 loci, with

some variance in genotyping success (Table 8, Appendix II). Genotyping of samples 1–928 for loci CK1B5D–PPi1 was done as described in Lillandt *et al.* (2002). Genotyping of all other markers and samples was done by Ecological Genetics Research Unit as described below.

For samples 929–1152, loci CK1B5D–PPi1 were amplified using PCR (4.57 µl water (MilliQ), 1 µl 10x BioTaq™ PCR Buffer (Bioline, Taunton, MA), 1 µl 100x BSA (NEB), 0.3 µl 50 mM BioTaq™ MgCl₂ (Bioline, Taunton, MA), 0.08 µl 25 mM (each) dNTPs (Finnzymes), 1 µl 10 µM primer F, 1 µl 10 µM primer R, 0.05 µl 5U/1 µl BioTaq™ DNA polymerase (Bioline, Taunton, MA) and 1 µl 1:10 diluted DNA sample). The thermal cycling conditions for loci CK1B5D–PPi1 were: 94°C for 3 min; 35 cycles of 94°C for 30 s, 50–60°C for 30 s, 72°C for 45s and 72°C for 10 min. For all samples, loci 2F9–5G10 were amplified using PCR with slightly varying conditions (5 µl 2x Multiplex Mastermix (Qiagen), 2 µl water (MilliQ), 1 µl Q-solution (Qiagen), 1 µl 2 µM primer F, 1 µl 2 µM primer R and 1 µl 1:10 diluted DNA sample). The thermal cycling conditions for loci 2F9–5G10 were: 94 °C for 15 min; 30 cycles of 94 °C 30 s, 56 °C for 1 min 30 s, 72 °C for 1 min, followed by 60 °C for 5 min and 20 °C for 5 min. PCRs were mainly carried out by laboratory technician Marika Karjalainen.

Amplified PCR products were diluted with MilliQ (1:50 dilution for samples 1–928 and 1:150 dilution for samples 929 and onwards) and 3 µl of the dilution was mixed with 12 µl of 1:47 dilution of either MegaBACE™ ET550-R Size Standard (GE Healthcare, Life Sciences) or MegaBACE™ ET400-R Size Standard (GE Healthcare, Life Sciences). The samples were analysed with MegaBACE™ 1000 capillary sequencer (GE Healthcare, Life Sciences). PCR product dilutions and addition of size standards was mainly done by laboratory technician Marika Karjalainen and the MegaBACE™ sequencer runs by laboratory technician Leena Liikanen.

The sizing of microsatellite alleles based on MegaBACE™ 1000 capillary sequencer results was carried out using FRAGMENT PROFILER 1.2 (GE Healthcare, Life Sciences) by PhD Sonja Jaari.

2.3.2 Sexing and analysis of family relationships

After genotyping, the sex of individuals, originally determined based on morphological characteristics, was genetically confirmed for 90.6 % (n=1158) of the birds; the success being higher (100 %) in birds that were successfully genotyped for at least 14 loci and lower (46.8 %) with the rest of the birds. Until year 2003 (samples 1–928), genetic sex identification was carried out by MSc Bo-Göran Lillandt (B.-G. Lillandt, unpubl., see Lillandt *et al.* 2001, 2003), and starting from year 2006 (samples 929–1152), based on a W-linked CHD1 locus (Fridolfsson & Ellegren 1999) by Marika Karjalainen.

Following sex identification, pedigrees — as comprehensive as possible — were constructed. DNA-based parentage analysis was necessary due to the complex social

behaviour of Siberian jays, and also due to the fact that most birds were ringed only after some of the juveniles had already dispersed from their natal territory. Parents were primarily searched among the territory-holding monogamous breeding pairs in the population, since nearly nonexistent extra-pair paternity rates have been assumed in the Siberian jay based on field observations, species ecology and genetic reverse analyses of attained pedigrees (Ekman *et al.* 1994, Lillandt *et al.* 2001, P. Gienapp & J. Merilä, unpubl.). Parentage probabilities first analysed by MSc Bo-Göran Lillandt, by counting allele mismatches of the microsatellite loci available at the time to alleles of known breeding pairs with a relevant life span (Lillandt *et al.* 2001, 2003). After this, Dr. Phillip Gienapp crosschecked parentage determination in Ecological Genetics Research Unit by analysing parentage likelihood scores with CERVUS 3.0 (Kalinowski *et al.* 2007). The pairs with highest likelihood were compared against field observations, and in cases without uncertainties, individuals were assigned to parents. Individuals with no probable parents in the study area were classified as immigrants. In the very few unclear cases, no parents were assigned to the given individual.

2.4 Production of ecological parameters and genetic samples for N_e estimation

2.4.1 AT and KT datasets

To take advantage of both the thirty-year time span of the total observation history and the wide scope of the observations in the later years, I focused on two datasets: Siberian jays observed in the original study area (AT) in 1974–2006 and those observed in the total study area (KT) in 1999–2006. I calculated ecological parameters needed for N_e estimation separately for AT and KT if possible. However, due to the limited size of the AT and KT datasets, I calculated most parameters based on other types of classifications of the data.

2.4.2 Annual population sizes

For the use of further calculations, e.g. for the calculation of average population size, adult sex ratio and offspring survival ratio, I estimated annual population sizes for AT and KT. The presence of individuals in KT was estimated based on ringing year and the year of last observation. If an individual was ringed during the year x , and the year of last observation was $x + g$, the individual was considered to be present during x to $x + g$, even if it was not sighted every year in between; Siberian jays are known to be loyal to their territories, and typically exhibit only juvenile dispersal until they find a permanent territory (Ekman *et al.*

1994, 2001, Lillandt *et al.* 2003). To determine if an individual was living in AT during any certain year, annual territory records were used.

While estimating annual population sizes, I made a distinction between those offspring that survived in the study area until adulthood and those that did not. This distinction allowed focusing some of the further analyses to those breeders and offspring that had a chance of contributing to the gene pool. This distinction gave three classes of annual population sizes:

- (1) Annual number of **adults**, N_{xA} , includes all individuals that were present in the area and which, based on their ringing year, ringing age and year of last observation were adults, i.e. were at least one year old during year x .
- (2) Annual number of **surviving offspring**, N_{xO} , includes individuals that were ringed as nestlings or juveniles in the area during year x and that were known to have survived until next year.
- (3) Annual number of **non-surviving offspring**, N_{xD} , includes individuals that were ringed as nestlings or juveniles in the area during year x and that were not sighted in any later year. This class may include individuals that survived until adulthood but migrated outside of study area as juveniles. The number of individuals in this class may be affected by whether nestlings were ringed during the year in question, but since the offspring survival ratio is always dependent on definition (e.g. Ridley 2007), this is not expected to influence further analyses.

Since there was no survival data for the offspring of year 2006, I estimated N_{xO} and N_{xD} for the year 2006 out of the total number of offspring in 2006 by using the average **offspring survival ratio** of all ringed juveniles of the previous years in the dataset. Individuals ringed as nestlings were excluded in the estimation of the offspring survival ratio.

I divided the annual numbers of adults further into subclasses, based on two classifications:

- (1) Annual number of **adult females** N_{xF} and annual number of **adult males** N_{xM} were estimated only for KT (1999–2006) where genetic sex identification success was 100 %, and thus, $N_{xF} + N_{xM} = N_{xA}$. These annual numbers were also used to estimate a general **adult sex ratio**, r , based on the weighted mean of N_{xM} / N_{xA} of KT 1999–2006.
- (2) Annual number of **breeding adults** N_{xB} is an estimate of the number of adults that produced surviving offspring in the study area during year x . As the parents of some surviving offspring had not been identified, I counted the parents of those surviving offspring whose parents had both been identified, and divided this number by the proportion of offspring used in this calculation out of all surviving offspring born during the

year, in order to account for the non-identified parents. If emigration and immigration are assumed to be equally large, then the total number of nestlings and juveniles ringed during the given year reflects the number of offspring born within the study area, even though it may include immigrant juveniles. The estimate neglects the parents of such individuals that are born in the area but only ringed as adults, but the error caused by undermining this possibility is expected to be very small. Annual number of **non-breeding adults** is simply $N_{xNB} = N_{xA} - N_{xB}$. The proportion of breeding adults was similarly estimated for the year 2006 as the proportion of surviving offspring.

I calculated annual total population sizes as $N_{xA} + N_{xO} + N_{xD}$. The average annual number of newborns was estimated with $N_1 = \overline{(N_{xO} + N_{xD})}$, acknowledging that this number neglects nestlings that died before ringing. In case of AT, regression analysis was used to test whether population size was linearly dependent on study year, i.e. whether population size was steadily increasing or decreasing. Other generalised measures of population size were estimated based on the numbers of adults and surviving offspring only, i.e. $N_{xA} + N_{xO}$, to capture only such changes that could affect the genetics of the population. The average population size was estimated with $N = \overline{(N_{xA} + N_{xO})}$. Annual population growth rates, λ_x , were estimated as the proportional change in $N_{xA} + N_{xO}$ compared to the previous year; the smallest and largest four-year average growth rates were recorded, to reflect the scale of population size fluctuations in one generation.

To retrieve information on how annual population size and its fluctuations affect gene flow from generation to generation, I estimated the average proportion of non-surviving offspring out of annual total population size for AT and KT. In the case of AT, regression analysis was used to test whether the proportion of non-surviving offspring and the proportion of breeding individuals in the population were linearly dependent on the total population size. KT time series was considered too short for similar analysis.

2.4.3 Immigration

I estimated annual immigration rates in AT and KT based on breeding data, which would reflect most realistically the effective annual rates of immigration from the population genetic viewpoint (Wang 2004, Kobayashi *et al.* 2008). To calculate a measure that would be commensurable with estimating immigration based on arriving immigrants, the annual percentages of immigrant males and females breeding in the study area *for the first time* were calculated out of all males and females breeding during the year (immigrants being those individuals that according to the pedigree analyses had no probable parents in the study area; see section 2.3.2). Breeding was estimated based on the production of surviving off-

spring. Individuals without DNA samples were not taken into consideration when calculating the percentage of immigration. Average immigration rates were separately calculated for females, males and both sexes. It should be noted that annual immigration rates may not be commensurable with estimates of immigration rate per generation (Wright 1943), but annual immigration rates are more easily compared with annual fluctuations of population size.

To verify the assumption concerning mainly juvenile dispersal among the Siberian jays, I calculated the proportion of immigrant males and females that arrived to the study area as adults. The analysis was carried out based on all immigrant males and females in the data for which sex was genetically verified.

2.4.4 Age specific survival and fecundity

I calculated **age-specific survival and fecundity values** based on individuals that were ringed as nestlings or juveniles in the study area and for which sex was genetically identified. For each of these individuals, the age at death was estimated based on ringing year and the year of last observation. Next, the numbers of all individuals that lived at least to age i were added to the age cohort i , and in this way virtual age cohorts were created. The survival and fecundity rates calculated with this dataset are thus generalisations over the whole data, and as such, do not specifically apply either to AT or KT. However, they should give an overall picture of the life history of the Suupohja Siberian jays.

Based on these virtual age cohorts, I calculated proportional drops in the sizes of successive age cohorts. The dependency of survivorship within adult age classes on age was tested with regression analysis. Since no significant dependency between age and survival was found, I estimated age-independent yearly survival rates for adult males (v_m) and adult females (v_f) by calculating a weighted average proportional decrease between successive age cohorts, in addition to estimating age-specific survival rates for males (v_{iM}), females (v_{iF}) and both sexes (v_i). This method of calculating v does not account for individuals that migrated from the study area. However, as individuals do not usually migrate after establishing a territory (Ekman *et al.* 1994, 2001, Lillandt *et al.* 2003), this should not have a large effect on the final estimate of v that is counted across age cohorts. The survival data was also used to estimate j , **maximum age of death**.

I estimated age-specific fecundities by first listing the parents of surviving offspring for whom the identity of both parents were known. Next, I calculated the ages of the mothers and fathers of these offspring. Age specific fecundities, i.e. births per individual in age class i , were estimated for males (l_{iM}), females (l_{iF}) and both sexes (l_i) such that the contribution of the age class i to the gamete pool is $l_i v_i$ and $\sum l_i v_i = 1$.

I used age-specific fecundities to estimate **generation times** for females (T_F) and males (T_M) separately with $\sum l_i v_i i$. This equation is essentially the same as the Charlesworth (1994) equation for calculating generation time independently of population growth rate. The average generation time for both sexes was estimated with $T = (T_F + T_M)/2$.

2.4.5 Variation in the number of progeny

I estimated the **sex ratio of breeding adults** as the ratio of breeding males (N_{mB}) per breeding females (N_{fB}), i.e. $a = N_{mB}/N_{fB}$, based on the dataset of breeding pairs produced in pedigree analysis. To test the assumption of monogamy, the number of individuals in this data that had produced offspring with more than one partner during one year was recorded.

Mean and variance of lifetime progeny number for females and males, $\bar{k}_f, \bar{k}_m, \sigma^2_{kf}$ and σ^2_{km} respectively, were estimated based on the progeny numbers of individuals that were ringed as juveniles in the study area between 1992 and 1999. These cohorts were chosen based on high genetic sex identification success (100 %), cohort size (≥ 37) and adequate time for the individuals to produce offspring until the end of the study period. Numbers of surviving offspring were recorded for these individuals. Number of genotyped loci was also recorded, to illustrate the reliability of parent-offspring pair matching. Since the sizes of the cohorts were small and cohort size affected the variance estimate, variances were calculated by creating samples of four cohorts combined, to mimic the number of offspring in one generation, as the generation time was estimated to be close to four years. Four successive cohorts were grouped (i.e. 1992–1995, 1993–1996, etc.), the first cohorts were used to complement the samples after reaching the last cohort (i.e. 1998–1999+1992–1993), and finally, the average variances were calculated based on the variances of these cohort-groups. The percentages of males and females in the dataset that did not produce any offspring during their lifetime were also recorded.

Annual distribution of the number of progeny was estimated based on the whole dataset, by calculating for each year the number of surviving offspring produced by each adult female. The data over the years was combined to create a virtual year with different numbers of surviving offspring produced. Based on this distribution, the proportion of females whose annual breeding success follows Poisson distribution (see Nunney & Elam 1994), α_f , was estimated: First, the mean expectancy of the Poisson distribution was set to be the mean number of surviving offspring produced by those females that bred successfully. Next, the POISSON function of MICROSOFT® EXCEL® 2004 FOR MAC 11.5.2 was used to calculate the proportion of females attempting to breed that were expected to be successful i.e. to produce at least one surviving offspring according to this Poisson distribution. Next, the number of successful females in the dataset was divided by the expected proportion of

successful females, to estimate the number of females attempting to breed. Finally, the proportion of females following Poisson distribution was calculated by dividing the number of females attempting to breed with the total number of adult females in the dataset.

2.4.6 Momentary genetic samples of AT and KT

To enable genetic analysis at several time points, I prepared a set of year-specific momentary samples of genotype data for AT and KT. In the case of AT, where cohort sizes were small, samples had to be based on individuals of different ages, so surviving offspring and adults of all ages living in AT during the year of virtual sampling were included in the sample. To minimise the noise caused by the age structure in further genetic analyses, two samples were taken temporally as far from each other as possible, while aiming at as large sample sizes as possible. As a result, years 1981 and 2002 were chosen for virtual sampling in AT. 14 loci were used in the analysis, since the rest of the loci had a low number (<19) of genotyped individuals per locus in the 1981 sample. In the case of KT, where the sample sizes were larger, samples were collected for each year, but only individuals of same age, i.e. offspring born during the year under study, were selected. The samples did not include non-surviving offspring born during the year, to avoid bias that might be caused by clutches of siblings with similar genes but no contribution to the population gene pool. Therefore, year 2003 and 2004 cohorts were excluded due to small size and the year 2006 cohort due to the lack of survival data. The KT samples consisted of 21 loci.

2.5 Effective population size estimation

2.5.1 Role of ecological factors in the formation of the N_e/N ratio

To test the role of various ecological factors on the resulting N_e/N ratio in the Siberian jay, I used four simple N_e estimators, each based on one ecological parameter, to calculate preliminary N_e/N ratio estimates. Since the values given by the estimators were dependent on population size (N), the estimates were derived for AT and KT datasets as if the two datasets represented true populations. Also, N_e estimates for these imaginary populations were calculated for comparison. All four of the estimators below were originally developed for populations with discrete generations, but Hill (1979) showed that the effective population size is the same for a population with overlapping generations as would be with discrete generations, providing equal lifetime variance of progeny production and number of individuals entering the population for each generation.

According to Waples (1990a, 1990b, see also Schmeller & Merilä 2007), effective population size can be approximated by multiplying the average annual **number of breeders** by generation time:

$$N_{eB} = \overline{N_{xB}}T. \quad (1)$$

Lehmann and Perrin (2006) suggest harmonic mean population size as an approximate N_e estimate in populations with **fluctuating population size** (see also: Allendorf & Luikart 2007:158). This was calculated based on the numbers of adults and surviving offspring, t being the number of years analysed:

$$N_{eN} = t / \sum \frac{1}{(N_{xA} + N_{xO})}. \quad (2)$$

The effect of **variance in lifetime progeny numbers** on the effective population size can be estimated with the following Allendorf & Luikart (2007:153–157) equation for populations with constant population size:

$$N_{eK} = \frac{8N - 4}{\sigma_{km}^2 + \sigma_{kf}^2 + 4}. \quad (3)$$

According to Wright (1938, Allendorf & Luikart 2007:151–153, Schmeller & Merilä 2007), the **reproductive skew between sexes** can be analysed with the following equation:

$$N_{eFM} = \frac{4N_f N_m}{N_f + N_m}. \quad (4)$$

Since the number of males and females can be expressed as $N_m + N_f = N$, and the proportion of males to females can be expressed as $N_m = aN_f$, the equation was modified into

$$\frac{N_{eFM}}{N} = \frac{4aN_f^2}{N^2} = \frac{4aN_f^2}{(1+a)^2 N_f^2} = \frac{4a}{(1+a)^2}. \quad (5)$$

I used equations (1) and (2) to calculate the respective N_e estimates and N_e/N ratios for AT and KT based on the annual values and the average N of the respective areas. Equation (3) was similarly used to calculate N_{eK} estimates and N_{eK}/N ratios for AT and KT, but only the average N was specific to the dataset in the equations, and the variance estimate calculated from the 1992–1999 dataset was used in both estimates. Equation (5) was used to calculate a general N_{eFM}/N ratio based on a calculated generally for the Suupohja Siberian jays; this ratio was then used to calculate N_{eFM} estimates separately for AT and KT based on the average N of the respective areas.

2.5.2 Demographic estimates of the N_e/N ratio

I estimated the N_e/N ratio in the Suupohja Siberian jays by using three demographic N_e estimators, each based on a different set on ecological parameters. Since the values given by the estimators were dependent on population size (N), I derived the estimates for AT and KT datasets, as if they represented true populations. Most variables required by the estimators were, however, estimated generally for the Suupohja Siberian jays. Also N_e estimates for these imaginary populations were calculated for comparison.

Nunney and Elam's (1994) "minimal method" is developed for the analysis of populations of conservation concern, which are most often diploid, two-sexed, long-lived species with overlapping generations — such as the Siberian jay. The method also contains specific adjustments for monogamous species. By Nunney and Elam's own estimates, the minimal method gives results close to those gained by using the Hill (1972) model, which is regarded as the most accurate demographic estimator of effective population size (Harris & Allendorf 1989, Nunney & Elam 1994), but with less work.

Nunney & Elam's equation contains factors that are calculated differently depending on mating pattern and age-dependent survivorship of the population in question. I first verified that the Siberian jay adult survival is not age-dependent (see section 2.4.4) and that the mating pattern is monogamous with a slight excess of adult males in the population (see sections 2.4.2 and 2.4.5). Next, the equation was reformulated accordingly into

$$\frac{N_{eNE}}{N} = 4r(1-r)T \left(\frac{r(1+v_f)}{1-v_f} + \frac{(1-r)(1+v_m)}{1-v_m} + (1-r) \left(1 - \frac{(1-r)\alpha_f}{r} \right) / \frac{(1-r)\alpha_f}{r} + r \frac{1-\alpha_f}{\alpha_f} \right). \quad (6)$$

The offspring survival rate, different from the adult survival rate, was not taken into account, since Nunney and Elam do not give an equation that utilises year-by-year-specific survival rates. The proportion of non-surviving offspring should affect the gene flow from generation to generation only through the variation in the number of progeny – which is not a factor in this equation. The given N_{eNE}/N ratio was multiplied with N of the respective study area to get an N_{eNE} estimate.

Lande & Barrowclough's (1987) demographic estimator takes in account the lifetime variance in the number of progeny, which in the Siberian jay with a long adult life span may be significant. By combining Lande & Barrowclough's (1987) formulas for accounting effects of adult sex ratio, variance in male and female lifetime progeny production and sex specific generation time, the equation turns into

$$N_{eLB} = 4 \left(T \left(\frac{N_m \bar{k}_m - 1}{\bar{k}_m + (\sigma_{km}^2 / \bar{k}_m) - 1} \right) T_m + T \left(\frac{N_f \bar{k}_f - 1}{\bar{k}_f + (\sigma_{kf}^2 / \bar{k}_f) - 1} \right) T_f \right)^{-1}. \quad (7)$$

I estimated the average numbers of males and females as $N_m = rN$ and $N_f = (1-r)N$, by using the N of the respective study area.

Lande & Barrowclough (1987) suggest the separate calculation of these parameters for various points of time where the average progeny number and its variance are retrieved with equations where the population growth rate is a factor. Finally, the harmonic mean of these momentary estimates is used as the overall effective population size estimate for a fluctuating population. However, due to limited cohort sizes and the effort required by this method, I used general parameters $\bar{k}_f, \bar{k}_m, \sigma^2_{kf}$ and σ^2_{km} instead of the momentary parameters. One general effective population size estimate was calculated for each study area. The N_{eLB} estimates were divided with the N of the study area to get an estimate of the N_{eLB}/N ratio.

Felsenstein's (1971) demographic estimator for populations that have overlapping generations and a constant or steadily changing population size was also applied. The estimator was originally developed for haploid populations, but it also gave accurate results in the analysis of Waples & Yokota (2007) with three simulated species that were diploid, iteroparous and random mating, and represented three different life history types. Felsenstein's equation is based on weighting individuals by their reproductive values, based on their age specific survival and fecundity. By accounting for yearly breeding seasons in the Siberian jay, and therefore using N_1 instead of B that was used by Felsenstein (1971) for humans, and after inserting Felsenstein's formula for calculating reproductive values (page 592), the equation turns into

$$N_{eF} = \frac{N_1 T}{1 + \sum_i v_i \left(\frac{v_{i+1}}{v_i} \right) \left(1 - \frac{v_{i+1}}{v_i} \right) \left(\frac{\lambda^i}{v^{i+1}} \sum_{j \geq i+1} v_j l_j \lambda^{-j} \right)^2 \lambda^i - \sum v_i l_i^2 \lambda^{-i}}. \quad (8)$$

Constant population size was assumed, i.e. $\lambda = 1.0$. To test the scale in which the effective population size would be dependent on population size fluctuations, the equation was tested for AT and KT with the minimum and maximum values of λ_x of the study area. The N_{eF} estimates were divided by the N of the study area to get an estimate of the N_{eF}/N ratio.

Based on the analysis of the calculated respective N_e/N ratios, I chose an overall estimate to be used as the N_e/N ratio of Suupohja Siberian jays in further analyses (see section 4.1.2). I multiplied the average population size (N) of both AT and KT by this ratio, in order to illustrate what the N_e in the Suupohja population would be if the population would diminish to either population size by losing its migration pathways to other populations. Similarly, this ratio was used together with the genetic N_e estimates to estimate the census size of the total population.

2.5.3 Genetic estimates of N_e , assuming an unstructured population

Due to the unexpectedly high proportion of immigrants among breeding individuals (see section 3.1.2), I tested the genetic differentiation of these immigrants compared to the individuals born in the study area by calculating an F_{ST} -estimate (Rousset 2008, GENEPOP ON THE WEB 4.0, <http://genepop.curtin.edu.au/>, 1 Feb. 2010) between the KT cohort 1999 and the breeding immigrants at KT during the KT study period. Since the genetic differentiation between immigrants and local individuals proved to be low (see section 3.2.3), the genetic N_e estimates were produced based on default assumptions that the individuals classified as immigrants would belong to the same population as the Siberian jays in the study area (i.e. that they would not be real immigrants). An additional assumption was that the total population, supposedly not much larger than that in the study area, would not be characterised by isolation by distance. Based on these assumptions, the KT genetic samples were considered a representative sample of the whole population (Leberg 2005, Fraser *et al.* 2007).

I calculated the average genetic N_{eG} estimate for the Suupohja Siberian jays by first generating annual N_e estimates based on KT data with a moment-based temporal estimator and a point estimator (see below), and then calculating an average value of the calculated mean temporal and point estimates. To test the reliability of the assumption that a geographically restricted sample would be representative of the whole population, I also calculated temporal and point estimates of N_e based on AT data.

To calculate a temporal N_e estimate for AT, **the traditional moment-based temporal method** as in Nei & Tajima (1981) was applied, resulting in N_{eNT} . The challenge in using the Nei & Tajima (1981) method is in its development for discrete generations: Jorde and Ryman (1995) demonstrated that in species with overlapping generations, the age structure of the population causes temporal fluctuations of allele frequencies that can lead to biased temporal effective population size estimates unless the wobbling effect is taken into consideration by the estimator. However, Waples and Yokota (2007) state that the relative strength of the wobbling effect diminishes as the time between samples increases, and finally the population age structure is not a relevant source of bias if the samples have been spaced 3–5 generations apart. However, this condition was met by the AT samples.

In the case of KT, the **Jorde–Ryman adjustment of the traditional temporal method** (1995) was applied. The Jorde–Ryman method is developed specifically for populations with overlapping generations; it estimates the strength of the wobbling effect between samples by incorporating demographic data in the analysis, and thus allows effective population size estimates from consecutive cohorts of newborn individuals. Waples and Yokota (2007) found the Jorde–Ryman method to be more accurate than traditional temporal methods when

generations are overlapping for populations with constant, growing or declining population sizes. The overall estimator is

$$N_{eJR} = \frac{C}{2T(F - \frac{1}{2n_t} - \frac{1}{2n_{t+1}} + \frac{1}{N_1})}, \quad (11)$$

where F is the variance of allele frequency change between two consecutive cohorts, n_t and n_{t+1} are the sample sizes of the two cohorts, and C is a population age structure specific correction factor that is calculated by iterating age specific survivorship (v_i) and gene pool contribution ($l_i v_i$) parameters with Jorde and Ryman's (1995) equations (10)–(13) and (23). Momentary N_e estimates were calculated for all consecutive cohort-pairs, and the final N_e estimate was calculated as the mean value of these momentary estimates. The Python 2.1 code used in the calculation is presented in Appendix IV (section 7.4).

Various authors, including Nei & Tajima (1981) and Pollak (1983) developed formulas for calculating F from allele frequency change and estimating N_e based on measures of F . Jorde & Ryman (1995) showed that their estimator gives relatively accurate results both with Nei & Tajima's (1981) F_c and with Pollak's (1983) F_k , but in their later publication, Jorde and Ryman (2007) suggested a less precise but more unbiased measure F_s . Both temporal analyses were calculated by using F_s as the estimate of F .

LDNE 1.31 (Waples & Do 2008) was applied to both AT and KT data, resulting in point estimates of N_{eLDNE} . Given a microsatellite data set, LDNE analyses linkage disequilibrium of unlinked loci by using the Burrow's method (Cockerham & Weir 1977, Weir 1979, Waples 2006, Waples & Do 2008) and a relevant bias correction developed by Waples (2006). LDNE assumes a closed population, discrete generations, unlinked loci and selective neutrality of loci, and can be set to assume lifetime monogamy. The LDNE estimates were calculated by using the same data sets for AT and KT as which were used for temporal analysis. Since LDNE assumed unlinked loci, only loci separated by at least 45 cM in each linkage group (see Jaari *et al.* 2009) were included in the LDNE analyses, narrowing the datasets of LDNE analyses to 12 loci in KT and 11 loci in AT. LDNE 1.31 was downloaded from <http://fish.washington.edu/xfer/LDNE/> (12 Dec. 2008) and run on MICROSOFT® WINDOWS® XP™ 5.1 with jackknifing-based 95 % confidence intervals and by using 0.01 as the lowest allele frequency included in the analysis. Lifetime monogamy was assumed. It should be noted that while LDNE 1.31 assumes discrete generations, its accuracy when used with populations with overlapping generations poorly known (Waples & Do 2008). In the case where samples contain individuals of single cohorts, as it is the case with the KT annual data, the LDNE 1.31

estimates are assumed to be closer to the genetic estimate of N_b than that of N_e (Waples 2006). This might cause a downward bias to the estimates of N_{eLDNE} for KT.

To estimate the amount of information revealed by N_e estimation compared to that of heterozygosity and allelic richness, I estimated observed heterozygosity and allelic richness for each annual sample in AT and KT. Allelic richness was estimated by using HP-RARE 1.0 software (Kalinowski 2005), which was downloaded from <http://www.montana.edu/kalinowski/Software/HPRare.htm> (7 Jun. 2009) and installed and run on a MICROSOFT® WINDOWS® XP™ 5.1. Allelic richness estimates were calculated for each temporal sample by using the smallest sample size of the respective study area.

2.5.4 Genetic estimates of N_e , assuming immigration or isolation by distance

Since the origin of the individuals immigrating to the study area was not known, and since the validity of the assumption on the population structure is crucial for the correct estimation of N_e , I also considered two alternative assumptions concerning the population genetic structure of the Suupohja Siberian jays were:

1. The Suupohja Siberian jays form a self-standing population within the study area, and the individuals classified as immigrants originate from another Siberian jay population.
2. The Suupohja Siberian jays are genetically more connected with other Siberian jays outside the study area than expected, creating a larger continuous population of Siberian jays where dispersal distances limit the distribution of allele frequencies. Immigrants to the study area may differ from the study area Siberian jays genetically because of isolation by distance.

To test the first assumption, I applied the **Wang & Whitlock's (2003) moment-based estimator** to calculate a genetic N_e estimate and a migration (m) estimate at the same time. Fraser *et al.* (2007) recommend the application of the Wang–Whitlock estimator alongside the application of estimators for closed populations in all cases where gene flow is expected; the analysis of the discrepancies of the results may help to understand the population structure. The Wang–Whitlock estimator, designed specifically for open populations experiencing immigration from another population, is based on an assumption that immigration from another population would develop the allele frequencies of the target population towards those of the source population, which could then be detected by a suitable estimator. The version of the estimator that is developed for one study population (B) and an infinite source population of immigrants (A) requires three genetic samples; two temporally spaced samples (0 and t) from the study population and one from the source. By incorporating

equations for estimating effective size and by estimating mean allele frequencies during the sampling interval, the estimator becomes

$$\frac{1}{2N_{eWW}} = \frac{\Delta x_{B,t}^2 - \left(\frac{x_{B,0}(1-x_{B,0})}{2S_{B,0}} + \frac{x_{B,t}(1-x_{B,t})}{2S_{B,t}} \right) - \Delta x_{AB}^2 (1 - (1 - \hat{m})^t)^2}{\left(\frac{1 - (1 - \hat{m})^{2t}}{(2 - \hat{m})\hat{m}} \right) \times \left(\frac{x_{B,0}(1-x_{B,0}) + x_{B,t}(1-x_{B,t})}{2} \right)}, \quad (9)$$

where Δx is the difference in allele frequency between two samples, t is the time point of the second temporal sample between t generations, S is the size of the sample in question and \hat{m} is the estimate of migration rate. For estimating migration rate from the same data, Wang & Whitlock (2003) give an estimator

$$\hat{m} = 1 - t \sqrt{1 - \left(\frac{1}{\sum_l (\Delta x_{AB})^2} \right) \sum_l \left((\Delta x_{AB})^2 \times \frac{\Delta x_{B,t}}{\Delta x_{AB}} \right)}. \quad (10)$$

where l is the locus being analysed.

The Wang–Whitlock analysis was based on KT data, but for comparison, a similar analysis was also carried out based on AT data. The sampling years in KT were as far temporally spaced from each other as possible, i.e. 1999 and 2005. Allele frequencies of the source population were determined based on individuals that bred in the study area in question between the sampling years and that had not been born in the study area in question. Immigration rate was calculated with Wang & Whitlock’s migration estimator, but due to a negative result, the analysis was carried out by using zero as the immigration estimate. Estimates of $1/(2N_{eWW})$ were calculated for each allele, the mean and 95 % CI were estimated over all alleles and loci, and these values were used to calculate the N_{eWW} estimates.

The second alternative assumption concerning the population genetic structure of the Suupohja Siberian jays could have been tested by either estimating the genetic neighbourhood size of the Suupohja Siberian jays (Rousset 2000, Rousset 2001, Sumner *et al.* 2001, Leblois *et al.* 2003, Leberg 2005), or by estimating N_e in Suupohja with an F_{ST} –based estimator of N_e developed for geographically structured populations (Wang & Caballero 1999), but since data regarding territory locations, population densities or dispersal distances was not adequate for such analyses, these estimates were not generated.

3 RESULTS

3.1 Ecological factors affecting N_e estimation

3.1.1 Annual population sizes

Annual population sizes in AT (1974–2006) varied from 6 to 53 individuals (Figure 2; Table 9 in Appendix III), and four-year population growth rates (λ) from 0.72 to 1.16. The annual population size did not show a significant increasing or decreasing trend (linear regression; $R^2=0.017$, $F_{1,31}=0.54$, $p=0.47$). The proportion of non-surviving offspring was on average 13.6 ± 1.4 (SE) % of the total population size, the proportion being higher when the population size was large ($R^2=0.34$, $F_{1,30}=15.38$, $p<0.001$). The average offspring survival ratio was 52.6 % of the juveniles. There was no significant relationship between the total population size and the proportion of breeders in the population when all data was analysed ($R^2=0.005$, $F_{1,30}=0.14$, $p=0.71$), but if years with zero offspring were excluded from the analysis, the proportion of breeding individuals showed a decreasing trend when the population size increased ($R^2=0.16$, $F_{1,24}=4.63$, $p=0.042$). The average population size (N) was 23.88 ± 1.50 (SE), the average number of newborns (N_1) was 7.79 ± 0.85 (SE) and the average number of breeding adults ($\overline{N_{xB}}$) was 5.48 ± 0.72 (SE).

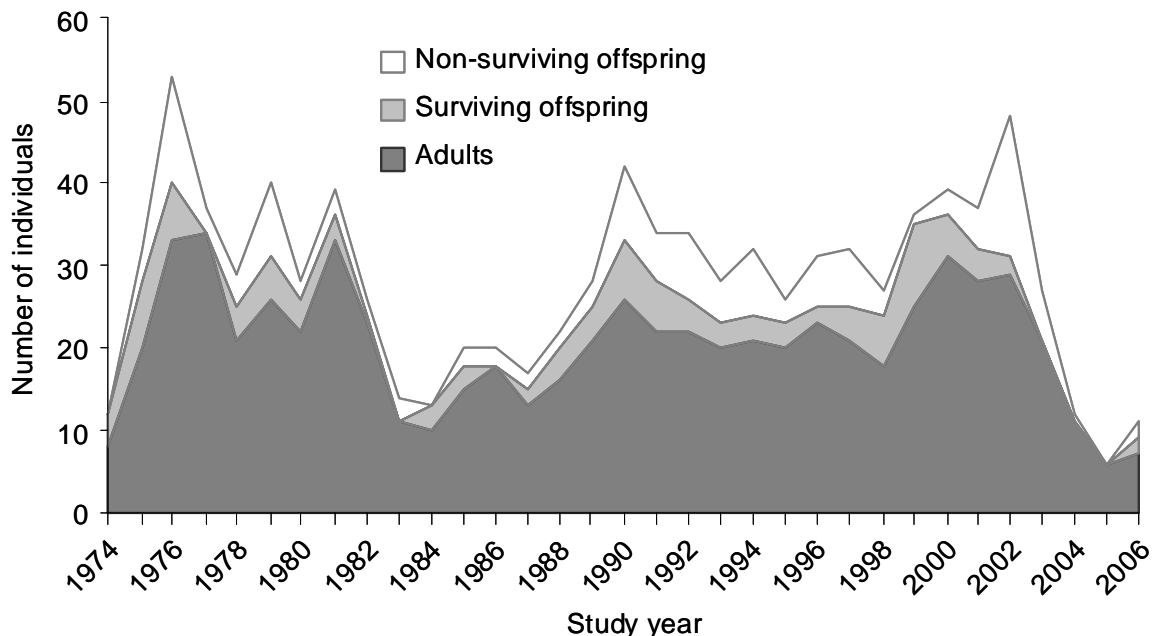


Figure 2: Population size fluctuations in the original study area (AT 1974–2006). The stacked area diagram depicts numbers of adults, surviving offspring and non-surviving offspring.

Annual population sizes in KT (1999–2006) varied from 263 to 131 individuals (Figure 3; Table 10 in Appendix III), and four-year population growth rate varied between 0.87–0.99.

The average offspring survival ratio was 42.8 % of the juveniles. The proportion of non-surviving offspring was on average 16.8 ± 1.4 (SE) % of total population size. The average population size (N) was 169.85 ± 14.0 (SE), the average number of newborns (N_1) was 59.63 ± 6.2 (SE) and the average number of breeding adults ($\overline{N_{xB}}$) was 37.70 ± 6.2 (SE). Adult sex ratio (males/all adults) was 0.513 ± 0.002 (95 % CI).

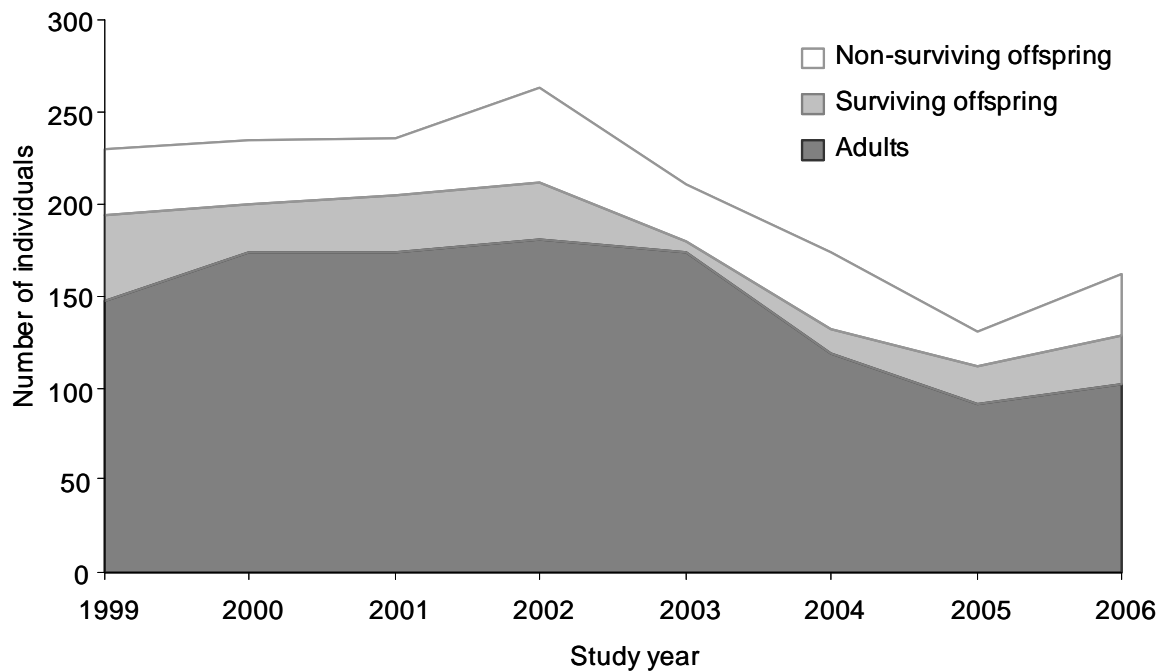


Figure 3: Population size fluctuations in the total study area (KT 1999–2006) The stacked area diagram depicts numbers of adults, surviving offspring and non-surviving offspring.

3.1.2 Immigration

Analysis of immigration rates in AT (1974–2006) was based on 31 breeding males and 28 breeding females. Immigration rate was 22.3 ± 6.2 (SE) % for males, 27.6 ± 6.1 (SE) % for females and 25.0 ± 4.5 (SE) % for both sexes combined. Annual percentages of first-time breeding immigrants out of all breeders are presented in Table 9 (Appendix III). Analysis of immigration rates in KT (1999–2006) was based on 78 breeding males and 83 breeding females. Immigration rate was 12.9 ± 4.1 (SE) % for males, 17.1 ± 4.1 (SE) % for females and 15.0 ± 3.9 (SE) % for both sexes combined. Annual percentages of first-time breeding immigrants out of all breeders are presented in Table 10 (Appendix III). Based on the whole dataset of immigrants, 82.7 % of immigrant males ($n=98$) and 70.8 % of immigrant females ($n=161$) had arrived to the study area as adults.

3.1.3 Age-specific survival and fecundity

Age-specific survival and fecundity was estimated based on records of 592 males and 554 females. Survival of adult Siberian jays is presented in Figure 4. Survival until adulthood was 47.0 % in males and 39.1 % in females. After reaching adulthood, there was no linear interaction between age and survival (males; $R^2=0.073$, $F_{1,9}=0.71$, $p=0.42$; females; $R^2=0.005$, $F_{1,9}=0.045$ $p=0.84$). The year-to-year survival rate was 69.6 ± 0.5 (95 % CI) % for males and 73.1 ± 0.5 (95 % CI) % for females, and 71.4 ± 0.3 (95 % CI) % for both sexes combined. The maximum age at death was in practice 12 years; only one individual in this dataset produced offspring after reaching this age.

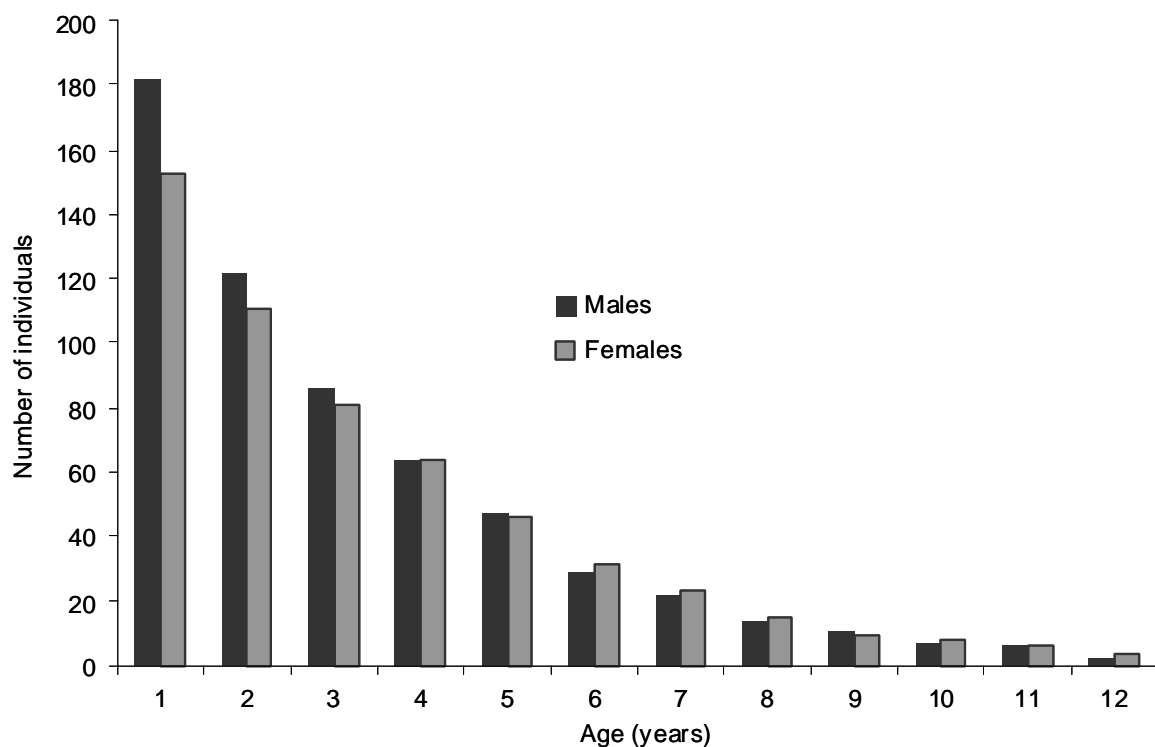


Figure 4: The distribution of age during last sighting of adult Siberian jay males and females in the 1992–1999 cohorts. Plotted are the numbers of individuals that reached a given age.

Age-specific contributions to the gene pool of adult Siberian jays are presented in Figure 5. The age of maximum relative production of surviving offspring was three years for females (21.0 % of offspring) and four years for males (20.7 % of offspring). Sex specific generation times (T) were 4.23 years for females, 4.20 years for males and 4.22 years for both sexes.

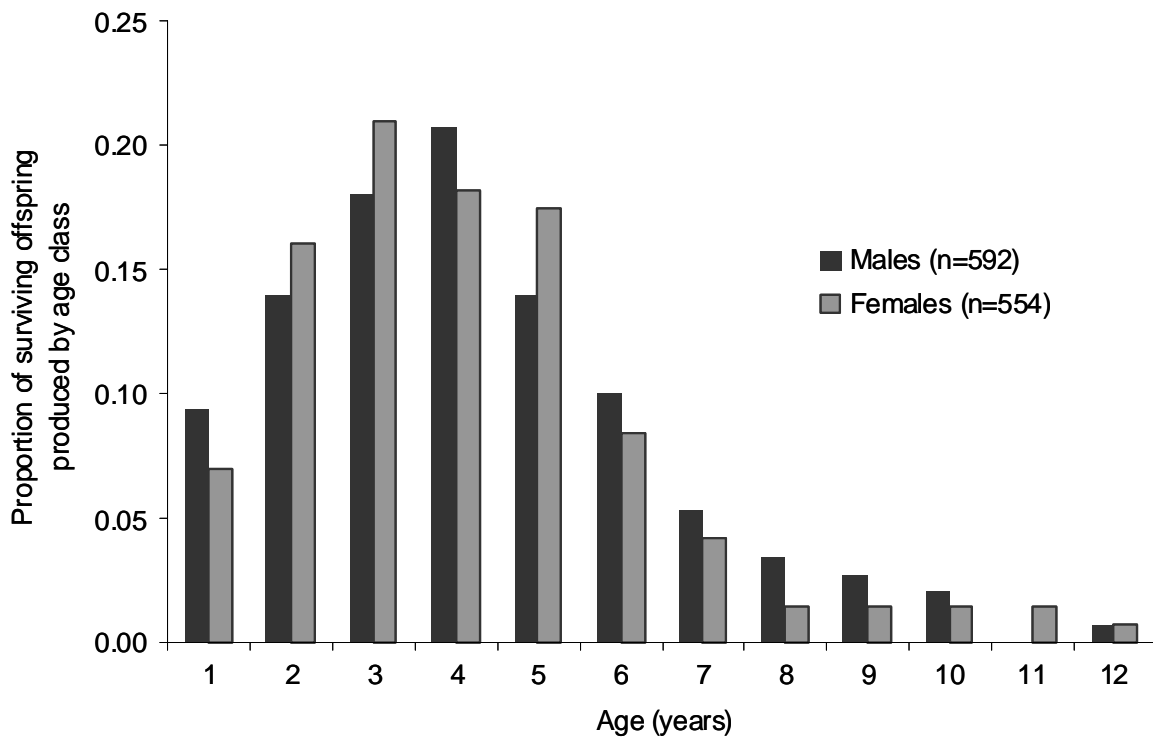


Figure 5: Proportions of surviving offspring produced by different age classes of male and female Siberian jays.

3.1.4 Variation in the number of progeny

Sex ratio of breeding adults was estimated based on 876 breeding pair-years, consisting of 276 males and 272 females. All breeding pairs that produced surviving offspring were monogamous, but individuals formed new breeding pairs after the disappearance of a lifetime partner. Thus, the lifetime sex ratio of breeding males to breeding females (a) was 1.01.

Lifetime variation in the number of progeny produced was estimated based on 63 females and 89 males in the cohorts covering years 1992–1999. Five of these individuals were genotyped for less than 14 loci, the lowest number of genotyped loci being eight. Offspring numbers up to seven offspring are presented in Figure 6. Yearly cohort size varied between four and 19 for females and between five and 27 for males. The average number of surviving offspring was 1.59 for females and 1.24 for males. The maximum number of offspring was nine for females and 13 for males. The average variance of breeding success within four successive cohorts was 4.09 ± 0.65 (95 % CI) for females and 4.60 ± 1.06 (95 % CI) for males. 41.3 % of females and 52.8 % of males did not produce any surviving offspring during their lifetime.

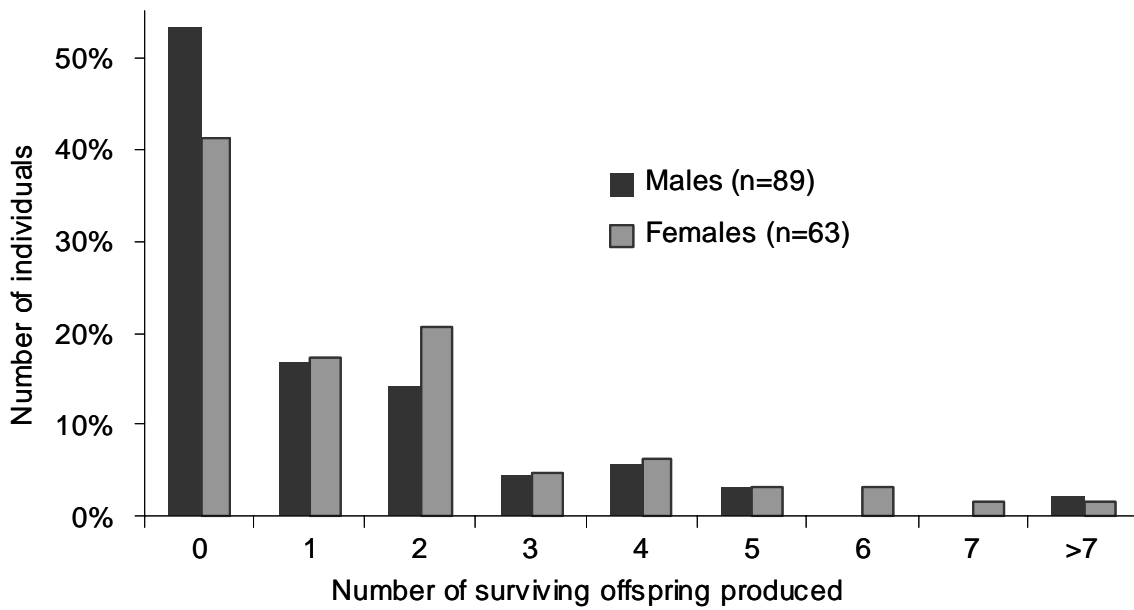


Figure 6: Variation in the number of surviving offspring produced by the males and females of the 1992–1999 cohorts of the Suupohja Siberian jays.

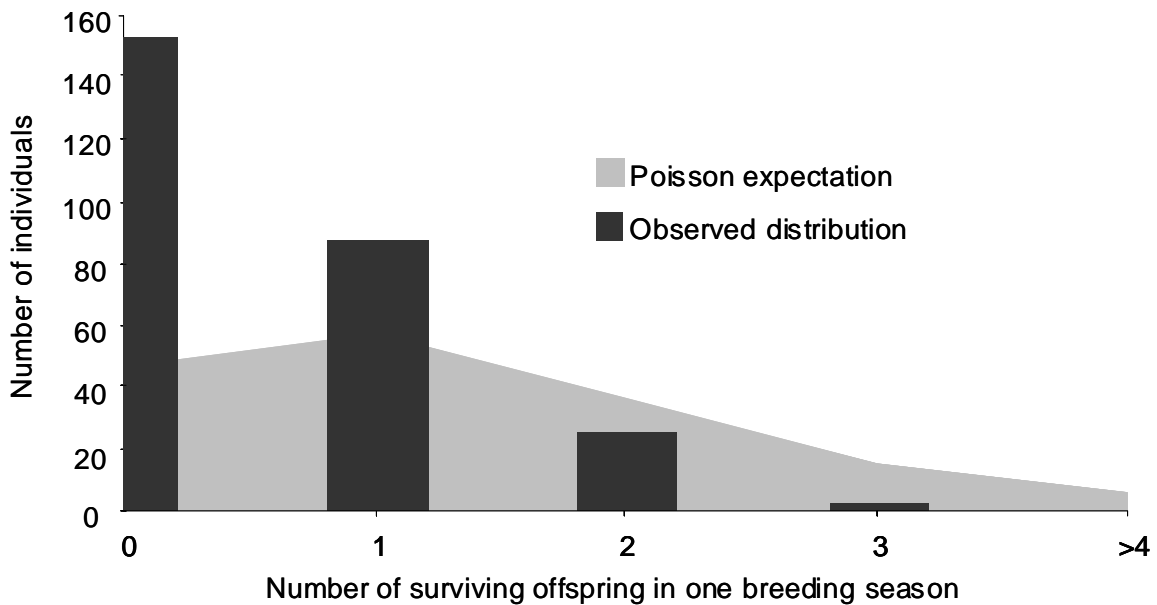


Figure 7: Realised yearly offspring numbers of adult Siberian jay females, together with expectation under Poisson distribution.

Annual variation of progeny number of females was estimated based on 267 females, out of which 114 had produced at least one surviving offspring during one breeding season. The average annual number of surviving offspring produced was 0.54 per female and 1.25 per each successful female. Based on a Poisson distribution of 1.25 surviving offspring per each female attempting to breed, 71 % of the females attempting to breed were expected to be successful, the rest producing zero surviving offspring. Since the number of unsuccessful

females in the dataset exceeded the expected number of unsuccessful females by 108, the proportion of adult females whose annual breeding success followed Poisson distribution, α_f , was 59.7 % (Figure 7).

3.2 Effective population size estimates

3.2.1 Role of ecological factors in the formation of the N_e/N ratio

The N_e/N ratios and the corresponding effective population size estimates in AT and KT, derived with estimators which are based on one ecological factor each, are presented in Table 2. The N_e/N ratio, based on the reproductive skew between sexes, was estimated based on a common dataset for both study areas, and is thus identical in AT and KT. N_{eK} estimates in AT and KT are calculated based on the same variance estimates, but different estimates of N , which partly explains the similarity of the N_{eK}/N ratios in AT and KT.

Table 2: Effective population size estimates (N_e) and the respective N_e/N ratios given by equations (1)–(3) and (5) from left to right for AT (1974–2006) and KT (1999–2006).

Study area specific estimate	Annual nr. of breeders (N_{eB})		Fluctuating pop. size (N_{eN})	Reproduct- ive skew (N_{eFM})	Variation in progeny number (N_{eK})	
	Mean	95 % CI			Mean	95 % CI
AT N_e	23.38	± 5.82	19.06	23.88	14.74	12.98–17.03
N_e/N	0.98	± 0.24	0.80	1.00	0.62	0.54–0.71
KT N_e	158.94	± 51.00	160.84	169.85	106.73	94.04–123.38
N_e/N	0.94	± 0.30	0.95	1.00	0.63	0.55–0.73

3.2.2 Demographic estimates of the N_e/N ratio

The N_e/N ratios and the corresponding effective population size estimates in AT and KT, derived with three demographic N_e estimators, are presented in Table 3.

Table 3: Average N_e estimates and the respective N_e/N ratios given by equations (6)–(8) from left to right for AT (1974–2006) and KT (1999–2006).

Study area		Nunney & Elam (1994, N_{eNE})	Lande & Barrow- clough (1987, N_{eLB})	Felsenstein (1971, N_{eF})
AT	N_e	14.88	14.43	7.45
	N_e/N	0.62	0.60	0.31
KT	N_e	105.84	106.45	57.03
	N_e/N	0.62	0.63	0.34

The Felsenstein (1971) method also allowed estimating effective population size in relation to population growth. In AT, varying the population growth rate factor (λ) by using the minimum and maximum four-year averages of the study period gave effective population size estimates in a range of 0.21–18.79. In KT, a similar procedure gave effective population size estimates in a range of 14.76–50.24.

The ratio $N_e/N = 0.6$ was chosen to be used in further calculations due to a possible bias in the Felsenstein method and to compensate the fact that population size fluctuations were neglected in the estimation (see section 4.1.2). Based on this ratio, a hypothetical Siberian jay population with the census size of KT would have an effective population size of $N_e = 101.8$, and a hypothetical population with the size of AT would have an effective population size of $N_e = 14.3$.

3.2.3 Genetic estimates of N_e , assuming an unstructured population

The F_{ST} -measure, estimated between KT cohort 1999 and the breeding immigrants at KT, was 0.01, suggesting a low level of genetic differentiation between immigrants and the individuals born in the study area.

When estimating N_e of the Suupohja Siberian jays, assuming an even population structure, the Jorde–Ryman (1995) temporal method for overlapping populations gave a correction factor $C = 9.42$ based on age-specific survival and fecundity parameters. Effective population size estimates, based on four successive year-pairs, are presented in Table 4. The average N_e estimate within the three other year-pairs was 99.6 ± 37.4 (SE). The point N_e estimates given by the LDNE software, plus yearly estimates of heterozygosity and allelic richness are presented in Table 5. The average point N_e estimate calculated based on LDNE estimates was 194.7 ± 112.0 (SE).

Table 4: F_s values and N_{eJR} estimates (Jorde & Ryman 1995, 2007), calculated based on successive cohorts in KT (1999–2006), together with sample sizes (S1 and S2) and estimates of total cohort size (N_1).

Year-pair	N_1	S1	S2	F_s		N_{eJR}	
				Mean	95 % CI	Mean	95 % CI
1999–2000	83	46	26	0.025	0.019–0.033	162.3	73.1–851.5
2000–2001	63	26	31	0.053	0.042–0.071	32.9	21.5–50.8
2001–2002	83	31	31	0.031	0.024–0.041	103.7	52.7–290.1

Table 5: Annual point-estimates of N_{eLDNE} (Waples & Do 2008), together with heterozygosity (H_o) and allelic richness (A) estimates, based on KT samples of 1999–2002 and 2005.

Year	S	N_{eLDNE}		H_o		A
		Mean	95 % CI	Mean	95 % CI	
1999	46	639.6	160.5–Infinity	0.610	± 0.097	4.78
2000	26	104.9	48.0–2024.6	0.551	± 0.083	4.81
2001	31	105.6	57.4–336.1	0.602	± 0.091	4.98
2002	31	89.2	48.9–258.8	0.568	± 0.090	4.87
2005	20	34.0	20.8–66.5	0.598	± 0.082	4.43

The overall mean of the genetic temporal and point N_e estimates was 147.2. Based on the N_e/N ratio of 0.6 for the Suupohja Siberian jays, and holding to the assumption that the genetic samples of the KT dataset were representative samples of the Suupohja population, the population size (N) of the Siberian jay population around Suupohja was estimated to be circa 245 individuals, which would exceed the average population size of KT by 44 %.

The moment-based temporal effective population size estimate for AT, calculated for comparison, was based on 20 and 31 individuals (years 1981 and 2002 respectively). F_s was 0.148 (0.108–0.215; 95 % CI), which gave a N_e estimate of 17.1 (12.0–23.2; 95 % CI). The point N_e estimates given by the LDNE software, plus yearly estimates of heterozygosity and allelic richness for AT are presented in Table 6.

Table 6: Annual point-estimates of N_e , heterozygosity and allelic richness values based on AT samples in years 1981 and 2002.

Year	S	N_{eLDNE}		H_o		A
		Mean	95 % CI	Mean	95 % CI	
1981	20	27.6	18.1–46.6	0.643	± 0.109	4.60
2002	31	58.3	37.9–103.4	0.673	± 0.099	4.96

3.2.4 Genetic estimates of N_e , assuming immigration or isolation by distance

The combined effective population size (N_e) and migration rate (m) analysis in KT was based on 96 individuals and 21 loci. The migration rate estimate given by the Wang–Whitlock (2003) estimator was negative (–19.5 %). Based on the 0 % immigration rate, the $1/(2N_{eWW})$ estimate was 0.0218 ± 0.0086 (95 % CI), which gave an N_{eWW} estimate of 23.0 ± 57.9 (95 % CI). Since the Wang & Whitlock (2003) estimator gave a negative immigration estimate, the alternative assumption on the population genetic structure, expecting clear signs of immigration in KT dataset, was considered void. It should also be noted that because the genetic samples used to analyse the KT data were only spaced ~1.5 generations apart, and the Wang–Whitlock estimator did not account for the population age structure, the resulting N_{eWW} estimate might also be biased downwards due to the yearly fluctuations in the allele frequencies.

The combined effective population size (N_e) and migration rate (m) analysis in AT was based on 68 individuals and 14 loci. The migration rate estimate given by the Wang–Whitlock (2003) estimator was negative (–6.8 %). Based on the 0 % immigration rate, the $1/(2N_{eWW})$ estimate was 0.0223 ± 0.010 (95 % CI), which gave an N_{eWW} estimate of 22.4 ± 49.8 (95 % CI).

4 DISCUSSION

4.1 The N_e/N ratio among the Suupohja Siberian jays

4.1.1 The role of ecological factors in the formation of the N_e/N ratio

There was much variation in the N_e/N ratios suggested by those four types of demographic N_e estimators that were based on a single ecological factor each, both in AT and KT datasets. However, since these four estimators are not expected to give an overall N_e/N ratio, but rather show the relative role of each ecological factor in the formation of the true N_e/N ratio, this variation was expected.

Since the Siberian jays practice lifetime monogamy, there is practically no reproductive skew between sexes that would restrict the passing of genes of either sex to the next generation. Thus, N_{eFM}/N suggests that N_e would be equal to N among the Suupohja Siberian jays if this were the only factor to separate the population from being an ideal population. Similarly, the annual proportion of breeding individuals multiplied by the generation length is also close to N , which indicates that if different individuals would breed in different years, nearly all adults would get a chance to breed during their adult life. Thus, N_{eB}/N is also close to one both in AT and KT.

N_{eK}/N was far lower in both AT and KT than the above mentioned estimates. This is due to a relatively large variance in progeny number in relation to the size of the population. This indicates that in practice, as also visualised by Figure 6, certain individuals pass their genes to the later generations with greater efficiency than others. The annual variation in progeny number (Figure 7) shows that this is not because some individuals would produce a large number of surviving offspring during any particular year — even the successful breeding pairs do not typically produce more than two surviving offspring per year. Rather, some long-lived individuals succeed in breeding in several years during their lifetime while others succeed less often or not at all. As a conclusion, the lifetime variation in progeny number has the largest lowering effect to the true N_e out of the three ecological factors considered (viz. gender-related reproductive skew, proportion of breeding individuals and variance in progeny number). As a result, the N_{eK}/N ratio can be said to get closest to the true N_e/N ratio among these three estimators.

N_{eN} suggests a very different kind of N_e/N ratio in AT as compared to KT. This is apparently due to the difference in the time span covered by these two datasets; the AT dataset contains more years and more variation in population size, which eventually creates a lower harmonic mean over the years. Nevertheless, the large difference in the ratios suggests that population size fluctuations could be an important factor in the formation of the

true N_e in the Siberian jay. But is this the case in AT? Based on the very high proportion of immigrants breeding in AT, one can regard AT as just a fraction of the larger Siberian jay population that is actively exchanging genes. Population size drops in AT do not create as severe bottlenecks as in a closed population – where the local survivors would be the only founders of the next generation – since mobility between different parts of the population helps to compensate for the loss of local individuals in AT. In fact, this compensation effect is possibly the reason for the gentler slopes of the population size curve in KT (Figure 3) compared to AT (Figure 2). It should also be noted that according to Bergholm (2007) there were fluctuations in the recapture rates of Siberian jays until late 1980s, which may have caused part of the population size fluctuations in AT during early study years. Consequently, the true amount of measured fluctuations may be a bit less than indicated by my results.

4.1.2 The N_e/N ratio in the Suupohja Siberian jays

The estimators developed by Nunney & Elam (1994, N_{eNE}) and Lande & Barrowclough (1987, N_{eLB}) suggest relatively similar N_e/N ratios as those suggested by N_{eK} . This is expected in the case of N_{eLB} , which utilises the same estimates of lifetime variation in the number of progeny as its main parameters as N_{eK} . It is of more interest that N_{eNE} , which is based on annual variation in the number of progeny and annual survival, also gives a similar estimate. This suggests that the lifetime variation in breeding success in the Siberian jay can be quite reliably estimated with these two annual factors: the most successful breeders are simply those that have managed to survive for many years and breed successfully several times. The adult sex ratio included in both N_{eNE} and N_{eLB} does not alter the ratio significantly, as the numbers of males and females are quite even in the population.

The Felsenstein's (1971, N_{eF}) estimator, which assumes constant population size, gives a much lower estimate of the N_e/N ratio (both in AT and KT) than the estimators discussed above. This estimator is based on age-specific survival and fecundity rates, and there is no obvious reason why these factors would capture some aspect of Siberian jay ecology that the factors used by e.g. N_{eNE} would not. The most apparent explanation lies in the fact that while the N_{eF} estimator focuses on age-specific survival rates, its expectation of the size of the adult population is dependent on the number of juveniles entering into the population during a given generation (N_1T). The number of recruits and the age-specific survival rates together give an impression of a small adult population, since they account for emigration and death of offspring through a small v_2 , but they do not account for immigration from other parts of the population – which is significant in both AT and KT. In fact, if the size of one generation would be estimated with N instead of N_1T , the N_{eF} estimates would give a higher N_e/N ratio (0.55) both in AT and KT. But as the true age structure of the adult

population is unknown due to the openness of the population (i.e. migration), the N_{eF} estimates should be considered void.

Do the three estimators — N_{eNE} , N_{eLB} and N_{eK} — then encompass the true N_e/N ratio? It should be noted that none of these three estimates take the population size fluctuations into consideration, and based on the difference of N_{eN} estimates in AT and KT, one can expect that population size fluctuations do have an effect on the N_e/N ratio in the long run. Even though the N_{eF} estimates as such may not be correct, the experimentation with the minimum and maximum values of λ and their effect on N_{eF} shows that population growth rate would have a noticeable effect on momentary N_e estimates. Similarly, experimentation shows that if N_{eLB} would be calculated as a harmonic mean of momentary estimates, the altogether harmonic mean would be affected not only by varying population sizes, but larger momentary variances of progeny numbers. Therefore, the true N_e would probably be somewhat smaller than the general estimates calculated here. It is reasonable to assume that the true N_e/N ratio is somewhat below 0.6 in the Suupohja Siberian jay, but as the exact value is not known, 0.6 was used in this study.

4.2 Implications to the conservation of the Siberian jay

4.2.1 Are the Suupohja Siberian jays less isolated than expected?

Since the genetic N_e estimators are sensitive to the violation of their underlying assumption concerning population structure, the observation of surprisingly high annual immigration rates, estimated based on the breeding data of the Suupohja Siberian jays, require further consideration. It is also noteworthy that the majority of both female and male immigrants in the breeding data were ringed in the study area as adults, even though juvenile dispersal was expected based on prior knowledge (Ekman *et al.* 1994, 2001, Lillandt *et al.* 2003).

The method of classifying immigrants based on pedigree data may contain errors, but in general, the results should be reliable compared to relying on sighting and ringing locations. Individuals ringed as adults are slightly more prone to false classification as immigrants, since the lack of knowledge of their birth year complicates pedigree analysis: the search of parents is limited to 2-3 study years taking place before the year of ringing of the adult bird, in order to avoid false associations due to a large number of possible parents (Lillandt *et al.* 2003). However, according to Lillandt *et al.* (2001, 2003), very few individuals were classified as immigrants due to an extended gap between them and the potential parents. False immigrant classifications of individuals ringed as adults would also have required that individuals would have been born within the study area but not sighted before

adulthood. Lillandt (2002) considers immigration by an adult bird more likely than the possibility that an individual would not have been sighted during its first year in the study area.

It should also be noted that estimating immigration rates based on breeding data gives estimates on effective, not actual, immigration. While this study provides no data on actual immigration rates to the Suupohja study area, the recorded high *effective* immigration rates are supported by the high N_{eG} estimate calculated for the Suupohja Siberian jays, suggesting that the genetic neighbourhood of the Suupohja Siberian jays was indeed larger than the study area during the study period. A possible explanation for the high effective immigration rates is provided by Lillandt (2009a), as he argues that individuals with the best breeding success in the Suupohja study area are typically those that have at least one immigrant as a parent. This raises a possibility that effective immigration rates were higher than actual immigration rates, if also immigrants themselves produced more surviving offspring than locally born individuals.

In conclusion, it seems plausible that constant effective immigration to the study area was a real phenomenon during the study period. It then remains to be considered why a noticeable number of Siberian jays would have immigrated to the study area as adults. One conceivable explanation would be that some Siberian jays would disperse during their second year in search of a permanent territory. Although still young for Siberian jays, two-year-old Siberian jays would no longer be classified as juveniles. It should also be considered whether the method of distinguishing adult Siberian jays from juveniles based on tail feathers was as reliable as expected.

4.2.2 The geographic and genetic structure of the Siberian jay population

The low F_{ST} -value measured between the KT cohort 1999 and the breeding immigrants at KT suggests that immigrants to the Suupohja study area were not significantly genetically differentiated from locally born individuals. The suggestion is also supported by the negative migration estimate given by the Wang–Whitlock (2003) estimator: The negative estimate was caused by the fact that KT allele frequencies did not shift towards the allele frequencies of the immigrants between the sampling years, but slightly to the opposite direction, indicating that allele frequencies of the immigrants did not differ significantly enough from those of local individuals to override local genetic drift. This suggests that at least the majority of the Suupohja immigrants did not originate in another (genetically differentiated) population, but that they would instead be members of the same population than the Siberian jays in the study area — just born outside of the study area. In other words, observed “immigration” might reflect mobility between different parts of a larger continually distributed population.

What is left unclear in this study is the true size and structure of the Siberian jay population that the Suupohja Siberian jays were part of. According to a collection of Siberian jay sightings in Finland in 1999–2005 (Ministry of the Environment 2006, Pihlajaniemi 2006), the Siberian jay sightings in the Suupohja study area were accompanied by a ca 350 km continuum of scattered Siberian jay sightings towards the Northeast along the coast of Ostrobothnia during the study period. Even though the average recorded dispersal distances within the study area is measured to be short (females: 5.0 ± 2.0 km SE, males: 5.0 ± 2.0 km SE; P. Gienapp, unpubl.), the longest recorded dispersal distances in the Siberian jay have been up to 79 km within the Suupohja study area (Lillandt 1993) and 290 km altogether (von Haartman *et al.* 1967). Since there is no data concerning the distribution of dispersal distances to and from the study area, the existence of regular long-distance dispersal events cannot be ruled out (Lillandt 1993). It is therefore possible that individuals sighted along the Siberian jay continuum of Ostrobothnia would be genetically connected to the Suupohja Siberian jays. The existence of isolation by distance or clear population structure along this speculated continuum would depend on the distribution of the dispersal distances and the dispersal barriers along the way (Masakado 1995). If there were genetic samples from the more Northern parts of the continuum, an isolation-by-distance test (Rousset 1997) could be used to verify the population structure.

The large-scale dynamics of the Siberian jay population around Suupohja also depend on whether or not there was emigration from the study area. The average number of surviving offspring per breeding adult estimated in this study for the Suupohja Siberian jays was less than two, which implies that the population size in the study area would have steadily declined without constant immigration. If the low number was mainly due to actual mortality, the numbers would imply a demographic deficit in the study area with supplementation from more well-off areas, i.e. a source-sink system. Source-sink dynamics have lately been reported in another Siberian jay population (Nystrand *et al.* 2010). On the other hand, it is also possible that part of the locally “non-surviving” juveniles have simply emigrated to other parts of the larger population just as the immigrants that have arrived in the Suupohja study areas. The first explanation sounds more conceivable, as constant emigration should have resulted in regular sightings of individuals with coloured rings outside the study area. On the other hand, as the coverage of bird distribution surveys in the coast of Ostrobothnia to the North of Suupohja is only classified as Satisfactory (Finnish Museum of Natural History 2010), it is possible that many such emigrants would go unnoticed.

4.2.3 Population structure and the concept of effective population size

Estimation of the effective population size of the Suupohja Siberian jays during the study period was complicated by the lack of clarity concerning the population size and structure of

the Suupohja Siberian jays, due to which the census population size that the average genetic N_e estimate is associated with cannot be precisely defined (Fraser *et al.* 2007).

The low geographic structuring indicated by the low F_{ST} -value gave reason to assume that the genetic estimates of N_{eG} would reflect the effective population size of the total Siberian jay population exchanging genes, instead of just that of the KT population used in sampling (Leberg 2005, Fraser *et al.* 2007). On the other hand, if the population would be effectively panmictic in its distribution of allele frequencies, the genetic N_e estimates based on the geographically more restricted AT dataset should not be very far from those calculated based on the KT dataset. As the genetic N_e estimates based on the AT dataset were lower than those based on the KT dataset, it must be concluded that at least AT was geographically too restricted to provide samples that would be representative of the whole Siberian jay population in Suupohja (see: Masakado 1995). The potential reasons for the lower scale of the N_e estimates in AT could be either small sample sizes, isolation by distance (Wright 1943, Rousset 1997) or regional variations in population dynamics.

For conservation purposes, however, a genetic N_e estimate based on a restricted study area may be valuable even if it does not reflect the genetic composition of the whole population — after all, what matters for the genetic viability of local individuals is the population genetic processes in its effective genetic neighbourhood (see: Luikart *et al.* 2010). It is also likely that the geographic range of the KT study area was wide enough to balance the regional variations of the smaller study areas, and thus the N_{eG} estimate would be close to that of the total population that was actively exchanging genes during the study period.

4.2.4 The genetic viability of the Suupohja Siberian jays

Assuming that the average genetic estimate of $N_{eG} \approx 147$ was in the right ballpark for the population exchanging genes around Suupohja, it would suggest that the Suupohja Siberian jays would not face immediate risks of inbreeding or mutation accumulation (Franklin 1980, Soulé 1980b) with the population sizes that they retained during the study period, but that the recorded population sizes or densities were neither adequate to secure the long-term genetic viability of the population (Lande 1995, Franklin & Frankham 1998). It should be noted that the LDNE estimates might be biased downwards due to the usage of single cohorts as annual samples. However, this does not seem likely, as the LDNE estimates were generally higher than those given by the Jorde–Ryman estimator, and the latter should be suited for the data used. Hence, it can be concluded that the genetic viability of the Suupohja Siberian jays is critically dependent on the demographic development of the population in the future.

What would happen if the Siberian jays in the study area would lose their connections to whatever are the origins of the majority of its immigrants? If migration to the study area would cease, and the Siberian jays of the study area would have to survive as an independent population, the consequences to the Siberian jay population dynamics would most likely be complex. It is possible that the closure of immigration pathways would decrease local competition, which would increase the proportion of juveniles that would find a territory within the closed population. In that case, the N_e of the population would fall close to 100. However, if the breeding success of individuals that had at least one immigrant as parent is indeed higher in the study area than that of local individuals (Lillandt 2009a) — a potential sign of heterosis (Keller & Waller 2002) — the cessation of immigration would eventually result into declining fitness in the study area. Taking into account that immigration has been associated with the demographic stability of the study area several times along the course of the study (Lillandt 2004, 2009a), it is possible that lack of immigration would initiate a circle of events where also the N_e would drop to an unsustainable level, and finally the demographic and genetic factors would interact with fatal consequences (cf. Giplin & Soulé 1986).

Several signs indicate that the preconditions for the overall viability of the Suupohja Siberian jays is already weakening: The juvenile survival rate in AT was zero during the last three study years, and according to Bergholm (2007), immigration was weakest during the last three years of the study period. Since the study period, further declines in KT population size have been reported, plus a finding that the decline is strongest in the southernmost study areas (Nousiainen 2008, Lillandt 2009b). The emergent withdrawal of the Siberian jays from these peripheral territories is a logical consequence of the factors that have been considered to threaten the viability of the Siberian jays: habitat loss, the weakening of dispersal pathways and the resulting decline in dispersal events are likely to increase inbreeding, reduce fitness and to decrease genetic and demographic **rescue events** (Brown *et al.* 1977, Ovaskainen & Hanski 2001) especially in the most peripheral areas. The ongoing process, therefore, underlines the role of gene flow in maintaining population viability.

4.2.5 Potentially relevant factors in conservation

The population size that can be considered large enough for sustaining an endangered population depends on the expected amount of disturbance experienced by the focal population. In zoos and reserves with strict management, some captive populations have been successfully managed for decades at very low population sizes (e.g. Milinkovitch *et al.* 2004). However, in these cases, the risks caused by unplanned population size fluctuations and genetic bottlenecks are minimised by intensive management of the captive population, e.g. via veterinary medicine and pedigree planning (Frankham *et al.* 2002). In the case of wild

populations, the unplanned factors caused by e.g. birth rate variation, climate variation, predation and infectious diseases cannot usually be controlled by managers. Therefore, the average population size needs to be higher, since the population size has to have a buffer to tolerate natural fluctuations in population size without the risk of extinction. For populations surviving in environments modified by human activity, human activity adds yet another source of disturbance to the population dynamics, requiring an even higher average population size.

Whereas it might be relatively easy to specify requirements for a strict reserve area for the Siberian jay, it is more difficult to define a credible conservation scheme for commercially exploited forests. This is, however, a relevant scenario for the Siberian jay conservation network within the METSO programme, since the network aims at the application of a diverse set of actions from full conservation to forest management changes (Finnish Association for Nature Conservation 2010). While the palette of conservation actions planned by the network is already diverse, this study highlights three additional factors that are relevant for the retention of genetic viability in small Siberian jay populations. The first two factors are the scale of population size fluctuations and the variation of the number of surviving progeny, which appear to be the main factors affecting the demographic N_e/N ratio in the Siberian jay. The estimated N_e/N ratio of 0.6 is relatively high compared to other species (see: Frankham 1995, Frankham *et al.* 2002, Palstra & Ruzzante 2008), so there is no specific need for actions to increase this ratio. If, however, actions will be taken to support the persistence of the Siberian jay in managed forests, some simple actions might help to prevent unnecessary loss of genetic variation.

For small populations, recurrent temporary drops in population size increase the risk of the population eventually collapsing due to its small size (Shaffer 1981, 1987). Population size fluctuations can also harm a small population by creating recurrent bottlenecks where genetic variation is lost, increasing genetic uniformity (Nei *et al.* 1975). Natural causes of population size fluctuations cannot easily be controlled, but if conservation is attempted in human modified landscapes, it might make sense to regulate the human-induced disturbance in order to prevent amplification of natural population size fluctuations. As forestry activities are considered an important factor affecting the population dynamics of the Siberian jays (Bergholm 2007, Griesser *et al.* 2007), it should be considered whether timber loggings and thinnings in Siberian jay areas could be regulated, for instance by postponing planned activities during years of low adult population size.

In small populations, strong variation in the number of surviving progeny may speed up the homogenisation of the gene pool, and lead to losses of adaptive genetic variation within unsuccessful breeders. The retention of genetic variation in a small population would benefit from a relatively even distribution of offspring survival (Frankham *et al.* 2002). This could be aided by utilising knowledge on the territory requirements of the Siberian jay in order to

improve conditions in inhabited but lower-quality territories. Such quality improvements might help more breeding pairs to produce surviving offspring, and thus transfer more genetic variation to the next generations. Interestingly, the current conservation plans are aiming at concentrating resources in the conservation of the territories that are producing the most offspring (Siberian jay conservation seminar 19 Oct. 2007, Finnish Association for Nature Conservation 2010). In the extreme cases of Parikkala and Virrat, this might be a rational choice in order to prevent the population from withering to demographically unsustainable numbers within a short timescale. However, maximising the progeny production of a narrow set of individuals might, if the genetic neighbourhood is restricted, have fatal results in a longer timeframe.

The third and the most important factor highlighted by this study is the importance of gene flow for the viability of the Siberian jays. In light of the N_e estimates calculated in this study, the small Siberian jay isolates of Southern Finland consisting of only a handful of territories do not appear to be genetically viable in a longer term as such, especially as even the relatively large Siberian jay population in the Suupohja area appears to be dependent on gene flow. While the average dispersal distances in the Siberian jay are considered short (Lillandt 1993, Pimenoff 2000), the less common long-distance dispersal events might have an important role in the retention of low levels of genetic structuring (see: Alberto *et al.* 2010). In addition to alleviating inbreeding and genetic unification in the isolated Siberian jay areas, dispersers may also be able to generate demographic rescue effects. Therefore, as also emphasised by Pimenoff (2000:37), studies on the long-distance dispersal behaviour of the Siberian jays in the Finnish landscape would be needed for the successful conservation of the Siberian jays of Southern Finland.

4.3 Suitability of different N_e estimator types for the Siberian jay

4.3.1 Comparison of the used demographic N_e estimation methods

The demographic N_e estimators used in this study require various types of demographic data, some of which are quite laborious to collect. In a long-lived species such as the Siberian jay, the data needed for estimating the lifetime variance or progeny number for estimating N_{eLB} requires that the population is monitored for several years, that the parents of juveniles born in the population during the monitoring period are identified and that the individuals belonging to the cohort under study can be identified throughout the study period. To follow the Lande–Barrowclough (1987) method to the full extent would also require performing such a study of several cohorts to obtain several momentary estimates of N_e . If there is emigration from the population, the survival of the juveniles should either be

confirmed before the age of dispersal, or monitoring of dispersing juveniles should be made possible. These requirements are most often unrealistic in the study of long-living species. The Lande–Barrowclough method is perhaps most suitable for the monitoring of N_e in laboratory or domestic populations with large cohorts.

The Nunney–Elam (1994) method contains in this respect an essential simplification compared to the Lande–Barrowclough method, since it requires estimates of annual survival rates and annual variance of progeny production. If adult survival rate can be assumed independent on age, and adults can be distinguished from juveniles, these two parameters can at best be estimated based on one year's monitoring period.

The Felsenstein (1971) method requires information on age-specific fertility rates. Getting this data does not necessarily require lifetime follow-up of certain individuals, in case the age distributions of breeding adults on any particular year studied can be defined by other means. For instance, in case of fish, age can sometimes be defined from otoliths and scales (e.g. Lai *et al.* 1996, Gunn *et al.* 2008). For some bird species, the existence of ringing programs may enable researchers to base a study on individuals ringed as nestlings. In many cases, age-specific data concerning breeding females is easier to collect than that of breeding males, unless there are DNA samples of all individuals in the population. Even if the Nunney–Elam and Lande–Barrowclough methods do not directly require the knowledge on age-specific fertility rates, generation length is a parameter in both estimators, and the definition of generation length requires knowledge on the average age of breeding.

As a conclusion, collecting data required by the demographic methods is relatively laborious with long-lived species that have large body size — and thus typically small cohort sizes — in a study area reasonably coverable by field studies. Of the methods tried here, the Nunney–Elam method is clearly the handiest. Despite the workload, demographic estimation of the N_e/N ratio has several benefits over genetic estimation: most demographic estimators are free from the complicated assumptions often restricting the use of genetic estimators (but see Luikart *et al.* 2010 for the downside), the results may be easier to interpret, and the N_e/N ratio of the population can be put to some kind of a scale even though the size and structure of the whole population is not known. As suggested by Harris & Allendorf (1989), knowledge on species ecology may sometimes also allow ecologists to make relatively accurate guesses of some hard-to-measure parameter values, which in some cases may be justified in order to get a handle on the range where the N_e/N ratio lies. In the case of the Siberian jay, a relatively good understanding on the N_e/N ratio formation would have been gained by just using the three simple N_e estimators based on sex ratio of breeding adults, variance of progeny numbers and fluctuating population size. It should be noted, however, that if the demographic parameters would have large confidence intervals, and if the subsequent calculations would be carried out by using the point estimates, the resulting confidence interval of the N_e estimate might appear more precise than it should.

4.3.2 Comparison of the used genetic N_e estimation methods

The data required by the genetic methods is straightforward to collect, provided that the questions regarding the population structure are solved and sampling locations can be justified. However, because the traditional temporal methods and the Wang–Whitlock method would need 3–5 generations between temporal samples, they are beyond the scope of most studies on long-living species such as the Siberian jay, unless long-term genetic data has been collected for other purposes. The Jorde–Ryman method solves this problem by allowing the study of successive cohorts, but then, also the demographic data is needed. Point estimators, therefore, are often the most practical option. However, point estimators still have weaknesses, such as strict assumptions of the mating pattern, or assumptions on the physical linkage of the loci used for the analysis. The latter types of assumptions are especially inconvenient from a conservation biologist’s point of view, since linkage maps are not usually available for species under monitoring. In the case of the Siberian jay, linkage analysis revealed several tightly linked loci in the developed microsatellite dataset (Jaari *et al.* 2009), and removal of these loci from N_e estimation resulted in clearly different annual LDNE estimates than those calculated based on the whole microsatellite dataset — although the average scale of the estimates stayed the same (H. Fabritius, unpubl.).

The large variance of the annual genetic N_e estimates shows that the genetic estimators used are quite sensitive to the sample size or to the variation of the parameters that they rely on. The Jorde–Ryman (1995, 2007) estimate, which estimates the extent of genetic drift based on changes in allele frequencies, is sensitive to immigration and is biased downwards if immigrants with different allele frequencies breed in the population. LDNE (Waples & Do 2008), on the other hand, being based on inbreeding effective population size, measures the effective population size of the *adults* of the cohort measured, and might also reflect longer timescales than just one generation (Luikart *et al.* 2010). Thus the yearly fluctuations of N_{eLDNE} may be different from those of N_{eJR} . An example of this can be seen in the AT results: N_{eNT} is lower than either of the LDNE estimates, and the LDNE estimates indicate that the N_e increased in AT over the years. What probably has happened is that the gene pool in AT widened over the years of study, and LDNE reacts to it as a sign of increased N_e , whereas the Nei–Tajima method reacts to the change in allele frequencies with a low N_e .

These considerations underline the fact that the N_e estimates may vary in time and according to the N_e estimation method used. Thus, the differing sensitivities of the methods should be taken into consideration and the number of samples should be large enough to balance annual variation — generally, taking an average value of the annual N_e estimates should work well as long as the variation of annual N_e estimates can be expected to fluctuate around the true value without bias. Hence, even if point estimators are used,

gathering samples from several large-enough cohorts in a row might be reasonable for the reliable overall estimation of N_e , unless the sample sizes are very large.

4.3.3 Does N_e estimation beat monitoring heterozygosity and allelic richness?

Heterozygosity and allelic richness estimates showed similar trends in AT and KT; both estimates increased with time in AT, while in KT, the estimates did not show either increasing or decreasing trend.

The increase in genetic diversity towards the later AT samples requires consideration. Neither the demographic development nor the immigration rates in AT give a clear explanation as to why genetic diversity would have increased. The AT population experienced a demographic bottleneck since the first sampling year (1981), but recovered until the latter sampling year (2002; Figure 2). The most plausible explanation is similar to the explanation of the differences of LDNE and Nei–Tajima N_e estimates for AT: if the gene pool in AT widened over the years during the immigration-aided recovery from the bottleneck (Lillandt 2000), new variation would have fused into the local gene pool, increasing genetic diversity. This, then, would not necessarily be a sign of improved genetic viability.

At the same time, the relatively stable genetic diversity estimates in KT did not indicate any issues with the genetic viability in the Suupohja Siberian jays. A few years' monitoring period might not be adequate to reveal declining trends in genetic diversity in species with a several years' generation length. In any case, monitoring heterozygosity and allelic richness of the Suupohja Siberian jays would so far have given no indication on a possibly compromised long-term genetic viability. The compromised genetic *viability* would only have been recorded while it would start showing symptoms via losses of genetic *variation* — in which phase conservation of the remaining habitat patches might no longer be adequate to sustain a viable population.

4.4 Concluding remarks

This study revealed high effective immigration rates to the Siberian jay study area in Suupohja, which was unexpected on this scale based on previous knowledge. The Siberian jays of the Suupohja study area seem to have been genetically connected to the Siberian jays within a larger geographic distribution in the past, despite their low average dispersal distances. One potential reason for the recorded low levels of genetic structuring is that the Siberian jay population in the Suupohja study area might have acted as a sink during the study period, which would widen the genetic neighbourhood reflected by the genetic samples. On the other hand, the occasional long-distance dispersal events might have also had an im-

portant role in the retention of low levels of genetic structuring in a Siberian jay population. However, in the light of the N_e estimates calculated in this study, the Suupohja Siberian jays would most probably not be able to retain their genetic variation if immigration to the study area would cease.

The estimated average N_e/N ratio of 0.6 for the Siberian jays indicates that Siberian jays generally retain genetic variation well. The most important factors related to the loss of genetic variation, besides population size, are the extent of gene flow, the scale of population size fluctuations and variation of progeny numbers between breeding individuals. As gene flow appears to be a crucial factor for the genetic viability of the remaining Siberian jay isolates in Southern Finland, the dispersal behaviour of Siberian jays in the Finnish forest landscape would merit further study.

This study highlighted the complexity of effective population size estimation in a species that has overlapping generations, a relatively long generation length, low population densities, and the ability for occasional long-distance dispersal events. The applied estimators gave wide-ranging estimates of N_e and N_e/N ratios, and the interpretation of the results was further complicated by the unavoidable violation of some of the assumptions of the estimators. In the end, however, the results of effective population size estimation provided a more profound perspective to the genetic viability of the Suupohja Siberian jays than what could have been retrieved by the mere monitoring observed heterozygosity and allelic richness: while the observed heterozygosity and allelic richness estimates indicated no losses of genetic variation in the Suupohja area during the study period, the comparison of the various N_e estimators indicated dependency on constant immigration from other parts of a larger population in order to retain genetic variation in the local population.

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7 APPENDICES

7.1 Appendix I: Microsatellite loci used in the analysis

Table 7: Characteristics of the 21 microsatellite loci in Suupohja population, including number of individuals genotyped (N), number of alleles (A_n), expected heterozygosity (H_e), observed heterozygosity (H_o), deviation from Hardy–Weinberg equilibrium (H-W), frequency of null alleles (F(Null)) and polymorphic information content (PIC; Jaari *et al.* 2008, Lillandt *et al.* 2001).

Microsatellite	N	A_n	H_e	H_o	H-W	F(Null)	PIC
CK1B5D	1003	2	0.50	0.55			
CK2A5A	1033	16	0.75	0.75			
CKL5	1010	11	0.82	0.85			
LTML7	1007	2	0.40	0.41			
LTML8	1022	14	0.84	0.86			
MJG1	1034	2	0.43	0.46			
PER1	1034	6	0.54	0.55			
PPi1	1008	4	0.54	0.60			
PPi2	1029	5	0.74	0.77			
2F9 (SJ103)	929	15	0.88	0.92	0.66	0.04	0.75
2G1 (SJ104)	950	6	0.54	0.50	1.00	−0.02	0.19
2G7 (SJ105)	909	6	0.60	0.46	0.52	0.13	0.40
3B12 (SJ106)	953	7	0.72	0.58	0.46	0.10	0.72
3C12 (SJ109)	926	5	0.78	0.88	0.97	−0.07	0.75
3G8 (SJ107)	951	5	0.63	0.75	0.07	−0.10	0.68
4B7 (SJ110)	934	3	0.55	0.67	0.37	−0.14	0.49
4C10 (SJ111)	921	4	0.54	0.58	0.09	−0.07	0.47
4D8 (SJ112)	895	4	0.38	0.25	0.50	0.22	0.24
4H9 (SJ114)	888	6	0.60	0.46	0.50	0.13	0.43
5G2 (SJ115)	872	3	0.51	0.29	0.83	0.25	0.44
5G10 (SJ116)	861	2	0.51	0.63	0.29	−0.11	0.40

7.2 Appendix II: Ringing statistics in the study area

Table 8: Yearly statistics of Siberian jay ringings in the study population in 1974–2006, including total number of ringed individuals, age distribution and geographic distribution of ringed individuals and the annual number and percentage of ringed individuals genotyped to at least 14 loci.

Year	Total	Age distribution			Geographic distribution								Genotyped 14+	
		Nest-lings	Juveniles	Adults	AT	PT	IT	ILA	ELA	NLA	KLA	Number	%	
1974	12	0	4	8	12	0	0	0	0	0	0	0	1	8
1975	21	2	10	9	21	0	0	0	0	0	0	0	3	14
1976	29	12	8	9	29	0	0	0	0	0	0	0	7	24
1977	5	0	3	2	5	0	0	0	0	0	0	0	1	20
1978	8	3	5	0	8	0	0	0	0	0	0	0	2	25
1979	16	0	14	2	16	0	0	0	0	0	0	0	6	38
1980	6	4	2	0	6	0	0	0	0	0	0	0	3	50
1981	13	0	6	7	13	0	0	0	0	0	0	0	10	77
1982	3	0	3	0	3	0	0	0	0	0	0	0	1	33
1983	3	3	0	0	3	0	0	0	0	0	0	0	0	0
1984	6	1	2	3	6	0	0	0	0	0	0	0	3	50
1985	8	0	5	3	8	0	0	0	0	0	0	0	6	75
1986	4	0	2	2	4	0	0	0	0	0	0	0	3	75
1987	13	3	4	6	6	7	0	0	0	0	0	0	9	69
1988	10	0	6	4	8	1	1	0	0	0	0	0	3	30
1989	17	3	8	6	10	6	1	0	0	0	0	0	4	24
1990	35	13	9	13	21	12	2	0	0	0	0	0	7	20
1991	29	10	10	9	13	12	4	0	0	0	0	0	10	34
1992	47	16	18	13	13	31	3	0	0	0	0	0	35	74
1993	39	12	21	6	11	21	7	0	0	0	0	0	19	49

Year	Total	Age distribution			Geographic distribution								Genotyped 14+	
		Nestlings	Juveniles	Adults	AT	PT	IT	ILA	ELA	NLA	KLA	Number	%	
1994	40	5	24	11	11	25	4	0	0	0	0	29	73	
1995	39	0	29	10	7	28	4	0	0	0	0	37	95	
1996	38	0	34	4	10	22	6	0	0	0	0	37	97	
1997	37	0	32	5	11	24	2	0	0	0	0	37	100	
1998	64	0	41	23	9	23	8	8	0	6	10	64	100	
1999	117	0	83	34	11	33	11	12	4	31	15	117	100	
2000	77	0	61	16	10	33	4	8	0	17	5	77	100	
2001	88	0	63	25	10	26	6	5	1	29	11	88	100	
2002	109	15	68	26	20	17	8	6	2	42	14	109	100	
2003	54	4	32	18	6	9	5	1	1	21	11	54	100	
2004	61	0	54	7	1	25	4	5	2	16	8	61	100	
2005	44	0	39	5	1	14	4	3	1	10	11	44	100	
2006	65	0	58	7	5	11	4	4	6	24	11	65	100	
Total	1157	106	758	293	328	380	88	52	17	196	96	952		

7.3 Appendix III: Annual population sizes in AT and KT

Table 9: Annual population sizes in AT (original study area) 1974–2006.

Year	Adults		Offspring		Population growth (λ)	Immigration
	Breeding	Non-breeding	Surviving	Non-surviving		
1974	5.3	2.7	4	0	--	100 %
1975	16	4	8	4	--	25 %
1976	12	21	7	13	--	33 %
1977	0	34	0	3	--	--
1978	6	15	4	4	--	50 %
1979	7.5	18.5	5	9	--	17 %
1980	6	16	4	2	0.84	0 %
1981	4	29	3	3	1.38	25 %
1982	2	21	1	2	0.67	0 %
1983	0	11	0	3	0.46	--
1984	4	6	3	0	1.18	50 %
1985	6	9	3	2	1.38	17 %
1986	0	18	0	2	1	--
1987	4	9	2	2	0.83	25 %
1988	8	8	4	2	1.33	50 %
1989	8	13	4	3	1.25	0 %
1990	11.2	14.8	7	9	1.32	25 %
1991	9.6	12.4	6	6	0.85	33 %
1992	5.3	16.7	4	8	0.93	25 %
1993	6	14	3	5	0.88	0 %
1994	4	17	3	8	1.04	0 %
1995	6	14	3	3	0.96	17 %
1996	4	19	2	6	1.09	25 %
1997	6	15	4	7	1	50 %
1998	8	10	6	3	0.96	13 %
1999	14	11	10	1	1.46	7 %
2000	8	23	5	3	1.03	14 %
2001	6	22	4	5	0.89	0 %
2002	4	25	2	17	0.97	50 %
2003	0	21	0	6	0.68	--
2004	0	11	0	1	0.52	--
2005	0	6	0	0	0.55	--
2006	2.1	4.9	2.1	1.9	1.46	--

Table 10: Annual population sizes in KT (total study area) 1999–2006.

Year	Adults				Offspring		Population growth (λ)	Immigration
	Breeding	Non-breeding	Females	Males	Surviving	Nonsurviving		
1999	64.2	82.8	78	69	46	37	--	15 %
2000	37.4	136.6	94	80	26	35	1.04	5 %
2001	50.9	122.1	86	87	31	32	1.02	29 %
2002	50.9	129.1	93	87	31	52	1.03	23 %
2003	10	164	91	83	5	31	0.85	0 %
2004	22	97	59	60	13	41	0.74	23 %
2005	29.5	62.5	42	50	20	19	0.85	11 %
2006	39.5	63.5	50	53	24.8	33.2	1.13	15 %

7.4 Appendix IV: A python 2.1 program for calculating N_{eJR}

This Python 2.1 code calculates the effective population size with the Jorde & Ryman (1995) method based on user input. Please note that the code does not check for errors in the user input.

```
#!/usr/bin/python2.1

# *-*-*-*-* FUNCTIONS *-*-*-*-*
# A FUNCTION THAT CHOOSES THE RIGHT FUNCTION BASED ON INPUT VALUES
def function_chooser(t, i, j):
    if t == 0: return 0.0
    elif i == j == 0: return f_1_1(t)
    elif i == j > 0: return f_i_i(t, i)
    elif i == 0: return f_1_j(t, j)
    elif j == 0: return f_1_j(t, i)
    elif i > j: return f_i_j(t, i, j)
    else: return f_i_j(t, j, i)

# FOUR FUNCTIONS THAT ARE NEEDED TO ITERATE A POPULATION SPECIFIC C VALUE
def f_1_1(t):
    x = 1.0
    for i in range(k):
        for j in range(k):
            x += p[i] * p[j] * f[t-1][i][j]
    return x

def f_1_j(t, j):
    x = 0.0
    for i in range(k):
        x += p[i] * f[t-1][i][j-1]
    return x

def f_i_i(t, i):
    x = 1/l[i] - 1/l[i-1] + f[t-1][i-1][i-1]
    return x

def f_i_j(t, i, j):
    x = f[t-1][i-1][j-1]
    return x
```

```

# *-*-*-*-* THE MAIN PROGRAM *-*-*-*-*
print """This program calculates an effective population size estimate
based on the Jorde & Ryman (1995) method for overlapping populations."""

# DEFINE POPULATION SPECIFIC PARAMETERS BASED ON USER INPUT
l = raw_input("Enter l (survival probability to each age, separated by a
comma, e.g. 1, 0.97, 0.95):").split(',')
p = raw_input("Enter p (the proportional contributions to gamete pool by
each age group, separated by a comma, e.g. 0.1, 0.27, 0.63):").split(',')
for x in range(len(l)): l[x] = float(l[x])
for x in range(len(p)): p[x] = float(p[x])
G = float(input("Enter G (generation length):"))
N1 = float(input("Enter N1 (n. of newborns entering population each
year):"))
n1 = float(input("Enter n1 (n. of individuals in the first sample:"))
n2 = float(input("Enter n2 (n. of individuals in the second sample:"))
F = float(input("Enter F (variance of allele frequency change:"))
k = len(p)

# INITIALIZE A 3D MATRIX FOR CALCULATING VALUES WITH PARAMETERS t, i & j
f = [[[0.0 for n in range(k)] for n in range(k)] for n in range(200)]

# ITERATE THE FUNCTIONS IN TIME (t) BASED ON USER-DEFINED PARAMETERS
for t in range(200):
    for a in range(k):
        for b in range(k):
            f[t][a][b] = function_chooser(t, a, b)

# INITIALIZE C
C = [0.0 for n in range(199)]

# ITERATE C THROUGH (t), 1-200
for t in range(199):
    C[t] = (f[t][0][0] + f[t+1][0][0] - 2.0 * f[t+1][0][1]) /
(f[t+1][0][0] - f[t][0][0])

# CALCULATE NE BASED ON ITERATED C
Ne = C[198] / (2 * G * (F - (1/ (2 * n1)) - (1/(2 * n2)) + (1/N1)))

# PRINT THE FINAL RESULT
print "The effective population size estimate is ", Ne, "."

```